W1: DIABETES

MoW1:1 Why is the protection provided by female gender wiped out in diabetes
B.V. Howard, MedStar Research Institute, Wash., DC, USA
Diabetes has been shown to be a significant independent risk factor for cardiovascular disease (CVD), especially in women. Review of population data reveals that the prevalence of type 2 diabetes is higher in women and it increases the risk of CVD in women to a greater extent than in men. The dyslipidemia characteristic of diabetes, hypertension and other adverse changes in CVD risk factors have been shown to play a major role in the development of CVD in individuals with diabetes. More adverse changes in several CVD risk factor occur in diabetic women; mechanisms for this may include the insulin resistance that accompanies type 2 diabetes, and diabetes-associated changes in sex hormones. These findings highlight the need for increased attention to CVD risk factors in the care of diabetic women and for further research into the mechanisms of the accelerated atherosclerosis in diabetes.

MoW2:1 Vascular effects of insulin
Hannele Yki-Järvelin, Department of Medicine, University of Helsinki, Helsinki, Finland
Insulin resistance has been associated with the development of cardiovascular disease and hypertension but the mechanisms are poorly understood. In vivo in normal subjects, insulin regulates vascular function at multiple sites. In peripheral resistance vessels, insulin acts as a slow vasodilator via a mechanism, which can be abolished by inhibiting nitric oxide synthesis, and is masked by sympathetic vasoconstrictor tone. Defects in insulin action on peripheral vasodilatation have been described in various insulin resistant states. These defects have, however, been observed at supra- rather than physiological insulin concentrations or after prolonged infusions of insulin, which make them unlikely to contribute to hemodynamic alterations such as increased peripheral vascular resistance and diastolic blood pressure in insulin resistant individuals. Another recently described action of insulin involves acute insulin induced decreases in arterial stiffness in pre-resistance vessels in normal subjects. This action of insulin is observed at physiological insulin concentrations within 30 min. A defect in insulin regulation of arterial stiffness characterizes insulin resistant individuals including those with insulin resistance secondary to obesity or chronic hyperglycemia. The ability of insulin to diminish stiffness is also blunted in insulin resistant subjects with untreated essential hypertension. The inability of insulin to decrease stiffness increases augmentation i.e. the pressure difference between the second and first systolic peak of the aortic pressure wave and thereby increases pre-load and decreases diastolic filling of the left ventricle. The mechanisms underlying these changes in humans remain unknown due to the inaccessibility of pre-resistance arteries. Taken together these data demonstrate the existence of resistance to insulin's antiatherogenic actions in arteries greater than those controlling peripheral vascular resistance.

MoW3:1 Plasminogen activator inhibitor type 1: Influence of metabolic factors and pharmacological control
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PAI-1 is a glycoprotein synthesized by cells of the vessel wall, adipose tissue and hepatocytes. Both environmental and genetic factors are involved in the biosynthesis of this glycoprotein. Recent data have demonstrated a crucial role of metabolic variables such as insulin and/or insulin resistance, obesity, triglycerides and blood pressure as determinants of circulating PAI-1 in healthy and diseased subjects, thus suggesting a link between the insulin resistance syndrome and fibrinolytic system. In support of this, in vitro studies have demonstrated that insulin and triglycerides stimulate PAI-1 biosynthesis by cultured endothelial and hepatic cells. Moreover, insulin and triglycerides, either directly or through an effect mediated by long-chain fatty acids act synergistically in inducing PAI-1 synthesis in these cells. Angiotensin II has also been shown to upregulate PAI-1 synthesis in human endothelial and smooth muscle cells. The mechanisms by which these metabolic factors influence PAI-1 have been recently elucidated and involve the activation of a complex network of signaling pathways leading to the activation of the MAP kinase cascade. The knowledge of the mechanisms involved in PAI-1 biosynthesis is of particular importance for the development of specific therapeutic strategies to decrease the cardiovascular risk in these patients. At present, triglyceride lowering drugs, statins, angiotensin converting enzyme inhibitors and/or angiotensin receptor type 1 inhibitors are under evaluation also for their potential effects in controlling PAI-1 biosynthesis.

MoW4:1 The atherogenic dyslipidemia of visceral obesity
J.P. Despres1, 1Quebec Heart Institute, Lipid Research Center, Laval University, Sainte-Foy, Canada
Studies published over the last 20 years have emphasized the role of body fat distribution, especially of visceral adipose tissue accumulation as a critical correlate of metabolic abnormalities that were in the past associated with excess fatness per se. Thus, excess visceral adipose tissue accumulation, which can be assessed by imaging techniques such as computed tomography, has been associated with hyperinsulinemia, insulin resistance, glucose intolerance which may lead to NIDDM among genetically susceptible individu- als, hypertriglyceridemia, elevated LDL particle concentration, increased proportion of small-dense LDL and HDL particles and reduced plasma HDL cholesterol concentrations leading to a substantial increase in the cholesterol/HDL-cholesterol ratio. Results of the prospective Quebec Cardiovascular Study have emphasized that this cluster of metabolic abnormalities is associ- ated with a substantial increase in the risk of coronary heart disease (CHD). As most visceral obese patients have rather "normal" cholesterol levels, it is suggested that CHD risk in these individuals should be assessed, in addition to conventional risk variables, by a triad of metabolic abnormalities which include hyperinsulinemia, hyperapo B and small-dense LDL particles. We have also developed a low cost screening approach where a high proportion of visceral obese individuals with the insulin resistance dyslipidemic syndrome could be identified. We have reported that more than 80% of men with waist circumference values above 90 cm and with fasting triglyceride levels above 2.0 mmol/L were characterized by the atherogenic metabolic triad (hyperin- sulinemia, elevated apo B and small dense LDL phenotype). It is proposed that simple and inexpensive variables such as the waist circumference and fasting triglyceride levels may be helpful markers for the screening of high risk patients.

MoW5:1 Postprandial hyperglycemia is a risk factor for cardiovascular death in newly diagnosed type 2 diabetes: The Diabetes Intervention Study (DIS)
M. Hunfeld1, U. Julius1, H. Schmelleck2, U. Schwanebeck1, 1Medical Faculty C. G. Carus Dresden, 2Weimar, Germany
Objective: Prospective studies have consistently shown that the risk of myocardial infarction (MI) and cardiovascular death (CD) in type 2 diabetes is 2-4 fold higher than in non-diabetic subjects matched for major risk factors. We therefore analysed the importance of fasting and postprandial blood glucose (ppBG) on the incidence of MI and CD.
Methods: 1139 newly diagnosed type 2 diabetics classified as diet controlled with no other severe diseases. Follow-up time ≥ 11 years. BG was measured after 4 weeks on diet before and one hour after breakfast, major risk factors were recorded under standard conditions. Statistics: Cox regression analysis and cumulative incidence stratified by gender and categories of quality of risk factor control according to NIDDM Policy criteria.
Results: 11 year follow-up data were available for 828 patients of which 71 (8.6%) died from CD. In multivariate analysis age, blood pressure, triglycerides and smoking were independent risk factors for MI and age, male sex, ppBG, triglycerides and blood pressure for CD resp. In Cox regression analysis comparing categories of quality control of risk factors smoking (relative risk RR vs. non-smokers 1.51), ppBG (RR poor vs. good control 2.09) and blood pressure (RR poor vs. good control 2.2) for subsequent MI and smoking (RR 1.65) poor ppBG (RR 2.24), poor blood pressure (RR 3.23) and poor
triglycerides (RR 2.18) for CD resp. were of significant importance. PpBG was a stronger predictor in females than males.

**Conclusions:** Our data show that pp hyperglycaemia is an independent risk factor for coronary heart disease in type 2 diabetes which could substantially add to the excessive cardiovascular mortality in these patients.

**W.2 THROMBOSIS AND FIBRINOLYSIS**

**MoW1.2**

**Thrombosis and fibrinolysis: Lessons from clinical trials**

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Clinical trials nearly always have their origin in observational studies showing associations between a medicine or a putative risk factor that it appears valuable to modify and the occurrence of clinical events such as myocardial infarction and coronary death. However, confounding and selection biases can never be excluded as explanations for the results of observational studies. A randomised controlled trial ensures that the groups under comparison are identical in all respects. A difference in outcome following an intervention can then confidently be attributed to the intervention. One objective of a randomised trial may be to try to confirm or refute the causal contribution of a risk factor. Most trials are of course undertaken to evaluate the place of treatments in preventive and clinical practice. Platelet-active agents and anticoagulants are of proven worth in primary prevention, the clinical management of acute events and in secondary prevention. The remarkable success of thrombolytic therapy in early suspected myocardial infarction, adds to the evidence that the level of fibrinolytic activity is also involved. There is increasing interest in the potential value of combination therapy, either with platelet-active agents and anticoagulants or with platelet-active agents with different actions – provided there is no unacceptable increase in the risk of bleeding. Many newer agents are under consideration, a major contrast compared with older treatments being that it will still be some years before their full safety profiles have been established. An important outstanding question is the value of lowering raised fibrinogen levels. Nearly all trials throw up unexpected findings. Whether these are chance observations or suggest real effects (e.g. greater benefit in one group than another) is difficult to judge but since there is a limit to the number of trials that can be carried out, the clinical significance or otherwise of results that were not anticipated has to be considered.

**MoW2.2**

**Functional mutations predisposing to arterial thrombosis**

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The human genome shows in individuals on average in one in 500 nucleotides a sequence variation some of which may be of significance for developing arterial disease or for the response to intervention or prevention. In addition to silent mutations, mutations in protein sequence with consequences for the function and mutations in the regulatory sequences for protein production do occur. Haemostatic factors are important in arterial thrombosis. In these factors genetic variation in function and regulation has been observed. They concern amongst others fibrinogen, factor VII, GP IIb/IIIa, t-PA, PAI-1, factor XII and hirudin. The genetic studies of fibrinogen have shown that both precursor and mature fibrinogen have an increased prothrombotic effect. The nature and consequences of these genetic polymorphisms are currently the subject of intense research.

**MoW7.1**

**Plasma cholesterol ester transfer and hepatic lipase activity are determinants of low high density lipoprotein cholesterol associated with insulin resistance in type 2 Diabetic and non-diabetic subjects**

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**Objectives:** To evaluate the hypothesis that rates of plasma cholesterol ester transfer (CET) and lipase activities are influenced by insulin sensitivity (IS) and contribute to the low high density lipoprotein cholesterol (HDL-C) levels observed in type 2 diabetic (DM) and insulin resistant non-DM subjects.

**Methods:** IS was measured as the glucose infusion rate (M-value) during the last 6 h of a 3 h euglycaemic hyperinsulinaemic clamp (150 mU/kg/h, blood glucose target 4.6 mM) in 16 DM and 16 non-DM men. DM and non-DM subjects were divided in equal groups with low or high IS. Post-heparin plasma lipoprotein lipase (LPL) and hepatic lipase (HL) activities were measured in plasma samples obtained 1–2 weeks before the clamp. CET was measured using a radioisotope method.

**Conclusions:** CET with high IS, plasma CET was lower than in the other groups (p = 0.05 for all). Plasma CET (R = −0.62, p < 0.001 in all subjects combined, n = 32) and HL activity (R = −0.49, p < 0.01, n = 32) but not LPL activity (R = 0.01, n = 32) were inversely correlated with the M-value. HDL-C was also inversely correlated with plasma CET (R = −0.48, p < 0.01, n = 32) and with HL activity (R = −0.47, p < 0.01, n = 32).

**MoW3.2**

**The endogenous fibrinolytic enzyme system: Regulation and significance for cardiovascular disease**

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Impaired fibrinolytic function secondary to elevated plasma plasminogen activator inhibitor-1 (PAI-1) activity is associated with precocious coronary heart disease (CHD) and with recurrent cardiovascular events in subjects with manifest CHD. Molecular explanations of clinically important gene-environment interactions that determine the regulation of PAI-1 are now emerging. From a clinical perspective elevated plasma PAI-1 activity can be considered as a component of the insulin resistance syndrome. A common
DNA-protein interactions at the t-PA promoter shear stress responsive element in intact human conduit vessels exposed to high shear stress


Objective: We recently showed that shear stress increases tissue-type plasminogen activator (t-PA) gene expression and protein synthesis in endothelial cells of intact human conduit vessels. We tested the hypothesis that this effect is mediated by interaction of NF-KB with the shear stress responsive element (SSRE) of the t-PA promoter, as shown for PDGF-B.

Methods: Human umbilical veins were perfused at high or low laminar shear stress (25 vs <4 dyn/cm²) and identical intraumbilical pressure (20 mmHg) for 1.5, 3, and 6 h in a computerized bio-mechanical perfusion system. Total RNA or nuclear proteins were extracted from explanted endothelial cells. t-PA mRNA was quantified after reverse transcription with TaqMan real-time PCR using GAPDH as endogenous control. DNA-protein interactions were studied by electrophoretic mobility shift assay (EMSA).

Results: t-PA mRNA increased by 54% (p = 0.002) relative to control after 6 h of high-shear perfusion. A distinct, transient SSRE interaction peaking after 1.5 h was observed with no difference in band shifts between high and low sheared vessels. Competition experiments indicated that NF1 but not NF-KB was involved in this interaction. With labelled consensus sequences, NF1-binding showed a shear-independent up-regulation with a similar time course as the SSRE interaction, whereas NF-KB complex formation was shear-dependently upregulated after 1.5 and 3 h.

Conclusion: Induction of t-PA gene expression by shear stress in intact pressurized vessels appears to be independent of interactions with its promoter SSRE. Since two binding sites for NF1 are present within and close to the t-PA SSRE, it is possible that the interaction of an NF1-like protein with this region may sterically hinder shear-induced NF-KB to interact with SSRE in this particular gene.
W:3 Imaging of Atherosclerosis

MoW1:3 Quantitative angiography and intravascular ultrasound as research tools for the imaging of atherosclerosis
P. J. de Feyter. Thoraxcentrum, Rotterdam, The Netherlands
Quantitative coronary angiography (QCA) has been used as a surrogate end-point to study progression/regression of coronary atherosclerosis in several (MARS, CCAT, MAAS, REGRESS, HARP, PLACI) intervention studies using cholesterol lowering treatment. Indeed progression of disease could be retarded but not stopped and occasionally a lesion regressed. However, QCA is luminography so that only the "fingerprints" of atherosclerosis can be detected if the disease is progressed so far that it encroaches upon the lumen. During the development of early lesions the coronary lumen is preserved due to remodelling of the coronary artery and thus is angiographically unnoticed. This is the main reason that angiography seriously underestimates the extent of CAD.
Intracoronary ultrasound (IVUS) is a tomographic technique which allows to study the coronary wall and the lumen.
User interactive border detection algorithms allow precise quantification of coronary plaque, and lumen whereas sequential measurements of a coronary vessel segment provide volumetric quantification of the plaque and permits investigations of the remodelling process. Various long-term randomized interventional studies are now being conducted which use both QCA and IVUS techniques to provide further insights into the mechanisms of plaque progression/regression and coronary artery remodelling.
The results of these studies will become available within a few years.

MoW2:3 Extravascular ultrasound and CT measures of coronary calcium as research tools to better understand early atherosclerosis/subclinical disease
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Objective: To describe the importance of different diagnostic tools used to evaluate atherosclerosis and subclinical disease.
Methods/Results: Non-invasive imaging of atherosclerosis/subclinical disease is essential to facilitate further reductions in cardiovascular disease (CVD) related morbidity and mortality. Carotid ultrasound, extravascular ultrasound and CT are a proven strategy for evaluation of intimal medial thickness and stenosis. Intimal medial thickness has been shown to be related to traditional CVD risk factors and is an independent predictor of CVD outcomes. Brachial artery ultrasound assessment of flow-mediated vasodilatation has been linked to CVD risk factors, however, less data is available on the association with CVD outcomes. CT Coronary artery calcium (CAC) is high correlated with coronary angiography findings and is associated with CVD risk factors and outcomes. CAC measures have the distinct advantage of evaluating atherosclerotic burden in the coronary bed. Conversely, much less is known about the importance of change in CAC (i.e., does it reflect true atherosclerotic progression or plaque stabilization?). Future opportunities include using MRI to evaluate plaque characteristics in the carotil and coronary beds.
Conclusions: The different subclinical disease measures may individually provide valuable information for the earlier detection of higher risk populations to facilitate more effective interventions for reduction of future CVD burden. Opportunities may exist to use a combination of these different subclinical measurements to better understand the etiology of early subclinical disease. New research studies are currently underway to better determine the incremental gain of using these subclinical measures in prediction of CVD outcomes beyond traditional CVD risk factors.

MoW3:3 Low dose metoprolol and fluvastatin slow progression of atherosclerosis: Main results from BCAPS
G. Berglund, J. Wikstrand, L. Jaxzon, H. Wedel, B. Hedblad. For the BCAP study group: University Hospital, Malmö, Sweden
Background/Objectives: Several trials have shown that statins can slow the progression of atherosclerotic events, sudden deaths and death from heart failure, but anti-atherosclerotic effects have not been shown in humans. The Betablocker Cholesterol-lowering Asymptomatic Plaque Study (BCAPS) aimed at assessing the effects of low dose metoprolol and fluvastatin alone and in combination on carotid atherosclerosis.
Methods: Degree of atherosclerosis was determined with B-mode ultrasound. Asymptomatic subjects with an atherosclerotic plaque in the right carotid bifurcation (n = 793) were randomly allocated to fluvastatin 40 mg once daily, metoprolol 25 mg once daily, the combination or placebo for 36 months. Subjects were seen every sixth month and ultrasound of the right carotid artery was performed initially and after 18 and 36 months. Mean intima-media thickness (IMTmean) over 10 mm in the common carotid artery and maximal intima-media thickness in the bifurcation (IMTmax), were the main effect variables. Death and cardiovascular events were monitored although insufficient statistical power was foreseen.
Results: Fluvastatin significantly reduced the rate of progression of the IMTmean (P < 0.001) compared to placebo. Metoprolol significantly reduced the progression of IMTmax (p = 0.003). The combination of the two drugs reduced both measures of progression compared to placebo. First cardiovascular event tended to be lower in the metoprolol (n = 6) than in the placebo group (n = 13), although not significant, p = 0.055.
Conclusion: This is the first evidence in humans that a betablocker can slow progression of carotid atherosclerosis. As previously shown the statin also slowly progression of carotid atherosclerosis. Further studies are of interest to clarify the different effect of the two drugs on signs of atherosclerosis in the common carotid and in the bifurcation.

MoW4:3 Coronary calcification score and predicted risk of coronary heart disease
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Objective: Lipid-lowering therapy is less cost effective in the primary prevention of coronary heart disease (CHD) than in secondary prevention because only a minority of those at high risk will sustain an event if left untreated. This study assesses quantification of coronary calcification by electron beam computed tomography (EBCT) as a means of discriminating between hypercholesterolaemic subjects with and without pre-clinical CHD.
Methods: Asymptomatic men aged 45-64 with a plasma cholesterol ≥ 6.5 mmol/l were further selected according to whether their absolute risk of CHD was low (≤10%/10 years) or high (≥20%/10 years), computed with the Framingham Equation. Of 286 eligible subjects, 223 underwent EBCT scanning.
Results: The mean log coronary calcification score was significantly higher in the 97 high risk men than in the 189 low risk men (1.58 ± 0.84 v 1.00 ± 0.85, P < 0.001), arithmetic means 158 ± 55, and the proportion with a high coronary calcification score (>400) was greater (11% v 2%, P < 0.01). However, 27% of the high risk group had a low coronary calcification score (<10), which is known to be associated with minimal angiographic abnormalities.
Conclusion: The observation that over a quarter of hypercholesterolaemic men with an absolute risk of CHD of ≥20%/10 years had a coronary calcification score indicative of a low likelihood of significant coronary artery disease is novel. However, uncertainties about the predictive power of coronary calcification for clinical events must be resolved before EBCT scanning can be validated as a means of screening asymptomatic subjects at high risk of CHD.

MoW5:3 Noninvasive imaging and quantitation of atherosclerosis with radiolabeled oxidation-specific antibodies
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Methods to detect preclinical, "high risk", atherosclerotic lesions are urgently needed. Current noninvasive methods do not directly assess the presence of oxidized LDL (OxLDL) which is enriched in vulnerable plaques. We have developed monoclonal antibodies that bind to epitopes of OxLDL. Intravenous injection of 125I-MDA2, a prototype oxidation-specific antibody that binds to malondialdehyde-lysine epitopes on LDL (MDA-LDL), resulted in 8-10 fold higher uptake in plaque vs. normal tissue within the same aortas in Watanabe rabbits and mice with atherosclerosis. Autoradiography showed that 125I-MDA2 accurately reflected the lipid-strained lesions whereas no signal was seen in areas with normal tissue. Aortic 125I-MDA2 uptake showed a linear correlation with traditional lesion parameters (% surface area covered by lipid stained lesions and aortic weight). In a dietary regression study in LDLR/−/− mice, in vivo uptake of 125I-MDA2 correlated well with the % surface area and aortic weight in progressing atherosclerosis but was more sensitive in detecting the depletion of OxLDL following a regression diet with or without antioxidants. Another antibody titers (IgM and IgG) to Cu-OxLDL and MDA-LDL increased up to 80% with the high fat diet but decreased with the

regression diet (chow or chow + vitamins E and C) and also correlated well with 125I-MDAa uptake. 99mTc-MDAa imaging of live Watanabe rabbits showed accurate detection of atherosclerotic lesions. Because murine antibodies have several disadvantages in human applications, we have cloned a human antibody to OXLDL in the 1K17, which binds to both the oxidized lipid and protein moiety of OXLDL and has similar in vivo aortic uptake patterns. These studies describe a novel approach that could lead to noninvasive imaging, quantitation and surveillance of OXLDL-rich lesions in humans.

MoW6.3 Are small lesions in atherosclerotic rabbit aorta shown by histology visible on magnetic resonance images?

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Objective: Our objective is to develop MRI methods to image the detailed structure of atherosclerotic lesions using a rabbit model of atherosclerosis.

Methods: 18 New Zealand White (NZW) rabbits were fed a diet supplemented with 0.2% cholesterol. The abdominal aortas of the rabbits were injured using a 4F Fogarty embolectomy catheter at a balloon pressure of 1 atmosphere. MRIs were acquired with a fast spin-echo technique either with or without fat suppression on a 2T Bruker Medspec at 20 to 30 weeks after injury. Image resolution was 250 × 250 × 2000 micrometer. After imaging the rabbits were culled and their aortas were examined by histology. MRI images were compared with histological sections.

Results: All 18 rabbits used in the study developed atherosclerosis shown by MRI and histological examination of the thickness of the lesions produced by balloon injury in 0.2% cholesterol fed NZW rabbits was variable. It was up to 1 millimeter, well within the resolution of MRI. MRI images showed marked thickening of the aorta wall. Histology confirmed the presence of extensive intima remodeling and enlargement in the same regions. We found that not only large but as small as 300 micrometer thick lesions are visible on MRI.

Conclusion: Small atherosclerotic lesions of 300 micrometer can be readily imaged by MRI in NZW rabbits.

W4 INFECTIONS, CHD, AND ATHEROSCLEROSIS

MoW1.4 Consistency between infection and atherosclerosis

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Already in 19th century inflammation caused by infection was proposed to be behind atherosclerosis and septic bacterial infections in experimental animals caused lesions resembling atherosclerosis. The idea was revived when herpesviruses were found to cause atherosclerosis in animals. Human herpesviruses, HSV-1 and CMV have been associated with atherosclerosis and restenosis, and enteroviruses have been mentioned. Also bacteria, like Helicobacter pylori and dental pathogens, are suspected, but the most compelling evidence is from Chlamydia pneumoniae, a small gram-negative intracellular bacterium, which is a common cause of respiratory tract infections worldwide. Its association with atherosclerosis was found with seroepidemiology in 1988 and now nearly 30 studies have confirmed the original observations.

Moreover, the presence of the agent in the lesions, discovered in 1992, has been verified in over 30 studies. Successful animal experiments have been reported from 1997 onwards and two out of the three preliminary intervention studies with antibiotics were positive. The presence of an infectious agent in the atherosclerotic lesions could easily explain their inflammatory nature, and in vitro several mechanisms how C. pneumoniae could cause these alterations have been demonstrated. When waiting the results of larger intervention trials we should find out in animal and in vitro experiments the pathogenetic mechanisms of chronic C. pneumoniae infections, how they diagnose them in patients, and what type of treatment is effective in eradicating them.

MoW2.4 Infections and atherothrombotic risk: The role of sero-epidemiology

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The possible role of infection in the etiology of atherosclerosis has long been of investigative interest. However, the contribution of sero-epidemiologic studies of infection and atherothrombotic risk remains a source of controversy, in part because the findings from prior studies appear inconsistent. While cross-sectional sero-epidemiologic studies have suggested possible associations of prior infection with atherothrombotic risk, recent reports from several prospective studies have failed to demonstrate associations between the presence of IgG antibodies to Chlamydia pneumoniae, herpes simplex virus, type 1 (HSV-1), and cytomegalovirus (CMV) and incident myocardial infarction. In general, evidence from pathologic, animal-experimental, and molecular studies support a possible etiologic role of infection in atherosclerosis. For these reasons, some have questioned the contribution of sero-epidemiologic studies. In this presentation, we review the major findings from sero-epidemiologic studies in the context of other research paradigms, explore alternative explanations for the inconsistent findings, and suggest a further role for sero-epidemiologic studies of infection and athero-thrombotic risk.

MoW3.4 Identifying and treating patients in the framework of infection and atherosclerosis

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The occurrence of inflammation in atherosclerotic lesions, which is mediated at least to some extent by cellular immune mechanisms, is now recognized. There are serological, pathological evidences that show an association between Chlamydia pneumoniae and acute myocardial infarction. These findings suggested a potential contribution of the cytopathologic effects of infection, including the inflammatory response of the endothelium to atherosclerosis. In addition, new data suggest that the HLA system and HSP-60 may be implicated in this process.

The ROKIS Pilot Study, was the first clinical trial with a primary clinical endpoint to prove the efficacy of antibiotics in coronary artery disease. Patients treated with roxitromycin had a significantly lower rate of combined clinical endpoints. These early results need confirmation by large scale studies not only to prove the efficacy of antibiotic treatment, but also to define the potential population to be treated and how long should be the optimal treatment period.

MoW4.4 Folate deficiency, hyperhomocysteinemia and Chlamydia pneumoniae: Who came first?

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Objective: We investigated a hypothesized interference of plasma homocysteine(eine (Hcy) concentrations and Chlamydia pneumoniae infection in patients with coronary artery disease (CAD).

Methods: Fasting plasma homocyst(eine and IgG antibody titers against Chlamydia pneumoniae (C.p.) were measured in 184 male CAD patients under 60 years of age.

Results: 35 patients were hyperhomocysteinemic (Hcy > 14 μmol/L) and Gp A vs 149 patients with Hcy levels < 14 μmol/L. Group B). Prevalence of IgG seropositivity against C. p. was significantly higher in patients of group A (66% vs. 41%, p = 0.007), as were also mean antibody titers (p = 0.026). Hcy was significantly associated with folate levels (p = 0.018) and hypertension (p = 0.007). Age, smoking, body mass index, vitamin B6 and B12, diabetes and lipids were not associated with either hyperhomocyst(eine or IgG seropositivity.

Conclusions: Elevated plasma Hcy levels are associated with chlamydial IgG seropositivity in patients with CAD. Causality remains elusive at present. Explanations for our findings include damage of vascular cells through high Hcy concentrations and/or cell lysis after replication of C. p. with increased mutual susceptibility. Folate deficiency increases Hcy, but may also impair cell-mediated immunity with higher rates of intracellular infections.

MoW5.4 Leucocytes count and fibrinogen level are associated with carotid and femoral intima-media thickness

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Objective: Inflammatory processes are supposed to play a role in atherogenesis. The intima-media thickness (IMT) of the carotid and femoral artery is an accepted marker of atherosclerosis. The aim of this study was to examine the relationship of leucocytes count and fibrinogen level to carotid and femoral IMT as well as to known risk factors for atherosclerosis.

Methods: A total of 597 subjects, aged 40 to 70 years, were analysed from the Risk factors in IGT for Atherosclerosis and Diabetes (RIAD) Study. Carotid and femoral IMT was determined by B-mode ultrasonography. A variety of cardiovascular risk factors were measured by established methods.

Results: In univariate analysis carotid and femoral IMT was significantly correlated to leukocytes count and fibrinogen level. Leucocytes count significantly correlated to blood pressure, body mass index, waist to hip ratio, triglycerides, high-density lipoprotein cholesterol, insulin resistance (HOMA), fibrinogen, plasminogen activator inhibitor, tissue plasminogen activator, mi-croalbuminuria, smoking and low physical activity as well as to fasting and postprandial levels of plasma glucose, proinsulin and specific insulin. Fibrinogen was significantly related to blood pressure, body mass index, total cholesterol, triglycerides, insulin resistance (HOMA), plasma glucose, von Willebrand factor, plasminogen activator inhibitor, tissue plasminogen activator, alcohol consumption and low physical activity. In multivariate analysis leukocytes count was an independent determinant of the maximal carotid IMT and fibrinogen level – of carotid and femoral IMT.

Conclusions: Our data support the hypothesis that inflammatory processes could contribute to carotid and femoral atherosclerosis.

MoW6:4 Antibodies to Chlamydia pneumoniae react non-specifically with lipid in macrophages in human atherosclerotic arteries
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Objective: To detect Chlamydia pneumoniae in the human atherosclerotic arterial wall by immunohistochemistry.

Methods: Avidin-biotin complex immunohistochemistry on 30 paraffin embedded carotid artery endarterectomy specimens with antigen retrieval by microwaving. Chlamydia pneumoniae antigens were recognised by antibodies to HSP60, MOMP, LPS, and MOMP/LPS. Controls were 5 mouse plasmacytoma immunoglobulins of the same Ig classes.

Results: Surprisingly, all Chlamydia antibodies gave extensive staining of lipid-laden macrophages in the atherosclerotic plaques of every specimen. The reactivity was too extensive to be explained by Chlamydia infection. A lymosomal granular pattern was obtained, similar to that produced by a CD68 antibody, but not to the distribution of lipofuscin, an insoluble advanced oxidation product of lipoprotein. Four of five control mouse immunoglobulins used at the same concentrations gave little or no reactivity, but one gave similar but weaker staining.

An ELISA assay for reactivity against human LDL showed increased binding by the Chlamydia antibodies compared to the control mouse immunoglobulins.

Conclusion: Paraffin section immunohistochemistry can show a novel ability of immunoglobulins to react with LDL in atheroma macrophages. The panel of Chlamydia antibodies all showed this reactivity. It can give positive results in the detection of arterial wall Chlamydiae by immunohistochemistry.

MoW7:4 Antibodies to Chlamydia pneumoniae relate to the intima-media thickness in the carotid artery and to an atherogenic fatty acid profile
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Dept of Internal Medicine; Geriatrics, University Hospital, Uppsala; Microbiology, Gävle Hospital; Medicine, Danderyd hospital, Sweden

Objective: To investigate the impact of IgA antibodies to Chlamydia pneumoniae in the intima-media thickness in the carotid artery and the lipid profile.

Methods: IgA antibodies to Chlamydia pneumoniae, the intima-media thickness in the carotid artery (far wall in the common artery), lipoproteins and the free fatty acid composition in cholesterol esters and phospholipids were investigated in a population sample of 56 healthy middle-aged subjects.

Results: Eight of the subjects showed detectable IgA antibodies to Chlamydia pneumoniae. These subjects showed an increased intima-media thickness in the carotid artery compared to the others (0.85 ± 0.15 SD vs 0.75 ± 0.17 mm, p < 0.05). Neither age, nor the lipoprotein pattern of the subjects differed, but the subjects with antibodies showed an increased proportion of dihydroxymalonic acid and a reduced arachidonic to dihydroxymalonic acid ratio (both p < 0.05) when compared to those without.

Conclusions: The occurrence of IgA antibodies to Chlamydia pneumoniae is related to the intima-media thickness in the carotid artery and to an atherogenic fatty acid profile in apparently healthy individuals.

MoW3:5 Role of shear and pressure in regulation of PDGF-B in intact human conduit vessels
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Objective: PDGF-B is a potent vascular growth factor involved in the vascular remodeling process. In the present study, we investigated the possible roles of combined shear and pressure on PDGF-B expression at a whole-veessel level.

Methods: Human umbilical veins were exposed to high vs low shear stress (25 vs -4 dyn/cm²) at identical intraluminal pressure (20 mmHg) or high vs low pressure (40 vs 20 mmHg) at identical shear stress (10 dyn/cm²)
for 1.5, 3 or 6 h in a new computerized perfusion system. Endothelial cells were cultured with collagenase treatment and total RNA was extracted. After reverse transcription, PDGF-B gene expression was quantified by real-time RT-PCR using Taqman probe and primers (reverse primer spanning exon junction). G3PDH was used as endogenous control. Localization and semi-quantification of PDGF-B protein expression was achieved by computer-aided semi-quantitative immunohistochemistry.

Results: PDGF-B protein expression was localized mainly in the vascular endothelium. Shear stress and pressure induced significantly different temporal regulation patterns of PDGF-B gene expression (ANOVA, p = 0.006). After an initial transient upregulation, the PDGF-B gene expression was significantly down-regulated by 39% after 6 h shear perfusion, while in pressure stimulated vessels an 89% upregulation was detected (contrast analysis, p = 0.01).

Conclusions: Shear stress and pressure exerts differential regulating effects on PDGF-B gene expression. This is of importance for understanding the function of PDGF in a complex hemodynamic environment.

MoW4.5

Elimination of PDGF-B from only the circulating cells of APOE(−/−) mice significantly impacts atherosclerotic lesion formation

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Platelet-derived growth factor (PDGF) is a potent stimulant of smooth muscle (SMC) migration and proliferation in culture. To test the role of PDGF in the accumulation of SMC in vivo, we evaluated ApoE(−/−) mice that develop complex lesions of atherosclerosis. Although targeted deletion of the PDGF genes is embryonic lethal, we have developed chimeric mice in which fetal liver cells from PDGF-B-deficient embryos were used to replace the circulating cells of lethally irradiated ApoE(−/−) mice. One month after transplant, all monocytes in PDGF-B(−/−) chimeras are of donor origin (lack PDGF), and no PDGF-BB is detected in circulating platelets, primary sources of PDGF in lesions. Although lesion volumes are comparable in the PDGF-B(+/+) and (−/−) chimeras at 35 weeks, lesions in PDGF-B(−/−) chimeras contain mostly macrophages, appear less mature, and have a marked reduction in fibrous cap formation as compared with PDGF-B(+/+) chimeras. Data from 45-week animals are being evaluated for the extent of SMC accumulation in lesions at a very late time point. Gene array analysis of peritoneal macrophages from PDGF-B(+/+) and (−/−) chimeras suggests that the absence of PDGF alters macrophage gene products that may contribute to modified lesion formation in PDGF-B(−/−) chimeras. Thus, elimination of PDGF-B from circulating cells in ApoE(−/−) mice is sufficient to significantly reduce SMC infiltration into lesions and delay lesion progression.

(Supported in part by NIH grant HL 18645 to EWR and RR and by grant Ka 1078/1 from the Deutsche Forschungsgemeinschaft to WEK)

MoW5.5

Proto-oncogene product Crk differentially interacts with PDGF α- and β-receptors

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Objective: To identify intracellular proteins which are involved in signal transduction pathway specific for the PDGF α-receptor.

Methods: Affinity purification using an immobilized synthetic peptide containing phosphorylated Tyr-762 in the PDGF α-receptor was performed. Wild-type or tyrosine-residue mutated PDGF α-receptors were stably transfected into porcine endothelial (PAE) cells. The cells were treated with PDGF-BB, lysed and subjected to immunoprecipitation followed by Western blotting. Tyrosine phosphorylated GST fusion protein of CrkII was produced in E. coli encoding inducible tyrosine kinase gene.

Results: Proteins in HeLa cell lysate of molecular sizes 27, 38 and 40 kDa bound to the phosphorylated but not to the unphosphorylated peptide. Partial amino acid sequences of the purified proteins indicated that they were identical to SH2-containing proto-oncogene products CrkI, CrkII and CrkL, respectively. CrkII bound to wild-type but not to Y762F PDGF α-receptor upon ligand-stimulation of PAE cells. In contrast, association between CrkII and PDGF β-receptor was negligible, whereas CrkL became prominently phosphorylated by the β-receptor. GST-CrkII fusion protein could bind activated PDGF β-receptor in vitro, and the association was diminished by tyrosine phosphorylation of the fusion protein.

Conclusion: Tyr-762 in the PDGF α-receptor serves as the binding site for CrkI. CrkII can also bind to PDGF β-receptor in vitro. However, CrkII can hardly associate with the β-receptor in vivo, because internal tyrosine phosphorylation of CrkII negatively regulates its binding to the target molecules. Differential binding of CrkII to the PDGF α- and β-receptors may be a rationale for functional diversity of the two receptors.

MoW6.5

Cytokine regulation of endothelial cell function: New molecules in an old paradigm

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IL-1 is a prototypic activator of endothelial cells which induces expression of a set of proinflammatory/proinflammatory functions. We found that vascular cells as well as cells of other origin, respond to IL-1 exclusively via the type I IL-1 receptor (R). The type II R acts as a decoy for IL-1, regulated by anti-inflammatory signals. Recent evidence, including gene transfer and identification of a novel rapid pathway of release is consistent with the decoy R concept. The IL-1 receptor family included the various Toll, some of which are expressed in endothelium. Their signaling pathway will be discussed. Among IL-1 inducible genes, we identified a new molecule related to pentraxins (PTX3). The sequence, genomic organization, predicted structure and in vivo expression was presented. PTX3 will be discussed.

MoW1.6

MoW1.6

W6 NEW ASPECTS OF STATIN TREATMENT

MoW1.6

Stats and the atherogenic process

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Statin, like most biologically active molecules, have multiple actions. Reduction in cardiovascular events following sustained statin therapy results from a combination of beneficial effects. Separating the relative contributions of these effects is as complex as determining causality in a multifactorial disease such as atherosclerosis. The complex etiology of atherosclerosis and recognition of the contribution of a wide spectrum of risk determinants in a given individual is readily accepted. In contrast, the notion that the effect of a drug is the algebraic sum of its many positive and negative effects is not given the attention it deserves in current practice. Many properties of statins could directly influence lesion formation. Statins have antioxidant effects that can be demonstrated both in vitro and in vivo. They take place at therapeutic plasma concentrations. They differ among statins, may characterize the parent compound or a metabolite, influence both LDL and HDL oxidation, impart a paraoxonase-sparring effect or be modified by a slight change in the molecular structure. Statins’ anti-inflammatory properties confirmed in clinical trials complement their plaque stabilizing effects. They inhibit inflammatory cytokines as well as proliferation of inflammatory cells. There is evidence that adhesion molecules may be reduced by statins. The effect on cell proliferation is a key feature of lipophilic statins. The significance of their ability to or not to inhibit smooth muscle cell proliferation is still a matter of controversy. Macrophages may be affected by statins so as to reduce foam cell formation. Statins inhibit the ability of macrophages to oxidize LDL, and reduce the expression of scavenger receptors including CD36, and LOX-1 on their surface. The effect of statins on apolipoprotein E production by macrophages is currently being investigated. A better understanding of the pleiotropic effects
influencing the atherogenic process could eventually allow for the refining of indications of the various statins and help in the appraisal of their individuality.

**MoW2.6** Statins and C-reactive protein (CRP)
T.E. Strandberg, Department of Medicine, University of Helsinki, helsinki, Finland

C-reactive protein (CRP) was first found in the 1930s in plasma of patients with pneumococcal pneumonia. Nowadays CRP is a widely used measure to evaluate the activity of inflammation and diagnose bacterial infections. CRP belongs to acute phase proteins, the concentrations of which in serum are increased by inflammatory cytokines (for example interleukin-6) up to 1000-fold. Conventionally, CRP concentrations below 10 mg/l have been interpreted as normal, and CRP of healthy individuals is generally below 2 mg/l. Results from several follow-up studies have revealed that CRP concentrations over 2 mg/l – but below 10 mg/l – predict future coronary heart disease events. This suggests that silent inflammation, either in the coronary arteries or elsewhere in the body, could play a role in the development of atherosclerosis. CRP may also have pathogenic significance, as low levels of CRP have been found in atherosclerotic arteries. Interesting new findings of CRP and coronary prevention are the following:

- Pravastatin seemed to prevent coronary events more effectively in those patients whose CRP was increased at baseline.
- In coronary patients with hyperlipidemia, both short-term and long-term statin treatment is associated with decreased CRP levels.
- CRP change is not associated with LDL reduction during statin treatment, but at least in short-term inversely with change in HDL cholesterol.

Practical consequences of the statin effects on CRP are currently obscure, but reduction of CRP and inflammation may play a role in the early stabilization of the atherosclerotic plaque.

**MoW3.6** Effect of statins on the synthesis of lipoprotein containing apo-B-100
A.L. Catapano, Institute of Pharmacological Sciences University of Milan, Italy

Statins effectively lower plasma LDL cholesterol. Their action takes place through the inhibition of the HMG-CoA Reductase, a key step in the cholesterol biosynthetic pathway. By inhibiting cholesterol synthesis statins induce a rapid regulation of LDL receptors, thus leading to a sharp decrease of plasma LDL levels. Recent data, however, suggest that statins may lower plasma lipids also by different mechanisms. In the liver the secretion of LDL depends on a finely tuned balance between synthesis and intracellular degradation of apoB-100. In the poorly lipidated form apo B is readily degraded intracellularly and this mechanism, rather than apolipoprotein synthesis, appears to be the major determinant of apolipoprotein B-100 secretion. Statins by lowering cholesterol availability (both free and esterified) may decrease apolipoprotein B-100 secretion. To address this question we studied the effects of NK-104, a competitive inhibitor of HMGCoA-Reductase on apoB-100 synthesis and secretion from the human hepatoma cell line HepG2. Cells were preincubated with NK-104 (0.01–5 μM) for 24 h. The incubation with the drug continued for further 4 h in the presence of absence of oleate (0.8 mM). ApoB-100 in the medium was determined by an ELISA assay. Incubation of HepG2 with NK-104 resulted in a marked inhibition of cholesterologenesis, determined as incorporation of 14C-acetate into sterol, and decreased apoB-100 secretion in a dose-dependent manner (about ~20% vs control), both in basal conditions and after incubation with oleate. Evaluation of the distribution of apoB-100 among different lipoprotein secreted showed a reduction of apoB-100 associated with lipoproteins in the LDL density range. Pulse-chase experiments demonstrated that NK-104 did not affect the synthetic rate of apoB-100 but increased intracellular degradation of newly synthesised protein; apoB-100 mRNA levels were not affected. These data, together with other in vitro and in vivo findings, suggest that statins may decrease lipoproteins secretion especially in patients with LDL overproduction.

**MoW4.6** Atorvastatin increases the catabolism of chylomicron remnants in normolipidemic subjects
K.G. Parholer1, P.H. R. Barrett2, P. Schwald1,1 Med. Dept. II, Klinikum Grosshadern, University Munich, Germany; 2Dept. Medicine, University Western Australia, Perth, Australia

Objective: Atorvastatin (atorva) is a potent HMG-CoA reductase inhibitor that also decreases fasting triglyceride concentrations. Because of the positive association between elevated triglycerides and CAD we investigated the effect of atorvastatin on postprandial lipoprotein metabolism.

Methods: We evaluated the effect of 4 weeks of atorvastatin (10 mg.d−1) on postprandial lipoprotein metabolism in 10 normallipidemic men (30±2 years, 22±3 kg.m−2, cholesterol (chol) 187±21, triglyceride (TG) 130±44, HDL-chol 45±7, LDL-chol 116±19 mg.dl−1). Postprandial lipoprotein metabolism was evaluated with a standardized fat load (1300 kcal, 8% fat, 7% carbohydrates, 6% protein, 80,000 IU Vitamin A) given after 12 h fast. Plasma was obtained every 2 h for 14 h. Chylomicrons (C) and chylomicron remnants (CR) were isolated by ultracentrifugation and chyl, TG, apoB, apoB-48 and vitamin A (vitA) was determined.

Results: Atorva significantly (p<0.001) decreased fasting chol (−28%), TG (−33%), LDL-chol (−41%) and apoB (−39%), while HDL-chol increased (4%, NS). The area under the curve for plasma-TG (−27%) and CR-TG (−40%), CR-Chol (−49%), CR-apoB-48 (−43%) decreased significantly (p<0.05), while CR-vitA decreased slightly (−34%, p=0.08). In contrast none of the C-parameters changed with atorvastatin therapy.

Conclusions: Atorva decreases postprandial CR but not C. This indicates, that atorva has no effect on hydrolysis, but induces an increase in CR clearance presumably due to decreased competition for receptors and/or because of an increased receptor activity or number. Thus, atorvastatin does not affect C formation, secretion or catabolism, but increases CR catabolism.

**MoW5.6** Pravastatin reduces mortality: The Prospective Pravastatin Pooling Project
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Objective: To definitely address the effects of pravastatin 40 mg daily on total mortality, CHD morbidity, and specific non-CHD events within important subgroups.

Methods: The Prospective Pravastatin Pooling Project was designed to address the effects of pravastatin 40 mg/day on all-cause and cause-specific mortality by combining data from three large prevention trials, the West of Scotland Coronary Prevention Study (WOSCOPS), Cholesterol And Recurrent Events (CARE), and Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID), according to objectives specified before the results of any trial were known. Combining these three 5-year trials yielded a database that included 19,765 patients and 112,230 person years of follow-up. In addition to all these trials, the combined results for the two trials in patients with prior CHD (CARE and LIPID) were compared to those of the trial in patients without prior CHD (WOSCOPS).

Results: Patients taking pravastatin (vs. placebo) had significantly lower all-cause mortality (relative risk reduction [RR] 20%, 95% confidence interval [CI] 12% to 27%; p<0.001), due largely to significantly reduced CHD mortality (RR 24%, 95%CI 14% to 33%). Differences in other vascular deaths and non-cardiovascular deaths were insignificant. The greatest reductions in absolute risk were estimated, however, in patients with a history of CHD.

Conclusions: Pravastatin is effective in the prevention of CHD mortality across a broad range of cholesterol levels, with the absolute benefit of treatment related principally to the baseline risk of CHD death.

**MoW6.6** A large, 36 week study of the HDL-C raising effects and safety of simvastatin versus atorvastatin
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Objective: This study evaluated the HDL-C, apo A-I raising effects and safety of simvastatin (S) and atorvastatin (A) at their upper dosage ranges.

Methods: In a double-blind, parallel, 36 week dose escalation study, 826 hypercholesterolemic patients (LDL-C > 4.14 mmol/L) were randomized to S (8, 20, 40 mg days 6 weeks, 80 mg 6 weeks, 80 mg 24 weeks) or A (20 mg 6 weeks, 40 mg 6 weeks, 80 mg 24 weeks). 88, 80 mg 24 weeks.

Results: S was superior to A at raising HDL-C (primary endpoint) and apo A-I at each dose comparison.
Both A and S lowered mean LDL-C substantially by 46 to 55% and 43 to 49%, respectively (p < 0.001 in favor of A at each dose comparison). During the 24-week A 80 versus S 80 mg period, a significantly larger number of patients (p = 0.004) were discontinued because of clinically meaningful (>3 x upper limit of normal) consecutive increases in hepatic transaminases with A (143/92: 3.6%) than with S (23/84: 0.5%).

Conclusions: S had superior HDL-C and apo A-1 raising effects across the doses studied, which was more prominent at the highest doses. At the 80 mg dose of each drug, S also had a better hepatic safety and tolerability profile.

MoW7.6: Cholesterol-lowering therapy with pravastatin in older patients (aged 65 to 75) in the lipido study

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1University of Melbourne; 2NHMRC Clinical Trials Centre, Sydney; 3Core Research Group, University of Queensland, Brisbane; 4National Heart Foundation, Melbourne, Australia

The LIPID (Long-Term Intervention with Pravastatin in Ischaemic Disease) study found that, for patients with coronary heart disease (CHD) (prior myocardial infarction (MI) or unstable angina) and cholesterol levels of 4-7 mmol/L, therapy with pravastatin reduced total and CHD mortality. Of the 9014 patients, 3514 were in prespecified older age group (aged 65–75 years). We aimed to ascertain the effects of pravastatin on total mortality and on CHD death or nonfatal MI in the older patients.

The older patients had a higher risk for the prespecified outcomes than the younger patients (both P < 0.001). The risk of an MI or fatal stroke was also higher for older patients (both P < 0.001), but they were less likely to undergo revascularization (P = 0.035). Pravastatin reduced the relative risk of the prespecified outcomes to a similar extent in the older and younger group; as the absolute risk of events was higher in the older age group, the absolute benefit of therapy was larger for them.

<table>
<thead>
<tr>
<th>Specified event</th>
<th>Age group (years)</th>
<th>Risk of event</th>
<th>Relative risk reduction (%)</th>
<th>Absolute risk reduction (%)</th>
<th>NNT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD death or nonfatal MI</td>
<td>31-64</td>
<td>13.4</td>
<td>22</td>
<td>5.0</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>65-75</td>
<td>19.7</td>
<td>21</td>
<td>4.2</td>
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<tr>
<td>Total mortality</td>
<td>31-64</td>
<td>9.8</td>
<td>22</td>
<td>2.2</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>65-75</td>
<td>20.6</td>
<td>20</td>
<td>4.1</td>
<td>25</td>
</tr>
</tbody>
</table>

* Numbers needed to treat over 6 years per 1000 patients

Conclusion: Treatment with pravastatin appears useful and effective in this older age group of patients with previous MI or unstable angina.
Monday June 26, 2000: How-to Session Abstracts

MoH2:1 Transgenic mouse models of atherosclerosis and lipoprotein metabolism

David S. Grass, DNX Transgenic Sciences, 5 Cedar Brook Drive 08512, Cranbury, New Jersey, USA

Two different transgenic approaches were taken to express genes that are involved in atherosclerosis and lipoprotein metabolism. In the first approach, transgenic mice expressing both human cholesteryl ester transfer protein and apolipoprotein B100 were developed. On a normal chow diet, these mice have a lipoprotein cholesterol profile similar to humans (high LDL-C, Low HDL-C) in contrast to non-transgenic mice which have predominantly HDL-C. When fed a high fat, high cholesterol diet (HFHC), these mice have significantly increased levels of serum cholesterol and an increased susceptibility to atherosclerosis compared to non-transgenic controls.

In the second approach, transgenic mice expressing extracellular (Group II) Phospholipase A2 (PLA2) were produced. These mice have significantly decreased levels of HDL-C and are highly susceptible to atherosclerosis both on the HFHC diet and a normal chow diet. Thus, two different transgenic mouse models of lipoprotein metabolism and atherosclerosis were produced, one which mimics normal human lipoprotein cholesterol distribution and second which alters normal HDL metabolism.

MoH3:1 The rabbit as a model for atherosclerosis and restenosis

J.H. Campbell, G.R. Campbell. University of Queensland, Brisbane, Queensland, Australia

Severe hypercholesterolemia in the rabbit, achieved through diet, leads to widespread lipid-rich lesions in the arterial intima of the aorta and larger arteries. Similar lesions occur in the Watanabe Heritable Hyperlipidemic (WHHL) rabbit. Histology of these lesions shows lipid-laden foam cells of monocyte/macrophage origin, but rarely other features of human atheroma such as necrosis, ulceration, thrombosis or the involvement of smooth muscle. Lesions in the carotid or iliac artery produced by balloon catheter injury, electrical stimulation or air desiccation in combination with hyperlipidemia creates a fibrous, smooth muscle-rich plaque overlaying a central core of lipid-laden cells, debris and extracellular lipid. These lesions more closely resemble human atherosclerosis and provide an appropriate substrate on which subsequent balloon angioplasty can be performed to study the process of restenosis.

Responses to the second injury in this double injury model include variable intimal hyperplasia, vessel expansion, dissection, thrombus formation, medial smooth muscle proliferation, collagen formation, wound contraction and vessel wall recoil. Medial thinning and medial macrophage infiltration are p5 dominant features. Multilinear step-wise regression of morphometric data has led to the conclusion that luminal restenosis is caused by vessel remodelling, not intimal hyperplasia, and is determined by the extent of medial thinning and vessel constriction following balloon angioplasty.

MoH4:1 Bone marrow transplantation studies in mice to determine the role of macrophage-derived apolipoprotein E and the macrophage LDL receptor in diet-induced atherosclerosis

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Objective: Determination of the role of macrophage-derived apolipoprotein E (apoE) as well as the macrophage LDL receptor (LDLr) in atherosclerosis.

Methods: Bone marrow from wildtype, apoE knockout, LDLr knockout, and apoE:LDLr double knockout mice was transplanted into irradiated C57Bl/6 mice and the effects on diet-induced atherosclerosis were established.

For in vitro studies, thioglycolate-elicited peritoneal macrophages were used.

Results: Macrophage apoE-deficiency in C57Bl/6 mice induced a 1.5-fold increase (p < 0.01) in the mean atherosclerotic lesion area (150 ± 13 × 10^2 μm^2, n = 8) as compared to controls transplanted mice (101 ± 8 × 10^2 μm^2, n = 13), whereas macrophage LDLr-deficiency induced a 3-fold increase (p < 0.001) in the lesion area (34.7 ± 6.2 × 10^2 μm^2, n = 13). Transplantation of apoE:LDLr double knockout bone marrow resulted in a lesion area of 80.7 ± 13.2 × 10^2 μm^2, n = 11. Remarkably, the increase in lesion area induced by macrophage apoE-deficiency is of a similar magnitude in the presence (49 ± 10^2 μm^2) and in the absence (46 ± 10^2 μm^2) of the macrophage LDLr, while the extent of the antiatherosclerotic action of the macrophage LDLr is also independent of the presence (66 ± 10^2 μm^2) or absence (69 ± 10^2 μm^2) of macrophage apoE. In vivo, we provide further mechanistic data demonstrating that the LDLr is involved in the βVLDL-induced accumulation of cholesterol esters by macrophages and the transformation to foam cells. Furthermore, we show that macrophage-derived apoE is an important determinant for cholesterol efflux.

Conclusions: Macrophage apoE production and LDLr expression have opposite independent effects on atherosclerotic lesion development, whereby macrophage apoE production appears to be protective, while the macrophage LDLr facilitates atherosclerotic lesion development.
MoP1:W1 DIABETES

Dissociation between the evolution of VLDL apo B and HDL apo A-I metabolism on insulin therapy in non-insulin-independent diabetes mellitus (NIDDM) patients

L. Duviollard, B. Vergès, F. Pont, E. Florentin, P. Gambert. Unité INSERM 498, Dijon, France

Objective: To study the influence of insulin therapy on VLDL apoB and HDL apoA-I metabolic abnormalities in NIDDM patients with a poor glycaemic control.

Methods: We performed an in vivo stable isotope kinetic study, using L-[1-13C] leucine, in 6 poorly controlled NIDDM patients, before and 2 months after the introduction of insulin therapy, and in 5 control subjects.

Results: On insulin therapy, fasting plasma triglycerides fell by 35% in the fasting state and by 24% in the fed state. Insulin treatment induced a decrease of VLDL apoB plasma concentration (121 ± 42 vs 158 ± 91 mg.l⁻¹, p < 0.05 (controls: 48 ± 20)), related to an increased catabolism of VLDL towards IDL or LDL (0.20 ± 0.08 vs 0.14 ± 0.07 pooh⁻¹, p < 0.05 (controls: 0.36 ± 0.10)). On the contrary, insulin therapy had no effect on the apoA-I hypercatabolism initially present in NIDDM patients (0.39 ± 0.11 vs 0.34 ± 0.05 pooh⁻¹, (controls: 0.23 ± 0.01)). HDL apoA-I fractional catabolic rate was significantly correlated to HDL triglyceride/cholesterol1 ester and triglyceride/protein ratios, which were significantly higher in NIDDM patients than in controls and were not modified by insulin therapy.

Conclusions: Insulin treatment partially corrects VLDL apoB metabolic abnormalities in NIDDM patients. Nevertheless, the improvement is not sufficient to slow down the high neutral lipid exchanges between triglyceride rich lipoproteins and HDL and to correct HDL apoA-I hypercatabolism.

MoP2:W1 Insulin therapy partially corrects the defect of LDL receptor expression in non-insulin-dependent diabetes mellitus (NIDDM) patients: Quantification by flow cytometry

L. Duviollard, G. Lizard, B. Vergès, E. Florentin, P. Gambert. Unité INSERM 498, Dijon, France

Objective: Low density lipoproteins (LDLs) play a major role in the development of atherosclerotic lesions. LDL particles are cleared from the plasma compartment mainly by the LDL receptor. In vivo kinetic studies have demonstrated that LDL catabolic rate was decreased in NIDDM patients, and that insulin therapy restores a normal LDL catabolic rate. We quantified LDL receptors on blood mononuclear cells in NIDDM patients to gain further insight into the mechanisms responsible for these metabolic modifications.

Methods: The erythrocytes of total blood samples were lyzed by an ammonium chloride solution. Leucocytes were successively incubated with a mouse anti-human LDL receptor IgG and with a goat fluorescein-labelled anti-mouse IgG. Fluorescence was quantified by flow cytometry and the number of cell surface LDL receptors was determined by using the QPIKIT calibration kit (Dako).

Results: In NIDDM patients, the number of mononuclear cell LDL receptors was decreased in NIDDM patients compared to healthy control subjects (5270 ± 1056 vs 9586 ± 1599, p < 0.05). A two-month insulin treatment induced an increase of the number of mononuclear cell LDL receptors (6895 ± 2473 vs 5270 ± 1036, p < 0.05).

Conclusions: In NIDDM patients, the expression of LDL receptors is decreased and this defect is partially corrected by insulin therapy. These modifications are likely to explain the variations of LDL catabolic rate observed in NIDDM patients.

MoP3:W1 Increased expression of LOX-1 in diabetic rat arteries

Mingyi Chen, Tatsuya Sawamura, Tomoh Masaki. National Cardiovascular Center Research Institute, Osaka, Japan

Objective: LOX-1 is a novel oxidized LDL receptor in endothelial cells which is potentially involved in the pathogenesis of atherosclerosis. Diabetes mellitus accelerated atherosclerosis via pathways not fully understood, therefore, we investigate the expression of LOX-1 in streptozotocin induced diabetes rat arteries.

Method: Male Sprague-Dawley rats in 12-week old were made diabetes by streptozotocin (50 mg/kg) injection. The vehicle injected rats were served as non-diabetic control. Northern and Western blot analyses were applied to examine LOX-1 expression in streptozotocin induced diabetic rat aortas compared with control. Immunostaining was performed to identify the precise localization of LOX-1. RT-PCR and immunostaining studies were used to examine the expression of LOX-1 induced by diabetes rat serum, hyperglycaemia, AGE-BSA and TNF in cultured aortic rings and endothelial cells.

Result: LOX-1 was significantly increased in diabetic rat arteries. The prominent staining was endothelial cells, particularly in the bifurcation areas of carotic, intercostal, renal and celiac arteries of diabetic rats. In cultured rat aortic tissues and endothelial cells, the diabetic rat serum and AGE-BSA induced LOX-1 expression, while the control rat serum in combination with high glucose did not. Moreover, we found that LOX-1 ligand activity was accumulated in the diabetic rat serum. Consistently, lipoprotein deprivation greatly attenuated the potentials of diabetic rat serum to induce LOX-1 expression.

Conclusion: Diabetes significantly upregulated LOX-1 expression in the endothelium which was mediated by both the lipid-dependent (probably modified lipoproteins) and lipid-independent mechanisms such as AGE-RAGE interactions. These findings suggest a potential role of LOX-1 in diabetic vascular complications.

MoP4:W1 Enhanced expression of osteopontin by high glucose: Involvement of osteopontin in diabetic vascular diseases

M. Takemoto, S. Mori, K. Yokote, S. Asami, Y. Saito. Second Department of Internal Medicine, Chiba University School of Medicine, Chiba, Japan

Objective: Atherosclerotic arteries in diabetic patients tend to undergo restenosis after balloon angioplasty and are often morphologically characterized by diffuse calcification of the medial layer. Osteopontin (OPN) is a 60-kDa phosphoglycoprotein originally purified from bone matrix, which turned out to be a ligand for α5β1 integrins. In addition to its well-described roles in tissue calcification, several studies have suggested that OPN is involved in atherosclerotic processes such as intimal thickening. However, little is known concerning the involvement of OPN in diabetic vascular complications. Therefore, we examined the expression of OPN in human artery and rats both in vivo and in vitro.

Methods: We examined in vivo expression of OPN in diabetic human arteries as well as rat arteries using immunohistochemical techniques. Then we examined the effect of high glucose on OPN expression at protein as well as transcriptional level using cultured rat aortic smooth muscle cells.

Results: OPN was expressed in tunica media of diabetic patient’s artery and streptozotocin-induced diabetic rat’s arteries but not in controls. Treatment of rat aortic smooth muscle cells with high glucose (25 mM) or glucosamine (10 mM) increased OPN expression significantly both at mRNA and protein levels. A protein kinase C inhibitor, GF109203X, as well as an inhibitor of glutamine-fructose-6-phosphate amidotransferase, azaerine, profoundly inhibited the high glucose-induced upregulation of OPN.

Conclusion: These results demonstrate the increased production of OPN in hyperglycemic state and propose its potential role in the development of diabetic vascular diseases.

MoP5:W1 Nitric oxide and cutaneous vasomotor response in coronary artery disease patients

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Objective: to evaluate the nitric oxide (NO) production after the bicycle exercise stress test and correlation with the postocclusive hyperemic response (PHR) in coronary artery disease (CAD) patients with/without HDL-hypocholesterolemia.
Subjects: 18 healthy controls (C), 16 CAD patients with HDL-hypercholesterolemia (wC) and 16 without HDL-hypercholesterolemia (nC). All subjects were without LDL-hypercholesterolemia, hypertriglyceridemia, peripheral vascular disease and hypertension. The groups were matched for age, sex, BMI. The diagnosis of CAD was substantiated by coronary angiography.

Methods: The level of NO end products NOx (NO2- plus NO3-) in plasma were measured using anion-exchange chromatography. We recorded changes in the cutaneous blood flow induced by 3 min arterial occlusion (cuff above the knee) on the dorsum of the great toe using Laser Doppler fluxmetry (Periflux 4001, Perimed).

Results: NOx was increased (mean ± SD; Mann-Whitney U test) after the exercise (wC 32.1 ± 4.0 vs 36.1 ± 5.3 μM/L, p < 0.05; nC 41.2 ± 3.1 vs 51.3 ± 7.8 μM/L, p < 0.01 and C 45.2 ± 2.3 vs 60.3 ± 7.4 μM/L, p < 0.001), and only wC patients had a lower increase of NOx in result of exercise than healthy subjects (wC vs C p < 0.01). PIR was prolonged only in wC patients (wC 56 ± 29 vs C 32 ± 8, p < 0.001) and the time to PIR maximum correlated with an enhanced NOx concentration after the exercise (r = -0.51, p < 0.05).

Conclusions: CAD patients with HDL-hypercholesterolemia show a decreased ability to produce endothelial-derived NO and it may contribute to a prolonged cutaneous vasoconstrictor response.

MoPbW11 Low density lipoprotein (HDL) and impaired glucose tolerance: Insights from stable isotope studies J. Pietrach, U. Julius, M. Hanefeld. Institute of Clinical Metabolic Research, Medical Faculty, Dresden, Germany

The in vivo kinetics of HDL apolipoproteins apoA-I and A-II (apo-A-I, apo-A-II) were studied in 13 non-obese subjects with impaired glucose tolerance (IGT) and 11 controls with normal glucose tolerance (NGT) using a stable isotope approach. During a 12 h primed, constant infusion of [13C]-phenylalanine tracer enrichment was determined in HDL apoA-I and apoA-II. Rates of protein synthesis and catabolism were estimated by model-based analysis. Triglycerides were higher in IGT (1.46 ± 0.35 vs 0.81 ± 0.26 mmol/L, p < 0.05) but in the upper normal range. HDL apoA-I level was significantly lower in subjects with IGT (0.92 ± 0.13 vs 1.39 ± 0.07 g/L, p < 0.01). The mean fractional catabolic rate (FCR) of HDL apoA-I was significantly higher in IGT (0.38 ± 0.06 vs 0.25 ± 0.06 d-1, p < 0.01) while the HDL apoA-I production rate as well as kinetic parameters of HDL apoA-II were not affected. Cholesteryl ester transfer protein (CETP) activity was significantly higher in IGT (36.4 ± 5.2 vs 25.8 ± 5.4 mmol/mL × h-1). Postheparin hepatic lipase (HL) activity was somewhat, but not significantly, higher in the IGT subjects (0.51 ± 0.22 vs 0.42 ± 0.24 μM/L × s-1). There were significant correlations between HDL apoA-I FCR and the following parameters: HDL apoA-I (r = -0.982, p < 0.01), plasma triglycerides (r = 0.752, p < 0.05), HDL triglycerides (r = 0.829, p < 0.05), insulin (r = 0.762, p < 0.01), proinsulin (r = 0.784, p < 0.01), CETP activity (r = 0.668, p < 0.01), and HL activity (r = 0.644, p < 0.01). These data support the hypothesis that low HDL in subjects with IGT is the result of an enhanced apoA-I catabolism, strongly modulated by the concerted action of lipid transfer proteins and lipolytic enzymes. Our in vivo results seem to be an early metabolic finding in insulin resistant states even when other lipid parameters, especially plasma triglycerides, still appear to be unaffected.

MoPbW21 Association of LDL size and coronary risk factors in postmenopausal women C. Posadas-Romero1, J. Zamora-González1, M. Cruz-G1, A. Hernández-Oroz1, G. Cardoso-Saldaña1, R. Posadas-Sánchez1

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Objective: To determine the association of LDL particle size with coronary risk factors in a population-based sample of postmenopausal women (PW).

Methods: Sampling design: A sample of 98 PW 50 to 65 years was randomly selected from the population of 5870 in the northeastern region of Mexico City. Risk factors: Lipids, lipoproteins, apolipoprotein A-I and B, insulin and glucose were quantified in a venous blood sample after a 12-hour fast. LDL size was determined by non-denaturing gradient polyacrylamide electrophoresis and visceral fat (VF) by computer axial tomography.

Results: There was no significant difference in lipids, lipoproteins and apolipoproteins between the age-matched subjects (HTDM) PW (n = 59) and healthy (C) PW (n = 99). HTDM showed smaller LDL size (26.1 ± 1.1 vs. 27.2 ± 1.1; p < 0.001) and higher prevalence of LDL B phenotype than C (36.2% vs. 5.1%; p < 0.001). LDL size was associated with HbA1C-C and TG in HTDM; and with TG, LDL-C, HDL-C, TG, ApoB, insulin and glucose in C VIF was associated with LDL size in C but not in HTDM PW. Multiple regression analysis showed LDL size was explained by TG and insulin (52%) in C and by HDL-C (19.2%) in HTDM.

Conclusions: LDL size was not associated to menopause but to its related diseases (hypertension and/or diabetes).

MoPbW10 Impact of glycation on lipoproteins-induced generation of fibrinolytic regulators from vascular endothelial cells G. Shen, S. Ren, J. Zhang. University of Minnesota, Winnipeg, Manitoba, Canada

Reduced fibrinolytic activity is frequently found in plasma of patients with diabetes mellitus (DM), which may be involved in the development of vascular complications in diabetes. Vascular cells-derived fibrinolytic regulators are regulated by plasma lipoproteins. Non-enzymatic glycation increases the oxidative stress of lipoproteins. Our group investigated the influence of glycation on lipoproteins-induced generation of fibrinolytic regulators from cultured vascular endothelial cells (EC). Glycation enhanced the production of plasminogen activator inhibitor-1 (PAI-1) in human umbilical vein and coronary artery EC induced by low density lipoprotein (LDL) or lipoprotein a [Lpa(a)]. The tissue type plasminogen activator (tPA) in EC was inhibited by glycyated LDL or Lpa(a) compared to native forms of the lipoproteins. Very low density lipoprotein and LDL but not high density lipoprotein (HDL) isolated from patients with Type 1 or Type 2 DM increased PAI-1 production and reduced tPA generation from EC compared to corresponding lipoproteins from controls. The presence of aminoguanidine, an inhibitor of the formation of advanced glycation end products (AGEs), during glycation inhibited glycyated LDL- or Lpa(a)-induced changes in PAI-1 and tPA in EC. Co-treatment with butylated hydroxyanisole, vitamin E or HDL reduced the formation of conjugated dienes in glycyated LDL and prevented the changes in the generation of fibrinolytic regulators from EC induced by glycyated LDL. The findings suggest that glycation of atherogenic lipoproteins may be implicated in the attenuated fibrinolytic activity in diabetic states. Oxidative modification and AGEs formation may contribute to glycoproteins-induced changes in vascular EC-derived fibrinolytic regulators (supported by Can. Diabetes Assoc.).

MoPbW17 The effect of beta-carotene and alpha-tocopherol supplemented accelerated atherosclerosis in diabetic apoe deficient mice J. Koren, A. Shaih, J. George, D. Harish. Institute of Lipid and Atherosclerosis Research, Sheba Medical Center, Tel-Hashomer; Sackler Faculty of Medicine, Tel-Aviv University, Israel

Objective: Augmented oxidative stress in diabetes has been suggested to contribute to the synergistic effects of hyperglycemia and hyperlipidemia on atherogenesis. The present study tested the hypothesis that dietary supplementation with anti-oxidents will inhibit diabetes-induced atherosclerosis in mice.

Methods: Female apoE-deficient mice were either fed a normal Chow diet (A) or a chow diet supplemented with 0.2% beta-carotene and 0.2% alpha-tocopherol (B). Both groups were injected with STZ to induce hyperglycemia that persisted until sacrifice, 10 weeks later.

Results: Plasma glucose, lipid profile and body weight were similar among the two experimental groups. LDL from anti-oxidant treated mice was more resistant to ex vivo oxidation as measured by conjugated diene formation. Yet, antioxidant antibody levels did not differ between the groups. Anti-oxidants reduced the proliferative capacity of sphenocytes to a non-specific mitogen without altering their cytokine secreting patterns. Treatment with b-carotene and a-tocopherol did not appear to influence aortic sinus atherosclerosis. In group A, fed with normal chow diet aortic sinus atheroma area was (274 ± 30 ± 105 mm²) as compared to (218 ± 30 ± 105 mm²) in group B fed with the antioxidant diet. Nor did it have an effect on total aortic surface atheroma coverage (5.98 ± 0.48% as compared to 3.34 ± 0.91% p = 0.29, respectively).

Conclusions: Despite the favorable effects of b-carotene and a-tocopherol combination on LDL susceptibility to oxidation ex vivo and lymphocyte reactivity, this treatment strategy does not appear to have a favorable impact on accelerated atherogenesis in diabetic apoE-deficient mice.
Remnant lipoprotein cholesterol levels are higher in Type 2 diabetic subjects with macrovascular complications

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Objectives: Dyslipidemia contributes significantly to the increased risk of cardiovascular disease in Type 2 diabetes. Hence, the aim of this study was to determine if levels of remnant lipoprotein cholesterol (RLP-C) are higher in Type 2 diabetic subjects with macrovascular complications (DM2-MV) compared to those without macrovascular complications (DM2) and matched controls (C).

Methods: Fasting blood was drawn at baseline from subjects in all 3 groups (n = 24/group), matched for age, gender and body mass index. RLP-C levels were measured by an immunofluorescence assay.

Results: Lipid levels were not significantly different between the 3 groups studied. When compared to C, RLP-C levels were significantly higher in DM2 and DM2-MV (median: 6.5 and 8.7 vs. 5.3 mg/dl; p < 0.01 and p < 0.0002 respectively). DM2-MV had significantly higher levels of RLP-C than DM2 (p < 0.05). RLP-C levels correlated significantly with plasma triglycerides (r = 0.7; p = 0.002). When comparing normotriglyceridemic patients, only DM2-MV had significantly greater levels of RLP-C compared to controls (p < 0.01).

Conclusions: Thus, using this novel assay, remnant levels are significantly higher in Type 2 diabetic subjects with macrovascular disease and may appear to be a more sensitive predictor of dyslipidemia than the standard lipid profile.

Angiographic severity and extent of coronary artery disease in type 1 diabetes

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Objective: To study characteristics of coronary artery disease (CAD) specifically in type 1 diabetes with computer-aided quantitative coronary angiography (QCA).

Methods: This retrospective study comprised 64 (24 female and 40 male) type 1 diabetic patients and 64 non-diabetic control subjects that were individually matched for sex, age (within 5 years), diabetes duration (within 3 years) and serum creatinine value (<100, 100–300, >300 μmol/l, or dialysis). To estimate the severity, extent, and overall "atheroma burden" of CAD, we used QCA-based segmental analysis of coronary angiograms.

Results: Type 1 diabetic patients had greater global severity, (48 ± 18 vs. 35 ± 22, P < 0.0001); global extent, (34 ± 16 vs. 19 ± 14, P < 0.001); and global atheroma burden indices, (22 ± 13 vs. 14 ± 12, P < 0.001), than non-diabetic control subjects. QCA-derived indices of CAD were on average 1.4 to 4.3 fold higher in diabetic than in non-diabetic patients. These differences were particularly marked in women.

Conclusions: We found that type 1 diabetic patients with a clinical indication for coronary angiography, especially women, have more severe, extensive, and distal type of CAD compared with individually matched nondiabetic control patients. The greater impact of type 1 diabetes in women is not explained by the established risk factors. Our present and previous results indicate that "diabetic" CAD is related to type 1 and to long-lasting type 2 diabetes.

Apo E2 is associated with severe coronary artery disease in type 2 patients but is protective against severe disease in non-diabetic patients

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Objective: Diabetes and apo E both influence triglyceride-rich lipoproteins which have recently been implicated in plaque progression. The present study examined the hypothesis that apo E2 is associated with more severe coronary disease in type 2 diabetic patients.

Methods: Patients were recruited from a university hospital cardiology department. All patients underwent a coronaryographic examination. Plasma lipids and apo E genotypes were analysed. Diabetic status was only ascertained after recruitment. The study was limited to patients with a positive arteriographic examination (491 patients: 295 without diabetes, 196 with diabetes (type 2)).

Results: In non-diabetic patients, E2 carriers had a significantly lower prevalence of triple vessel disease than E3/3 carriers (16.2 vs 36.6%; OR 0.33 (0.13–0.84), p < 0.05). With type 2 patients, E2 carriers had an excess of triple vessel disease compared to E3/3 genotypes (72.0 v 41.3%; OR 3.3 (1.20–9.15), p < 0.05). Differences were independent of other variables, notably E2 homozygosity and fasting plasma lipids by regression analysis. The apo E2 subgroup was not associated with disease severity.

Conclusions: Diabetic apo E2 carriers had more severe coronary artery disease than diabetic patients with other apo E isoforms. In non-diabetic patients the E2 allele was protective against severe coronary disease. We hypothesise that interaction between the diabetic milieu and the E2 allele accelerates plaque progression even in E2 heterozygotes. This may involve modulated postprandial metabolism of triglyceride-rich lipoproteins.

Effects of atorvastatin vs. fenofibrate on lipoproteins, LDL-subfraction distribution and hemorheology in type 2 diabetic patients with mixed hyperlipoproteinemia


Dyslipoproteinemia is a strong risk factor for cardiovascular disease in patients with diabetes mellitus. It is unclear whether fibrate or statin therapy is more effective in these patients. We compared atorvastatin (AS) (10 mg/d) with fenofibrate (FF) (250 mg/d) each for 6 weeks separated by a 6 week washout phase in 11 patients (4 m, 7 w; 60.0 ± 6.8 yrs; BMI 30.0 ± 3.0 kg/m²) with type 2 diabetes mellitus (HbA₁c, 7.3 ± 1.1%) and mixed hyperlipoproteinemia (LDL-cholesterol 164 ± 36 mg/dl; triglycerides (TG) 260 ± 107 mg/dl; HDL-cholesterol 49 ± 11 mg/dl) using a randomised, crossover design. Lipid profiles, LDL-subfraction distribution (density gradient ultracentrifugation), plasma viscosity (viscometer) and red cell aggregation (RCA) (erythrocyte aggregometer) were determined before and after each drug, fibrinogen concentrations (immunonephelometry) in one assay with deep frozen plasma at the end of the study. LDL-cholesterol was determined significantly reduced only after AS therapy (AS: −39%, p < 0.01; FF: −14%, p = 0.07), however TG reduction was only significant with FF (AS: −21%, p = 0.51; FF: −40%, p < 0.005). Both drugs increased HDL-cholesterol (AS: +9%, p = 0.05; FF: +49%; p = 0.06). AS did not affect LDL subfraction distribution, but FF induced a shift from small dense LDL (1.040–1.064 g/ml) to intermediate-dense LDL (1.027–1.040 g/ml) by 31% (p < 0.03). FF lowered fibrinogen concentration by 19% (4.04 ± 0.57 vs. 3.28 ± 0.25 g/l, p < 0.01) associated with a decrease in PV by 3% (p < 0.02) and improved RCA by 15% (p < 0.02), while AS did not affect fibrinogen, PV or RCA.

We conclude, that FF improves TG-concentration, LDL-subfraction distribution and hemorheological parameters in type 2 diabetes, while AS is considerably more potent in LDL-cholesterol reduction.

Insulin enhances platelet activation and aggregation in vitro

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Objective: Diabetes mellitus is accompanied by platelet hyperreactivity and an increased risk of thrombotic complications. Previous data on insulin effects on platelets have been less than consistent. We therefore investigated the influence of insulin on platelet activation and aggregation.

Methods: Fasting venous blood was collected from young, healthy males, using citrate and hirudin as anticoagulants. The effect of insulin on platelet activation was measured as P-selectin expression and fibrinogen binding using whole blood flow cytometry in unstimulated and ADP-stimulated samples, incubated with 0–10,000 μU/mL insulin for 20 min. The effect of insulin on EP50 for ADP-induced aggregation was studied using Born aggregometry in platelet rich plasma (PRP) and collagen-induced aggregation using impedance aggregometry in whole blood, incubated with insulin 30 and 300 microU/mL for 3 min.

Results: Insulin dose-dependently enhanced platelet P-selectin expression and fibrinogen binding in unstimulated and ADP-stimulated samples (p < 0.001 by ANOVA for all; n = 20). Insulin (30 and 300 microU/mL) also enhanced ADP-induced aggregation in PRP (p < 0.01; n = 14) and collagen-induced aggregation in whole blood (p < 0.01; n = 14). To investigate implications of low calcium concentrations in citrated blood, the effect of insulin was verified in hirudinized samples (n = 12).

Conclusions: Insulin dose-dependently induces platelet activation and aggregation, independently of extracellular calcium. Thus, beneficial effects of insulin treatment on platelets may be related to indirect, rather than direct effects.
Apo E phenotype is associated with coronary artery calcification in the general population but not in type 1 diabetic patients

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**Objectives:** To examine the association between CAC and Apo E phenotype in 196 type 1 diabetic (DM) men and women and 198 non-diabetic men and women (aged 30–55 yrs).

**Methods:** Electron beam CT scanning was used to quantify CAC. Apo E phenotype was carried out using a rapid micromethod.

**Results:** The frequency of apo E phenotypes was as follows: E3 (3 3) –60%, E2 (3 2 or 2 2) –15%, E4 (4 3 or 4 4 or 4 2) 25%. Phenotype did not differ by sex or DM. In the non-DM group, compared to those with E3, HDL-cholesterol was slightly lower in those with E4 (–0.13 mmol L–1, p = 0.05) and LDL-cholesterol was lower in those with E2 (–0.9 mmol L–1, p = 0.001). In the DM group, compared to those with E3, HDL-cholesterol (HDL-C) was non-significantly lower in those with E4 (–0.05 mmol L–1, p = 0.5) and LDL-cholesterol (LDL-C) was lower in those with E2 (–0.7 mmol L–1, p = 0.001). Triglycerides (TG) did not vary by phenotype in either group. In the non-DM group, compared with E3 phenotype, those with E4 phenotype had an elevated odds of calcification (51% vs. 33%, OR = 2.7, 95% CI 1.3–5.8, p = 0.01). This was independent of LDL-C, HDL-C and TG (OR = 3.0, p = 0.009 on adjustment) and of apo A and apo B. In DM subjects no association was seen (OR = 0.91, p = 0.8, p = 0.05 for the interaction between diabetes and apoE phenotype). The prevalence of CAC was similar in those with E2 and E3 phenotype in both groups. There was no evidence of any modulating effect of ApoE phenotype on the effect of other risk factors on CAC in either group.

**Conclusion:** Apo E 3 phenotype is protective for coronary calcification compared with Apo E 4 phenotype. This is not due to differences lipid levels between phenotypes. The protective mechanism of E3 or the detrimental effect of E4 is not apparent in type 1 diabetic patients.

Glycemic control and plasma lipoproteins in menopausal women with type 2 diabetes treated with oral and transdermal combined hormone replacement therapy

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**Objective:** To compare the effect of a fixed combination of an oestrogen (17β oestradiol) with a synthetic progestagen (norethisterone) on glycemic control and plasma lipoproteins in women with type 2 diabetes.

**Methods:** Oral and transdermal HRT were compared to no HRT treatment in 33 postmenopausal women with type 2 diabetes, in a 12 week prospective open parallel group study.

**Results:** In the 11 women who received 12 weeks of oral HRT there was a significant fall in total cholesterol [5.9 ± 1.0 (SD) to 4.9 ± 1.1 mmolL–1, p = 0.004], low density lipoprotein (LDL) cholesterol [3.44 ± 0.89 to 2.77 ± 0.92 mmolL–1, p = 0.004] and triglyceride values [median (range)], [2.46 (0.96–5.52) to 2.29 (1.00–3.87) mmolL–1, p < 0.05]. Oral HRT improved HbA1c [7.4 ± 1.4 to 6.8 ± 1.2%, p = 0.005]. No improvement in these metabolic parameters occurred in the 9 women receiving transdermal HRT or the 13 controls randomised to no treatment.

**Conclusion:** In women with type 2 diabetes, cyclcic oestrogen and progestagen taken orally for 12 weeks significantly improved glycemic control and lipoprotein concentrations. These metabolic benefits were not apparent when a similar HRT preparation was administered transdermally.

Cholesteryl ester transfer is associated with HDL size and coronary artery calcification in type 1 diabetic and non-diabetic subjects

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**Objectives:** We compared plasma cholesteryl ester transfer (CET) and protein activity (CETP-a) in type 1 diabetic (DM) and non-diabetic (non-DM) subjects, and (ii) examined the association of CET with HDL-cholesterol (HDL-C), HDL particle size and coronary artery calcification (CAC), a measure of atherosclerosis.

**Methods:** Electron beam CT scanning was used to quantify CAC in 199 DM patients and 196 non-DM subjects (age 30-55 yrs; 50% female). CET was measured using a radioisotope method. CETP-a was measured using an exogenous substrate assay, as an estimate of its mass. HDL size was measured by NMR-spectroscopy. Analyses were adjusted for age and sex.

**Results:** CET was positively associated with triglycerides (TG) (p < 0.001) and CETP-a (p < 0.001). TG were 0.2 mmol/L lower in those with than without DM (p = 0.001). CETP-a was higher in those with DM, independently of HDL-C. LDL-C and TG (4% higher = e3.7 arbitrary units, SD = 18, p = 0.027). CET was lower in those with DM (10% lower = 0.7 mmol/L/hr, SD = 12, p = 0.003) but not independently of TG (0.3 mmol/mL/hr, on adjustment, p = 0.7). In both groups, a higher CET was associated with lower HDL-C (0.14 mmol/L lower per 10 mmol/L/hr of CET, p < 0.001) and smaller HDL size (0.17 nm lower per 10 mmol/L/hr of CET, p < 0.001). CET was associated with CAC (Covariances Odds ratio OR for CAC per 10 mmol/L/hr of CET = 1.45, 95% CI 1.2–1.7, p < 0.001 adjusted for DM). The association was independent of HDL-C and HDL size but not TG (OR = 1.2, p = 0.1).

**Conclusions:** i) DM patients have elevated CETP activity, but their CET is lower than non-DM subjects because of their lower TG. ii) Higher CET is associated with lower HDL size, lower HDL-C and with increased CAC. These data support the concept that the atherogenicity of TG is mediated by increased CET, coinciding with a reduction in HDL size and HDL-C.

Low paraoxonase in small dense HDL may predispose to coronary heart disease in type 1 diabetes

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**Introduction:** High incidence of coronary heart disease (CHD) in type 1 diabetes, despite increased HDL cholesterol, may reflect low activity of paraoxonase, an enzyme closely associated with apo A1.

**Objectives:** We aimed to assess the ratio of paraoxonase activity to apo A1 concentration (PON/A1) in HDL subfractions of type 1 diabetic and control subjects, and to relate the findings to CHD risk, assessed by measurements of carotid and femoral artery intima-media thickness (IMT).

**Method:** Paraoxonase activity was measured using phenylacetate as substrate. We used single vertical spin density gradient ultracentrifugation to isolate HDL subfractions from serum, and measurement of PON/A1 activity. Mean (SD) retrieval of paraoxonase activity in subfractions was 87 (12%). Carotid and femoral IMT were measured using β-mode ultrasound.

**Results:** Thirty-five type 1 diabetic (duration of diabetes 22 (13) years, HbA1c 7.67 (1.17%) and 24 control subjects, matched for age, sex and BMI, were studied. HDL cholesterol was higher (1.53 (0.36) v. 1.32 (0.34) mmol/L, p < 0.05) in diabetic subjects; mean IMT was also higher (0.92 (0.03) v. 0.55 (0.13) mm; p < 0.005). Apo A1 (1.64 (0.35) v. 1.50 (0.26) g/L), NS, serum paraoxonase activity (121 (28) v. 120 (36) μmol/min/mg apoA1; NS) and serum PON/A1 (75 (19) v. 80 (20) μmol/min/mg apoA1; NS) were similar. In both groups, PON/A1 increased progressively as HDL subfraction density increased. PON/A1 was therefore highest in the smallest, densest HDL subfraction, but significantly lower in this subfraction in diabetic subjects (164 (61) v. 217 (117) μmol/min/mg apoA1; p < 0.05). Correlation of PON/A1 in this subfraction against mean IMT was of borderline significance (r = –0.24; p = 0.06).

**Conclusion:** Low PON/A1 in small dense HDL may predispose to CHD in type 1 diabetes.
**Results:** HDL Ox was higher in D than C. Mean ± SD: TBARS (nmol MDA/mg protein) 5.17± 9.2 vs. 38.1 ± 10.9, V_max (nmol/mg P/min) 5.1 ± 1.0 vs. 3.1 ± 1.1 and D_max (nmol/mg P) 232 ± 62 vs. 151 ± 53, respectively, p < 0.005. Lag (min) was shorter in HDL from D than C. 1.5 ± 1.8 vs. 6.4 ± 8.2, p < 0.05. HDL CC (%) was not different between D (Cho 20.2 ± 2.0, Tg 4.3 ± 1.4, P 53.4 ± 4.3) and C (Cho 20.2 ± 1.3, Tg 3.4 ± 1.5, Ph 22.9 ± 3.8, P 53.5 ± 1.3). HDL FR and α-1 (nmol/mg P) were higher in D: 9.2 ± 0.5 and 5.7 ± 2.0, than in C: 7.6 ± 0.3 and 3.5 ± 1.3, respectively, p < 0.05. ON activity was not different between D and C. Median (Range), PON (nmol/mg/min) 121 (50-300) and 190 (50-380), PON 1M (nmol/mg/min) 170 (60-570) and 386 (70-790), ARE (nmol/mg/min) 120 (80-119) and 134 (90-230), respectively. Interestingly, α-1 correlated negatively with lag (r = -0.52, p < 0.005) and positively with TBARS (r = 0.47, p < 0.05) and D_max (r = 0.67, p < 0.005). No correlations were found between FR and Ox parameters.

**Conclusions:** D showed increased HDL Ox. This was directly associated with α-4, according to the tocoferol mediated peroxidation and not related to HDL glycation or CC. HDL oxidation could impair its anti-atherogenic role.

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**MoP20:W1**

**Homocysteine augments impaired endothelium-dependent relaxation and cGMP formation in aorta of diabetic rats**


**Objectives:** Elevated levels of homocysteine (HC) may increase the incidence of angiopathy in patients with diabetes mellitus (DM). Since arterial nitric oxide (NO) formation is impaired in both DM and by HC, the HC and DM may interact to further reduce NO formation. The effect of HC on endothelium-dependent relaxation and cGMP formation by aorta from diabetic rats was therefore investigated.

**Methods:** Rats were rendered diabetic (plasma glucose > 20 mmol/L) with streptozotocin and after 2 weeks aorta excised and acetycholine-stimulated relaxation and cGMP formation assessed using an organ bath and radiolimunoassy, respectively. The effect of pre-incubation with exogenous HC (±superoxide dismutase [SOD]) for 30 min on these parameters was then investigated.

**Results:** Acetylcholine-stimulated relaxation was significantly impaired in aorta from diabetic rats ($4.1 ± 5$ [% inhibition; mean ± SEM; n = 6]) compared to controls which was further reduced by the addition of 10 μM (61 ± 6) and 100 μM HC (93 ± 7). These effects were completely reversed by SOD (300 U/ml) in both the presence and absence of HC. Changes in cGMP were identical.

**Conclusions:** HC enhances the generation of superoxide in arterial tissue from diabetic animals which reacts with NO to form peroxynitrite thereby reducing the bioavailability of NO. The increased incidence of angiopathy in diabetic patients with elevated HC plasma levels may be due, in part, to the augmentation of superoxide generation by HC and therefore of impaired NO actions.

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**MoP21:W1**

**Prevalence of impaired glucose tolerance in selected FCH patients**

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**Objectives:** to evaluate the prevalence of impaired glucose tolerance (IGT) and to test the presence of hyperglycemia in selected Familial Combined Hyperlipoproteinemia (FCH) affected patients.

**Methods:** 37 FCH kindreds (N = 156; M = 88; F = 68; mean age = 50.6 ± 18.1 years) and a control normolipidemic healthy population (N = 155; M = 94; F = 61; mean age = 48.7 ± 15.9 years) were considered. The FCH diagnostic criteria were 1) intravascular 2) atherogenicity and 3) coronary heart disease or cerebrovascular disease (4140 or 4430 codes by ICD 10) coronary heart disease and/or hypertension. An OGTT was performed on 10 normolipidemic FCH patients and 10 healthy subjects. Results were statistically processed by Student's t-test for independent samples.

**Results:** The prevalence of IGT and type 2 Diabetes Mellitus in FCH patients was 9% and 20%, respectively. No significant differences was found about fasting glucose levels (FGL) between men and women (t = 2.0, p = 0.47) and between younger (≤65 years) and older (>65 years) FCH patients (t = 1.74, p = 0.86). Prevalent IIB and mixed IIA/IIB phenotypes showed significant higher FGL than those with normal phenotype (p < 0.05). FGL levels in total FCH patients and in FCH patients with normal phenotype were higher compared with control subjects (t = 8.24, p < 0.01; t = 4.6, p < 0.01). In addition, plasma glucose levels were significantly higher in FCH patients than in control subjects at 30, 60, 90 and 120 minutes after oral glucose load (212 mg/dl vs. 105; 191 ± 78 vs. 139 ± 56, respectively, p < 0.05).

**Conclusion:** FCH patients show significantly higher fasting and after OGTT glucose levels compared to healthy controls.

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**MoP22:W1**

**Paraoxonase activity and genetic variation in two paraoxonase genes (PON1 and PON2) in type 2 diabetes**

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**Serum PON** is an HDL-associated enzyme which is believed to inhibit LDL oxidation and this may provide protection against the risk of coronary heart disease. Three PON like genes designated PON1, PON2 and PON3 have been identified and mapped to chromosome 7q21-q22. Polymorphism in the PON1 (Gln 192 Arg and Leu 55 Met) and PON2 (Ser 311 Cys) have been described. In this study we investigated serum PON activity and PON polymorphism in 54 controls and 175 Caucasian type 2 diabetic patients. Serum PON activity in the controls (288.57 U/l (70-950)) was significantly higher (p < 0.001) than in diabetic patients (218.07 U/l (25-810)). The gene frequency for the PON1 192 and PON2 55 polymorphism was not statistically different (p = NS X^2 test) between cases and controls (A = 0.77; B = 0.23 vs A = 0.73; B = 0.27) and (L = 0.64; M = 0.36 vs L = 0.61; M = 0.39). The same result was obtained when we compared in both groups the gene frequency for the PON2 311 polymorphism (S = 0.71; C = 0.29 vs S = 0.70; C = 0.30). These results suggest that genetic distribution of PON may not be only determining factors but that other as yet undetermined factors could be involved in the enzyme activity changes.

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**MoP23:W1**

**Diabetes and obesity in CHD patients in the Asia-Pacific region: The ASPAC study**

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**Objectives:** To determine the prevalence of diabetes and obesity among 4,112 patients presenting to hospital with acute myocardial infarction or unstable angina in 10 countries in the Asia-Pacific region.

**Methods:** The medical records of consecutive patients have been evaluated over 6 months following hospital admission. Diabetes was defined as a measured random blood glucose (RBO) of ≥11.1 mmol/L, or use of oral hypoglycaemic agents and or insulin at any time. Obesity was defined as either a documented history in the medical record or measured body mass index (BMI) ≥30 kg/m².

**Results:** Diabetes and obesity assessment, prevalence and treatment*  

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Assessment rate (%)</th>
<th>Prevalence (%)</th>
<th>Drug Rx Advise (%)</th>
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<tr>
<td></td>
<td>M</td>
<td>F</td>
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<td>15-31</td>
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<td>Mean</td>
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</tr>
<tr>
<td>Mean</td>
<td>46</td>
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* all results for the range of rates seen across countries; Rx = treatment

**Conclusion:** Diabetes prevalence among CHD patients appears much higher than in many Western countries and must be tackled urgently. Screening for diabetes and treatment with drugs and/or insulin is well established in clinical practice. In contrast, rates of measurement of BMI are much more variable and formal dietary advice less consistently given. Morbid obesity is considerably less frequent than in many Western countries suggesting a genetic susceptibility to diabetes in Asia.

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**MoP24:W1**

**Oxidative stress in families of type 1 diabetic patients**

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It is still discussed whether oxidative stress precedes or merely reflects diabetic complications. We investigated indices of glucose and lipid metabolism, markers of lipid and protein oxidation, and the effects of oxygen radicals on erythrocytes of type 1 diabetics and their relatives. We recruited 30 type 1 diabetics (10 without diabetic complications, 10 with retinopathy, 10 with...
Moderately elevated triglycerides are associated to insulin resistance (evaluated by HOMA) in normal-weight subjects with normal glucose tolerance

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Objective: To study whether plasma triglycerides (>400 mg/dl) affect insulin resistance estimated by homeostasis model assessment (HOMA)_IR_ in subjects with normal body weight and normal glucose tolerance.

Methods: We retrospectively evaluated 440 normal weight subjects (BMI 20–25), who underwent an OGTT; subjects with age >20–64 and fasting triglycerides >400 mg/dl were considered for analysis. For the 216 subjects with these characteristics, triglyceride was the index of insulin resistance: Insulinogenic Index and HOMA_IR_ [(fasting IRI (mU/ml) × FBG (mmol/l)/2.5). Data were analyzed both on the entire group by multiple regression analysis and by comparing HOMA_IR_ values in tertiles of triglycerides, by non parametric analysis of variance (Kruskal–Wallis test).

Results: Subjects in the lower tertile of triglycerides levels (24–81 mg/dl) had a lower HOMA_IR_ as compared to the upper 2 tertiles, while no difference was observed between the upper 2 tertiles (p of KW = 0.0011). There was no difference for Insulinogenic Index. Multiple regression analysis showed a highly significant independent direct correlation between HOMA_IR_ and triglycerides (both in absolute values and log-transformed: p = 0.0007 and p = 0092 respectively) and an inverse association with HDL-C (p = 0.0003). Age just reached to reach statistical significance, and BMI was not an independent determinant in multiple regression analysis. Total and LDL-C had no relation to HOMA_IR_.

Conclusions: Triglycerides levels are associated to insulin resistance as measured by HOMA_IR_ also in normal-weight subjects with normal glucose tolerance. Values of triglycerides 81–133 mg/dl are associated to insulin resistance, with no further increase in resistance as the values increase in the range 134–389 mg/dl. HDL-C is inversely and independently predictive of insulin resistance by multiple regression analysis2, while total and LDL-C do not interact with insulin activity, as measured by HOMA_IR_.

Delayed activation of nitric oxide synthase II in the aorta of streptozotocin-diabetic rats

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After tissue damage or inflammatory stimuli, smooth muscle cells express the inducible isoform of nitric oxide synthase (NOS II), which has also been detected within the atherosclerotic plaque. In diabetes, NOS II induction has been postulated to be involved in β-cell destruction but could also be a mechanism for countering diabetes-related enhanced NO inactivation by free radicals. The aim of the present work was to investigate the expression and activity of this rat liver endothelial cell-derived proinflammatory stimul of nitric oxide synthase (VSMC) or i) aortic SMCs were isolated from streptozotocin-diabetic rats and ii) after in vivo treatment of diabetic rats with lipopolysaccharide (LPS). NOS II protein and activity were measured by immunoblotting and Griess reaction, respectively. After incubation with a cocktail of cytokines (IL-1β, INF-γ, TNF-α, and LPS) for 24 or 48 h, NOS II protein content was maximal at 24 h and then decreased in control VSMC. In contrast, the enzyme was detectable after 24 h but reached the synthesis peak after 48 h in diabetic VSMC. When measuring NOS II activity after 24-h stimulation, the levels of nitrite significantly increased in the culture medium of control but not in that of diabetic VSMC. After a 48-h challenge, however, nitrite production was considerably enhanced in both control and diabetic VSMC. The addition of L-arginine to cells treated for 24 h with cytokines did not influence nitrite accumulation in the medium. Similarly, after a 24-h challenge of cells with cytokines in the presence of antioxidant compounds such as ascorbic acid, l-carnitine or leucine, nitrite production was unchanged. Interestingly, however, nitrite formation in diabetic VSMC after 24-h cytokine treatment was similar to that in control cells when the incubation was carried out in medium deprived of any exogenous activity. Similarly to what is observed in primary cultures, the peak of NOS II production in aortic rings incubated with either cytokine mix or LPS alone occurred after 24 h in control and after 48 h in diabetic tissues. Accordingly, in vivo treatment with LPS for 24 h significantly reduced aortic production of NOS II in diabetic with respect to control animals. In conclusion, a delayed NO production by NOS II was observed in the aorta of diabetic rats. In the case of VSMC, this delay was reversed by removal of estrogen-like compounds from the culture medium. While the nature of this block is currently being investigated, these results, together with the well-known impairment in endothelial NOS III activity, point to a general impairment of the NO pathway in diabetes.

Homocysteine and lipid metabolism in children with type 1 diabetes

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Adults with micro- and macro-vascular complications of type 1 and type 2 diabetes have increased Hcy and plasma lipids. As the processes leading to diabetes complications begin early in life, we aimed to investigate Hcy and lipid metabolism in children with type 1 diabetes.

Fasting blood samples were collected from 75 children (aged 13.6 ± 2.6 years) with type 1 diabetes for 5.4 ± 3.7 years and 52 matched controls (aged 13.4 ± 2.5 years). Total plasma Hcy: MTHFR genotype (677 and 1298 polymorphisms); HbA1c; serum vitamin B12, serum folate, and red cell folate; plasma triglycerides, total and HDL-cholesterol; and plasma creatinine were measured. LDL-cholesterol and creatinine clearance were calculated.

Fasting plasma total Hcy was reduced in patients compared with controls, whereas serum vitamin B12 and red cell folate were increased. Using stepwise regression analysis, after correction of Hcy levels for age, serum folate, serum B12 and creatinine clearance, a significant difference between patients with type 1 diabetes and controls persisted (p = 0.001). There was no difference in the frequency of MTHFR polymorphisms. Plasma total Hcy did not correlate with triglycerides, LDL-cholesterol or HDL-cholesterol. LDL-cholesterol was significantly higher in patients than controls, HDL cholesterol was also higher, borderline on significance. HbA1c correlated significantly with LDL cholesterol (r = 0.412, p < 0.001) and triglycerides (r = 0.474, p < 0.001) but did not correlate with Hcy (r = 0.1, p = 0.4) or HDL-cholesterol (r = 0.1, p = 0.39).

Hcy metabolism is altered in children with type 1 diabetes. Higher values for serum folate and vitamin B12 reflecting differences in dietary intake between children with diabetes and controls, partially account for this difference. Our data do not suggest an interaction between Hcy and lipid metabolism in children with type 1 diabetes.

Predictors of LDL particle size in type 2 diabetes


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Objective: To evaluate LDL/apolipoprotein B ratio and other easily measured biological and clinical parameters, previously described in non-diabetic subjects as predictors of LDL particle size, in a group of type 2 diabetic patients.

Methods: 45 type 2 diabetic patients (34 male, age 56.8 ± 11.6 years, body mass index 28.8 ± 4.0 kg/m², diabetes duration 11.7 ± 6.6 years and HbA1c 8.3 ± 1.8%) were studied. Systolic blood pressures were obtained after a 10–12-hour fast. Total cholesterol (c) and triglyceride (t) (mg/dl) were measured. LDL(C) and triglyceride (t) (mg/dl) were measured. LDL(C)/apoB(B) ratio and non-HDL were calculated. Univariate correlations and multiple regression analysis were applied.
We conclude that: 1) in healthy, male gender and smoking are associated with high tHcy levels; 2) tHcy is lower early in the course of type 1 diabetes mellitus (accelerated hepatic transsulfuration?); 3) increased tHcy levels are associated with diabetic nephropathy.

**MoP31:W1**

Fasting dysglycemia and coronary artery disease: Contribution of abdominal obesity and hyperglycemia

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**Objective:** To assess whether the association of fasting glucose with angiographically proven coronary artery disease (CAD) is modified by the presence of abdominal obesity and hyperglycemia.

**Methods:** In this cross-sectional study, multiple logistic regression models were tested in order to determine the relationship of fasting glycaemia to angiographically assessed CAD among 584 men aged 18-69 years who were stratified according to waist circumference (<100 vs ≥100 cm), fasting triglyceridemia (TG) (<2.0 vs ≥2.0 mmol/L) and fasting glucose concentration (<5.0 mmol/L, 5.0-6.9 mmol/L, ≥7.0 mmol/L). By convention, "dysglycemia" was defined as a fasting glucose concentration between 5.0 and 6.9 mmol/L.

**Results:** As observed in other populations, univariate analysis revealed increased odds of finding CAD among subjects with elevated glucose levels: dysglycemic group (5.0-6.9 mmol/L, odds ratio: 1.64 (p = 0.16) and diabetic group (≥7.0 mmol/L, odds ratio: 4.14 (p = 0.001)) compared to normoglycemic controls. Compared to non obese (waist < 100 cm) and normotriglyceridemic (TG < 2.0 mmol/L) controls, the risk of CAD associated with dysglycemia was significantly higher among subjects with both an increased waist line and hyperglycemia (odds ratio: 4.55; 95% CI: 1.07—19.21).

**Conclusion:** Results of the present study emphasize the importance of the atherogenic dysglycemia resulting from abdominal obesity in the modulation of the CAD risk associated with elevated glucose levels.

**MoP32:W1**

B vitamins substantially reduce plasma homocysteine in patients with diabetes mellitus

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**Objective(s):** Accelerated atherosclerosis leads, through mechanisms not fully recognized, to premature cardiovascular morbidity in patients with diabetes mellitus (DM). As homocysteine may amplify injury to vascular tissue in these patients, we have followed its response to supplementation with B vitamins.

**Methods:** Thirty-one patients with DM (Hb A1C = 8.6 ± 1.8%), 60 ± 10 y, with normal creatinine (0.95 ± 0.19 mg/dL) were supplemented with B vitamins – pyridoxine, B12 and folic acid (FA) – or placebo for 4 weeks. Plasma homocysteine was measured by an enzymatic acid analysis.

**Results:** B vitamin supplementation resulted in a significant 80% and 147% increase in both plasma and red blood cells (RBC-FA), from 9.3 ± 4.2 to 15.3 ± 3.8 and from 395 ± 187 to 727 ± 273, respectively (p < 0.0001, in ng/mL). Subsequently, there was a substantial decrease in plasma homocysteine from 10.6 ± 3.9 to 8.6 ± 1.4 (p < 0.05, mmol/L) in the patients with DM. In the placebo-treated group, average plasma and RBC-FA were not altered and plasma homocysteine did not show any significant change throughout the study (9.8 ± 3.6 vs. 11.6 ± 5.7 mmol/L, p > 0.05). Plasma B12 was not consistently altered in either treatment group.

**Conclusions:** Taken the potential harmful effect of homocysteine in vascular biology, the feasibility of its reduction by short-term B vitamin supplementation, may prove to be protective against cardiovascular morbidity in patients with DM.
**Materials and Methods:** 100 patients with NIDDM (30 male and 70 female) aged 40–65 (55 ± 2) were examined. The criteria of metabolic syndrome (MS) components were the following: AH-BP 140–169 mm Hg and/or DBP 90–99 mm Hg. Abdominal obesity (AO)-waist to hip ratio > 1.0 for men and >0.85 for woman if BMI > 25 kg/m²; hyperlipidemia (HLP)-TG level 150–400 mg/dl and/or LDL Ch > 135 mg/dl; compensatory mild and moderate NIDDM with basal glucose level > 126 mg/dl and ≥200 mg/dl 2 hours after 75 g glucose load with absence of urine glucose and ketone bodies. Global coronary events risk was calculated by method based on the PROCAM study.

**Results:** In the presence of MS components patients were divided into the following groups: isolated NIDDM — 4 males (4%), NIDDM with AO — 4 males and 10 females (12%), combination of NIDDM with AO and AH — 5 males and 11 females (16%), combination of NIDDM with AO and HLP-4 males and 6 females (10%), NIDDM with AH and HLP-5 males (5%). Full metabolic syndrome in 8 males and 43 females (51%). Global coronary events risk was distributed in the groups as follows: I group 2.4%, II 5.9%, III 16.6%, IV 28%, V 32.9% and patients with full metabolic syndrome 31%.

**Conclusion:** In the study group half of the patients with NIDDM had full metabolic syndrome. Patients with metabolic syndrome and those with a combination of NIDDM with AO and HLP and NIDDM with AH and HLP had a high global coronary events risk.

**MoP34:W1** Effect of doxazosin, a selective α1-adrenergic blocker, on the size of LDL particle size, in the type2 diabetic patients with hypertension

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**Objective:** Hypertension is common in patients with type 2 diabetes mellitus. We investigated the effect of doxazosin on the lipid metabolism in hypertensive patients with type 2 diabetes, especially LDL particle size which is associated with the insulin resistance syndrome.

**Methods:** Cross-sectional study was designed to determine whether doxazosin, administered with an angiotensin II converting enzyme inhibitor and a Ca antagonist, affects LDL particle size. As a follow-up study, lipid and glucose metabolism and LDL particle size were followed for 12 weeks before and after the initiation of doxazosin administration (1–4 mg/day).

**Results:** The average size of LDL particle was significantly larger in the patients treated with doxazosin (LDL-migration index: 0.348 ± 0.027) than those in the patient treated without doxazosin (0.378 ± 0.035), although low-density lipoprotein cholesterol levels did not differ between the two groups. The plasma glucose and HbA1c levels remained unchanged. Lipid profile showed normolipemia throughout the period of the study. However, LDL particle size demonstrated to change larger during the following period. Small LDL fraction diminished remarkably and large LDL increased on the polyaerylamid gel electrophoresis LDL system (LipoPrint).

**Conclusion:** It was concluded that doxazosin is a useful antihypertensive agent for hyperpressive type 2 diabetic patients in improving the size of LDL particle.

**MoP11:W2** Unsaturated, but not saturated, dietary fatty acids are associated with plasminogen activator inhibitor-1 (PAI-1) activity in men

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**Objective:** To investigate the cross-sectional association of dietary intake of nutrients and specific fatty acids with PAI-1 activity in men.

**Methods:** The PAI-1 activity was measured in 871 men aged 70, participating in a population-based longitudinal study in Uppsala, who underwent metabolic investigations and completed a 7-day food record and a medical questionnaire. The intake of the different nutrients was adjusted for the total energy intake prior to correlation analyses.

**Results:** The activity of PAI-1 was 17 ± 14 U/ml (mean ± SD). The total energy intake (7.3 ± 1.9 MJ) was inversely associated with PAI-1 (r = -0.12, p < 0.001). The intake of carbohydrates and fibre was inversely associated with PAI-1 activity whereas the intake of protein, alcohol, cholesterol, and dietary fat was positively associated with PAI-1. When the fat intake was divided into intake of saturated, mono- and polyunsaturated fat (15%, 12%, and 5% of energy, respectively), there was no correlation between the intake of saturated fat and PAI-1 (r = 0.02, n.s.) whereas the intake of both mono- and polyunsaturated fat was positively associated with PAI-1 activity (r = 0.10, p = 0.003 and r = 0.13, p < 0.001, respectively). The same pattern was present when the dietary intake of separate fatty acids was investigated. The associations were independent of body mass index, waisthip ratio, fasting insulin and serum triglycerides, smoking, physical activity, and use of lipid-lowering or anti-hypertensive medication.

**Conclusions:** The dietary intake of unsaturated fatty acids was associated with PAI-1 activity in men, whereas the intake of saturated fatty acids was not. These results are compatible with a recent in vitro study demonstrating increased production of PAI-1 when unsaturated, but not saturated, fatty acids were added.

**MoP2:W2** Mechanisms of smooth muscle cell migration into fibrin gels

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**Objective:** Fibrin may have an important role in the organization of thrombus, the development of atherosclerosis and the restenosis after PTCA. In this study, we examined the mechanisms of SMC migration into fibrin gels.

**Methods:** Migration of cultured SMCs into fibrin gels was assayed using the methods as reported previously (Thromb Res 84: 129. 1996).

**Results & Discussion:** SMCs migrated into fibrin gel prepared with thrombin without other chemotactic stimuli. Fibrin gels prepared with batroxobin, which cleaves only fibrinopeptide A, with ACTE, which cleaves only fibrinopeptide B, and with protamine sulfate, which cleaves nothing, forms fibrin-like gel, similarly induced the migration of SMCs compared to the gel prepared with thrombin, suggesting that the cleavage of fibrinopeptides is not involved in the migration of SMCs. Both anti-fibrin fragment D and E antibodies inhibited the migration of SMCs into fibrin gels, suggesting that both D and E domains of fibrin are involved in the migration of SMCs into fibrin gels. The migration of SMCs into fibrin gel was inhibited by GRCDS and an anti-integrin αvβ3 antibody, indicating that it is dependent on RGD-containing region of α chain and integrin αvβ3. Both fibrinogen fragments D and E inhibited the migration of SMCs into fibrin gels, suggesting that these fragments may be involved in the regulation of SMC migration into fibrin gels as the result of fibrinolysis.

**MoP3:W2** Protein kinase C-β mediates the production of plasminogen activator inhibitor-1 in vascular endothelial cells induced by native or oxidized low density lipoprotein and lipoprotein(a)

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Increased levels of low density lipoprotein (LDL) and lipoprotein(a) [Lp(a)] have been considered as strong risk factors for atherosclerotic cardiovascular diseases. Previous studies indicate that LDL, Lp(a) and their oxidized forms stimulate the production of plasminogen activator inhibitor-1 (PAI-1) in human umbilical vein endothelial cells (HUVEC). The present study examined the involvement of protein kinase C (PKC) and its isoform in LDL, Lp(a) and their Cu++-oxidized forms on PAI-1 generation in HUVEC and human coronary artery EC (HCAEC). Treatment with 10 nM Lp(a) or 100 nM LDL transiently increased PKC activity in cell lysates at 15 min and 5.5 h after the addition of the lipoproteins. In oxidized LDL or Lp(a)-treated HUVEC, the increases in PKC activity at 15 min and 5.5 h were significantly greater than in native Lp(a)-treated cells. Additions of 1 μM calphostin C, a PKC inhibitor, at the beginning or 5 h but not ≥9 h after the initiation of lipoprotein treatment blocked native and oxidized LDL or Lp(a)-induced increases in PKC activity and PAI-1 production. Treatment with LDL, Lp(a) or their oxidized forms induced translocation of PKC-β1 in EC. Treatments with 60 μM 379916, a PKC-β-specific inhibitor (Eli Lilly), prevented native or oxidized LDL- and Lp(a)-induced PAI-1 production. The production of PAI-1 induced by the lipoproteins in HCAEC was also blocked by 379916. The findings suggest that a delayed activation of PKC-β isoform may contribute to LDL, Lp(a) and their oxidized forms induced PAI-1 production in vascular EC (supported by MRC of Canada, University of Manitoba, MHRD and Canadian Diabetes Association).
MoP4W2

Platelet PAI-1 release and 4G/5G polymorphism of PAI-1 gene promoter in patients with coronary artery disease
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Objective: To compare the platelet plasminogen activator inhibitor-1 (PAI-1) content and release and the 4G/5G polymorphism of the PAI-1 gene promoter between patients with coronary artery disease (CAD) and normal control subjects, and to explore the possible influence of 4G/5G genotypes on the platelet PAI-1 content and release.

Methods: This study included 206 Chinese patients with angiographically proven CAD and 412 normal healthy controls. Platelet PAI-1 release was measured as the plasma PAI-1 antigen in whole blood treated with 10 U/ml thrombin at 37°C minus that in the untreated sample. Total PAI-1 content in platelet was determined from the PAI-1 antigen concentration in whole blood lysed with 1% Triton-X 100 minus that in the untreated plasma sample adjusted by hematocrit. The 4G/5G genotype was detected by polymerase chain reaction with single-strand conformation polymorphism and verified by direct sequencing.

Results: The CAD patients showed significant greater 4G allele frequency (0.604 vs. 0.532, p = 0.015) and higher 4G/4G genotype percentage (34.5% vs. 24.5%, p = 0.025) than normal control subjects. The total PAI-1 content in platelet (187 ± 66 vs. 156 ± 54 ng/ml, p < 0.001) and platelet PAI-1 release (97 ± 58 vs. 60 ± 39 ng/ml, p < 0.001) were significantly elevated in CAD patients than in controls. Patients with 4G/4G genotype had significantly higher total PAI-1 content in platelet (202 ± 64 vs. 182 ± 67 and 167 ± 59 ng/ml, p = 0.01) and greater platelet PAI-1 release (121 ± 55 vs. 92 ± 61 and 77 ± 39 ng/ml, p = 0.039) than those with 4G/5G and 5G/5G genotypes. A similar association between the 4G/5G genotypes and total PAI-1 content in platelet (167 ± 52 vs. 157 ± 59 and 138 ± 30 ng/ml, p = 0.041) and platelet PAI-1 release (73 ± 36 vs. 58 ± 42 and 51 ± 30 ng/ml, p = 0.023) were also observed in normal controls.

Conclusions: The study showed that the 4G/4G genotype of the PAI-1 gene promoter was associated with coronary atherothrombosis and elevated platelet PAI-1 content and release in a Chinese population. It suggested that the 4G/4G PAI-1 gene variant could be a transmissible coronary risk factor that would worsen the fibrinolytic defect by increasing the platelet PAI-1 release during a thrombotic event.

MoP5W2

Novel method for determination of lower molecular weight fibrinogen in human plasma
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Determination of fibrinogen (Fbg) by the method of Clauss gives significantly lower values in EDTA than in citrated plasma. This is due to the fact that lower molecular weight (LMW) Fbg forms fibrin clot only in the presence of magnesium (Mg) ions. Thus, measurement of thrombin-clotting times in EDTA plasma with and without Mg ions allows for a rapid determination of LMW-Fbg in human blood. Mean ± SD values for LMW Fbg in healthy subjects, patients with diabetes mellitus (DM) and without nephropathy were found to be 40 ± 6, 79 ± 14 and 165 ± 49 mg/dl, respectively. Concentration of LMW Fbg was inversely correlated with blood fibrinolytic activity, confirming previous findings that degradation of Fbg is not plasmin dependent. A positive correlation with leukocyte count and their activation suggests that the formation of LMW Fbg is a result of action of elastase and/or other proteases released from leukocytes during the process of chronic inflammation in DM.

MoP6W2

Effect of pyridoxal-5-phosphate on platelet aggregation in relation to intracellular calcium mobilization and adenosine-3’-5’-cyclic monophosphate accumulation
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Objective: To investigate the signaling of PAP on ADP-induced platelet aggregation in relation to intracellular calcium [Ca2+]2 mobilization and adenosine-3’-5’-cyclic monophosphate (cAMP) level.

Methods: Washed human platelets loaded with Fura-2/AM and treated with different concentrations of PLP (100, 200, 300, 500 or 600 nM) for 20 min were used to monitor [Ca2+]2 mobilization by 4-4500 spectrophotometer (HTIAC/HP). In the presence or absence of 1 μM calcium, ADP-induced platelet aggregation of washed human platelet treated with PLP (100, 200, 300, 500 or 600 nM) for 20 min in the presence of 2 mg/ml fibrinogen and 1 mM calcium was determined by Pack-4 aggregometer (Beaumont, Texas). Intracellular cAMP level of platelets was also analyzed in the platelet-rich plasma (PRP) treated with PLP (100, 200, 300, 500 or 600 nM) for 20 min by ELASA.

Results: ADP-induced platelet aggregation and [Ca2+]2 mobilization were decreased with increasing concentrations of PLP in both absence and presence of 1 mM calcium. Intracellular cAMP level was increased with increasing concentration of PLP. Platelet intracellular cAMP level was negatively correlated to aggregation (r = -0.88, P < 0.02) and [Ca2+]2 (r = -0.87, P = 0.02). However, platelet aggregation was positively correlated to [Ca2+]2 (r = 0.97, P = 0.001).

Conclusions: We found that PLP may exert its antiplatelet aggregation by increasing intracellular cAMP levels which inhibit [Ca2+]2 mobilization.

MoP7W2

Plasmin cleavage sites not detectable in fibrin within foamy macrophages of most human plaques
W.D. Thompson, 1 R. Stretton, 1 J. Seath, 1 S.J. McNally, 2 A. Reid, 3 E.B. Smith, 1 C.M. Stirk, 1,2 W.T. Melvin, 1 P.J. Gaffney, 3 1 Dept of Pathology; 2 Molecular and Cell Biology, University of Aberdeen, AB9 2ZD; 3 National Institute for Biological Standards and Control, Potters Bar EN6 3EQ, UK

Objective: To characterise fibrin within plate macrophages and in cultured macrophages using novel anti-fibrin antibodies.

Background & Methods: Fibrin has been demonstrated to be present in the foamy macrophages in nearly all human plaques by immunostaining with a specific antifibrin antibody (SF3, P.J. Gaffney). Two rabbit polyclonal antibodies were raised against synthetic peptides directed at sequences adjacent to the early and late plasmin cleavage sites on fibrin. Extracellular fibrin was only weakly immunostained, but fibrin in macrophages in chronic inflammatory lesions such as abscesses was strongly stained using the anti-β 15-27 and anti-γ 54-62 antibodies. In contrast, the plaque foamy macrophage intracellular fibrin was labelled strongly in only 4 of 40 lesions. These findings were explored further by partial enzyme digestion of fibrin clotted on glass slides and by short term culture of purified human monocytes on fibrin layers and within fibrin clots.

Results: Fibrin on slides was negative with the anti β and γ antibodies unless treated by partial digestion with plasmin, but not cathepsins B and D. Monocytes cultured on or within fibrin, developed a positive rim of adjacent immunostained fibrin within 1 hour using both antibodies, and phagocyted positive fibrin within 4 hours. This was reduced by inclusion of aprotinin to neutralise plasmin.

Conclusions: These results imply that the foamy macrophages within the core of the plaque phagocytose fibrin that has not been exposed to plasmin digestion, consistent with earlier studies of plaque extracts showing a relative deficiency of plasminogen in many advanced plaques.

MoP8W2

Vitamins C and E reduce vascular PAI-1 expression after angioplasty in a model of porcine hypercholesterolemia
J.A. Rodríguez, 1 J. Orbea, 2 A. Grau, 1 J.A. Badarme, 2 D. Martín-Caro, 1
1 Dept. of Cardiology; 2 Laboratory of Vascular Biology, School of Medicine, University of Navarra, Pamplona, Spain

Objective: Plasminogen activator inhibitor type 1 (PAI-1) increases alters fibrinolytic balance, contributing to fibrin and extracellular matrix accumulation. We hypothesized that antioxidant vitamins C and E could favorably modify PAI-1 expression after angioplasty in hypercholesterolemic pigs.

Methods: 36 animals were divided into three groups: a normal-cholesterol (NC) control group (n = 12); a high-cholesterol (HC) group (n = 12); and a high-cholesterol plus vitamins C and E (HCY) group (n = 12), supplemented last week with 1 g vitamin C and 1000 IU vitamin E/day. In all animals, vascular injury was induced in the right internal iliac artery 4 weeks after initiation of either dietary regimen; the left internal iliac artery was left intact, to be used as control. Plasma samples and vascular sections were obtained at different time points, to measure PAI-1 activity and analyze vascular morphometry and PAI-1 expression (in situ hybridization and immunohistochemistry).

Results: 4 weeks after vascular injury HCY group showed a 30% reduction in the number of arteries with neointima and a significant reduction in the intima area (vH HC group). Both plasma (P < 0.001) and vascular PAI-1 increased significantly after angioplasty, the mRNA expression being significantly higher as compared with non-injured arteries (P < 0.05). When analyzing the effect of vitamins on PAI-1 expression we found a marked decrease in plasma PAI-1 activity 28 days after injury (P = 0.018), as well as a reduction of vascular PAI-1 expression in the vascular wall (P = 0.036).
Conclusions: These results lead us to propose that antioxidant vitamins have a beneficial effect in this porcine model of angioplasty by reducing local and systemic PAI-1 levels, contributing to a decrease in fibrin and matrix accumulation.

Objectives: Fibrinolytic activity has been reported to be decreased in atherosclerosis. Recently, annexin II is identified as a co-receptor on endothelial cells for plasminogen and tissue plasminogen activator. In this study, we generated a recombinant annexin II protein and examined whether recombinant annexin II protein can modulate fibrinolytic activity on vascular endothelium.

Method: A full length annexin II cDNA was inserted into a histidine-tagged protein expression vector, pQE (pQE-AN II) and the cell lysate prepared from overnight culture of pQE-AN II transformed JM109 was purified using nickel column. The fibrinolytic activity of recombinant annexin II was analyzed by measuring fluorescent activity using fluorescently labeled plasmin substrate.

Results: Recombinant annexin II (RAN II) exhibited significantly higher plasmin generation compared to control protein (BSA) when coated on platelet-derived growth factor (RAN II: 1202 ± 51.07 R.U., BSA: 197.3 ± 35.92, p < 0.005). To investigate the fibrinolytic function of recombinant annexin II on endothelial cell, we subjected recombinant annexin II on human umbilical vein endothelial cell (HUVEC). Fluorescent immunolassay using anti-annexin II mAb showed specific binding of recombinant annexin II on HUVEC. Plasmin generation assay revealed that recombinant annexin II significantly enhances plasmin generation on HUVEC compared to HUVEC treated with human annexin II (RAN II: 124 ± 5.48 R.U., BSA: 62.5 ± 8.35, p < 0.005). Addition of EGF but not heparinase inhibited binding of RAN II as well as plasmin generation.

Conclusion: Recombinant annexin II enhances plasmin generation on vascular endothelium. This effect of annexin II seems calcium dependent but not sensitive to heparinase treatment. Enhancement of endothelial fibrinolytic activity by annexin II could modulate hypercoagulable state in atherosclerosis.

Objectives: Plasma AFT is correlated with triglycerides (TG) and may be increased by a preponderance of very low density lipoprotein (VLDL) particles. This study investigated the association of AFT with VLDL particle size distribution and whether AFT is associated with coronary calcification, a measure of atherosclerosis burden.

Methods: Coronary artery calcification (CAC) was quantified using electron beam computed tomography, in 190 type 1 diabetic patients (50% female) and 195 non-diabetic controls aged 30-55 yrs. AFT was assayed by ELISA. VLDL particle size was measured by NMR-spectroscopy. Five non-DM subjects with TG < 6 mmol/l were excluded.

Results: AFT was positively associated with BMI (partial correlation coefficient r = 0.2) and TG (r = 0.33), both at p < 0.01 after adjustment for age, sex and diabetes. AFT was also associated with the proportion of VLDL that was large (≥60 nm) independently of VLDL-TG levels (r = 0.16, p = 0.002), consistent with factor XIIB being activated by large TG-rich lipoproteins. Mean AFT was lower in type 1 diabetic patients compared to controls (1.55 ng/ml vs. 1.91 ng/ml, SD = 0.75 ng/ml, p < 0.001 adjusted for age and sex). This was independent of TG, BMI and proportion of VLDL that was large (difference = 0.34 ng/ml on adjustment, p < 0.001). Higher AFT was associated with an increased odds of any CAC (odds ratio per ng/ml increase in AFT = 1.5, 95% CI 1.1-2.1, p = 0.002), to a similar extent in diabetic and non-diabetic subjects. This association was not independent of BMI and TG (odds ratio on adjustment = 1.2, 95% CI 0.8-1.7, p = 0.4).

Conclusions: AFT is lower in Type 1 diabetic patients. This is only partly explained by their lower TG. Elevated AFT is a marker of increased TG and larger VLDL size. Elevated AFT is associated with increased CAC, but this association is largely explained by the association between AFT and triglyceride metabolism.
MoP14:W2 Influence of the fibrinolytic system in the regulation of extracellular matrix metalloproteinases
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The activity of the fibrinolytic system acts in the inside of the vessel wall, contributing to the remodeling of the extracellular matrix (ECM) through direct or mediated attack on the matrix metalloproteinases (MMPs).

Purpose: To study the fibrinolytic activity of the human arterial wall and its metabolic role in MMP production and activation.

Methods: 58 arterial samples from the circular piece obtained from the insertion of a femoral, aortic, or carotid bypass were immediately frozen in liquid nitrogen and maintained at −70 °C until they were studied. Arterial fragments were incubated in suitable medium for 120 minutes at 37 °C. tPA, uPA and PAI-1 were evaluated by ELISA (ng/mL); plasmin-generating ability (PGA) on fibrin plates with and without plasminogen against the standard for urokinase (PU 10−2/100 mg dry tissue); and MMP-2 and -9 by gelatinase activity as determined by zymography on polyacrylamide gel following electrophoresis, reading by scanning densitometry (OD = mm2/100 mg dry tissue). MMPs were identified against specific patterns, phenolntrol inhibition, MW determination and Western blot.

Results:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>tPA</th>
<th>uPA</th>
<th>PAI-1</th>
<th>PGA</th>
<th>MMP2</th>
<th>MMP2Ac</th>
<th>MMP9</th>
<th>MMP9Ac</th>
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<td>4.7</td>
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<tr>
<td>SD</td>
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<td>3.1</td>
<td>92.1</td>
<td>65.7</td>
<td>28.0</td>
<td>4.3</td>
<td>32.5</td>
<td>9.4</td>
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<tr>
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</table>

Correlation study: MMP9Ac-tPA: r = 0.46, p = 0.0003; MMP9Ac-tPA: r = 0.91, p = 0.0005; MMP2-Ac-tPA: r = 0.31, p = 0.024. MMP9 and MMP2 showed no correlation with PGA or the other parameters.

Conclusions: The arteries released into the incubation medium tPA, uPA, PAI-1, active plasmin, MMP9 and MMP2 as zymogens and in activated form. These results reveal a possible relation or activation of MMPs by uPA and exclude the possible role of plasmin in the activation of the MMPs in the human arterial wall.

Study sponsored by CICYT; project no. SAF96-0313.
Results: α-Thrombin time-dependently and concentration-dependently increased VEGF mRNA levels, mainly that coding for the soluble splice variant VEGF165, and stimulated the release of VEGF protein. These effects required the proteolytic activity of thrombin and were mimicked by a thrombin receptor activating peptide and reduced by inhibitors of either protein kinase C, protein tyrosine kinases or phosphatidylinositol 3'-kinase, and by antioxidant treatments. Upregulation of VEGF expression was also induced by conditioned medium from α-thrombin-stimulated SMC. Both a PDGF-neutralizing antibody and a TGF-β-neutralizing antibody significantly attenuated the α-thrombin-induced release of VEGF.

Conclusions: Thrombin upregulates the expression of VEGF in vascular SMC through a direct effect, which is dependent on the activation of protein kinase C, protein tyrosine kinases and phosphatidylinositol 3'-kinase, and the formation of reactive oxygen species, and an indirect effect, which is dependent on the endogenous formation of PDGF and TGF-β.

MoP18:W2 Effects of homocysteine on platelet aggregation in vitro
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Objective: High levels of plasma homocysteine are considered to be an independent risk factor for atherothrombotic disease. One possible mechanism may be through activation of platelet aggregation. This study was performed to explore the effects of homocysteine on platelet aggregation using various concentrations of homocysteine.

Methods: Platelets were obtained from 16 healthy subjects not taking drugs known to modify platelet activity. Platelet rich plasma (PRP) and whole blood, were incubated with homocysteine at concentrations of 30 μM, 0.5 mM and 1 mM at 37°C for 15 min before aggregation was assessed with and without the addition of other agonists. Changes in impedance were measured over 8 min and expressed as amplitude (ohms) and slope (ohms/min).

Results: Homocysteine alone failed to induce platelet aggregation, both in the whole blood and PRP. Homocysteine at 30 μM slightly increased (p < 0.05) platelet aggregation induced by ADP 20 μM in whole blood (slope 3.0 ± 0.8 vs 5.0 ± 2.0, ohms/min, n = 9) and by collagen 2 μg/ml in PRP (slope 8.5 ± 1.5 vs 11.2 ± 2.5, ohms/min, n = 6). However, homocysteine at 1 mM decreased (p < 0.05) platelet aggregation induced by ADP 10 μM and 20 μM in whole blood (amplitude 7.0 ± 4.0 vs 3.6 ± 2.5, 6.8 ± 2.2 vs 4.1 ± 3.3 ohms respectively, n = 9).

Conclusion: Homocysteine alone did not induce platelet aggregation in vitro. However, at 30 μM, a concentration similar to moderately elevated in vivo levels, homocysteine potentiated agonist-induced platelet aggregation in whole blood and PRP, but very high concentrations of homocysteine appeared to decrease platelet aggregation in vitro.

MoP19:W2 Adrenomedullin modulates the expression of tissue factor and factor VIIa pathway inhibitor by human aortic endothelial cells
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Objective: Adrenomedullin (AM) is a potent hypotensive peptide, recently isolated from pheochromocytoma tissue. Recent reports have shown some physiological roles of AM in the cardiovascular system. However, there is no report concerning the role of AM on blood coagulation system. We examined the effect of AM on expression of tissue factor (TF) and tissue factor pathway inhibitor (TFPI) in cultured human aortic endothelial cells (HAoECs).

Methods: HAoECs were originally isolated from a 52-year-old caucasian female. The subconfluent cells, between passages 4 to 6, were exposed to various concentrations of AM with serum-free medium. Cell growth was examined by colorimetric assay and apoptosis by TUNEL assay. TF and TFPI antigen levels in conditioned medium (CM) and cell lysates (CL) were measured with ELISA. The effects of AM receptor antagonists (AM22-52, AM1-25, CGP41251) on cell viability, AM-modulating monoclonal antibodies, and inhibitors of second messengers for AMP; Rp-8-Br-CAMP and for MAP kinase; PD98059 on AM action were also examined.

Results: Apoptosis was rapidly induced by serum-free condition, which was prevented by AM with dose dependent manner. Increase of TF antigen levels in CL, probably, was suppressed by AM. TFPI antigen levels in CM increased rapidly after AM exposure and also gradually until 24 hr in time- and dose-dependent manner. These effects of AM were inhibited by AM receptor antagonists, anti-AM antibodies, and inhibitors of cAMP and MAP kinase.

MoP20:W2 Risk of ischaemic heart disease or ischaemic cerebral stroke and levels of fibrinogen and von Willebrand factor
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Objective: To study levels of fibrinogen (Fb), von Willebrand factor (vWF) and arterial blood pressure in patients with ischaemic heart disease (IHD) or ischaemic cerebral stroke (ICS) and their children.

Material and Methods: The study was performed in 50 families (100 parents and 90 children) with a history of IHD (36 parents, 27 children; fathers with myocardial infarction, mothers healthy), ICS (30 parents, 34 children; one parent father or mother with cerebral stroke) or as healthy controls (34 parents and 29 children). The mean age of parents and children was 43.5 ± 6.1 and 12.2 ± 3.1 years, respectively.

Results: Fb and vWF levels in children of the IHD and ICS groups did not differ significantly from control values. Systolic (SP) and diastolic (DP) blood pressures in children of the IHD and ICS groups were significantly higher (IHD: 119 ± 19/76 ± 8; ICS: 108 ± 127/73 ± 8; control 98 ± 963 ± 8). Parents of the ICS group had elevated levels of Fb, vWF, SP and DP, as compared with controls (Fb: mothers 3.6 ± 0.84 vs 2.98 ± 0.35 g/l, fathers 4.02 ± 0.78 vs 3.08 ± 0.54 g/l; vWF: mothers 110.7 ± 2.0 vs 88.2 ± 21.5%, fathers 118.4 ± 4.5 vs 82.9 ± 23.7%; SP: mothers 140 ± 25 vs 116 ± 15, fathers 151 ± 28 vs 127 ± 18 mmHg; DP: mothers 90 ± 13 vs 74 ± 9, fathers 98 ± 14 vs 79 ± 13 mmHg).

A positive correlation was noted only for vWF levels in children and their mothers, being strongest in the ICS group (r = 0.43, 0.48 and 0.55 for control, IHD and ICS group, respectively).

Conclusion: It appears from our results that Fb and vWF are risk factors of particular importance for the progression of ICS, chiefly in females with accompanying arterial hypertension.

MoP21:W2 Procoagulant activity of T lymphocytes due to exposure of negatively charged phospholipids – Role of lipid oxidation
T.W. Barrowcliffe, M. Jardí, N. Rodriguez-Lambies, P. Fabregas, J. Felez. NIBSC, Potters Bar, UK; IRO, Barcelona, Spain

Our previous studies have described procoagulant activity (PCA) of a variety of cell lines due to exposure of negatively charged phospholipids, as confirmed by binding studies with Annexin V and coagulation FVIII. In the present study the ability of two T-lymphoblastoid cell lines, Molt 4 & Jurkat, to promote generation of Factor Xa (FXa) was compared to that of other cell lines, to normal lymphocytes and neutrophils, and to a standard procoagulant phospholipid (PL).

Cell lines Molt 4, Jurkat, Nalm 6, THP-1, U-937 & NB4 were cultured under standard conditions, washed in RPMI, and suspended in 0.05 M Tris/0.15 M NaCl, pH 7.4 at a concentration of 4 × 10^6/ml. Factor Xa generation was measured by incubating purified human FX, FXa, FVIII with CaCl2 & cells or PL, and subsampling at intervals into S2765. The activity of the cells, measured as initial rate of FX activation, was converted to phospholipid units (PLU) by comparison with a PL dilution curve.

The T-lymphoblastoid cell lines Molt 4 (28 ± 6 PLU/ml) and Jurkat (17 ± 2 PLU/ml) had most activity; the other cell lines gave activity ranging from 5.6-16 PLU/ml. Normal fresh lymphocytes and neutrophils had no detectable activity but both cell types developed activity (6-12 PLU/ml) on incubation in serum-free medium. Incorporation of vitamin E into cell cultures inhibited by 68% the increase in PCA on incubation of neutrophils but had no effect on the activity of normal lymphocytes or Molt 4.

These results demonstrate that T lymphocytes can develop potent PCA due to exposure of negatively charged phospholipids and this is unlikely to be due to lipid oxidation. T lymphocytes occur in atherosclerotic plaques and it is possible that their PCA could play a role in thrombotic episodes associated with plaque rupture.
MoP22.W2

Impairment of prostacyclin-induced platelet inhibition in free radical-treated platelets

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Objectives: Prostacyclin (PGI2) is a potent vasodilator, cytotoxic and platelet inhibitor. Hyperactive platelets are known to play an important role in atherothrombogenesis. Oxidant stress has also been proposed as a factor in atherothrombosis. We examined the effect of an oxidant stress directed in vitro against platelets on their sensitivity to the PG2 analogue carbacyclin (CBC).

Methods: Rat platelets have been exposed to sublytic concentrations of an azo-type free radical generator. Subsequently, the thrombin-induced platelet aggregation, the sensitivity to the inhibitory effect of CBC have been determined as well as the binding characteristics of PGI2.

Results: Oxidant stress resulted in a reduced inhibition by CBC which was correlated with the degradation of platelet unsaturated fatty acids which was opposed by antioxidants. The inhibition of platelet aggregation by PGI2 is known to be achieved by increasing the intracellular level of cAMP. We thus examined the effect of the oxidant stress on the CBC-induced cAMP synthesis. We found that the prostanoïd-induced cAMP concentration was decreased in mildly oxidized platelets. Since PGI2 and PGE1 bind to the same receptor on the platelet surface, [3H]PGE1 was used as a stable probe to determine the PGI2 receptor binding. Equilibrium binding showed a significantly reduced binding of [3H]PGE1 to the PGI2 receptors on platelet surface of oxidant-treated platelets when compared with controls.

Conclusions: These results demonstrate that mild oxidant stress induced a loss of the inhibitory effect of PGI2 analog on treated platelets. According to the known beneficial effects of PGI2, impairment of the inhibitory effect of this prostanoïd by oxidant stress may represent a new mechanism contributing to an increased atherothrombosis.

MoP23.W2

Pioglitazone upregulates the expression of thrombospondin in THP-1 cells

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Porosomial proliferator-activated receptor y (PPARy) regulates the differentiation of the monocytes/macrophages as well as adipocyte. Thrombospondin (TM) acts as a major anti-thrombotic mechanism by forming a complex with thrombin. It is assumed that TM may have anti-inflammatory properties as well as its anticoagulant properties in monocytes/macrophages.

Objective: Since the expression of thrombospondin (TM) by monocytes is upregulated during its differentiation into macrophages, we investigated the effect of pioglitazone, a thiazolidinedione that is a synthetic ligand of PPARy, on the expression of TM by THP-1 monocytic cells.

Method: THP-1 cells and human umbilical vein endothelial cells (HUVECs) were incubated with pioglitazone. Levels of TM antigen were measured by enzyme immunoassay. TM cofactor activity for thrombin-dependent protein C activation was determined by measuring the amidolytic activity of the activated protein C. The expression of TM mRNA was determined by Northern analysis.

Results: The incubation of THP-1 cells with pioglitazone treated to a dose-dependent increase in cellular TM antigen levels up to 2.7 times the control level, accompanied by an upregulation of TM cofactor activity. The expression of TM mRNA in pioglitazone-treated THP-1 cells was 7.2 times that in untreated cells. In contrast, the expression of TM antigen and the levels of mRNA in HUVECs were unaffected by such treatment.

Conclusion: PPARy regulates TM expression in THP-1 monocytic cells and that thiazolidinediones may prevent atherogenesis through an increase in the anticoagulant and/or anti-inflammatory properties of TM.

MoP3.W3

Effect of smoking cessation on arterial wall dynamics

E. van den Berkmoortel1, H. Wollersheim1, H. van Langen2, Th. Thien1.

Objective: To study the influence of smoking cessation on cross-sectional compliance (CC; mmHg/kPa) and distensibility coefficient (DC; 10^-5/kPa) of the right common femoral (CF) and both common carotid arteries (CCA).

Methods: In 55 non-smokers (NS), 57 smokers who persisted smoking (PS) and 37 smokers who stopped after inclusion (SS), CC and DC were calculated out of (changes in) diameters and pulse pressures. These were measured at baseline (b) and after 2 years follow-up (f) by Wall Track System® and Dinamap® [1]. Step smoking was controlled by urinary cotinine levels (<300 ng/ml). None of the participants suffered from other cardiovascular risk factors or overt atherosclerotic disease.

MoP1.W3

Carotid plaque measurement: An important tool for research as well as clinical management of atherosclerosis

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Objectives: For 6 years we have performed annual measurement of carotid plaque in our patients in the Premature Atherosclerosis Clinic, and in the Stroke Prevention Clinic, for both research and clinical purposes.

Methods: Cross-sectional area of all plaques in the common, internal and external carotid arteries on both sides are measured by tracing the plaques with a cursor in a magnified longitudinal view chosen to represent the largest extent of each plaque; the sum of all plaques is Total Plaque Area (TPA).

Results: 927 patients have been measured at baseline, 615 at 2 years, 393 at 3 years, 283 at 4 years, 234 at five years, and 139 at six years.2415 at 2 years, 393 at 3 years, 283 at 4 years. Multiple regression with age, sex, blood pressure, cholesterol, and smoking gives an R-squared of 0.515 for prediction of TPA; this gives a quantitative trait, unexplained atherosclerosis. To date 8 genetic association studies have been performed using this quantitative trait. Homocysteine independently predicts plaques, and vitamin therapy regresses it; a polymorphism in mannose binding lectin associated with increased chlamydia infection is an independent predictor of plaque area. Plaque area identifies patients at high risk for coronary disease: the top quintile of plaque area has an unadjusted odds ratio (OR) of 8.5 (95% CI 2.5 to 29.6); after adjustment for age, sex, blood pressure, lipids and smoking, the OR is still 8.1 at 8.5 (p < 0.01).

Conclusion: Measurement of carotid plaque area is a powerful tool for research and clinical management. Trying to manage atherosclerosis without knowing how the arteries are doing is like trying to manage hypertension without measuring the blood pressure.

P.W3 IMAGING OF ATHEROSCLEROSIS

MoP2.W3

Carotid and femoral intimomedial thickness and plaques in patients with familial hypercholesterolemia

T. J. Smidlev1, M.D. Trip2, H. Wollersheim1, S. van Wissen2, J.J.P. Kastelein2, A.E.H. Stalenhoef1, 3Academic Hospital Nijmegen; 2Academic Medical Center, Amsterdam, The Netherlands

Objective: To assess intimomedial thickness (IMT) and plaques prevalence in the carotid and femoral arteries in patients with familial hypercholesterolemia (FH) with and without manifest cardiovascular disease (CVD).

Methods: In patients with FH, participating in a 2 year double blind trial, lipids and IMT were assessed after a placebo run in period. IMT's (both right and left) of the common carotid artery (CCA), bulb (BUL) and internal carotid artery (ICA), together IMT carotid, and common carotid artery (CCA) were measured in mm by high resolution ultrasound. Plaque was defined as an IMT > 1.3 mm and/or wall interface displacement.

Results: (Table) 326 patients with definite FH, mean age 48.5 ± 10.5 yr were included.

<table>
<thead>
<tr>
<th>CVD</th>
<th>(m)</th>
<th>CVD+</th>
<th>(m)</th>
<th>CVD+</th>
<th>(m)</th>
<th>CVD+</th>
<th>(m)</th>
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<th>(m)</th>
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<tr>
<td>TC</td>
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<tr>
<td>LDL-C</td>
<td>8.04 ± 1.90</td>
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<td>8.14 ± 1.87</td>
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<tr>
<td>HDL-C</td>
<td>1.07 ± 0.27</td>
<td>1.09 ± 0.28</td>
<td>1.25 ± 0.31</td>
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<tr>
<td>TG</td>
<td>1.96 ± 0.93</td>
<td>1.98 ± 0.87</td>
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<tr>
<td>IMT carotid</td>
<td>0.94 ± 0.20</td>
<td>1.02 ± 0.22</td>
<td>0.92 ± 0.26</td>
<td>0.97 ± 0.31</td>
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<tr>
<td>IMT CFA</td>
<td>1.63 ± 0.75</td>
<td>2.03 ± 0.85</td>
<td>1.50 ± 0.66</td>
<td>1.85 ± 1.21</td>
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<tr>
<td>Plaques cca</td>
<td>12.1%</td>
<td>42.4%</td>
<td>12.8%</td>
<td>25.9%</td>
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<tr>
<td>Plaques bula</td>
<td>54.5%</td>
<td>63.6%</td>
<td>46.6%</td>
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<tr>
<td>Plaques cfa</td>
<td>56.6%</td>
<td>93.9%</td>
<td>48.1%</td>
<td>70.5%</td>
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</table>

Conclusion: The IMT in this group of relatively young FH patients is largely increased. Plaques prevalence is high even in patients without manifest CVD indicating a strongly elevated risk for CVD.

MoP3.W3

Effect of smoking cessation on arterial wall dynamics

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Objective: To study the influence of smoking cessation on cross-sectional compliance (CC; mmHg/kPa) and distensibility coefficient (DC; 10^-5/kPa) of the right common femoral (CF) and both common carotid arteries (CCA).

Methods: In 55 non-smokers (NS), 57 smokers who persisted smoking (PS) and 37 smokers who stopped after inclusion (SS), CC and DC were calculated out of (changes in) diameters and pulse pressures. These were measured at baseline (b) and after 2 years follow-up (f) by Wall Track System® and Dinamap® [1]. Step smoking was controlled by urinary cotinine levels (<300 ng/ml). None of the participants suffered from other cardiovascular risk factors or overt atherosclerotic disease.
MoP4:W3  Macrophages in carotid plaques predicted by ultrasound B-mode imaging

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Objective: Atherosclerosis may be regarded as an inflammatory disease dominated by macrophages. We tested whether macrophages in carotid atherosclerotic plaques can be predicted by echolucency on ultrasound B-mode imaging, elevated acute phase reactants, or elevated levels of lipoproteins.

Methods and Results: We studied 106 patients with ≥50% carotid stenosis and previous ipsilateral hemispheric neurological symptoms undergoing carotid endarterectomy. Macrophage density in carotid plaques classified by ultrasound B-mode imaging as echolucent (n = 56), intermediate (n = 25), or echogenic (n = 25) was 1.7% ± 0.3%, 1.5% ± 0.4%, and 0.7% ± 0.2% (x ± SE), respectively (ANOVA: p = 0.01). A computer-generated measure of plaque echolucency, gray scale median, also predicted increased macrophage density (r = -0.29; p < 0.005). In patients with macrophages present (n = 101) or absent (n = 5) in carotid plaques, plasma levels of C-reactive protein (CRP) were 8.6 ± 1.5 and 1.0 ± 0.1 mg/L (t-test: p < 0.01); equivalent values for von Willebrand factor were 120 ± 3 and 80 ± 10 mg/L (p < 0.005). Furthermore, total and LDL cholesterol levels in plasma predicted carotid macrophage density (r = 0.26; p = 0.008; r = 0.23, p = 0.02). Finally, macrophage density in carotid plaques of users (n = 57) and non-users of aspirin (n = 52) was 1.2% ± 0.2% and 1.7% ± 0.2% (t-test: p = 0.02).

Conclusions: Increased macrophage density in carotid atherosclerotic plaques is predicted by plaque echolucency and increased plasma levels of total and LDL cholesterol. Moderately elevated levels of CRP or von Willebrand factor in plasma seem to predict presence of macrophages, while use of aspirin is associated with reduced macrophage density in carotid plaques.

MoP5:W3  4D flow patterns in normal subjects vs patients with coronary artery disease

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Objective: To test the hypothesis that factors in the aorta may influence coronary artery flow.

Methods: Cardiac gated 3-dimensional time resolved velocity data obtained with MR velocity encoded phase contrast sequences were used. Normal subjects were studied earlier, one group of young people and one group of elderly and 12 patients with aortic grafts. Patients with CAD are being studied.

Results: Blood flows in a right-handed helix in the ascending aorta and the aortic arch. In young people blood passes from the ascending to the descending aorta in one heart beat. In people in their 60 s it takes two and above 78, 3 beats (p = 0.04). Systolic anastogate velocities are higher (p = 0.0003) and retrograde flow is slower in young vs elderly people. Age-matched patients with CAD have abnormal aortic flow patterns. All aortic grafts in the ascending or descending aorta had irregular abnormal flow.

Conclusions: Patients with CAD have abnormal blood flow patterns in the aorta compared to age matched normal subject. All aortic grafts have abnormal energy losing flow patterns.
with two-way ANOVA (family number as a random factor). Age was used as a covariate. Partial correlations (controlling for age) were calculated between IMT and different lipid parameters.

Results: Age averaged 39 ± 10 and 41 ± 12 years in FCHL patients and not affected relatives, respectively, (P = 0.064). The groups did not differ from each other as regards the number of smokers or blood pressure. The mean of maximal IMTs was 1.02 ± 0.25 in FCHL patients and 0.95 ± 0.21 in not affected relatives (P = 0.33). In the whole material IMT did not correlate significantly with any lipid parameters. When maximum IMTs of the carotid artery segments: common carotid artery (CCA), carotid bulb (CB) and internal carotid artery (ICA) were observed separately, ICA correlated significantly with apo B (r = 0.312, P = 0.016).

Conclusions: In this preliminary study, the significant correlation between ICA-IMT and apo B may indicate that apo B may not be only an important lipid marker for FCHL but could also predict degree of early atherosclerosis in these patients.

MoP10:W3 Early and intermediate lesions of atherosclerosis in the human carotid artery: Visualization by magnetic resonance imaging and assessment of imaging reproducibility

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Objective: To assess the reproducibility of high resolution magnetic resonance imaging (MRI) in identifying and quantifying early and intermediate lesions of atherosclerosis in human carotid arteries.

Methods: Nineteen subjects with <75% stenosis by duplex ultrasound were recruited for the study. Two independent MRI scans were conducted within 2 weeks using a high resolution carotid imaging protocol developed in our lab on a 1.5T scanner (SIGMA, GE Medical Systems). The imaging protocol features multiple-contrast black-blood imaging (T1, T2, proton density weighting), as well as a time-of-flight imaging. The in-plane pixel size was 250 µm. Lesion types (according to AHA definition) were identified and vessel wall volumes were measured using a blinded protocol.

Results: Among these patients, 36 carotid arteries were available for lesion type (LI) study, the LI agreed in 89% of cases, Kappa = 0.79. Vessel wall volume was measured in 24 pairs of arteries (available) with a mean volume difference of 6.55 ± 61.31 mm³. The Spearman's correlation coefficient was 0.94.

Conclusions: High-resolution MRI can be used to identify early and intermediate lesions of atherosclerosis in the human carotid arteries. In vivo MRI identification of plaque type and quantitative measurement of plaque volume was highly reproducible.

MoP12:W3 Association of coronary risk factors and early stage of atherosclerosis in men

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Objective: This study was undertaken to compare the vascular endothelial function in the brachial artery and IMT in the carotid artery with regard to the association of coronary risk factors.

Methods: Eighty one men with one or more coronary risk factors (hyperlipidemia, hypertension, diabetes mellitus and current smoking habits) without clinical atherosclerosis aged 50 ± 10 years and 18 age-matched control subjects without coronary risk factors were examined. Flow-mediated vasodilatation (%FMD) in the brachial artery was measured using high-resolution ultrasonography. IMT was also measured as the same probe in the right carotid artery.

Results: %FMD in the subjects with one or more coronary risk factor(s) was significantly lower than that of the subjects with control subject (4.3 ± 3.0 vs. 6.0 ± 0.66, P < 0.01). Subjects with one or more coronary risk factor(s) had significantly greater IMT than the control group (0.04 ± 0.03 mm vs. 0.07 ± 0.06 mm, P < 0.01). IMT was inversely related to %FMD (r = −0.22, P < 0.05). However, among the subjects with one or two risk factor(s) only %FMD showed significant difference compared to the control group.

Conclusions: Accumulation of the coronary risk factors significantly related to the impairment of endothelial function and carotid intima-media thickness. %FMD rather than IMT might reflect well with the early stage of atherosclerotic process.

MoP13:W3 Asymmetry of common carotid artery velocity is a marker for cardiovascular disease

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Objective: The aim of this study is to clarify the clinical usefulness of the high to low velocity ratio between the two carotids (velocity ratio), measured by ultrasonic quantitative flow measurement system (QFM).

Methods: We studied 287 Japanese outpatient (149 males and 138 females), aged 25 to 91 years (average 67.6 ± 11.0 years). Carotid blood velocity was measured using QFM-1100 ultrasonic blood flow meter. We divided the subjects into two groups depending on the velocity ratio; high (H) group (≥1.4) and low (L) group (<1.3). The end point of the study was the occurrence of angina pectoris, nonfatal myocardial infarction, or death from coronary heart disease. The average follow-up period was 3.4 ± 1.4 years.
**MoP14:W3**

**Carotid atherosclerosis by B-mode ultrasound in the centenarians and the younger healthy subjects**

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**Objective:** To investigate the relationship between atherosclerosis and aging, we sonographically examined the carotid arteries of the Japanese subjects over the broad generation of adults and elderly persons including centenarians.

**Methods:** We studied the 29 centenarians (7 men and 22 women) and the 274 healthy younger subjects (140 men and 134 women) aged 21–96 years, both of those had no history of hypertension, diabetes mellitus or any atherosclerotic diseases. Evaluation of mean intima media thickness (IMT) of common carotid arteries at sites free of plaque defined as locally raised lesions and occurrences of plaque were made by B-mode ultrasound.

**Results:** The intima-media complexes were diffusely thickened in the centenarians, and their average IMT at the six determined sites of bilateral common carotid arteries was 1.01 mm. The average IMT of the common carotid arteries increased linearly with age in all decades including centenarians (IMT = 0.009*Age + 0.107, R = 0.83). Although the prevalence of plaque increased consecutively up to the tenth decade, the occurrence of plaque in the centenarians (58.6%) was smaller than that in the tenth decade (83.3%, N = 24). Furthermore, the close associations between increasing IMT and prevalence of plaque were not shown over the ninth decade.

**Conclusions:** These data suggest that the pathogenic significance of increase in IMT differs from that of plaque formations. Most of increasing IMT may be caused by physiological aging process as diffuse intimal thickening especially in very elderly persons.

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**MoP15:W3**

**Femoral atherosclerosis has a higher predictive value than carotid for detection of coronary heart disease**


**Objective:** Carotid ATIS is considered as a surrogate for coronary heart disease (CHD). However, the correlation between femoral ATS and CHD has not been established yet. The aim of this study was to evaluate the prevalence of femoral ATIS (FATIS) as compared to carotid (cATS) in patients undergoing their first coronary angiography.

**Method:** Hundred sixty seven individuals [117 men and 50 women, mean age 57.8 (34-75) were included in the study. Coronary angiography was carried out in 119 (71%) for suspected CHD, 27 (16%) before surgery for valvulopathy, and 21 (13%) for other reasons. ATIS plaques were detected by high resolution B-Mode ultrasound (US) in the left and right carotid and femoral arteries before coronary angiography. Plaques were defined as a focal intima-media thickness ≥ 1200 μm. Patients were categorized into 2 groups with coronary stenosis (CAD) < 30% or ≥ 30%. Among the 110 individuals with stenosis ≥ 30%, 93 (85%) had a stenosis ≥ 70%.

**Results:**

- **Prevalence of ATIS plaques on peripheral arteries**
  - Sites: CAD < 30% (n = 57)  CAD ≥ 30% (n = 110)
  - Carotid: 32 (56%) 93 (84%)
  - Femoral: 34 (60%) 105 (95%)
  - p value <0.001

The positive predictive value of cATS detection (76%) was similar to that of FATIS (74%). However, the negative predictive value was significantly higher (p < 0.04) on femoral (82%) than on carotid arteries (60%).

**Conclusion:** These results suggest that US imaging of femoral arteries provides a better predictive value for CAD than that of carotids.

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**MoP16:W3**

**Coronary calcification detected by mobile helical CT unit in a mass screening: The frequency and relationship to coronary risk factors and coronary artery disease**

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**Objectives:** It is well known that there is strong relationship between coronary artery disease (CAD) and coronary calcification detected by electron beam CT or helical CT. But the actual condition of coronary calcification is unknown in general inhabitant spending everyday life. Therefore, we studied the frequency of coronary calcification and a relationship between coronary calcification and coronary risk factors and CAD in the mass screening using a mobile helical CT unit.

**Methods:** Sample population of 10008 persons (5321 males and 4687 females, mean age 58.5 ± 12.5 years) underwent plain helical CT examination with 10 mm slice thickness from the cardiac basis to the diaphragm. CT value of coronary arteries (LMT, LAD, LCX, and RCA) were measured on CRT monitor after observing whether the calcification was or not. The threshold of CT value for calcification was setting +110 HU and over. We studied the frequency of coronary artery calcification and relationship between calcification and age, gender, coronary risk factors (hypertension, hypercholesterolemia, diabetes mellitus) (DM), smoking, and drinking, past history of CAD.

**Results:** The frequency (16.0%) of coronary calcification was significantly higher in male than female (20.6% vs 10.7%, P < 0.001), and increased by age in both sexes. A frequency of the LAD artery calcification was the highest (85.0%). DM and hypertension related to coronary calcification. CAD had significant relationship with coronary calcification in male (odds ratio 3.95, P < 0.0001), and an odds ratio rise to 13.22 in male under 60 years old.

**Conclusions:** The frequency of coronary calcification was 16.0% in general inhabitant. It was shown that there was close relationship between coronary calcification and CAD in male under 60 years old.

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**MoP17:W3**

**Long term change in atherosclerosis of human abdominal aorta observed by enhanced CT**


**Objective:** To investigate long-term change in human atherosclerosis, non-invasive assessment system was developed and the effects of risk treatment in hyperlipidemic patients was observed.

**Methods:** Thirty male hyperlipidemic patients aged 40–55 years at entry (50.0 years) were treated and followed for 61–167 months (9.4 years). In whom 7 were suffering from myocardial infarction (MI), 5 stroke, 9 type 2 diabetes mellitus, and 9 hypertension. As for the noninvasive diagnostic method of premature atherosclerosis, the simple and the enhanced CT were performed 4 times or more per person and assessed as the wall thickening calcification volume (AWCV) by using the our developed Image Colour Analysis Program (Am J Hypertens.7:15S-4, 1994).

**Results:** Mean reduction of LDL-C was 9%, triglyceride 31%, SBP 7.2%, and DBP 8.6% during observation. Mean AWCV changed from 27.0% to 31.1% (0.4% progression per year), and the progression rate inclined to be lower than that in control patients treated with diet only (0.7%/y). But in 5 familial hypercholesterolemic patients with MI, AWCV progression was 0.8%/year and about twice higher.

**Conclusion:** Atherosclerosis assessment by CT is a good reproducible method and in long term observation, strict control of hyperlipoproteinemia may protect the progression of atherosclerosis of abdominal aorta.
MoP18:W3
Atherosclerotic plaque area by transesophageal echocardiography (TEE) related to coronary artery disease (CAD) and risk factors
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Objective: Previous studies have shown the value of plaque thickness in the thoracic aorta for predicting coronary artery disease by TEE. This study was conducted to examine whether measurements of plaque area by TEE is better predictor for CAD and risk factors compared with plaque thickness.

Methods: Sixty-nine consecutive patients (mean age 58 ± 8) who underwent coronary angiography for diagnosis of heart disease were evaluated by TEE. CAD (+) (n = 15) was defined as ≥50% stenosis of ≥1 major artery and the rest of patients were CAD (-) (n = 54). In descending thoracic aorta, we measured the plaque thickness (PT) and the ratio of the plaque area to the cross-sectional area of aorta (% plaqe area: %PA) at three predefined portions. Max PT, max %PA and mean %PA were used for analysis.

Results: The mean %PA and the max %PA in CAD (+) were significantly higher than in CAD (-) (11.6 vs 7.2%, p = 0.04:13.9 vs 8.8%, p = 0.04), although the max PT was not significantly different. Among conventional risk factors, HDL cholesterol showed inverse correlation with mean %PA (p = 0.03), but not with max %PA and max PT.

Conclusion: These results suggest that measurement of plaque area may be a better predictor for CAD than plaque thickness by TEE.

MoP19:W3
The relationship of flow-dependent dilatation of the popliteal artery to common carotid artery intima media thickness
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Objective: Common carotid artery intima-media thickness and flow-dependent dilatation of peripheral artery has been used to assess preclinical stages of atherosclerosis. We studied their relationship.

Methods: High-resolution ultrasound was used to determine flow-mediated dilatation of the popliteal artery in a 5-min. arterial occlusion and also to assess intima-media thickness of the common carotid artery (IMT CCA) in 26 healthy subjects, in 27 patients with hyperlipidemia and in 10 patients with coronary artery disease (CAD).

Results: Healthy Hyperlipidemia CAD

<table>
<thead>
<tr>
<th>IMT CCA (mm)</th>
<th>Healthy</th>
<th>Hyperlipidemia</th>
<th>CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.57 ± 0.12</td>
<td>0.69 ± 0.17</td>
<td>1.05 ± 0.26</td>
<td></td>
</tr>
</tbody>
</table>

* a significant change against healthy

By multiple and stepwise regression analysis we found a significant negative correlation of the change in diameter of popliteal artery and IMT CCA (in all subjects r = -0.57, p < 0.001 in subgroup of healthy subjects r = -0.52, p = 0.03). There was no correlation in patients with hyperlipidemia and in small subgroup of patients with CAD.

Conclusions: In patients with hyperlipidemia and with CAD we demonstrated impaired vasodilatation of popliteal artery during reactive hyperemia. In all subjects we found significant negative correlation between flow-mediated changes in popliteal artery and intima-media thickness of common carotid artery.

MoP20:W3
ACE and ATIR gene polymorphism in patients with ischaemic heart disease
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Polyorphism of ACE [I/D] and ATIR [A1166C] genes has been implicated in genetic risk for ischaemic heart disease (IHD) and myocardial infarction (MI). We determined genotype and allele frequencies for ACE and ATIR genes and searched for an association between ACE gene polymorphism and ACE activity and between ACE and ATIR gene polymorphism and left ventricular mass index as assessed by echocardiography in 100 patients (mean age 54.2 ± 9.2 y) with a history of myocardial infarction (MI) and in 100 healthy controls (mean age 52.3 ± 10 y). Genomic DNA was PCR amplified using two pairs of primers flanking the polymorphic regions. Subsequently, I/D ACE gene polymorphism and A to C transition at nucleotide position 1166 of the ATIR gene were studied. ATIR genotypes were identified after digestion with HaeII.

Our groups did not differ as to ACE genotype frequencies (31% II, 51% ID, 18% DD in the study group vs 30% II, 57% ID, 13% DD in the control group) or ATIR genotype distribution (AA 53%, AC 41%, CC 6% in the study group, vs AA 58%, AC 37%, CC 5% in the control group). Neither polymorphism was associated with mean age at incidence of myocardial infarction or with left ventricular mass index. These results seem to rule out an association between DD ACE or CC ATIR genotype and increased risk for myocardial infarction in the local Polish population.

MoP21:W3
Influence of plasmatic lipid levels on coronary atherosclerosis and remodeling as determined by 3D intravascular imaging
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Objective: Atherosclerotic arteries tend to undergo compensatory vascular enlargement (CE) to accommodate increasing plaque burden during the early stages of atherosclerosis leading to an increased proportion of restenosis after angioplasty. In some cases, inadequate compensatory contraction (IC) may occur. The aim of this study is to evaluate the influence of lipid disorders on arterial remodeling.

Methods: Volumetric reconstruction was carried out using a computerized intravascular ultrasound (IVUS) analysis in 100 pts undergoing coronary angioplasty. Theoretical average of cross sectional area (CSA) was determined at the site of minimal lumen area using proximal and distal reference CSA. Relative CSA (%) was used to classify pts in 2 groups: CE (53 pts, 60 ± 10 years) and IC (47 pts, 63 ± 9 years). Lipid profile was performed immediately prior to IVUS.

Results: Relative CSA was significantly different in both groups: CE 40 ± 23% and IC 30 ± 11% (p = 0.01). Lipid profile was similar (see table).

<table>
<thead>
<tr>
<th></th>
<th>CE</th>
<th>IC</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.8 ± 1.1</td>
<td>5.6 ± 1.1</td>
<td>0.30</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3.9 ± 1.1</td>
<td>3.7 ± 1.1</td>
<td>0.32</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>0.56</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>3.3 ± 1.7</td>
<td>3.1 ± 1.2</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Conclusion: Our findings suggest that coronary remodeling is not affected by lipid profile. Further sequential IVUS imaging studies are required to elucidate the mechanism of differential coronary remodeling.

P:W4
INFECTIONS, CHD, AND ATHEROSCLEROSIS

MoP1:W4
Elevation of anti-helicobacter pylori antibody is a predictor of ischamic events in patients with coronary heart disease
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Objective: Helicobacter pylori (HP) infection was reported to be associated with coronary heart disease (CHD). We studied the anti-HP IgG antibody titers in patients with CHD and evaluated whether this titer can serve as a risk factor of subsequent ischemic events.

Methods: Eighty-six consecutive patients (mean age 62 ± 10 years, 64 males) who were admitted for coronary angiography were enrolled in this study. All the patients had significant stenosis in at least one coronary vessel and they were followed up in our clinic after appropriate treatments (angioplasty, bypass graft surgery, or medication only). Titters of anti-HP IgG antibody were measured using enzyme-linked immunosorbent assay method. The study end points of ischemic events were unstable angina, acute myocardial infarction, or any new revascularization procedures.

Results: After 24 ± 12 months, 30 patients developed ischemic events. Risk factors, lipid profiles, medications, left ventricular ejection fraction, incidence of previous myocardial infarction, numbers of coronary vessels involved were similar between patients with or without ischemic events. Titters of anti-HP IgG antibody were significantly higher among patients with ischemic events (371.1 ± 441.5 vs. 153.0 ± 228.0 U/mL; p = 0.003). Univariate Cox regression analysis identified titters of anti-HP IgG antibody (hazard ratio 2.68: 95% CI 1.37–5.23; p = 0.004) and coronary artery bypass graft surgery (hazard ratio...
0.39; 95% CI 0.16–0.95; p = 0.039) as significant factors associated with ischemic events. In multivariate analysis, only titers of anti-HP IgG antibody (hazard ratio 3.36; 95% CI 1.41–8.01; p = 0.006) were independent predictor of future ischemic events.

Conclusions: Elevation of anti-HP IgG antibody titers is a predictor for subsequent ischemic events in CHD patients.

MoP2.W4
Association of chlamydia pneumoniae infection with diabetes mellitus in patients with coronary artery disease

Objective: To elucidate clinical and angiographic characteristics of patients (pts) with coronary artery disease (CAD) who had Chlamydia pneumoniae (CP) infection.

Methods: We evaluated clinical and angiographic characteristics and serum IgG antibody to CP in 131 consecutive pts who had cardiac catheterization. The cut off index of IgG titer was measured by enzyme-linked immunosorbent assay, the index > 1.10 being considered positive.

Results: Of the 131 pts, 95 had significant CAD (>50% stenosis). The prevalence of CP IgG seropositivity was higher in pts with CAD (46%) than in those without CAD (28%), and CP IgG titer was higher in pts with CAD (1.23 ± 0.76) than in those without CAD (0.86 ± 0.52) (p < 0.01). Of the 95 pts with CAD, 44 were positive for CP. Compared between CAD pts positive and those negative for CP, we did not find any difference in the prevalence of myocardial infarction and those of eccentric and calcified lesions on coronary arteriograms. Of note was that diabetes mellitus was more prevalent in CAD pts positive for CP than in those negative (50% vs 18%, p < 0.005). Multivariate analysis revealed that CP IgG seropositivity was significantly associated with diabetes in CAD pts, but it was not an independent risk factor for CAD.

Conclusion: CP infection was prevalent in pts with CAD, but it was not independently associated with CAD. CP infection was associated with diabetes mellitus which is one of classical risk factors for CAD.

MoP3.W4
Chlamydia pneumoniae seropositivity and Carotid Atherosclerosis – A Japanese population-based study
N. Maeda, J. Hayashi, Y. Sawayaama, C. Shimizu, K. Kashiwagi, H. Nakashima, S. Kashiwagi. Department of General Medicine, Kyushu University Hospital Fukuoka, Japan

Objective: The purpose of this study was to determine the association between Chlamydia pneumoniae (C. pneumoniae) infection and carotid atherosclerosis in a Japanese population-based sample.

Methods: In 560 Japanese individuals aged 25 to 85 years (177 men and 383 women, mean age 56.6 ± 13.1 years), we investigated the association between seropositivity to C. pneumoniae infection and carotid atherosclerosis. We measured intima-media thickness (IMT) of the common carotid artery and the presence of carotid plaque by carotid B-mode ultrasound. Plaque was defined as a clearly identified area of focal increased IMT (≥1.3 mm). Anti-C. pneumoniae IgA and IgG antibodies were measured by ELISA (HITAZYME C. pneumoniae).

Results: The seroprevalence of anti-C. pneumoniae IgG was detected in 43.9%, whereas the seroprevalence of anti-C. pneumoniae IgA was detected in 52.3% of the population. The seroprevalence of anti-C. pneumoniae IgA increased with age, but not anti-C. pneumoniae IgG. There was no difference in seroprevalence levels between men and women. Seropositivity to anti-C. pneumoniae IgA was associated with mean maximum IMT and the presence of carotid plaques, but there was no such associations with anti-C. pneumoniae IgG, by univariate comparison. There was an association between increased carotid plaque and hypertension, diabetes mellitus, smoking, and hyperlipidemia.

Conclusions: Seropositivity to anti-C. pneumoniae IgA was associated with carotid atherosclerosis.

MoP4.W4
Simvastatin reduces acute phase response markers
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Objective: Inflammation recently gains increasing interest in the process of atherogenesis. The influence of statins on this mechanism is widely unknown.

Methods: We examined acute phase response markers (C-reactive proteins, blood sedimentation rate [BSR], fibrinogen, interleukin-6 [IL-6], serum amyloid A and P) as well as the LPS (4 μg/ml)-induced monocyte expression of TNFα and interleukin-β (IL-1β) in 57 patients suffering from severe familial heterozygous hypercholesterolemia (FH) and 42 patients suffering from familial combined hyperlipidemia (FCH). Values were obtained before and 4 weeks after dietary counselling, after 1 month simvastatin (S) therapy, as well as after 3 and 6 months (either 20 or 40 mg S as necessary to achieve the target goals).

Results: S therapy did not but dietary intervention reduced monocyte TNFα and IL-1β significantly, the decrease being more pronounced in FCH as compared to FH. CRP, BSR and IL-6 were significantly decreased (FCH > FH), fibrinogen only borderline (∼11%). No change in SAA and SAP was detected. No difference between patients on 20 or 40 mg S was seen.

Conclusions: This study demonstrates that S dose-independently shows an antiinflammatory effect via reduction of proinflammatory cytokines expressed by monocytes and acute phase response markers, being apparently more pronounced in FCH.

MoP5.W4
The association between antibody titers to helicobacter pylori, chronic coronary heart disease and acute myocardial infarction
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Objective: To study the possible relation between Helicobacter pylori (H. pylori) that may act either acutely (eg. Precipitating plaque rupture) or chronically (eg. promoting plaque growth) and acute myocardial infarction (AMI) or chronic coronary heart disease (CHD).

Methods: Two groups of patients and a control group were investigated between May, 1997 to Feb, 1999 at Isfahan Cardiovascular Research Center. The AMI group consisted of 52 consecutive patients (mean age 54.8 ± 8 years) admitted because of an AMI with a diagnosis based on WHO Criteria. The CHD group consisted of 51 consecutive patients (mean age 52.8 ± 11 years) admitted for coronary angiography because of chronic symptoms of angina pectoris and had at least single vessel significant lesion ≥ 70% lumen narrowing in the coronary angiography report. The controls were random sample of 55 men and women from the same area as the patients, matched for sex, age and time. The controls underwent diagnostic coronary angiography and none had any lesion to be reported in each of coronary vessels. Serum samples were drawn for measurement of H. pylori antibodies using Eliza method and fibrinogen using fibrinometric method.

Results: AMI patients had significantly higher H. Pylori Ab levels compared to control group with an odds ratio of 4.95 (95% CI 1.7 to 7.4; P = 0.004). The CHD group showed similar findings with adjusted odds ratio of 3.3 (95% CI, 1.1 to 6.6; P = 0.01) while The comparison between AMI and CHD groups showed no significant difference with adjusted OR of 1.1 (95% CI 0.3 to 4 p = 0.08). The prevalence rates for H. Pylori Ab among cases and controls is shown in the following table:

<table>
<thead>
<tr>
<th>Case and Control Groups</th>
<th>No.</th>
<th>No. Positive Samples for H. Pylori</th>
<th>Crid Date</th>
<th>Adjusted for</th>
<th>(95% CI)</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMI Group</td>
<td>52</td>
<td>15</td>
<td>27.5</td>
<td>(25.5–29.5)</td>
<td>26.6</td>
<td>(24.8–28.7)</td>
</tr>
<tr>
<td>CHD or Coronary</td>
<td>51</td>
<td>8</td>
<td>9.8</td>
<td>(7.8–11.8)</td>
<td>8.3</td>
<td>(8.1–12.3)</td>
</tr>
<tr>
<td>Angina (+) Group</td>
<td>55</td>
<td>3</td>
<td>5.5</td>
<td>(3.5–7.4)</td>
<td>5.1</td>
<td>(3.1–7.1)</td>
</tr>
<tr>
<td>Control or Coronary Angin (+) Group</td>
<td>y2</td>
<td>2.43</td>
<td>0.08</td>
<td></td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion: These results do not support the hypothesis that H. Pylori may induce the acute coronary events.

MoP6.W4
Randomized double-blind, placebo-controlled study of azithromycin in C. pneumoniae positive postmyocardial infarction patients (CROAATS)

Objective: Inflammation recently gains increasing interest in the process of atherogenesis. The influence of statins on this mechanism is widely unknown.

Methods: We examined acute phase response markers (C-reactive proteins, blood sedimentation rate [BSR], fibrinogen, interleukin-6 [IL-6], serum amyloid A and P) as well as the LPS (4 μg/ml)-induced monocyte expression of TNFα and interleukin-β (IL-1β) in 57 patients suffering from severe familial heterozygous hypercholesterolemia (FH) and 42 patients suffering from familial combined hyperlipidemia (FCH). Values were obtained before and 4 weeks after dietary counselling, after 1 month simvastatin (S) therapy, as well as after 3 and 6 months (either 20 or 40 mg S as necessary to achieve the target goals).

Results: S therapy did not but dietary intervention reduced monocyte TNFα and IL-1β significantly, the decrease being more pronounced in FCH as compared to FH. CRP, BSR and IL-6 were significantly decreased (FCH > FH), fibrinogen only borderline (∼11%). No change in SAA and SAP was detected. No difference between patients on 20 or 40 mg S was seen.

Conclusions: This study demonstrates that S dose-independently shows an antiinflammatory effect via reduction of proinflammatory cytokines expressed by monocytes and acute phase response markers, being apparently more pronounced in FCH.
niae and coronary heart disease (CHD). Therefore, the effect of azithromycin, an antimicrobial macrolide antibiotic, was assessed in a multicenter study of 332 post-myocardial infarction (MI) patients to prove whether it can prevent or reduce major ischemic events in these patients. The study started in August 1998 and the predicted closing date is July 2001. Blood samples for C. pneumoniae testing were collected at the beginning and at the end of the 2-month pre-treatment period. Patients with titres ≥ 1:20 IgG or IgA in both tests were considered seropositive. They were randomised to receive: azithromycin 3 cycles of 500 mg once daily for 3 days, starting on days 1.10 or 20 or placebo at the same regimen. Following the initial examination patients are seen 8 times at regular intervals and blood samples are collected for haematology, biochemistry, lipids, fibrinogen, CRP, TNF-alfa and C. pneumoniae IgG and IgA titres. Most of the 22 patients who were withdrawn from the study so far because of adverse cardiovascular events (ACEVE) were seropositive. Due to the relatively early phase of the study it is still impossible to draw any conclusions on the efficacy and safety of azithromycin in treating C. pneumoniae positive post-MI patients. However, the observed distribution of ACEVE speaks in favor of the proposed hypothesis that C. pneumoniae positive patients have a higher risk of recurrent cardiovascular events.

MoP7:W4 C-reactive protein: Associations with cardiovascular risk factors and all-cause mortality in elderly

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Objective: To determine correlates of C-reactive protein (CRP) in a population of healthy elderly and to study if CRP levels are associated with all-cause mortality.

Methods: Baseline data of a prospective study were used for the analyses of correlates. Data on 471 elderly (234 men and 237 women) with no history of cardiovascular disease were available. A follow-up on mortality was performed from January 1992 until now.

Results: The mean CRP was higher in men than in women (3.2 vs 2.5 mg/L). Age turned out not to be a major determinant of CRP (men: 0.11; women: 0.03). In men the strongest correlates of CRP were systolic blood pressure and packyears of smoking. In women the strongest correlate was BMI. All-cause mortality during follow-up was 31% for men and 18% for women. For men the mortality rate increased over time of tortles of CRP (p = 0.0001), but this was not found for women (p = 0.170). Adjustment for age did not alter these results.

Conclusion: CRP levels were associated with several cardiovascular risk factors. The associations are different for men and women. CRP was associated with all-cause mortality in men, but not in women.

MoP6:W4 Changes in plasma levels of inflammation markers, lipid profile and homocysteine following treatment with antibiotic in patients with chronic Chlamydia pneumoniae infection operated for ischaemic heart disease

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Objective: To assess the results of treatment of chronic infection caused by Chlamydia pneumoniae in patients with ischaemic heart disease.

Material and Methods: 50 patients with elevated titres of antibodies against Chlamydia pneumoniae before coronary bypass surgery were treated with roxithromycin 2 × 150 mg during 30 days.

Results:

<table>
<thead>
<tr>
<th>Marker</th>
<th>Before therapy</th>
<th>After 30 days</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>335.4 mg/dl</td>
<td>293.7 mg/dl</td>
<td>0.0001</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>155.2%</td>
<td>140.2%</td>
<td>0.0162</td>
</tr>
<tr>
<td>o-1 acid glycoprotein</td>
<td>90.6 mg/dl</td>
<td>75.1 mg/dl</td>
<td>0.0002</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>17.4 mmol/l</td>
<td>15.5 mmol/l</td>
<td>0.0062</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>224.4 mg/dl</td>
<td>209.9 mg/dl</td>
<td>0.0458</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>40.6 mg/dl</td>
<td>37.1 mg/dl</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

Conclusions: Reduced levels of inflammatory markers may be attributed to eradication of Chlamydia pneumoniae or other microbes sensitive to the antibiotic. Lower levels of lipids and homocysteine after treatment suggest that chronic infection with Chlamydia pneumoniae leads to increased levels of some risk factors of ischaemic heart disease.
Endogenous neutralising antibodies against Platelet-Derived Growth Factor-AA inhibit arterogenesis in the cholesterol-fed rabbit

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Objective: To investigate the contribution of platelet growth factors, specifically the PDGF-A chain, in cholesterol-induced arterogenesis in the New Zealand White rabbit.

Methods: High titer of antibodies to PDGF-AA, or platelet cytosolic protein (PCP) were induced in these animals by immunising against recombinant human PDGF-AA or human PCP. Rabbits were then fed a 0.25–1% cholesterol-containing diet for 10 weeks to induce atherosclerotic lesions, and were then killed, perfusion fixed and their aorta removed. The extent of arterogenesis in the thoracic aorta was determined by quantitative morphometry after staining with oil red O. The intimal and medial areas in histological sections taken at the level of the first intercostal branch were quantified by image analysis.

Results: Immunisation against PDGF-AA and PCP, but not adjacent alone, resulted in rising titers of antibodies within 2 weeks, the levels of which reached a plateau by 8 weeks. The antibodies to PDGF-AA were isoform specific, and neutralised the biological activity of PDGF-AA in vitro. Integrated plasma cholesterol levels were similar in both groups. Compared to non-immune rabbits (n = 10), animals immunised against PDGF-AA (n = 10) or PCP (n = 10) had significantly smaller areas of the aorta covered by artherosclerotic lesions (24.6 ± 5.1% and 18.7 ± 4.2% respectively vs 34.4 ± 4.3% (p < 0.05)). This was associated with a reduced aortic intima:media area ratio in PDGF-AA immunised (0.009 ± 0.006) and PCP immunised rabbits (0.025 ± 0.017) than from non-immune animals (0.159 ± 0.066) (p < 0.05).

Conclusion: These data suggest that PDGF-AA is actively involved in cholesterol-induced arterogenesis in the rabbit.

Angiotensin II increases macrophage uptake of Ox-LDL and CD36 mRNA expression: Possible role of IL-6 and inhibitory effects of fosinopril and losartan

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Angiotensin II (Ang II) plays an important role in the development of arterogenesis, and using ACE inhibitors or Ang receptor blockers was shown to prevent the development of arterogenesis in hypercholesterolemic animal models.

Objective: To investigate the mechanism responsible for the atherogenicity of Ang II.

Methods and Results: Ang II was injected intraperitoneally into apo E deficient (E²) mice once a day for two weeks. Degradation of Ox-LDL and CD36 mRNA expression by peritoneal macrophages (MΦ) harvested from the Ang II-treated mice increased by 28% and 53%, respectively. Administration of the ACE inhibitor, Fosinopril or the Ang II receptor antagonist, Losartan, for a period of two weeks significantly inhibited Ox-LDL degradation by 25% and 40%, respectively. CD36 mRNA expression in MΦ derived from Fosinopril- or Losartan-treated mice, showed a 30 ± 4% and a 76 ± 30% reduction compared to controls, respectively. A dose-dependent increase in serum IL-6 levels was noted in response to increasing concentrations of Ang II (10⁻¹²M–10⁻⁷M) injections. The highest induction resulted in a 93% increase in serum IL-6 levels compared to the placebo-treated mice (125 ± 19 pg/ml vs. 64.5 ± 1 pg/ml, respectively). Serum IL-6 levels tested in mice administered with Fosinopril or Losartan were almost undetectable. Ang II effect on in vitro Ox-LDL degradation by MΦ was minimal. However, IL-6 demonstrated a dose-dependent increase in Ox-LDL degradation by up to 57%, as well as in CD36 mRNA expression (by up to 150%), compared to control.

Conclusion: Ang II effect on CD36 expression at the mRNA level, and on Ox-LDL degradation is mediated by Ang II-induced IL-6 production in the apo E² mice.
Carbon monoxide and nitric oxide-mediated induction of VEGF synthesis in vascular smooth muscle cells

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Objective: Vascular endothelial growth factor (VEGF) is synthesised by VSMC stimulated with cytokines, growth factors or hypoxia. The same treatments up-regulate heme oxygenase-1 (HO-1), which breaks down heme to carbon monoxide (CO), iron and biliverdin, quickly metabolised to bilirubin.

Under hypoxic conditions, HO-1 expression is partially dependent on the preceding induction of inducible nitric oxide synthase (iNOS). IL-β-mediated enhancement of VEGF synthesis by VSMC is also dependent on the NO synthesis (Dulak et al., ATVB, in press). Therefore, we decided to investigate the link between NO, CO and VEGF.

Results: Stimulation of rat VSMC with IL-1 β (10 ng/ml) resulted in generation of NO, increased HO-1 expression and strongly enhanced VEGF production. Inhibition of NO synthesis by L-NAME (2 mM) caused a significant reduction in VEGF synthesis. Inhibition of HO-1 activity by tin protoporphyrin (SnPP, 1–10 μM) dose-dependently up-regulated cytokine-induced NO generation, but it concomitantly decreased VEGF synthesis, even more potently than L-NAME. Treatment with heme (10 μM), a HO-1 activator, enhanced basal and augmented IL-1β-induced VEGF production. SnPP and zinc deuteroporphyrin IX (another HO-1 inhibitor) abolished heme-induced VEGF release. Hypoxia (24 h, 2 mm O2) strongly up-regulated VEGF synthesis which was dose-dependently inhibited by SnPP, but not by L-NAME. Bilirubin did not influence VEGF production, deoxideroxane (iron chelator) increased VEGF synthesis while hemoglobin (CO scavenger) decreased both basal and heme-induced VEGF production.

Conclusion: These results indicate that NO plays a role only in cytokine-induced VEGF production, whereas HO-1 significantly contributes to both IL-1β- and hypoxia-mediated VEGF synthesis. We propose that CO might be an important determinant of angiogenesis.

Association of IL-1RA gene polymorphism to future stroke

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Objective: Polymorphisms of genes of pro-inflammatory cytokine, such as IL-1β and its antagonist IL-1Ra is associated to autoimmune/inflammatory disorders. We here investigated the relation to future stroke.

Methods: As a part of the population-based WHO organized MONICA project, blood was sampled from individuals free from cardiovascular events. After an average period of 34.1 months, 113 individuals developed stroke and 226 healthy individuals served as controls. PCR amplification was used for analysis of the polymorphism of the IL-1β and IL-1Ra genes.

Results: There was an association between allele A2 of the IL-1Ra gene and stroke. The effect of hypertension was powerful if male patients carried IL-1Ra allele A2. In contrast, IL-1Ra allele A1 was related to stroke in hypertensive female patients.

Association of monocyte cytokine gene expression

D.P. Ramji, T.R. Hughes, J.R. Mead, S.A. Irvine, A. Cayer, Cardiff School of Biosciences, Cardiff University, Cardiff, UK

Objective: Macrophage lipopolysaccharide (LPS) has been implicated to play a
key role in the pathogenesis of atherosclerosis. The regulation of macrophage LPL expression by mediators that are known to be present in the lesion is likely to play a role in the inhibition of the progression of atherosclerosis. The objective of this study was to analyse the regulation of macrophage LPL expression by such mediators, and investigate their mechanism of action.

Methods: A range of macrophage cell lines and primary isolated macrophages were exposed to the mediators, and the LPL activity, mRNA levels and protein content were determined by enzymatic assays, Northern blotting/RT-PCR and Western blot analysis, respectively. For promoter-dissection, a range of DNA constructs, containing 5' truncations of the LPL promoter that are linked to the luciferase gene, were transfected into U937 promonocytic cells and both the constitutive and mediator-responsive reporter gene activity was determined. The interaction of transcription factors with the various regulatory sequences was investigated using gel retardation assays.

Results: Macrophage LPL expression was decreased following exposure of the cells to oxidized LDL and several cytokines and increased by a range of growth factors, with paired combinations of some mediators producing a synergistic action. The regulation of LPL was mediated predominantly at the transcriptional level and, in the case of IFN-γ and TGF-β, we have identified the cis-acting elements and the trans-acting factors that are involved in the response.

Conclusions: The studies provide a detailed understanding of the regulation of macrophage LPL expression by a range of extracellular mediators, and the mechanisms by which their actions are mediated.

MoP14: W5 Exaggerated vascular remodeling in the mice lacking angiotensin II type 2 receptor

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1Department of Geriatric Medicine, Graduate School of Medicine, University of Tokyo, Tokyo; 2Department of Medical Biochemistry, Ehime University School of Medicine, Ehime, Japan; 3Cardiovascular Research, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA

Objective: Angiotensin II type 2 (AT2) receptor counteracts the AT1 receptor signaling and inhibits cell growth in vitro. AT2 receptor is present scantily in the adult vasculature but is reexpressed after vascular injury. Thus, we tested the hypothesis that AT2 receptor could exert growth inhibitory effects in two different models of vascular diseases using AT2 receptor null (AT2KO) mice.

Methods and Results: Firstly, we performed polyethylene cuff placement around the femoral artery for up to 4 weeks, which lead to the vascular expression of inflammatory cytokines and AT1 receptor in wild-type (WT) and AT2KO mice. Additionally, AT2 receptor expression was induced predominantly in the intima/media in WT mice. Correlated with this, the neointimal lesion size and the smooth muscle cell proliferation, assayed by bromodeoxyuridine uptake, were twice greater in AT2KO than in WT mice. Secondly, we performed abdominal aortic banding in the two strains for 6 or 12 weeks. Carotid arterial pressure or the measure of cardiacmyocyte hypertrophy, heart weight/body weight ratio and the cross-sectional area of cardiomyocytes, was not different between the strains even after aortic banding. In contrast, coronary arterial thickening and perivascular fibrosis, determined by the media/lumen area ratio and the collagen/vessel area ratio, respectively, were 50% greater in AT2KO mice than in WT mice after banding, whereas these parameters were similar in the sham-operated mice.

Conclusion: These results suggest that AT2 receptor mediates the inhibitory effects on inflammation-induced and pressure-induced vascular remodeling.

MoP15: W5 Chylomicron remnant induces monocyte chemotactant protein-1 expression in rat vascular smooth muscle cells through an activation of p38 MAPK

K. Demoto1, T. Taniguchi1, T. Takahashi1, S. Kawasaki1, M. Taguchi1, Y. Matsu1, Y. Takahashi1, Y. Fujisaka1, Y. Ishikawa1, M. Yokoyama1, Y. Isikawa1, M. Yokoyama1, Y. Ishikawa1, M. Yokoyama1, Y. Isikawa1
1First Dept. of Int. Med., Kobe University School of Medicine, Kobe; 2First Dept. of Int. Med., Hyogo College of Medicine, Nishinomiya, Japan

Objective: Chylomicron remnant (CR), a major component of postprandial lipoproteins, seems to be related to the development of atherosclerosis, but the mechanisms are poorly understood. Therefore we investigated the effect of CR on monocyte chemotactant protein-1 (MCP-1) expression in cultured vascular smooth muscle cells (VSMCs).

Methods: CR was isolated from plasma of the functionally hepatectomized male rats injected with chylomicon which was collected from lymph ducts of male rats. The cytotoxic effects of CR on VSMCs were determined by WST-1 assay. The expression of MCP-1 mRNA was assessed by northern blot analysis, and the secretion of MCP-1 into the media was estimated by ELISA. An activation of p38 MAPK was assessed by immunoblot analysis with a phospho-specific p38 antibody.

Results: WST-1 assay revealed that no cytotoxicity was observed at the concentrations used in this study. CR stimulated the expression of MCP-1 mRNA and the secretion of MCP-1 to the media in a time- and dose-dependent fashion as shown by Northern blot analysis and ELISA, respectively. Treatment of VSMCs with CR resulted in a rapid and transient activation of p38 MAPK. Further, the pretreatment of VSMCs with p38 MAPK inhibitors, SB203580 and SB202190, dose-dependently inhibited MCP-1 mRNA expression and MCP-1 secretion in response to CR.

Conclusion: CR induces MCP-1 expression in VSMCs through an activation of p38 MAPK, implying that CR has a proinflammatory property leading to the progression of atherosclerosis.

MoP16: W5 Requirement of intact actin cytoskeleton for Angiostatin II-stimulated tyrosine kinase pathways in vascular smooth muscle cells

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Objective: Since morphological changes induced by growth factors are suggested to be required for efficient transduction of growth factor signals, we examined if the actin cytoskeleton played a role in Ang II-stimulated signal transduction pathways in VSMCs.

Methods: Cultured rat aortic VSMCs were used for experiments. Stress fibers and focal adhesions were visualized by double-staining with phalloidin and anti-vinculin antibody. Tyrosine phosphorylation of paxillin, PYK-2, p130Cas, and epidermal growth factor (EGF) receptor was analyzed by immunoblotting of anti-phosphotyrosine immunoprecipitates.

Results: Stimulation of cells with Ang II resulted in an increase in the formation of stress fibers. Treatment with cytochalasin D, an actin depolymerizing agent, completely disrupted stress fibers and focal adhesions. Pretreatment with cytochalasin D resulted in a significant inhibition of Ang II-induced tyrosine phosphorylation of paxillin, PYK-2, p130 Cas, and EGF receptor. EGF receptor inhibitor AG-1478 did not suppress Ang II-induced tyrosine phosphorylation of paxillin, PYK-2, and p130Cas, suggesting that EGF receptor is not an upstream regulator of tyrosine phosphorylation of these substrates. Further, treatment with colchicine, which disrupts the microtubules, did not affect Ang II-induced tyrosine phosphorylation of paxillin, PYK-2, p130Cas, and EGF receptor.

Conclusions: The maintenance of intact actin cytoskeletal, but not polymerized microtubules is essential for Ang II-induced tyrosine kinase pathways in VSMCs, implying the important role of the actin cytoskeleton for Ang II actions in VSMCs.

MoP17: W5 Regulation of PDGF-Rb mRNA expression by IFNγ and IFNα

P. Moretti, S. Pettersson, F. Fager, G. Bondjers, F. Lustig, Wallenberg Laboratory, Göteborg University, Göteborg, Sweden

Objective: The accumulation of monocytes at the sites of inflammation and endothelial injury is regarded as one of the initial steps in atherogenesis. We have previously found that IFNγ increases the expression of PDGF-a receptor (Rα), but not PDGF-Rβ receptor (Rβ), in human monocyte-derived macrophages (hMDM) and that this leads to an increase of hMDM migration towards PDGF. Here we have studied the mechanism behind the effect of IFNγ on hMDM and THP-1 cells. We have also investigated the effect of IFNα on Rb mRNA expression in THP-1 cells.

Methods: The mRNA expression of Rb was examined by RT-PCR. The DNA binding of nuclear proteins to the Rb-promoter was examined by electrophoretic mobility shift assay (EMSA).

Results: As in hMDM, IFNγ increases the mRNA expression of Rb but not of Rα in THP-1 cells. This increase is transient with an observed maximum after 4 h. We have found that incubation with IFNγ results in the activation and subsequent DNA binding of the transcription factor STAT1 to the Rb promoter in both THP-1 cells and hMDM. We have seen this binding of STAT1 already after 45 min. IFNα also increases the mRNA expression of Rb in THP-1 cells although to a lesser extent than IFNγ.

Conclusions: IFNγ induces binding of the transcription factor STAT1 to the Rb promoter. This could contributes to the increase in Rb mRNA expression. IFNα also increases the Rb mRNA expression. The stimulatory effect of IFNα
MoP18:W5
25-Hydroxycholesterol increases a hypoxia induced IL-8 protein secretion in human macrophages
E. K. Rydberg, L. Salomonsen, T. Björnheden, O. Wiklund, G. Bondjers, B. G. Ohlsson. Wallenberg Laboratory, Sahlgrenska Univ Hosp, Göteborg, Sweden

Objective: IL-8 has mitogenic and chemotactic activities toward smooth muscle cells and may contribute to the development of atherosclerosis. IL-8 is secreted from macrophages containing oxidized LDL. These macrophages are found in the hypoxic area of the atherosclerotic plaque. The purpose of this study was to investigate whether 25-hydroxycholesterol (25-OH), in oxidized LDL (oxLDL), together with hypoxia would increase the IL-8 secretion.

Methods: Human macrophages were incubated at 2% 3%, 2% 1% and 0% O2 with and without 25-OH at increasing concentrations up to 5 μg/mL. The IL-8 protein secretion was measured with ELISA. Nuclear protein extracts were assayed for binding to DNA by EMSA.

Results: Both hypoxia and 25-OH increased the IL-8 protein secretion. A higher IL-8 secretion was found in macrophages incubated at hypoxia together with 25-OH, than in cells incubated at only hypoxia. The highest increase of the hypoxia-induced IL-8 secretion was found when low concentrations of 25-OH were used. A high 25-OH secretion was already found at normoxia in macrophages incubated with 25-OH at 4 and 5 μg/mL. Hypoxia did not further increase this IL-8 secretion. The increased IL-8 secretion in cells incubated with 25-OH and hypoxia coincided with an increased binding of AP-1 to the IL-8 promoter.

Conclusion: 25-OH together with hypoxia increased the IL-8 protein secretion in macrophages. These observations may suggest that hypoxia may further increase the IL-8 secretion in foam cells located to a hypoxic area in the atherosclerotic lesion.

MoP19:W5
Oxidised LDL increases the VEGF expression in human macrophages
L. Salomonsen, O. Wiklund, G. Bondjers, B. G. Ohlsson. Wallenberg Laboratory, Sahlgrenska University Hospital, Göteborg, Sweden

Objective: Intimal neovascularisation in atherosclerotic plaques may cause complications such as intimal hemorrhage and plaque rupture. Vascular Endothelial Growth Factor (VEGF), found in atherosclerotic plaques, may contribute to this neovascularisation. The purpose of this study was to investigate whether oxidised LDL (oxLDL) may induce expression of VEGF in human macrophages.

Methods: Human differentiated macrophages were incubated with low, medium and highly oxLDL, corresponding to TBARS of 17, 34 and 55, or with various constituents in oxLDL. The VEGF mRNA expression was followed by RT-PCR and the VEGF secretion was detected by ELISA. The VEGF secretion was compared with the VEGF mRNA expression.

Results: Oxidised LDL induced VEGF expression in macrophages. The VEGF mRNA expression increased with increased oxidation of LDL, subsequently followed by an augmentation of the VEGF protein secretion. The VEGF secretion was dependent on the concentrations of oxLDL. We found that the increased VEGF expression is partly due to an oxLDL dependent stabilisation of the VEGF mRNA. When we used different components that may form with oxidation of LDL we found that 25-hydroxy cholesterol and the linoleic acid derivative 9-(S)-HODE (9-hydroxy-10,12-octadecadienoic acid) significantly increased the VEGF mRNA expression.

Conclusions: OxLDL increases the VEGF expression in a time and concentration dependent manner in macrophages and this might partly be due to stabilisation of the VEGF mRNA.

MoP20:W5
Disruption of tumor necrosis factor-α gene prevents the development of atherosclerosis in apoE-deficient mice
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Objective: Inflammatory cytokines, including tumor necrosis factor-α (TNF-α) have been implicated in atherogenesis. We examined the effect of TNF-α gene disruption on atherogenesis in apolipoprotein E-deficient mice (apoEKO), an animal model for atherosclerosis.

Methods: The TNF-α gene-deficient mice (TNF-αKO) were produced by gene targeting. TNF-αKO were bred to ApoEKO mice to generate the F1. The F1 offspring of the F1 intercrossing were crossed with wild-type mice (apoEapoE). Total serum concentrations of total cholesterol (TC) and triglyceride (TG) were measured enzymatically. Lipoprotein cholesterol distributions were evaluated by high performance liquid chromatography. Quantitative analysis of atherosclerotic lesions at 12 weeks of age was performed on the section where the valves are visible. The lesion size for each mouse was measured by NIH image.

Results: Although total cholesterol levels in serum were markedly elevated in both mice deficient in the TNF-α and apoE (TNF-α/apoEKO) (537 ± 28 mg/dl, n = 15) and apoEKO mice (468 ± 21 mg/dl, n = 13) compared with those of wild-type mice (70 ± 2 mg/dl, n = 11), no differences were observed between the two genotypes. Of particular note in the present study is the finding that the average size of the atherosclerotic lesions in TNF-α/apoEKO mice (5.1 ± 2.7 × 10^3 mm^2, n = 10) was significantly smaller than that of apoEKO mice (7.0 ± 2.8 × 10^3 mm^2, n = 11; p < 0.0001) despite the lack of difference in serum cholesterol levels between them.

Conclusion: This study provided direct evidence that TNF-α promotes atherogenesis, at least under a severe hypercholesterolemia condition.

MoP21:W5
Binding of platelet derived growth factor (PDGF) isoforms on Chinese hamster ovarian (CHO) K1 cells and human smooth muscle cells (hSMC)
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Introduction: PDGF is an important growth factor implicated in the proliferation and migration of SMCs from the media to the intima during the atherosclerotic process. PDGF is a dimer of A and/or B chains which can be present as long and short isoforms. Long isoforms have higher affinity to glycosaminoglycans (GAGs) than short ones. hSMC show differential expression of PDGF specific receptors depending on the cell phenotype whereas CHO K1 cells do not express them. Moreover, proliferating or quiescent hSMC express different GAG species. Binding of PDGF isoforms to the cell surface GAGs can modulate PDGF interaction with its receptor and therefore, PDGF effects on the cells.

Objective: To quantitatively compare the binding of PDGF long to short isoforms in CHO K1 cells and in spreading and quiescent hSMC.

Methods and Results: Binding of PDGF isoforms (0.4–40 μg/ml) to the cells was quantified by immunocytocchemistry with the use of specific antibodies anti-PDGF-AA/BB. Images were taken at confocal microscopy and fluorescence intensity of 30–50 cells were quantified by Methamorph Analysis System. Our results show that long isoforms bind with higher affinity to the cell surface of CHO K1 cells and hSMC than short isoforms. They also indicate that short isoforms have higher binding capacity than long isoforms in CHO K1 cells and in quiescent but not in spreading hSMC.

Conclusions: Altogether these results suggest that long and short PDGF isoforms can differentially interact with the cell surface GAGs and consequently affect their accumulation and activity.

MoP23:W5
A common 936 C/T mutation in the gene for vascular endothelial growth factor (VEGF) is associated with VEGF plasma levels
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Background: Vascular endothelial growth factor (VEGF) is an important regulator of angiogenesis. Strong inter-individual variations of VEGF plasma levels have been reported previously. Aim of the present study was to search for mutations in the 3’ untranslated region (3’ UTR) of the VEGF gene and to analyze their relation to VEGF plasma levels.

Methods: The complete 3’ UTR (nucleotide 700–2622) of the VEGF gene was screened for sequence variations by single strand conformation polymorphism (SSCP) analysis. Frequencies of mutated alleles were determined in 119 healthy subjects, VEGF Plasma levels were analyzed in a subgroup of 23 healthy men aged 18 to 36 years.

Results: Three novel mutations (702 C/T, 936 C/T, 1612 G/A) were found, allele frequencies of 702 T, 936 T and 1612 A were 0.007, 0.160, and 0.471, respectively. VEGF plasma levels were significantly lower in carriers of the 936 T allele (9.1 ± 2.7 pmol/ml mean ± SEM) than in non-carriers (28.0 ± 5.5 pmol/ml, P = 0.033), whereas the 702 C/T and the 1612 G/A mutations showed...
no association with VEGF plasma levels. The 936 CT exchange lead to the loss of a potential bonding site for transcription factor AP-4, although the functionality of this binding site remains unclear.

**Conclusion:** We have found three new mutations in the VEGF gene, one of them, a 936 CT exchange, may be an important determinant of VEGF plasma levels.

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**MoP24:WS**

*Atorvastatin reduces NF-κB activity and the expression of Interleukin-8, Metalloproteinase-3 and Cyclooxygenase-2 in a rabbit model of atherosclerosis*


**Fundación Jiménez Díaz, Autonomous University; Hospital Clinico San Carlos; Parke-Davis, Madrid, Spain**

**Objective:** To study the effect of the HMG-Co A reductase inhibitor Atorvas- tatin on the inflammatory activity in a rabbit model of atherosclerosis.

**Methods:** Atherosclerosis was induced in 14 New Zealand rabbits by femoral endothelial desiccation and atherogenic diet. After 2 weeks, they were treated with receive either 5 mg/kg/d Atorvastatin (n = 8) (ATV) or no treatment (n = 0) (NT) during 4 more wks. We studied arterial NF-κB activation (Southwestern histochemistry), macrophage infiltration, and expression of some NF-κB-regulated genes (immunohistochemistry and in situ hybridization). The results were expressed as the ratio of the intima staining positive.

**Results:** ATV reduced total cholesterol (2730 ± 690 vs 4140 ± 479 mg/dl, p = 0.001), VLDL-c (1317 ± 502 vs 2006 ± 422 mg/dl, p = 0.020) and chylomicrons (548 ± 373 vs 1430 ± 582 mg/dl, p = 0.022). ATV animals showed a reduction of intima (12699 ± 11795 vs 650047 ± 601147 μm², p = 0.005) and media (222194 ± 57360 vs 597075 ± 544724 μm², p = 0.008) size, and of macrophage infiltration (1 ± 1 vs 19 ± 12%, p = 0.001). The activity of NF-κB (358 ± 802 vs 8696 ± 2305 μm², p = 0.001) as well as the expression of IL-8 (23 ± 25 vs 63 ± 265, p = 0.015), MMP-3 (19 ± 7 vs 42 ± 28%) and COX-2 (32 ± 6 vs 60 ± 22%, p = 0.019) were reduced in ATV compared with NT animals, while there were no differences in the expression of IP-10 and MIP-1-2. Also, the activity of NF-κB in circulating mononuclear cells was reduced in ATV animals (2966 vs 17130 a.u.; EMSA).

**Conclusions:** ATV, through the inhibition of NF-κB activity and the down- regulation of a large group of genes, could reduce the inflammation within the atherosclerotic lesion. The anti-inflammatory properties of ATV, together with its hypolipemic effect, could contribute to the plaque stabilization in humans.

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**MoP25:WS**

*Peroxisome proliferator-activated receptors-γ (PPARγ) Increase the generation of VEGF*

A. Jozkowicz, W. Plachta, J. Dulak, E. Piatkowski, A. Dembinska-Kiec.

**Department of Clinical Biochemistry; Department of Medical Biochemistry, CMUJ, Krakow, Poland**

**Background:** PPARγ are transcriptional factors involved in development of adipose tissue, regulation of glucose homeostasis and suppression of macrophage-mediated inflammatory response. Two PPARγ agonists have been approved as the insulin-sensitizing drugs in humans. We checked the effect of PPARγ activation on generation of vascular endothelial growth factor (VEGF), one of the major angiogenic agents.

**Methods:** Rat vascular smooth muscle cells (VSMC) and mouse macrophages RAW264.7 were incubated for 24 h with PPARγ activators; prostaglandin J2 (PGJ2, 0.1–10 μM) and cigitazone (0.3–10 μM). PPARγ and VEGF mRNAs were detected by RT-PCR, VEGF-protein release was measured by ELISA. PPARγ activity was assessed using reporter plasmid containing PPAR responsive element (gifted by Dr. L. Fajas), while transcriptional activation of VEGF was assayed using reporter plasmid containing VEGF promoter (gifted by Dr. H. Kimura).

**Results:** PPARγ mRNA was barely detectable in VSMC, while it was highly expressed in RAW cells. PPARγ agonists did not change PPARγ mRNA expression, but significantly increased PPARγ activity. VEGF mRNAs was regural detected in VSMC and RAW, and its expression was higher in the presence of PPARγ ligands. Accordingly, PPARγ activators significantly increased the expression of VEGF mRNA in both types of cells, and this influence was even stronger in the presence of IL-1 or LPS. Preliminary results show, that the up-regulation of VEGF synthesis by PPAR agonists is at least partially mediated by transcriptional activation of VEGF promoter.

**Conclusion:** Activation of PPARγ increases the synthesis of VEGF both in VSMC and RAW cells. The augmented VEGF production in VSMC and macrophages may be of clinical relevance for patients enduramed with diabetic retinopathy who are treated with thiazolidinediones. (grants: 501/P/KLJ/14/L, KBN: 4 P05A 131 14, 4 P05A 108 17).

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**MoP26:WS**

*The Jagged/Notch expression in the VEGF165, bFGF and leptin induced angiogenesis in 3D collagen I model*


**Introduction:** VEGF is the inflammatory angiogenic cytokine expressed in atherosclerotic plaques. The Notch/Jagged transmembrane protein signalling pathway, an evolutionarily conserved cell-to-cell communication system is utilized by many cells to suppress the differentiation during development. Angiogenesis involves the regulation of endothelial cell (EC) migration growth and differentiation and is regulated by several cytokines including VEGF and FGF. Zimin et al. (1996) demonstrated that Jagged is an angiogenesis-induced gene, and the antiangiogenic Jagged oligomer resulted in the FGF but not VEGF-induced tube formation in the 2D model of angiogenesis. Since also leptin was recently suggested to be an angiogenic and promoting cell differentiation factor, the influence of VEGF, bFGF and leptin on Jagged/Notch gene expression and tube formation in collagen 3D model was compared.

**Methods:** The human HUVEC were isolated and cultured in Human Endothelial Growth Medium (Lonza) supplemented with 5% FBS. For the VEGF, bFGF or leptin-induced in vitro angiogenesis (1–30 ng/ml) the 3D model of tube formation in collagen I and Endothelial Serum-Free Medium (Sigma) was performed according to (Gottlieb et al. 1999) The expression of Jagged and Notch in HUVEC was analysed by reverse transcription and polymerase chain reaction (RT-PCR) amplification (Zimin et al. 1995) after the incubation of HUVEC with the above cytokines for 24 hours.

**Results:** Notch was basally express in the HUVEC, when any Jagged was found in non-stimulated growing cells. On the contrary to leptin, bFGF and the high (30 ng/ml) concentration of VEGF165 stimulated the Jagged mRNA expression. The ability to in vitro tube formation was in the following order: bFGF < VEGF165 >> > leptin.

**Conclusion:** The Jagged/Notch expression seems to be specific for bFGF and to the lower extend for VEGF signalling. This cytokines but not leptin may involve the Notch/Jagged mechanisms in the endothelial cell migration and differentiation (tube formation) during angiogenesis.

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**MoP27:WS**

*Interleukin-6 (IL-6) stimulates LDL-receptor gene expression via activation of the SREBP and the Sp1 family of transcription factors in HepG2 cells*


**University Hospital Freiburg, Dep. of clinical chemistry, Freiburg, Germany**

**Objective:** Inflammatory diseases are associated with elevated levels of cytokines and abnormal LDL-cholesterol metabolism. Previous work of our group showed that the pro-inflammatory cytokine IL-6 stimulates the LDL-receptor (LDL-R) via repeat 2 and 3 of the LDL-R promoter in HepG2 cells. In the present study we investigated the IL-6 dependent activation of the family of sterol responsive element binding proteins (SREBPs). Furthermore we elucidated the signal pathway with the use of genistein, a tyrosine kinase inhibitor, and PD 98059, an inhibitor of the mitogen activated protein (MAP었습니다) signal regulated kinase (ERK) kinase (MEK).

**Methods:** HepG2 cells were stimulated with IL-6 (25 ng/ml) for up to 90 minutes before isolation of whole cell extracts for Western blot analysis. For Northern blot analysis cells were preincubated for 2 hrs with genistein (20–500 μM) or PD 98059 (10–100 μM), followed by an incubation with IL-6 (25 ng/ml) for up to 7 hrs before preparation of the total cellular RNA.

**Results:** IL-6 stimulates the release of mature SREBP1a and SREBP2. The Sp1 content of the cells remains unchanged. Northern blot experiments show that the IL-6 dependent induction of the LDL-R mRNA is prevented by preincubation with genistein and not by an preincubation with the MEK inhibitor PD98059, so that the IL-6 signal is transduced via a tyrosine phosphorylation, and does not involve an activation of the MEKs and serine/threonine phosphorylations.

**Conclusion:** The IL-6 signalling normally involves two pathways. After tyrosine phosphorylation of the signal transducer gp130, the Janus Kinases activate the MEK/MAPK pathway and the signal transducer and activators of
transcription (STATs). Our results show that the IL-6-dependent activation of the LDL-R gene expression is not mediated via the MEK/MAPK pathway but probably via the Jak/STAT cascade.

MoP28.W5 The upregulation of the MCP-1 in the vascular wall can be prevented by statins independently of plasma cholesterol levels
J. Martinez-González, J. Alfón, L. Badimon. Cardiovascular Research Center, CSCI/JBBB-HSCSP, Barcelona, Spain
Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase are widely used in the treatment of dyslipidemias and have shown beneficial effects in primary and secondary prevention of cardiovascular diseases. There is new information that seems to suggest that the beneficial effects observed may be not solely attributable to plasma cholesterol reduction.

Objective: our objective was to evaluate the effect of two statins at similar dose, although unequal plasma lipid lowering potential, on vessel wall expression of Monocyte Chemotactic Protein-1 (MCP-1) and the inducible form of Nitric Oxide Synthase (NOS II).

Methods: atherosclerosis was induced in pigs by feeding a high cholesterol and saturated fatty acid diet for 50 days. Mild atherosclerotic lesions were found at this early stage of induction. Animals were treated with either atorvastatin (3 mg/kg/day), pravastatin (3 mg/kg/day) or placebo. MCP-1 and NOS II mRNA levels in carotid, femoral and thoracic aorta were analyzed by RT-PCR.

Results: plasma total cholesterol was reduced by 28% with Atorvastatin and 17% with Pravastatin. Average MCP-1 expression in carotid, femoral and thoracic aorta were significantly reduced with both statins by 37% (p < 0.05), while NOS II expression was unaffected.

Conclusions: MCP-1 expression induced by atherogenic diet is prevented by statins, regardless of the systemic cholesterol levels. Down-regulation of MCP-1 can attenuate the development of atherosclerosis and contribute to stabilization of the plaques.

MoP1.W6 NEW ASPECTS OF STATIN TREATMENT

MoP1.W6 Pravastatin prevents postprandial hyper- and dislipidemia in patients with CHD
D.M. Aronov, M.G. Bubnova, N.B. Perova, V.G. Zhasminova, M.A. Golubev, Russian State Research Center for Preventive Medicine, Moscow, Russia

Objectives: This study was aimed to evaluate Pravastatin influence on postprandial lipemia.

Patients: We enrolled 44 pts with known CAD, mean age 55 ± 1.5 years, and total cholesterol mean level > 225 mg/dl.

Methods: All patients were on a hypolipidemic diet AHA step I for a certain period, afterwards they were randomized to two groups, 22 in each: first group received the diet plus Pravastatin 20–40 mg/day, and second received the diet for 3 months. A standart fat load consisted of 20% milk cream in a dose 65 g of pure fat per 1 sq. m of body surface (milk dose-639 ± 7 ml of milk cream) was given at the beginning and at the end of the follow up period. Blood samples were collected 3 and 6 hours after the fat load.

Results: In Pravastatin group we observed a significant reduction in postprandial levels of total cholesterol by 25%, LDL-C by 38%, TGs by 41%, apolB by 25%, apoB/apolA I ratio by 32%, and significant increase in postprandial levels of LDL-C by 31%. Control group patients results did not show any significant changes.

Table 1. Pravastatin influence on postprandial lipids (6 h after the fat load).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Beginning of follow-up</th>
<th>End of follow-up</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Chol</td>
<td>252 ± 6.8</td>
<td>190 ± 5.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tg</td>
<td>356 ± 18.7</td>
<td>210 ± 31.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C</td>
<td>189 ± 5.7</td>
<td>110 ± 6.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>35 ± 1.5</td>
<td>46 ± 1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApolB</td>
<td>163 ± 7.3</td>
<td>116 ± 7.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApolB/ApolA1</td>
<td>1.27 ± 0.08</td>
<td>0.87 ± 0.08</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Conclusion: Pravastatin treatment improves atherogenic lipemia in fasting condition as well as after the fat load.

MoP2.W6 The effect of fluvastatin on catheter-induced intimal thickening in normocholesterolemic rabbits
P. Ye, D.C. Yu, L.G. Song. Division of Geriatric Cardiology, Chinese PLA General Hospital, Beijing 100853, China

Objective: The anti-atherosclerotic effect of fluvastatin at doses insufficient to lower serum cholesterol on the catheter-induced intimal thickening and possible mechanism were investigated in abdominal aorta of rabbits.

Methods: Fifty-six rabbits were randomly divided into eight groups (n = 7, each). Fluvastatin was given mixed with food at daily dose of 8 mg/kg starting 5 days before catheterization. Light microscope, immunohistochemistry, transmission electron microscope and RT-PCR assay were applied to assess vascular smooth muscle cell (VSMC) proliferation and apoptosis, as well as oncogene expression in vascular wall.

Results: At day 10 and day 15 after catheter-induced denudation intima/media (I/M) thickness ratio was obviously higher, and also the number of PCNA-positive cells and TUNEL-positive cells in media was significantly more compared with controls. The intimal hyperplasia was mostly composed of a-SM-actin-positive cells. In rabbits given fluvastatin 1M ratio and the number of these positive cells significantly decreased compared with those without fluvastatin. The overexpression of protooncogene H-ras mRNA and decreased expression of anti-oncogene P53 mRNA were found after vascular injury, whereas fluvastatin significantly reduced H-ras mRNA and increased P53 mRNA expression.

Conclusion: Proliferation of VSMC in the media and the migration to the intima can be inhibited, and apoptosis of VSMC be induced by short-term use of fluvastatin after balloon catheter denudation, independent of serum lipid change. The mechanism underlying is presumably associated with the influence of fluvastatin on oncogene expression in the injured vascular wall.

MoP3.W6 Pravastatin stabilize fibrous caps of coronary plaques of WHHL rabbits
M. Shiomi1, T. Ito1, Y. Hirouchi2, M. Enomoto2, 1Kobe University School of Medicine, Kobe; 2Biosafety Research Center, Fukuoka, Japan

Objectives: We examined effects of hypolipidemic therapy on plaque stability.

Methods: We orally administered pravastatin, an inhibitor of HMG-CoA reductase, at a dose of 50 mg/kg to 9 WHHL, from 10 month-old to 22 month-old. Placebo rabbits were given carboxymethylcellulose with the same manner. The plasma lipid levels were monitored every 4 weeks. At the end of drug administration, rabbits were anesthetized by intravenous injection of pentobarbital sodium, and then were perfused with saline. Aortic atherosclerosis was evaluated as % of surface lesion area and lesion thickness. Coronary atherosclerosis was evaluated as score calculated with stenosis measured using histopathological sections. Composition of plaques or the fibrous caps were evaluated regarding macrophages (MCP), smooth muscle cells (SMC), extracellular lipid deposits (ECLD), and collagen fibers (CLG). Instability of plaques or fibrous cap were evaluated as a ratio of (MCP + ECLD)/(SMC + CLG).

Results: Compared to the placebo group, the serum cholesterol levels, intimal thickening of aorta, and coronary stenosis score were tended to decrease by 25%, 13%, and 27%, respectively. In the lesional composition, ECLD and/or MCP + ECLD was significantly decreased in the pravastatin group. Regarding the instability score, the pravastatin group was lower by 28% (P = 0.027) in the aortic plaque and by 61% (P = 0.051) in the fibrous cap of coronary lesions than those of the placebo group.

Conclusion: Our results suggest that pravastatin can prevent instability of plaques and the fibrous cap.

MoP4.W6 Statin therapy in hyperlipidemia induces reduction of coagulation activation in the first month of therapy
W. Korte1, W. Riesen1, G. Noseda2, 1Kantonsspital St. Gallen; 2OSP. Beata Vergine, Mendrisio, Switzerland

Objective: Recent evidence suggests that increased D-dimer concentrations are a risk marker for (recurrent) cardiac events in patients with CAD. Statins stabilize plaques and reduce the need for revascularization. We postulated that consequent lipid lowering therapy results in early reduction of coagulation activation.

Methods: 135 randomly chosen patients from the SWITCH (Swiss Intervention Trial on Cholesterol) study were evaluated. 122 were evaluable. In this study, patients with elevated total cholesterol (TC) concentration or elevated cholesterol/HDL-ratio (TC/HDL) and indication for a lipid lower-
ing therapy were treated with Atorvastatin to reach TC concentrations ≤ 5.2 mmol/L or cholesterol/HDL-ratio ≤ 5. Patients were stratified according to their D-dimer at baseline (highest quartile (Q4) vs. lower quartiles (Q1–3)). Patients were followed up at 1 and 3 months during therapy. Serum D-dimer concentrations (proven to be identical to plasma concentrations in a pilot study) were measured using the Tiasquant D-Dimer on a Hitachi 917 analyzer (Roche Diagnostics).

Results: Median cholesterol in Q4 fell from 8.15 (baseline) to 5.65 and 5.10 mmol/L in Q1–3 from 7.70 (baseline) to 5.60 and 5.05 mmol/L at 1 and 3 months (all p < 0.001). Median D-Dimer in Q4 fell from 755 μg/L to 590 μg/L and 380 μg/L, respectively (1 and 3 months, p to baseline < 0.001, Wilcoxon Signed Rank Test). Median D-Dimer in Q1–3 were unchanged (240 μg/L at baseline, 1 and 3 months, ns).

Conclusions: We show here that therapy with Atorvastatin in patients with hyperlipidaemia and increased coagulation activation (uppermost quartile) diminishes coagulation activation within the first 4 weeks of therapy. This suggests that the protection of further atherothrombosis and thus cardiac events begins within the first month of therapy.

MoP5:W6 Influence of simvastatin on LDL-subtypes in patients with heterozygous familial hypercholesterolemia and combined hyperlipidemia
H.C. Geiss, K.G. Parhofer, P. Schwandl. Med. Dept. II, Klinikum Grosshadern, University of Munich, Germany

Low density lipoprotein (LDL)-subtypes differ concerning their atherogenic potential. Small-dense LDL are more atherogenic than large-buoyant LDL. Statins, such as simvastatin (simva), are used to reduce elevated levels of LDL-chol in patients with heterozygous familial hypercholesterolemia (FH) or combined hyperlipidemia (CHLP). We investigated the effect of simva on LDL-subtype distribution in patients with FH and CHLP.

In 16 patients (14 male, 12 female, age 44 ± 22) suffering from FH (n = 9, LDL-chol. 275 ± 41 mg/dL, TG 119 ± 35 mg/dL or CHLP (n = 7, LDL-chol. 183 ± 29 mg/dL, TG 229 ± 100 mg/dL) simva (20 mg/day) was given for a period of 4 weeks. Plasma lipids and 7 LDL-subtypes (density gradient ultracentrifugation, density range 1.019–1.063 g/mL) were determined before and during simva treatment; the absolute and percentage differences in the LDL-subtype distribution were evaluated by the Wilcoxon-test.

Simva reduced LDL-chol. by 31% (FH: -34%, CHLP: -29%) and TG by 11% (FH: -29%, CHLP: -29%). In the entire group and in patients with CHLP all LDL-subtypes were reduced (p < 0.01), whereas in patients with FH the amount of LDL-7 (1.054–1.063 g/mL) did not decrease significantly, while all other subfractions decreased. Furthermore, during simva patients with FH showed an increase in the relative amount of LDL-7 (p < 0.01) compared to less dense LDL-subtypes, whereas in patients with CHLP all LDL-subtypes were reduced to a similar degree.

Thus, simva reduces LDL-chol. by decreasing all subfractions. In CHLP this reduction was more uniform than in FH, where small-dense LDL were reduced to a lesser degree than large-buoyant or intermediate-dense LDL. These differences may reflect differences in HDL-chol. as well as differences in the metabolic response to simvastatin treatment.

MoP6:W6 Lipid-lowering therapy with cerivastatin improves endothelial function after 2 weeks
Roland E. Schmieder, Johannes Jacobi, Christian Delles, Markus P. Schlaich, Markus Schneider, Stefan John. Dept. of Medicine IV, University of Edangen-Nürnberg, Germany

Aim: To investigate the effect of short-term cerivastatin therapy on endothelial function. Improved endothelium-dependent vasodilation (EDV) plays a central role in the development of atherosclerosis and acute coronary syndromes in hypercholesterolemia.

Methods: This was a double-blind, placebo-controlled study involving 35 patients (52 ± 11 years) with low-density lipoprotein cholesterol (LDL-C) > 160 mg/dL (mean 197 ± 44 mg/dL) randomized to receive cerivastatin 0.4 mg or placebo. EDV was measured by plethysmography and i.a. infusion of acetylcholine (ACH 12 and 48 μg/min). Endothelium-independent vasodilation was measured by i.a. infusion of nitroprusside (NP 3.2 and 12.8 μg/min).

Results: After 2 weeks treatment, LDL-C (±SEM) decreased by 33 ± 8% in the cerivastatin group and increased by 2 ± 4% in the placebo group (p < 0.001). Endothelium-dependent increases in forearm blood flow improved after 2 weeks cerivastatin therapy, relative to baseline (ACH 12 μg/min: 22.3 ± 5.2 vs 11.2 ± 1.9 ml/min/100 ml; 48 μg/min: 31.2 ± 6.3 vs 19.1 ± 3.1 ml/min/100 ml; see Figure: cerivastatin □ before, ● after; placebo ○ before, ● after). Similar results were found when EDV was expressed as % change from baseline. The cerivastatin group had an improvement in EDV after 2 weeks compared with placebo: +203 ± 85% vs −26 ± 71% (p < 0.05) for ACH 48 μg/min.

Conclusion: Cerivastatin rapidly improved NO bioavailability, suggesting a role for lipid-lowering therapy in the treatment of acute coronary syndromes.

MoP7:W6 Efficacy and safety of cerivastatin/bezafibrate and cerivastatin/fenofibrate combination therapies
M. Farner1, R. Espe2,1. Point Médical, Dijon, France; 2Hospital Militar Central, Buenos Aires, Argentina

Aim: Comparison of data from 2 studies in which cerivastatin (C) was combined with bezafibrate (B) and fenofibrate (F), respectively, for the treatment of primary hypercholesterolemia.

Methods: Both studies were randomized, multinational and double-blind. All patients had LDL-C > 4.12 mmol/L (or ≥3.5 mmol/L if they had CHD or 2 or more cardiovascular risk factors) on entry. In the C/B combination study, patients were randomized to one of 3 groups following a placebo run-in. Group 1 received C 0.3 mg/d for 16 wk. Group 2 received B 400 mg/d for 16 wk. Group 3 received C 0.3 mg/d and B 400 mg/d for a further 8 wk. The same design was employed in the C/F combination study, with fenofibrate micronized 200 mg/d replacing B. A double-dummy technique was employed in both studies, using C or信息服务 placebos to ensure double-blinding.

Results: % changes in efficacy variables from baseline to wk 16 (ITT):

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C/B Combination</th>
<th>C/F Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C</td>
<td>-42.6%</td>
<td>-40.3%</td>
</tr>
<tr>
<td>Total chol.</td>
<td>-29.2%</td>
<td>-29.3%</td>
</tr>
<tr>
<td>HDL-C</td>
<td>+33.8%</td>
<td>+12.4%</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-44.1%</td>
<td>-37.2%</td>
</tr>
</tbody>
</table>

* Significantly (P < 0.01) greater improvement than seen with C or B monotherapy (MT); ** except for B vs combination (com). Significantly (P < 0.001) greater improvement than seen with C or F MT.

Both combinations had a good safety profile, with no marked differences from the safety profiles of the respective MTs.

Conclusions: Cerivastatin combined with bezafibrate or fenofibrate produced significantly greater LDL-C and total cholesterol decreases than seen with any of the drugs alone, while maintaining the benefits of fibrate therapy for reducing triglycerides and increasing HDL-C.

MoP8:W6 Determination of mRNA levels of cholesterol regulatory proteins in cultured human cells using ribonuclease protection assay
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Objective: To determine mRNA levels of cholesterol regulatory proteins in cultured human cells.

Methods: 1) We designed a rapid method for determining mRNA content of cholesterol regulatory proteins in cultured human cells, Hep G2 cells, using a ribonuclease protection assay. [23P] labeled RNA fragments for genes of human low density lipoprotein (LDL) receptor, 3-hydroxy-3-methylglutaryl
coenzyme A (HMG-CoA) reductase, sterol regulatory element binding proteins 2 (SREBP-2), SREBP cleavage-activating protein (SCAP), site-1 protease (SIP) site-2 protease (S2P) were prepared in vitro transfection. 2) The human hepatoma cell line Hep G2 was cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FBS with or without itavastatin. mRNA levels were determined by ribonuclease protection.

**Results:** Itavastatin induced mRNA of HMG-CoA reductase, LDL receptor and SREBP-2 in Hep G2 cells. On the other hand, itavastatin did not induce neither SCAP, SIP nor S2P in this condition.

**Conclusion:** Ribonuclease protection assay is very useful for determining the mRNA levels of cholesterol regulatory proteins and their differential regulation.

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**MoP09-W6**

The relative induction of mRNA for HMG-CoA and LDL receptor by five different HMG-CoA reductase inhibitors in cultured human cells

S. Morikawa1, 2, M. Umetsu1, S. Nakagawa2, H. Yamasaki3, H. Suganami2, K. Inoue1, 3, M. Kitahara1, T. Hamakubo1, T. Kodama1, Y. Saito1, 1Department of molecular Biology and Medicine, Research Center for Advanced Science and Technology, The University of Tokyo; 2Tokyo Research Laboratories, Kowa Company Ltd.; 3Department of Cardiology, Juntendo University; Shiraoka Research Station of Biological Science, Nissan Chemical Industries, Ltd.; Second Department of Internal Medicine, School of Medicine, Chiba University, Japan

**Objective:** To compare the relative induction of messenger RNA for 3-hydroxy-3-methylglutaryl coenzyme A reductase and low density lipoprotein receptor by five different HMG-CoA reductase inhibitors in cultured human cells.

**Methods:** The human hepatoma cell line Hep G2 was cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (FCS). The medium was replaced with 10% LPDS-DMEM with or without chemicals and cells incubated for the indicated time. At each time point, total RNA was isolated and hybridized with [32P] labeled riboprobe. mRNA levels were determined by ribonuclease protection assay.

**Results:** When cells were treated with a 200-fold excess of the IC50 concentration of each inhibitor, itavastatin was able to induce LDL receptor mRNA most effectively.

**Conclusion:** The effect of HMG-CoA reductase inhibitors on the upregulation of mRNA for HMG-CoA reductase and LDL receptor are different from each other and among these lipophilic inhibitors itavastatin is most effective in inducing LDL receptor mRNA in Hep G2 cells.

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**MoP10-W6**

Pravastatin use and risk of coronary events and cerebral infarction in Japanese men with hypercholesterolemia: The Kyushu Lipid Intervention Study (KILIS)

J. Sasaki, K. Arakawa, On behalf of the KILIS group; Fukuoka University School of Medicine, Fukuoka, Japan

**Objective:** To examine whether pravastatin use confers reduction in coronary heart disease (CHD) events and cerebral infarction (CI) in Japanese men with hypercholesterolemia.

**Methods:** A total of 5640 men aged 45–74 years with serum total cholesterol (TC) of 220 mg/dl or greater and without a history of CHD event or stroke were recruited to undertake either pravastatin or conventional treatment. After exclusion of men with prescription of drugs excluded in protocol and others, 2219 men in the pravastatin group and 1634 in the conventional treatment group remained in the analysis. Average follow-up period was approximately 5 years. The Cox hazards model was used to calculate relative risk (RR) adjusted for coronary risk factors at the baseline.

**Results:** RR for pravastatin use were: CHD 0.86 (one-sided p = 0.23), CI 0.78 (p = 0.13), and CHD + CI 0.81 (p = 0.08). RS for a good compliance with pravastatin were: CHD 0.75 (p = 0.11), CI 0.74 (p = 0.13), and CHD + CI 0.73 (p = 0.04). 52% in the pravastatin group and 18% in the conventional treatment group experienced a 15% or greater decrease in serum TC on average in the follow-up, and they showed an approximately 50% reduction in CHD and CI combined, as compared with men with less than 5% decrease in serum TC.

**Conclusions:** Pravastatin use reduces both CHD events and CI in Japanese men with hypercholesterolemia as well.

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**MoP11-W6**

Plasma fibrinogen behavior during treatment of hypercholesterolemia with simvastatin

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**Objective:** To assess plasma fibrinogen behavior in hypercholesterolemic patients treated with simvastatin for a 12-week period.

**Material and Methods:** Patients with type IIa hypercholesterolemia who met the guidelines of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II) for pharmacological intervention, were assessed. Patients were given 10 mg simvastatin daily for 12 weeks. Plasma fibrinogen levels were determined at baseline and at 12 weeks after the start of drug treatment. Plasma fibrinogen assays were done according to Clauss quantitative method with normal values ranging from 200 mg % up to 400 mg %. Results were assessed by the Student’s t-test for paired samples. Significance level was p < 0.05

**Results:** 1,000 patients were screened from May 98 to November 99. Mean basal fibrinogen levels were 292.56 (SD 64.71). At 12 weeks, mean plasma fibrinogen levels of 268.06 (SD 49.53) were observed. Mean decrease of plasma fibrinogen level was 8.38%, which was statistically significant (p < 0.001).

**Conclusions:** Recent studies show conflicting results related to the effect of statins on free plasma fibrinogen levels of patients with atherosclerotic vascular disease. Uncontrolled studies have underlined its relevance as a predictor of vascular events. The present study shows that the use of simvastatin for the treatment of hypercholesterolemia provides a statistically significant reduction in plasma fibrinogen levels.

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**MoP12-W6**

Reduced expression of soluble markers of endothelial dysfunction after 1 year treatment with atorvastatin and simvastatin in patients with coronary heart disease

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**Objective:** To compare possible effects of simvastatin and atorvastatin on circulating inflammatory and haemostatic markers of endothelial dysfunction in atherosclerosis.

**Methods:** The effects were studied in patients with established CHD (aged 40–76 yrs), 30 pts. (76% women) randomised to simvastatin (S-group) and 28 pts. (24% women) to atorvastatin (A-group). Fasting blood samples were collected before and after 12 months treatment for determinations of tissue plasminogen activator antigen (tPAG), von Willebrand factor (vWF), thrombomodulin (TM), and the soluble forms of P-selectin, E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).

**Results:** (median values): There were equally significant reductions in the levels of total cholesterol, LDL cholesterol and triglycerides and a rise in HDL cholesterol in both groups. In the A-group significant reductions were seen in ICAM-1 and P-selectin (266 to 203 ng/ml (p < 0.001) and 101 to 84 ng/ml (p < 0.01), respectively). In the S-group there was significant decrease in P-selectin (109 to 96 ng/ml (p < 0.05)) and in E-selectin (49.9 to 44.8 ng/ml (p < 0.05)) whereas an increase was observed in VCAM-1 (398 to 455 ng/ml, p < 0.05). There were, however, no differences in changes between the groups in any variable. No changes were observed in the haemostatic variables.

**Conclusions:** The reduction in proinflammatory markers of endothelial function, i.e., ICAM-1, E-selectin and P-selectin, may be indicative of a less activated state of the endothelium after 1 year treatment with both simvastatin and atorvastatin. The rise in VCAM-1 on simvastatin might point to different regulatory pathways of the adhesion molecules.

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**MoP13-W6**

ApoA-1, but not total cholesterol and LDL-C, is predictive for myocardial infarction (MI) and death in patients with coronary artery disease (CAD) and treated lipid levels

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**Objective:** To investigate the predictive value of treated lipid levels on MI and death in a large cohort of patients with CAD who were treated to target lipid levels.
Methods: 848 Hyperlipidemic patients (675 men and 173 women) with angiographically defined CAD received statin therapy. The first complete lipid profile (total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG), apolipoprotein A-1 (ApoA-1)) and apolipoprotein B (ApoB)) that demonstrated >30% decrease of baseline TC level before statin therapy, was included for analysis. An age-adjusted Cox regression model was used to test the effect of TC, LDL-C, HDL-C, TG, ApoA-1, ApoB, diabetes and smoking on subsequent myocardial infarction and all cause mortality.

Results: Treated target lipid levels, TC (4.4 ± 0.7), LDL-C (2.6 ± 0.6), TG (1.6 ± 0.8), ApoB (0.91 ± 0.19), diabetes and smoking did not predict subsequent events. The only significant predictor in both men and women was ApoA-1 (1.31 ± 0.24) (P = 0.026 and P = 0.002 respectively). HDL-C (1.1 ± 0.3) was predictive in women exclusively (P = 0.004).

Conclusions: Under hypotensive treatment, levels of Apo A-1 were predictive for mortality and death for men and women and HDL-C only for women. Since treated TC, LDL-C and TG were not predictive anymore, value of longterm treatment is sustained.

MoP14.W6 Predictors of glucose intolerance (GI) in the West of Scotland Coronary Prevention Study (WOSCOPS)

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Objectives: To establish baseline predictors of GI in WOSCOPS.

Methods: GI was defined as at least two post-randomisation measurements of fasting glucose >6.5 mmol/L and at least one post-randomisation measurement 1.5 mmol/L greater than baseline, or the prescription of hypoglycaemic drugs post-randomisation. Self-reported diabetics and those with a baseline glucose >6.5 mmol/L were excluded. GI developed in 192 of the 5,940 eligible randomised subjects.

Results: Baseline body mass index (BMI), triglyceride (TG), white cell count (WCC), systolic blood pressure, total cholesterol and glucose were significant positive predictors of GI in an univariate analysis. HDL cholesterol and pravastatin treatment were significant negative predictors. In a multivariate model BMI (odds ratio [OR] 1.32 P < 0.001), WCC (OR 1.17 P = 0.02), TG (OR 1.47 P < 0.001) and pravastatin therapy (OR 0.74 P = 0.037) remained significant. Baseline glucose was a significant (P < 0.001) predictor with greatest risk associated with the two highest quintiles (4.7-5.0 mmol/L OR 1.81; >5.0 mmol/L OR 5.07).

Conclusions: Markers of metabolic syndrome BMI, TG and glucose were strong predictors of GI in middle-aged men. The data supports the association of inflammation with the development of GI. Pravastatin appeared to protect against the development of GI possibly via its TG-lowering action. However the influence of pravastatin on inflammation and endothelial function cannot be ruled out as modes of action.

MoP15.W6 Muscle pains and/or CK-elevation during HMGC-CoA-reductase inhibitor therapy are frequently associated with increased isoprostane

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Background: Muscle pains with or without CK-elevation are among the most frequent side effects on statins. The pathophysiological background, however, still remains obscure.

Methods: The isoprostane (IP) 8-epi-PGF2 alpha as a marker of in-vivo oxidation injury was determined in plasma, serum and urine in 31 patients when muscle problems manifested and at different time intervals after withdrawing the respective statin by means of a specific immunoassay. 37 healthy people of comparable age and sex and 11 patients suffering from FH without any treatment yet served as controls.

Results: More than the half of patients (19 out of 31) with muscle problems showed elevated IP-values (by >50%) in plasma and urine, while serum values were elevated to a lesser extent. Stopping the respective statin or successfully changing to another member of this family of compounds resulted in rapid IP-normalization within almost 1 week. An increase in IP was noted with all statins available.

Conclusions: These findings indicate a significant involvement of oxidation injury in muscular side effects of statins in patients suffering from FH.

MoP16.W6 Effect of simvastatin on remnant lipoprotein in heterozygous familial hypercholesterolemia

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Objective: To investigate the effects of simvastatin on triglyceride-rich lipoproteins (TRL’s) in familial hypercholesterolemia (FH).

Methods: We carried out a sequential intervention trial of 4 week treatment periods with 40 mg simvastatin, placebo and 40 mg simvastatin in 15 subjects with heterozygous FH. At the end of each treatment, fasting concentrations of apolipoprotein B-48 (apo B-48), remnant-like particle cholesterol (RLP-C), total apolipoprotein B-100 (apo B-100), LDL cholesterol, total cholesterol, HDL cholesterol and triglyceride were measured and Apo B-48 concentration was determined using SDSPAGE with enhanced chemiluminescence, RLP-C using an immuno-separation assay. Apo B concentration was measured by an immuno- nephelometry.

Results: 

Conclusions: In FH, simvastatin decreases the plasma concentration of TRL remnants including those of intestinal origin. The mechanism may include increased expression of the LDL-receptor and/or decreased competition for high affinity clearance pathways.

MoP17.W6 –491A/T polymorphism in the apo E gene promoter region modulates the lipid lowering response to atorvastatin and bezafibrate in patients with mixed dyslipidemia

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Objective: To compare the incidence of common genetic polymorphisms in the apo E gene in the response to atorvastatin and bezafibrate in patients with mixed dyslipidemia.

Methods: 116 subjects who participated in the ATOMIX Study (Atorvastatin in Mixed Dyslipidaemia) were selected. Their response to atorvastatin and bezafibrate treatment in a randomized double blind trial was considered after a year of treatment. Total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol and non-HDL-cholesterol change concentrations were evaluated. Apo E genotype and two common promoter region polymorphisms (−491A/T and −219T/G) of the apo E gene, were determined by PCR amplification and restriction isotyping. Statistical analysis was performed and significance level was considered when p ≤ 0.05.

Results: Apo E genotype and −219T/G polymorphisms did not appear to influence the response to atorvastatin neither bezafibrate treatments. However −491T carriers showed a significant enhanced response effect of atorvastatin respect to non-carriers in total cholesterol (−35% vs. −27%), LDL-cholesterol (−42% vs. −33%) and non-HDL-cholesterol (−42% vs. −34%) decrease, while these lipid changes were not affected by the polymorphism in the bezafibrate group. Moreover, −491T carriers showed a significant lower response to triglyceride decrease effect of bezafibrate (−23% vs. −39%) and a parallel lower increase in HDL-cholesterol (−21% vs. −25%).

Conclusions: The common −491A/T polymorphism in the apo E gene promoter region seems to affect the efficiency of atorvastatin and bezafibrate treatment in patients with mixed dyslipidemia. Although this observation should be confirmed in more extensive studies, it can be taken into account in order to evaluate the convenience of a drug treatment in patients with this dyslipidemia.
**MoP18:W6** Pharmacodynamics of new HMG-CoA reductase inhibitor ZD4522 in patients with primary hypercholesterolemia

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**Objective:** To assess the effects of once-daily oral doses of ZD4522 (rosuvastatin) and atorvastatin - both synthetic inhibitors of HMG-CoA reductase - on lipids and apolipoproteins (apo) in hyperlipidemic patients.

**Methods:** This randomised, placebo-controlled, parallel-group dose-ranging trial (4522IL/I0008) was conducted in patients with elevated LDL-C (>4.14 mmol/L (>160 mg/dL) but <6.21 mmol/L (<240 mg/dL)) and TG < 3.39 mmol/L (<300 mg/dL). After a 6-week dietary run-in period (18-70 years) and post-menopausal women (50-70 years) received double-blind placebo or ZD4522 (1-40 mg) or open-label atorvastatin (10 or 80 mg) during a 6-week treatment period. Percentage change (%A) from baseline to wk 6 in lipid parameters was analysed by ANOVA for ZD4522 and placebo groups only, with informal comparisons made across treatments. Dose response for ZD4522 was analysed by linear regression.

**Results:** 142 patients entered 6-wk treatment period (124 patients provided data in efficacy analysis). Compared with placebo, all doses of ZD4522 significantly lowered LDL-C, TC and apo B in a dose-dependent manner (p < 0.001); all ZD4522 doses lowered TG and raised HDL-C in these patients. Similar results were generally seen with low- and high-dose atorvastatin, although ZD4522 showed greater improvements on a mg-per-mg basis. A linear regression fit suggested each doubling of ZD4522 dose resulted in a further 5.2% reduction in LDL-C.

**Conclusions:** ZD4522 showed clinically relevant, dose-related reductions in LDL-C and beneficial effects on other lipid parameters. Safety profile of ZD4522 compared favorably with those of atorvastatin and placebo.

**MoP19:W6** Single- and multiple-dose pharmacokinetics and safety of the new HMG-CoA reductase inhibitor ZD4522

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**Objective:** To examine the pharmacokinetics (PK) and safety of single and repeated oral doses of ZD4522 (rosuvastatin) - a synthetic inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.

**Methods:** Three randomised, double-blind, placebo-controlled trials (4522IL/I0001, 4522IL/I0002 and 4522IL/I0011) were conducted in healthy male volunteers. Subjects in Trial 1 received placebo or single doses of 5, 10, 20 or 40 mg ZD4522 on 2 dosing days separated by a 1-wk washout period; subjects in Trials 2 and 11 received placebo or single doses of ZD4522 (20 or 40 mg in Trial 2, 80 mg in Trial 11), followed by a 4-day washout period, then repeated doses of the same treatment for 7 days. ZD4522 plasma concentrations were measured before and during each dosing period and at intervals up to 96 h after the final dose. Safety was assessed by adverse events, laboratory tests and physical and electrocardiographic examinations.

**Results:** 37 subjects (18-50 yr) were recruited into these trials (n5 = 13; n11 = 16; n11 = 8); all but 1 (protocol noncompliance) completed double-blind treatment. Cmax and AUC(0-24) were approximately linear over the multiple-dose range (Table). Moderately rapid absorption of ZD4522 was observed, without relevant accumulation at steady state. Similar results were seen after single oral doses. Repeated doses up to 80 mg ZD4522 were well tolerated, with no evidence of clinically relevant liver function abnormalities or myopathy.

**Conclusion:** PK and safety data in healthy volunteers support long-term dosing of ZD4522 in subsequent trials in hyperlipidemic patients.

**MoP20:W6** Pharmacological properties of ZD4522 - A new HMG-CoA reductase inhibitor


**Objectives:** To measure the in vitro and in vivo potency of ZD4522 (rosuvastatin) as an inhibitor of rat liver HMG-CoA reductase in comparison with 5 other statins and to determine the duration of inhibition in vivo.

**Methods:** ZD4522 was compared with atorvastatin, cerivastatin, fluvastatin, pravastatin and simvastatin. IC50 values were measured in rat hepatic microsomes and the effect of varying substrate concentrations was also examined. To determine ED50 values, compounds were orally dosed to rats and, 2 hr later, hepatic cholesterol synthesis measured by incorporation of [14C]acetate. The duration of inhibition by ZD4522, atorvastatin, cerivastatin and simvastatin was also measured 3–9 hr after a single oral dose corresponding to a 10-fold multiple of the ED50.

**Results:** ZD4522 was found to be a potent inhibitor of HMG-CoA reductase activity in rat hepatic microsomes, IC50 = 12 nM (95% confidence limits 9–16 nM), compared with other statins, range 13–55 nM (cerivastatin and pravastatin, respectively). The IC50 of ZD4522 was found to be inversely related to HMG-CoA but independent of NADPH concentrations from 0.3–5 mM. The ED50 of ZD4522 after oral administration was 1.2 mg/kg (0.5–3 mg/kg), intermediate between cerivastatin and pravastatin, 0.01 and 18.0 mg/kg, respectively. The time-course of inhibition after oral administration showed that ZD4522 had a prolonged effect on hepatic cholesterol synthesis with 62% inhibition at 7 hr, compared with 7%, 31% and 13% for atorvastatin, cerivastatin and simvastatin, respectively.

**Conclusions:** ZD4522 is a potent inhibitor of HMG-CoA reductase in rat liver with a relatively long duration of action.

**MoP21:W6** Uptake of HMG-CoA reductase inhibitor ZD4522 into hepatocytes and distribution into liver and other tissues of the rat


**Objectives:** To investigate the mechanism and kinetics of uptake of ZD4522 (rosuvastatin) and pravastatin into hepatocytes and to determine the distribution of ZD4522, pravastatin and simvastatin in liver and other tissues in the rat.

**Methods:** Initial rates of uptake of [14C]-labelled compounds into isolated rat hepatocytes were measured and kinetic parameters were determined. After iv administration of radiolabelled compounds to adult male rats, uptake clearance rates were determined from plasma and tissue radioactivities.

**Results:** ZD4522 and pravastatin were shown to be taken up into hepatocytes by active transport and passive diffusion. The rate of active uptake clearance (Vma/Km) of ZD4522 into hepatocytes was significantly greater than pravastatin (p < 0.001). The initial rates of uptake of both compounds were independent of Na+ or Cl− but were reduced by metabolic inhibitors. ZD4522 competitively inhibited the uptake of pravastatin with a Ki value close to its Km value for uptake. After iv administration to the rat, the uptake clearance rates of ZD4522 into liver, kidney and other tissues were 0.9, 0.2 and <0.02 mL/min/g, respectively. Pravastatin was also selective for liver uptake but simvastatin showed high rates of uptake into liver and some non-hepatic tissues such as adrenal and spleen. This result and the intracellular localisation of radiolabelled compound was confirmed using microautoradiography.

**Conclusions:** Both ZD4522 and pravastatin are taken up into hepatocytes partly by a Na+-independent, high-affinity, active uptake process but ZD4522 had the greater affinity for this system. Both ZD4522 and pravastatin were selective for hepatic uptake in vivo but simvastatin appeared to be less selective.

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*XIIth International Symposium on Atherosclerosis, Stockholm, Sweden, June 25-29, 2000*
MoP22-W6

Effect of simvastatin on plasma C-reactive protein and fibrinogen: A randomised controlled trial


Objective: There is a correlation between the risk of coronary and cerebrovascular disease and both plasma C-reactive protein (CRP) and fibrinogen (F) concentrations. We measured CRP and F in a single centre study with simvastatin in patients with primary hypercholesterolaemia in preparation for the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEASHO).

Methods: After a 2-week diet/placebo run-in, 141 patients were randomised to one of three double-blind treatments for 6 weeks: simvastatin 80 mg/day (slow release formulation) and folic acid 2 mg/vitamin B12 0.8 mg daily (S80/folic + B12); or S80 alone; or Folic + B12 alone.

Results: None of the plasma F changes from baseline were significant (mean % changes from baseline were: 2.2% - 3.8% and 2.3% for the S80/folic + B12, S80, and folic + B12 groups, respectively). CRP levels were very variable at baseline, but there was a significant decline with simvastatin alone or in combination, and the between-group comparison for S80/folic + B12 versus folic + B12 was highly significant (p = 0.001).

CRP, Change from Baseline at Week 6 (N per group = 45)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median baseline (mg/L)</th>
<th>Median change (mg/L, SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S80/folic + B12</td>
<td>1.3</td>
<td>-0.2 (0.8)</td>
<td>0.007</td>
</tr>
<tr>
<td>S80</td>
<td>1.3</td>
<td>-0.4 (1.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>Folic + B12</td>
<td>1.8</td>
<td>0.2 (1.0)</td>
<td>0.042</td>
</tr>
</tbody>
</table>

Conclusions: Simvastatin reduces CRP but not F. The mechanism, and whether this effect contributes to the demonstrated reduction in cardiovascular events produced by simvastatin, remain to be determined.

MoP23-W6

Acute inhibition of VLDL-apoB secretion by atorvastatin in primary hamster hepatocytes


Objective: To investigate the intracellular mechanisms of action of atorvastatin on VLDL-apoB secretion, in primary hamster hepatocytes freshly isolated from livers of Syrian golden hamsters. This animal model shares considerable similarities with humans in hepatic cholesterol and apolipoprotein B (apoB) metabolism.

Methods & Results: Treatment of hamster hepatocytes with atorvastatin caused a significant decrease in the synthesis of cholesterol and cholesterol ester but did not alter triglyceride synthesis. In contrast, the secretion of total VLDL was reduced by 44%. A significant decrease in the number of VLDL particles secreted by hamster hepatocytes was confirmed by sucrose density fractionation of culture media and micromolar luminal contents which showed a significant decline in the number of VLDL particles assembled in the lumen and secreted into the media. The decline in VLDL-apoB secretion was accompanied by enhanced intracellular degradation of apoB as demonstrated in intact cells as well as by a greater accumulation of degradation intermediates in permeabilized hamster hepatocytes. Interestingly, atorvastatin treatment did not appear to significantly alter the translocaional status of apoB, based on protease protection assays.

Conclusions: Taken together, the present data suggest that the assembly and secretion of large apoB-containing VLDL particles in primary hamster hepatocytes can be acutely inhibited by atorvastatin by a process involving reduced cholesterol ester synthesis and enhanced intracellular apoB degradation. The data also support the usefulness of the hamster as an experimental model to investigate the mechanisms of action of hypolipidemic agents.

MoP24-W6

Effect of fluvastatin on antioxidant activity of plasma in patients with stable angina with average cholesterol levels

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Lowering the blood cholesterol level, particularly low-density lipoprotein cholesterol, is associated with reduction in cardiovascular morbidity and mortality. Beneficial effect of statins is thought to result from their non-lipid mechanisms.

We investigated an effect of fluvastatin on antioxidant plasma activity in patients with stable angina with average cholesterol levels. The patients were randomly allotted into two groups. The study group consisted of 12 patients who were administered fluvastatin orally 40 mg once daily at bed-time, and the control group of 10 patients with no drug administration. Our study has been approved by the local Ethics Committee: Blood samples were collected for examination from cubital vein after and before 1-month treatment period on fluvastatin. Antioxidant activity of blood plasma was determined by a modification of Pellegrini et al method, based on reduction of preformed cation radical of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) by blood plasma. In the study group fluvastatin significantly (p < 0.05) increased antioxidant activity of plasma after 30 days of the treatment, in comparison to the control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Antioxidant activity [μmole equivalent of ascorbic acid] before treatment</th>
<th>after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluvastatin 40 mg/d</td>
<td>197.8 ± 47.5</td>
<td>210.8 ± 45.9</td>
</tr>
<tr>
<td>Control group</td>
<td>191.0 ± 42.6</td>
<td>198.6 ± 43.1</td>
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</tbody>
</table>

The results of our studies have demonstrated that fluvastatin has an additional mechanism independent on the effect on cholesterol concentration.

MoP25-W6

Simvastatin (Zocor) induces the expression of apolipoprotein AI in HepG2 cells and primary hamster hepatocytes

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Objective: We have investigated the effect of simvastatin, a potent inhibitor of HMG-CoA reductase, on the expression of apoAI in two model systems, HepG2 cells and primary hamster hepatocytes.

Methods & Results: HepG2 or hamster hepatocytes were incubated with different doses of simvastatin (0.1-10 mM) for a period of 18 hours. Cells were then pulsed with [125I]methionine for either 15 min (HepG2) or 60 min (hamster hepatocytes), and chased for various periods of time (15-120 min). Cells and media were collected at various periods of time, solubilized, and immunoprecipitated. A dose-dependent increase in apoAI levels was observed in both cells and media when either HepG2 or primary hamster hepatocytes were treated with various doses of simvastatin. There was a significant increase in the synthesis of apoAI in HepG2 cells (44.3 ± 12.1%), and hamster hepatocytes (212 ± 2%) after treatment with 10 mM simvastatin. The increase in apoAI expression appeared to result in a higher level of apoAI secreted into the culture media in both cell types (49.2 ± 7.8% in HepG2, 197 ± 0.2% in hamster hepatocytes). ApoAI mRNA levels were also significantly increased in both cell types. Control experiments with transferrin confirmed specificity of the effect on apoAI secretion. Time-course experiments suggested that simvastatin does not affect intracellular turnover of apoAI in either of the two cell models.

Conclusions: Acute treatment of cultured hepatocytes (both transformed as well as primary) results in a significant upregulation of apoAI gene expression, causing oversecretion of apoAI extracellularly. The stimulatory effect of simvastatin on apoAI synthesis and secretion may thus explain the clinical observation of an elevated HDL-cholesterol level in hyperlipidemic patients treated with simvastatin.

MoP26-W6

The association between LDL-C and outcome: The pravastatin pooling project

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Objective: Epidemiological data suggest a law of diminishing returns in the clinical benefit achieved when LDL-C levels are reduced. The long-term risks and benefits associated with aggressive reductions in LDL-C remain controversial. The investigators for the 4S, WOSCOPS and CARE studies have published results describing the relationship between clinical outcome and...
changes in, and achieved levels of, LDL-C, in the subjects treated with a statin. The methodology used by each study was different making comparisons difficult. Here, the Prospective Pravastatin Pooling project investigators present the results for WOSCAPS, LIPID and CARE using the 4S methodology.

Methods: In the pravastatin treated groups, subjects were divided into quintiles of achieved levels of and % changes in LDL-C at 12 months. Future coronary event rates were calculated and compared statistically after adjusting for baseline risk factors and lipids on and treatment LDL cholesterol and triglycerides.

Results and Discussion: There was no evidence of heterogeneity of risk associated with variations in % change in LDL cholesterol. In CARE achieved LDL-C was not associated with risk of a coronary event. In LIPID and WOSCAPS, where baseline LDL-C levels were higher, there was a positive association, primarily due to higher risk in subjects in the quintile with highest on-treatment LDL-C (>126 mg/dl in LIPID and >164 mg/dl in WOSCAPS).

MoP27:W6 Prospective pravastatin pooling project: Relative risk reduction by baseline HDL and TG concentrations


For the Pravastatin Pooling Project Investigators: *Harvard Medical Sch Boston, MA; †Nairi Heali Foundation, Melbourne; ‡Univ Glasgow, UK

1Univ Texas, Houston, TX; 2Univ Sydney, Australia; 3Wake Forest Univ, Winston-Salem, NC, USA; 4Univ Texas, Houston, TX.

Objectives: To study the relationship between baseline plasma HDL-C and triglyceride (TG) levels, recurrent coronary events, and the efficacy of pravastatin 40 mg in reducing coronary events in 3 major studies, WOSCAPS, CARE, and LIPID.

Methods: Plasma HDL and TG were divided into prespecified categories, and into quintiles.

Results: Pravastatin produced a uniform, significant relative risk reduction for the primary end points (EP), CAD death and nonfatal MI and the secondary EP, CAD death, nonfatal MI, and CABG/PTCA, in prespecified categories of baseline HDL (<1 mmol/l), >1 mmol/l), and of TG (<1.5 1.5-2.5, >2.5 mmol/l). Baseline HDL and TG had a similar relationship to coronary events in patients treated with pravastatin or placebo, and there was no evidence of modification of event reduction.

Conclusions: Pravastatin effectively reduces coronary events across a wide range of baseline HDL and TG concentrations.

MoP28:W6 Simvastatin increases plasma levels of the anti-oxidant enzyme paraoxonase by PON1 gene activation

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Objectives: To investigate the effects of simvastatin on biosynthesis and plasma levels of the antioxidant enzyme paraoxonase (PON).

Method I: The PON1 gene promoter region was cloned and reporter gene constructs containing promoter fragments of different lengths were transfected into HepG2 cells. The effects of simvastatin on transcriptional activities were analysed.

Method II: Plasma levels of PON1 were measured in patients (n = 25) before region which affects a transcription factor binding site. Simvastatin treatment was associated with a significant increase in serum PON activity (292.2 (30.0) vs 267.6 (35.9) U/ml; p < 0.01).

Conclusion: Simvastatin increased plasma PON activity through transcriptional mechanisms. The study demonstrates a novel anti-atherogenic mechanism of simvastatin linked to a beneficial influence on serum levels of an anti-oxidant enzyme.

MoP29:W6 Selectivity of ZD4522 for inhibition of cholesterol synthesis in hepatic versus non-hepatic cells


Objectives: To measure the potency and selectivity of ZD4522 (rosuvastatin) – a new HMG-CoA reductase inhibitor – as an inhibitor of cholesterol synthesis in hepatic and non-hepatic cells in comparison with 5 other statins.

Methods: Primary rat hepatocytes, a rat fibroblast cell line (NRK-49F) and human umbilical vein endothelial cells (HUVECs) were used. The statins, at a range of concentrations, were pre-incubated with the cells in serum-free medium for 30 min before addition of 14C-acetate for 3 h and measurement of the incorporation of 14C into cholesterol. IC50 values were calculated from the dose-response curves.

Results: In primary rat hepatocytes, ZD4522 was the most potent inhibitor of cholesterol synthesis. This result was confirmed using shorter incubation times to minimise any effect of differential metabolism of the compounds. Both ZD4522 and pravastatin were selective for hepatocytes but the others were relatively non-selective between the cell types. The IC50 of ZD4522 in hepatocytes was unaffected by the presence of lipoprotein-deficient serum.

<table>
<thead>
<tr>
<th></th>
<th>Hepatocytes</th>
<th>NRK-49F</th>
<th>HUVECs</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZD4522</td>
<td>0.30</td>
<td>310</td>
<td>41</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>0.82</td>
<td>540</td>
<td>5.5</td>
</tr>
<tr>
<td>Cerivastatin</td>
<td>2.5</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Fluvastatin</td>
<td>4.8</td>
<td>3.4</td>
<td>0.56</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>5.0</td>
<td>14.0 x 10^3</td>
<td>1.9 x 10^3</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>5.2</td>
<td>7.9</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Conclusions: ZD4522 is a very potent inhibitor of cholesterol synthesis in hepatocytes and is highly selective for liver cells, consistent with its relatively hydrophilic properties.

MoP30:W6 Increased levels of oxidized LDL in obese patients are normalised by treatment with atorvastatin

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1Center for Molec. and Vasc. Biol.; 2Dept. Endocrinol; Univ. of Leuven, Belgium

Objectives: Obesity is a major risk factor for cardiovascular disease (CVD). The incidence of obesity is gradually increasing. Previously, the association between CVD and the oxidation of LDL has been demonstrated. Therefore, the association between obesity and circulating oxidized LDL has been studied.

Methods and Results: In a blinded cohort study 518 patients (73 male/125 female) without clinical evidence of CVD were included. Mean body mass index (BMI) was 28 ± 9 kg/m². Plasma levels of oxidized LDL were 0.74 ± 0.48 mg/dl in patients with BMI < 25 kg/m² (n = 90), 1.02 ± 0.87 mg/dl in patients with BMI 25–29.9 kg/m² (n = 50) and 1.46 ± 0.72 mg/dl (p < 0.001) in patients with BMI > 30 kg/m² (n = 58). Levels of total cholesterol were 157 ± 35 mg/dl, 172 ± 33 mg/dl (p < 0.05) and 174 ± 32 mg/dl (p < 0.01), respectively. Levels of HDL cholesterol were 52 ± 14 mg/dl, 49 ± 13 mg/dl and 43 ± 12 mg/dl (p < 0.001), respectively. Levels of triglycerides were 93 ± 32 mg/dl, 116 ± 85 mg/dl and 140 ± 69 mg/dl (p < 0.001), respectively. In a multivariate stepwise regression model BMI (F = 62; p < 0.001), LDL cholesterol (F = 12; p = 0.001) and male gender (F = 6.9; p = 0.009) predicted independently levels of circulating oxidized LDL.

Subsequently, in a double-blinded intervention study, 40 consecutive female patients with BMI > 30 kg/m² and with a similar lipid profile as above were...
**MoP31:W6**

**No effect of simvastatin treatment on C-reactive protein in patients with familial hypercholesterolemia**

**M.F. Motschridel,1 M.P.M. de Maat2 R.G.J. Westendorp3 A.H.M. Smelt1**

1Leiden University Medical Center, Dept. of General Internal Medicine; 2Ghentius Laboratory, TNO-PG, Leiden, The Netherlands

**Objective:** To evaluate the effect of simvastatin treatment on serum levels of C-reactive protein (CRP) in patients with familial hypercholesterolemia (FH) and to assess the influence of cardiovascular disease (CVD) on CRP concentrations.

**Methods:** We measured baseline CRP levels and lipid profiles in 346 patients with FH (179 females and 167 males), aged 14–81 years (mean age 48 years). CVD was present at baseline in 34% of the patients. We also measured CRP levels and lipid profiles in a second blood sample, collected after one-year treatment with simvastatin (20 mg once daily) in a subgroup of 129 patients.

**Results:** Patients with CVD present at baseline (119 of the 346 patients) had significantly higher baseline CRP levels (2.30 mg/L versus 1.53 mg/L, P < 0.001). Other factors influencing CRP levels were smoking, body mass index, low density cholesterol, and triglycerides. In the subgroup of treated patients (n = 129) therapy significantly improved lipid profiles. There was a small, but non significant decrease of CRP levels upon therapy. CRP declined from 1.51 mg/L median (IQR 0.76–3.41) to baseline at 1.24 mg/L median (IQR 0.72–2.92) after treatment, (P = 0.33).

**Conclusions:** In patients with FH the presence of CVD is associated with higher CRP concentrations. Simvastatin therapy has no effect on CRP levels in these patients.

**MoP32:W6**

**Alleles of high active paraoxonase RQ192 polymorphisms are associated with HDL-cholesterol increasing effect of pravastatin**

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**Objectives:** To study the effect of human paraoxonase gene (PON) polymorphisms on pravastatin-induced changes in lipids and lipoproteins.

**Methods:** Fifty-one men (aged 35 ± 4 y) with mild hypercholesterolemia participated in a randomized, placebo-controlled and double-blind study. They received either placebo or pravastatin (40 mg/day) for six months. PON genotypes, ML55 and RQ192, were determined by PCR and restriction enzyme digestion. Subjects were divided into low active groups: QQ (n = 28) or MM, ML (n = 29) and high active groups: LL (n = 22) or RQ, RR (n = 23).

**Results:** In PON R/Q192 groups pravastatin induced differences between high and low active groups in plasma HDL-C (p = 0.0079) and in plasma apoA1 (p = 0.018). At baseline HDL-C concentrations were 1.27 ± 0.26 mmol/L and 1.35 ± 0.30 mmol/L in low and high active groups, respectively. ApoA1 concentration was at baseline 1.38 g/L in both groups. At the end of the follow-up mean HDL-C was 1.26 ± 0.31 mmol/L in low active QQ homozygotes and 1.50 ± 0.13 mmol/L in high active R-allele carriers (p = 0.038). Corresponding mean apoA1 concentrations were 1.37 ± 0.20 g/l and 1.57 ± 0.13 g/l (p = 0.012). Similar differences were not seen either in the placebo group or in PON ML55 genotypes. Serum total cholesterol, LDL-cholesterol and triglyceride concentrations were not affected by PON polymorphisms.

**Conclusions:** This study suggests that PON R/Q192 polymorphisms might influence changes in serum HDL-C and apoA1 concentrations during pravastatin treatment.

**MoP33:W6**

**HMG-CoA reductase inhibitor reduces monocyte adhesion to vascular endothelium under physiological flow conditions**

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1Tokyo Med Dent Univ., Tokyo, Japan; 2Mass. Gen. Hosp., Charleston; 3Brigham and Women’s Hosp., Boston, USA

**Objective:** To critically assess the direct effect of HMG-CoA-RI upon monocyte-endothelial interaction under physiological flow conditions.

**Methods:** A monocytes cell line U937 was incubated in the presence of cerivastatin for 48 hrs. Adhesive interactions of statin-treated U937 cells were then analyzed using activated (IL-18/1 0 U/ml, 4 hrs), human umbilical vein endothelial cells (HUVEC) under laminar flow conditions. Flow cytometric analysis of U937 cells was carried out using mAbs against CD11a, CD11b, CD18 and VLA-4. F-actin content of U937 after cerivastatin was quantified using FITC-labeled phalloidin.

**Results:** Preincubation of U937 with cerivastatin significantly decreased U937 adhesion to activated HUVEC (40.3 ± 12.9 vs 5.3 ± 1.5 adherent U937 cells/HIFP, P < 0.005). Interestingly, U937 rolling on activated HUVEC was not significantly decreased. FACs analysis of U937 after cerivastatin treatment revealed down regulation of CD11a, CD18, and VLA4. Cerivastatin-induced changes in U937 adhesion was reversed by treatment with 10 μM mevalonate acid, suggesting that mevalonate pathway is involved in this effect. We also quantitated the F-actin content of U937 after cerivastatin treatment. Cerivastatin significantly reduced F-actin content in U937 (147 ± 15.8 vs 26 ± 22.5 RFU, P < 0.005), that was completely reversed when co-incubated with mevalonate acid.

**Conclusions:** We conclude that cerivastatin reduces monocyte adhesion to vascular endothelium under physiological flow condition via down regulation of members of the integrin family of adhesion molecules. The effect of cerivastatin on U937 may involve the inhibition of actin polymerization. Our findings have potentially important implications for lipid-independent effect of HMG-CoA-RI that may be beneficial in patients with atherosclerosis.

**MoP34:W6**

**A new method to evaluate the lipid lowering effect of drugs on lipoprotein metabolism using agarose gel electrophoresis**

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1Jikei Univ. School of Med.; 2National Institute of Health and Nutrition; 3Helena Laboratory; 4Ochanomizu Univ, Tokyo, Japan

**Objective:** In this study, we report an easy method to measure plasma lipid levels and evaluate lipid lowering effect of drugs on lipoprotein metabolism in detail using agarose gel electrophoresis (Rapid Electrophoresis System; REP/Helena Laboratories).

**Methods:** Fasting blood was sampled from hypertensive patients at the beginning and 6 months after administration of lipid lowering agents. 1 μl of serum was applied on agarose gel and electrophoresed at 400 V for 15 minutes. After electrophoresis, cholesterol and triglyceride in each lipoprotein fraction were measured after densitometric scanning the intensity of the stained lipoproteins.

**Results:** HMG-CoA reductase inhibitors was significantly decreased LDL-C level, together with increasing HDL-C level, and VLDL-TG and CH/TG ratio in VLDL were significantly decreased by additional EPA treatment, in type 1b hyperlipoproteinemic patients VLDL-TG, VLDL-C, LDL-C and LDL-TG levels were significantly decreased by bezafibrate treatment in type IV and V patients. Moreover, the electrophoretic mobilities of their LDL were improved by some HMG-CoA reductase inhibitor and bezafibrate treatment.

**Conclusions:** The analysis using agarose gel electrophoresis brings us to further and detailed recognition about lipoprotein metabolism.

**MoP35:W6**

**Effects of fluvastatin on angiotensin II induced superoxide formation in human aortic smooth muscle cell**

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1Department of Internal Medicine, 2Department of Laboratory Medicine, Fukuoka University, Fukuoka, Japan

**Objective:** The effects of fluvastatin on formation of superoxide in human aortic smooth muscle cells by angiotensin II (Ang II) were investigated.

**Methods:** Different concentration of fluvastatin, simvastatin and trolox which is a water-soluble a-tocopheryl derivative were added in the culture
medium of human aortic smooth muscle cells with 100 nM Ang II for 2 to 24 hours, respectively and intracellular superoxide anion formation were detected by lucigenin assay.

Results: Treatment of human aortic smooth muscle cells with 100 nM Ang II for 2 to 24 hours caused a 3.2 ± 0.5-fold increase in intracellular superoxide anion formation were detected by lucigenin assay. Addition of 50–100 nM fluvastatin or trolox to the human aortic smooth muscle cells inhibited 30–52% of superoxide formation by Ang II. But similar concentration of simvastatin did not inhibit the superoxide formation in the human aortic smooth muscle cells by Ang II.

Conclusions: These results indicate that fluvastatin might prevent LDL oxidation by additional mechanisms besides its hypcholesterolemic activity.

MoP36/W6
Differences in effects of pravastatin and simvastatin (40 mg/day) on total serum cholesterol, 24s-hydroxycholesterol and 27-hydroxycholesterol
S. Locatelli, D. Lütjohann, K. von Bergmann. Department of Clinical Pharmacology, University of Bonn, Bonn, Germany

Objective: We investigated the influence of pravastatin and simvastatin on concentrations of total serum cholesterol (TC), 24s-hydroxycholesterol (24s-OH-Chol) and 27-hydroxycholesterol (27-OH-Chol) in 13 patients with primary hypercholesterolemia.

Methods: TC was measured enzymatically, 24s-OH-Chol and 27-OH-Chol were measured with an isotope dilution method using gas-chromatography/mass spectrometry, 5 women and 8 men (age 24–70 yrs, mean 51 yrs) were included in a cross-over study with administration of 40 mg/day simvastatin and pravastatin for 6 weeks each.

Results: During treatment with pravastatin a reduction of 29% was observed for TC (288 ± 41 to 206 ± 35 mg/dL (mean ± SD); p < 0.001), 24s-OH-Chol and 27-OH-Chol were reduced by 15% (86 ± 17 to 73 ± 17 mg/mL; p < 0.001) and 19% (207 ± 58 to 161 ± 40 mg/mL; p < 0.001), respectively. Treatment with simvastatin lead to a TC reduction of 35% (252 ± 40 to 163 ± 33 mg/dL; p < 0.001), as well as to a reduction of 24s-OH-Chol and 27-OH-Chol of 29% (95 ± 24 to 68 ± 19 mg/dL; p < 0.001) and 27% (205 ± 51 to 148 ± 28 mg/mL; p < 0.001), respectively. There was a significant difference in reduction of 24s-OH-Chol between the two statins (p = 0.034), but not for TC and 27-OH-Chol.

Conclusions: These results suggest that the greater reduction of 24s-OH-Chol under simvastatin therapy in these hypercholesterolemic patients is probably not only due to the decrease of TC. Since 24s-OH-Chol is mainly produced in the brain, lipophilic simvastatin may have an influence on extrahepatic cholesterol synthesis.

Supported by a grant of BMBF (01EC9402).

MoP37/W6
Effect of high dose simvastatin on plasma concentrations of homocyst(e)ine
D. Lütjohann, K. von Bergmann. Department of Clinical Pharmacology, University of Bonn, Bonn, Germany

Objective: Elevated plasma levels of homocyst(e)ine were found to be an additional risk factor for thrombotic and atherosclerotic vascular diseases. The effect of HMG-CoA reductase inhibitors on serum cholesterol and reduction of atherosclerotic events has been proved in several prospective studies, but their effect on homocyst(e)ine concentrations is controversial. Therefore, a prospective study on the effect of a high dose of simvastatin (80 mg at night) on plasma levels of homocyst(e)ine was performed on 18 patients with hypercholesterolemia (LDL > 160 mg/dL).

Methods: Blood samples from 7 women (age 30–65 yrs, mean 51 yrs) and 11 men (age 27–77 yrs, mean 48 yrs) were taken after an overnight fast before, 6, and 24 weeks after intake of simvastatin. Plasma concentrations of homocyst(e)ine were measured by an enzyme-immunoassay, folate by an ion-capture assay, and vitamin B12 by a microparticle-enzyme-intrinsic-factor-assay.

Results: Plasma concentrations of homocyst(e)ine decreased significantly after treatment with simvastatin within 6 weeks from 13.0 to 11.7 μmol/L (p < 0.002) and thereafter to 10.6 μmol/L (p < 0.02). A non-significant increase of the plasma concentrations of folate (from 9.6 to 11.0 and 11.4 nmol/L, respectively) and vitamin B12 (from 217 ± 240 and 226 pmol/L, respectively) could be observed.

Conclusions: The results of the present study indicate that simvastatin in a dose of 80 mg/day decreases homocyst(e)ine plasma concentrations significantly after 6 and 24 weeks, respectively. The decrease in homocyst(e)ine observed during the present study might contribute to the positive effect of simvastatin on the reduction of cardiovascular events.

Supported by a grant of BMBF (01EC9402).

MoP38/W6
Effect of atorvastatin on platelet function in patients with hypercholesterolemia
V. Sanguigni, D. Caccese, R. Magnantera, E. Ferrara, S. Mazzolini, F. De Vito, F. Violi1, R. Lauro. Department of Internal Medicine, University of Rome "Tor Vergata", Chair of Internal Medicine University of Rome "La Sapienza", Italy

Objective: Activation of platelets occurs in hypercholesterolemic patients. Nevertheless the biochemical substrate for such an activation is still unclear. Oxygen free radicals (OFR), like super oxide anion (O₂⁻), play a determining role in stimulating platelet function by acting as second messenger. The purpose of the present study was to evaluate the effect of a new statin, Atorvastatin, on platelet function in patients with hypercholesterolemia, in relation to OFR production from platelets.

Methods: Atorvastatin 10 mg/daily was given to 21 patients with hypercholesterolemia and no history of coronary artery disease, diabetes and hypertension, for up to 8 weeks. Normal subjects act as controls. O₂⁻ was determined by chemiluminescence using a Bio-Oxit 1251. Studies on platelet aggregation were performed following the Born method.

Results: The following table shows the results:

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Atorvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total-Cholesterol (mg/dL)</td>
<td>270.38</td>
<td>204.83</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dL)</td>
<td>185.62</td>
<td>129.22</td>
</tr>
<tr>
<td>O₂⁻ production</td>
<td>2.02</td>
<td>1.35</td>
</tr>
<tr>
<td>pmol/mg protein x 19 cells/min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: Atorvastatin therapy for a 8 weeks period resulted in 25% and 30% reduction in plasma levels of total cholesterol and LDL cholesterol. Platelet production of O₂⁻ was significant (p < 0.01) reduced by 33% after Atorvastatin treatment. This inhibitory effect of Atorvastatin on platelet activation is considered to be an important antiatherogenic intervention on platelet hyperaggregability observed in hypercholesterolemia.

MoP39/W6
Treatment of simvastatin in hypercholesterolemia induces changes in the coagulation system
J. Chojnowska-Janicka, M. Broncel1, M. Michalska, B. Kostka2
1Department of Clinical Pharmacology, Medical Military Academy; 2Department of Biochemistry, Medical University, Lodz, Poland

Objective: The aim of the study was to evaluate the effect of simvastatin therapy on serum lipids, thrombin generation, factor X activity and fibrinogen level in patients (pts) with hyperlipidemia (hipl) type II.

Material and Methods: The study involved 20 persons: 10 pts (mean age 58.9 ± 7.06) with the initial total serum cholesterol (TC) > 250 mg/dL, LDL-cholesterol (LDL-C) > 170 mg/dL and triglycerides (TG) < 400 mg/dL; 10 healthy individuals (mean age 55.4 ± 7.03 – control group. Pts were treated with simvastatin (20 mg/24 h) for 12 weeks. Lipids serum, the thrombin generation, factor X activity and fibrinogen level were measured before and after 12 weeks of active treatment. Lipids serum were estimated by enzymatic method. The thrombin generation was assessed by method of Sloan and Firkin using chromogenic substrate S-2238. Factor X amidolytic activity was determined as described by Colucci at al. using substrate S-2222. In both cases absorbance was measured spectrophotometrically at a wave length of 405 nm. Fibrinogen level was examined by Clauss method.

Results: Before treatment factor X activity and thrombin generation were higher in pts with hip in comparison with the control group (by 13.4%; by 12.7% respectively). 12-weeks therapy of simvastatin decreased significantly concentration of TC by 25.1%, LDL-C by 35.3%; the thrombin generation by 53% and factor X activity by 49.1%. The increase of fibrinogen level after simvastatin therapy was not significant (338.1 ± 68.4 vs 369.8 ± 74.1).

Conclusion: In patients with hyperlipidemia type II, lowering level of serum lipids by a 12-weeks simvastatin treatment is accompanied by a marked reduction of thrombin generation and factor X activity.

MoP40:W6  Effect of simvastatin on serum lipids and erythrocyte membrane in patients with hypercholesterolaemia type II

M. Broncel, J. Chojnowska-Jezierska1, M. Koter, I. Fruwiak2 1Department of Clinical Pharmacology Medical Military Academy; 2Department of Environment Pollution Biophysics, University of Lodz, Poland

Objective: The aim of the study was to evaluate the hypolipidemic efficacy and effects of simvastatin treatment on the fluidity and peroxidation of erythrocyte membrane in patients (pts) with hyperlipidaemia (HLP) type II.

Method: The study involved 31 pts (mean age 57.8 ± 4.5) with the initial serum total cholesterol (TC) > 250 mg/dl, LDL-cholesterol (LDL-C) > 170 mg/dl, triglycerides < 400 mg/dl. The control group consisted of 10 healthy individuals. Pts were treated with simvastatin (20 mg/24 h) for 3 months. Lipids serum and erythrocyte membrane fluidity, peroxidation were measured before, after 1 and 3 months of active treatment. Lipids serum were estimated by enzymatic method. The fluidity was determined by electron paramagnetic resonance (EPR) spectroscopy, using two labels: 5-doxylstearic acid (5-DSA) and 16-doxylstearic acid (16-DSA). The order parameter S was estimated using 5-DSA and the correlation times τ0 and τ1 by 16 DSA. The peroxidation of erythrocyte lipids was measured by the thiobarbituric acid technique.

Results: After 3-months of active treatment a significant decrease in the concentration of TC (by 26%), LDL-C (by 37%) was found. The order parameter S decreased significantly (0.757 ± 0.007 vs 0.745 ± 0.01) showing the membrane fluidity increase. The changes of the correlation times τ0 and τ1 were not significant. Simvastatin treatment induced a marked decrease of lipid peroxidation by 56%.

Conclusion: Simvastatin not only decreases concentration of cholesterol, but also is antioxidant and modifies the membrane structure of erythrocytes.

MoP41:W6  Prognostic assessment of patients with coronary heart disease: Results from the LIPID study

D. Colquhoun1, I. Marschner2, J. Simes3, P. Glasziou4, A. Keech2. 1University of Queensland Core Research Group, Brisbane; 2NHMRC Clinical Trials Centre, Sydney, Australia

Objective: Prognostic assessment of patients with coronary heart disease (CHD).

Method: LIPID was a placebo-controlled double-blind trial assessing the efficacy of pravastatin over 6 years in 9014 patients with CHD and baseline cholesterol of 4.7 mmol/L. Data on 8557 patients were used to quantify risk. A multivariate risk factor model was developed using the outcome of CHD death or nonfatal myocardial infarction (MI).

Results: In addition to the randomised treatment group, the following baseline characteristics were independently significant risk factors in the multivariate model: total cholesterol, HDL cholesterol, age, sex, smoking status, nature of CHD (MI or UA), prior revascularization, diabetes, hypertension and prior stroke. Based on the magnitude of its adjusted relative risk, each risk factor was assigned risk points, and the aggregate risk score was calculated for each patient. Risk levels were defined by categorizing the scores into quartiles. The predicted 5-year coronary event rates (%) for each risk level were:

<table>
<thead>
<tr>
<th>Risk Level</th>
<th>Low risk</th>
<th>Medium risk</th>
<th>High risk</th>
<th>Very high risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>5.8</td>
<td>10.3</td>
<td>13.5</td>
<td>20.2</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>4.6</td>
<td>8.1</td>
<td>16.7</td>
<td>16.1</td>
</tr>
</tbody>
</table>

Pravastatin therapy was associated with a significant reduction in coronary events across the range of risk levels. However, the absolute risk of an event was still high in treated patients with unfavorable risk factor profiles (table).

Conclusion: Statin therapy unequivocally prevents recurrent CHD events. This risk stratification identifies patients at continuing very high risk. New treatment strategies need to be developed for these patients.

MoP42:W6  Reduction in coronary events with pravastatin and proportion of treatment benefit explained by on-study LDL levels in the LIPID study

J. Simes1, I. Marschner2, D. Hunt4, D. Colquhoun3, P. Glasziou4, W. Hague1, S. MacMahon1, A. Keech2, A. Tonkin3. 1On behalf of the LIPID Study Group; 2NHMRC Clinical Trials Centre, Sydney; 3Royal Melbourne Hospital, Melbourne; 4University of Queensland, Brisbane. Institute of International Health, Sydney; 5National Heart Foundation, Melbourne, Australia

Among 9014 patients with prior myocardial infarction (MI) or unstable angina randomised to pravastatin or placebo the LIPID study showed highly significant reductions in fatal coronary heart disease and nonfatal MI (P < 0.00001). We investigated possible treatment mechanisms by analysing the proportion of the treatment effect (PTE) on coronary events explained by differences in on-study lipid levels. The proportion attributable to differences in lipids was estimated by Cox regression, after adjustment for baseline risk factors and lipid measurement error.

Risk reduction was reduced after adjustment for on-study lipid levels, indicating that changes in lipids can explain some treatment benefit. On-study levels of LDL cholesterol and apolipoprotein B accounted for the greatest PTE.

<table>
<thead>
<tr>
<th>Lipid parameter</th>
<th>Treatment effect with pravastatin after year</th>
<th>Risk reduction (%) due to pravastatin</th>
<th>P</th>
<th>PTE % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HDL cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein A-I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusion: The confidence intervals suggest that the data are consistent with all or with only part of the treatment effect being due to differences in lipids.

MoP43:W6  Effects of pravastatin on cardiovascular endpoints in patients with previous unstable angina

A. Tonkin1, S. Becker2, D. Hunt4, A. Keech2, G. Lane5, H. White5, W. Hague4, J. Simes2, On behalf of the LIPID Study Group; 1National Heart Foundation, Melbourne; 2NHMRC Clinical Trials Centre, Sydney; 3Royal Melbourne Hospital, Melbourne; 4Fremantle Hospital, Perth, Australia; 5Green Lane Hospital, Auckland, New Zealand

The long-term Intervention with Pravastatin in Ischaemic Disease (LIPID) study was the only secondary prevention trial with an HMG-CoA reductase inhibitor to deliberately include patients with unstable angina. In all, 3260 patients (2605 men, 566 women, aged 31–75 years) hospitalised for unstable angina 3–36 months previously were randomised to pravastatin or placebo and followed up for a median of 5.9 years.

Event rates for prespecified cardiovascular endpoints for patients stratified at randomisation with unstable angina as their qualifying event are shown in the table.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Placebo n (%)</th>
<th>Pravastatin n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD mortality</td>
<td>108 (6.6)</td>
<td>81 (5.0)</td>
<td>0.04</td>
</tr>
<tr>
<td>Total mortality</td>
<td>211 (13.0)</td>
<td>158 (9.7)</td>
<td>0.004</td>
</tr>
<tr>
<td>Total myocardial infarction</td>
<td>154 (9.5)</td>
<td>100 (6.1)</td>
<td>0.0003</td>
</tr>
<tr>
<td>CABG</td>
<td>179 (11.0)</td>
<td>144 (8.8)</td>
<td>0.03</td>
</tr>
<tr>
<td>PTC</td>
<td>116 (7.1)</td>
<td>103 (6.3)</td>
<td>0.28</td>
</tr>
<tr>
<td>Stroke</td>
<td>84 (5.2)</td>
<td>71 (4.4)</td>
<td>0.25</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>460 (28.3)</td>
<td>444 (27.0)</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Conclusion: Pravastatin reduced the risk of major endpoints in patients with previous unstable angina. The effects in patients with previous unstable angina were similar to those in patients with previous MI.

MoP44:W6  Effect of different doses of atorvastatin and atorvastatin/pravastatin in patients undergoing regular LDL-Apheresis

V. Schettler, A. Blankenberg, E. Wieland, P. Schaff-Werner1, G.A. Müller. Center of Internal Medicine, Georg-August-University, Göttingen; 1Institute of Clinical Chemistry and Pathobiology, University of Rostock, Germany

In hypercholesterolemic patients, mostly refractory to diet and drugs, LDL-Apheresis is a selective and effective lipid-lowering treatment. More aggressive lipid-lowering therapy may further slow the progression of atherosclerosis. This investigation compares the effect of different atorvastatin loses (40 and 60 mg) alone or 40 mg in combination with 20 mg pravastatin (Prav.) in hypercholesterinemic patients (n = 17), suffering from coronary heart disease and undergoing regular LDL-Apheresis for more than 6 months. All patients received atorvastatin (Ator.) at dose of 40 mg/d for 6 months which was increased to 60 mg/d for another 6 months followed by a combination of 40 mg/d Ator. plus 20 mg/d Prav. for the next 6 months. Cholesterol,
triglyceride, LDL, HDL, Lp(a) were determined before every LDL-apheresis treatment and were as follows:

<table>
<thead>
<tr>
<th>Cholesterol</th>
<th>Triglyceride</th>
<th>LDL</th>
<th>HDL</th>
<th>Lp(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mmol/L)</td>
<td>(mmol/L)</td>
<td>(mmol/L)</td>
<td>(mmol/L)</td>
<td>(g/L)</td>
</tr>
<tr>
<td>40 mg/dl Ator. (n = 17)</td>
<td>6.2 ± 0.5</td>
<td>1.7 ± 0.4</td>
<td>4.2 ± 0.5</td>
<td>12.0 ± 0.2</td>
</tr>
<tr>
<td>60 mg/dl Ator. (n = 9)</td>
<td>5.8 ± 1.2</td>
<td>1.6 ± 0.1</td>
<td>3.7 ± 0.1</td>
<td>12.0 ± 0.3</td>
</tr>
<tr>
<td>40 mg/20 mg/dl Ator/Prav. (n = 17)</td>
<td>6.2 ± 1.3</td>
<td>1.7 ± 1.1</td>
<td>4.3 ± 1.0</td>
<td>13.0 ± 0.3</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. 40 mg/dl Ator and 40 mg/20 mg/dl Ator/Prav. 1 patients discontinued.

Even though 60 mg/dl Ator. was most effective in lipid-lowering 8 out of 17 patients developed severe side effects such as muscle pains, CK elevation were therefore discontinued. The combination of two CSE inhibitors was not superior to mono-therapy with 40 mg/dl Ator. It is therefore suggested that 40 mg/dl Ator. are a safe and very efficient regimen in combination with LDL-Apheresis to reduce athrogenic lipids profiles. Neither a combination with a second CSE inhibitor nor increasing the dose to 60 mg/dl is recommended.

MoP45:W6  Potent inhibition of human LDL and membrane oxidative damage by the hydroxy metabolite of atorvastatin
R.P. Mason. Cardiovascular and Pulmonary Research Institute, MCP Hahnemann University, Allegheny Campus, Pittsburgh, PA, USA

Objective: Oxidative modification of lipids associated with low-density lipoproteins (LDL) and vascular cell membranes contributes directly to atheroma development and thus, agents that increase lipid resistance to oxidative damage are considered antiatherogenic. In this study, rates of lipid peroxidation were evaluated in isolated membranes and human LDL following treatment with various statins, including atorvastatin and its hydroxy metabolite.

Methods: Peroxidation was systematically measured by spectrophotometric analysis under physiologic-like conditions.

Results: The results demonstrated that the atorvastatin metabolite significantly (p < 0.001) inhibited oxo-radical damage (>10^7 μM) at low, nanomolar levels in isolated membranes enriched with polysaturated fatty acids. Membrane antioxidant activity was also demonstrated for atorvastatin, but not with lovastatin, mevastatin, pravastatin or simvastatin. The activity of atorvastatin metabolite was dramatically greater than that observed for the endogenous antioxidant, vitamin E. In human LDL, the atorvastatin metabolite was also very effective in inhibiting oxo-radical damage following stimulation with CuSO4.

Conclusions: The antioxidant mechanism of action for the hydroxy metabolite of atorvastatin is attributed to specific physico-chemical interactions with lipid molecules, as measured by x-ray diffraction analyses. In addition to its favorable effects on lipid metabolism, the potent lipid antioxidant effects of atorvastatin and its active metabolite represent a novel antiatherogenic mechanism of action.

MoP46:W6  Effect of atorvastatin (Liptor™) on VLDL-apoB and VLDL-triglyceride overproduction in vivo in an insulin resistant hamster model
I. Mangalolu, S. Van-Iderstein, B. Chen, C. Taghibiglou, R. Cheung, K. Adeli. Dept. of Clin. Biochem., Univ. of Toronto & Chemistry & Biochemistry, Univ. of Windsor, Canada

Objective: A novel animal model of insulin resistance, the fructose-fed Syrian golden hamster, was employed to investigate the effect of atorvastatin, a potent HMG-CoA reductase inhibitor, on hepatic VLDL overproduction.

Methods & Results: Fructose feeding for a two week period induced significant hypertriglyceridemia and hyperinsulinemia, and the development of whole body insulin resistance. Fructose feeding also induced a significant increase in hepatic secretion of VLDL-triglyceride and VLDL-apoB. In vivo feeding experiments were also performed in which several groups of three hamsters were fed a fructose-enriched diet for 14 days to induce the state of insulin resistance, followed by a fructose-enriched diet supplemented with 40 mg/kg atorvastatin for 7 to 14 days. Feeding protocol was as follows: Day 1-14, fructose feeding; Day 15-28, fructose + atorvastatin. Fructose feeding in the first two weeks caused a significant increase in plasma total cholesterol and triglyceride in both groups. However, there was a significant decline in plasma triglyceride levels following supplementation of the fructose-enriched diet with atorvastatin. This decline was not observed in control animals receiving a fructose-enriched diet without the drug. In addition, there was an average 45% decrease in VLDL-apoB production in hepatocytes isolated from hamsters fed atorvastatin compared to the control group. Intracellular apoB turnover was also significantly increased in livers of drug treated hamsters.

MoP47:W6  Stimulation of nitric oxide synthase by HMG-CoA reductase inhibitors
W. Mraz, U. Tisjar, H. Scharnagl, H. Gierens, H. Wieland. Medical University Clinic, Dept. Clinical Chemistry, Freiburg, Germany

Objective: Beyond the lowering of serum cholesterol HMG-CoA reductase inhibitors may have other beneficial effects in atherosclerotic patients such as stimulation of nitric oxide release. The aim of this study is to compare this positive effect with potentially inhibitory effects caused by various statins in cell culture experiments.

Methods: We incubated human umbilical vein endothelial cells with atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin, or simvastatin in the concentration range between 10^-5 and 10^-3 M and assayed the release of nitric oxide into the culture medium. To compare the effects of statins on cellular metabolism, [3H]-hypoxanthine, 5-bromodeoxyuridine, and MTT were used.

Results: All HMG-CoA reductase inhibitors tested in this investigation stimulated the release of nitric oxide. In their presence we measured up to 236% of activity compared to untreated cultures. In Northern blot analysis we detected an increase of the eNOS mRNA after 24 h of incubation with statins in a final concentration of 10^-4 M. The effect was observed with all statins except pravastatin which caused no effect under this condition. Cholesterol biosynthesis, cell proliferation and MTT metabolism were inhibited by all statins in a concentration dependent manner. However, in all three tests the inhibitory effect of pravastatin was much less predominant compared to the other statins.

Conclusion: In cell culture pravastatin stimulates nitric oxide synthesis but has less inhibitory effects on endothelial cells compared to the other HMG-CoA reductase inhibitors tested in this investigation.

MoP48:W6  Effect of statin therapy on HDL-C levels in patients with type IIa and type IIb hyperlipidemia
J.W. Nawrocki, T.K. Peters. 1, Parke-Davis Pharmaceutical Research, Ann Arbor, MI, USA; 2 Parke-Davis Company, Freiburg, Germany

Background: High levels of LDL-C and/or low levels of HDL-C considerably increase a patient’s risk of cardiovascular disease. Atorvastatin has been shown to provide significantly greater reductions in LDL-C and its starting dose of 10 mg/day than milligram-equivalent doses of other statins. To examine the changes in HDL-C levels observed with atorvastatin 10-80 mg/day, data from 25 clinical studies was examined. HDL-C data from patients receiving simvastatin 10 mg/day or pravastatin 20 mg/day were also assessed.

Methods: 1871, 368 and 171 patients receiving atorvastatin, simvastatin and pravastatin, respectively, were identified. Frederickson type IIa and IIb patients were included in the analysis and treatment periods ranged from 8 weeks to 12 months.

Results: At all doses, atorvastatin increased HDL-C from baseline levels. Moreover, the increase in HDL-C observed with atorvastatin 10 mg (+6.7%) was similar to HDL-C increases seen with simvastatin 10 mg/day (+7.6%) and pravastatin 20 mg/day (+5.8%).

MoP49:W6 Measurement of HDL-C (mg/dl (mmol/l))

<table>
<thead>
<tr>
<th>Dose of atorvastatin</th>
<th>10 mg</th>
<th>20 mg</th>
<th>40 mg</th>
<th>80 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>48 (1.24)</td>
<td>48 (1.24)</td>
<td>48 (1.24)</td>
<td>48 (1.24)</td>
</tr>
<tr>
<td>After treatment</td>
<td>50 (1.35)</td>
<td>52 (1.34)</td>
<td>52 (1.35)</td>
<td>48 (1.24)</td>
</tr>
<tr>
<td>Mean % change</td>
<td>+6.7</td>
<td>+8.2</td>
<td>+8.6</td>
<td>+7.0</td>
</tr>
</tbody>
</table>

Atorvastatin 10 mg/day, simvastatin 10 mg/day and pravastatin 20 mg/day increased HDL-C by +12.0%, +12.9% and +7.5%, respectively, in a subgroup of patients with baseline HDL-C ≤ 35 mg/dl (0.9 mmol/l).

Conclusion: In addition to being highly effective at reducing LDL-C levels in hyperlipidemic patients, atorvastatin increases HDL-C levels in a non-dose related manner.

MoP49:W6
Effect of atorvastatin and simvastatin in obtaining targets for lipids in secondary prevention. The treat-to-target study (3T)

A.G. Olsson, M. Eriksson, O. Johnson, T. Kjellström, J. Lanke, M. Lyken Larsen, T.R. Pedersen, M.J. Tikkanen, O. Wiklund. For the 3T Investigators; Faculty of Health Sciences, University of Linköping, Sweden

Objective: Statin studies in secondary prevention have shown that less than half of the patients reached the NCEP LDL-cholesterol (LDL-C) goal. The aim of this double-blind study was to compare Atorvastatin (A) and Simvastatin (S) to achieve the following lipid goals for secondary prevention: LDL-C ≤ 2.6 mmol/L (100 mg/dl) and serum triglycerides (S-TG) ≤ 1.5 mmol/L (133 mg/dl) in a hyperlipidemic population.

Methods: 1093 men (76%) and women (24%) with coronary heart disease and hypercholesterolemia were randomized to A or S for 52 weeks. Daily dose for both statins was 20 mg. If the LDL-goal was not reached at 8 weeks the daily dose was doubled at 12 weeks.

Results: 55% of patients treated with A and 77% treated with S needed doubled dose. At 52 weeks the LDL-C target was reached in 61% of those treated with A and in 41% treated with S (p < 0.00005). The combined target for LDL-C and S-TG was reached in 47% treated with A and in 27% treated with S (p < 0.00005). LDL-C was decreased by 49% with A and 44% with S (p < 0.00005); S-TG by 24% with A and 16% with S (p < 0.00005). HDL-C increased by 6% with A and 8% with S (p = 0.0034). The LDL/HDL ratio decreased by 51% with A and 48% with S (p = 0.0007). Both drugs were well tolerated and no drug related serious adverse events were observed.

Conclusions: A was more effective in helping patients to reach desired LDL-C and S-TG goals than S with the dosage regimen used. Fewer patients on A required dose titration compared to S.

MoP50:W6
Effects of HMG-CoA reductase inhibitors on endothoxin induced release of TNF and IL-6 in whole blood system

P. Fraunberger, B.R. Jueger, E. Faisal1, H.J. Groome1, A.K. Walli, D. Seidel. Institut of Clinical Chemistry, 6 Surgical Clinic, Grosshadern University Hospital, Munich; 1 Department of Pathology, German Cancer Research Center (DKFZ), Heidelberg, Germany

Objective: The enzyme HMG-CoA reductase generates mevalonate which is a precursor of a series of isoprenoids. HMG-CoA reductase inhibitors reduce mevalonate synthesis with subsequent diminished isoprenylation of proteins such as ras and reduced activation of transcription factors such as NF-kappaB. Therefore an antiinflammatory effect of statins by lower expression and release of inflammatory mediators has been postulated. The aim of our study was to investigate the effect of statins on the release of inflammatory cytokines induced by endotoxin in a whole blood system.

Methods: Whole blood from healthy controls and 5 patients treated with simvastatin or atorvastatin was stimulated in-vitro with endotoxin. After 24 hours the concentration of TNF and IL-6 release in plasma was measured. In addition whole blood from healthy volunteers was incubated in the presence and absence of 10 µM simvastatin or lovastatin for 20 hours at 37°C and the release of TNF and IL-6 after endotoxin stimulus for 4 hours was measured.

Results: Endotoxin induced TNF and IL-6 release in whole blood was slightly lower in patients treated with statins compared to healthy controls. However these differences were not statistically significant. In contrast preincubation of whole blood with simvastatin and lovastatin decreased the LPS induced release of TNF and IL-6 to about 60%.

Conclusions: Our data show, that statins suppress endotoxin-induced release of inflammatory cytokines in an human in-vitro whole blood system. Investigation on a larger number of patients treated with or without statins is required to establish in vivo significance of these in-vitro observations.

MoP51:W6
Simvastatin treatment lowers postprandial lipemia in CHD patients

I. Mezö, I. Reiber. Dept. Intern. Medicine, St. George Hospital, Székesfehérvár, Hungary

Objective: Postprandial lipemia has been implicated as a risk factor in the pathogenesis of atherosclerosis, so we investigated the effect of simvastatin on postprandial lipemic response.

Methods: 11 patients from the outpatient lipid clinic were treated with 20-80 mg simvastatin during 16 weeks. Oral fat loading tests and fasting lipid parameters were performed before and after treatment. Blood samples were taken fasting and after the test meal 2, 4, 6, 8 and 10 hours.

Results: The fasting total-C, LDL-C, triglycerides and apoB levels decreased by -27%, -39%, -13%, -36%, respectively and HDL-C, apoA-I concentrations were elevated by 11% and 7%. The maximum triglyceride values appeared 2 hours earlier (4 vs. 6 hours) than before the simvastatin therapy and the area under curve (AUC) was lowered by ~11% postprandially.

Conclusion: Our results suggest that simvastatin therapy effectively lowers not only the fasting cholesterol-rich lipoproteins but also the triglyceride-rich species in the postprandial state.

MoP52:W6
Effects of atorvastatin versus fenofibrate on lipoprotein production and catalytic rate in patients with combined hyperlipidemia

S. Blied1, S. Wagner2, A. Bedynke3, T. Debrand4, U. Keller5. 1 Dep. of Internal Medicine, University Hospital Basel, Switzerland; 2 Inst. of Clinical Chemistry, University Hospital Grosshadern, Munich, Germany

Objective: To assess the impact of atorvastatin and fenofibrate on VLDL, IDL and LDL metabolism in patients with mixed hyperlipidemia.

Methods: In a randomized cross-over study the impact of atorvastatin (80 mg) and fenofibrate (200 mg) on the metabolism of VLDL, VLDL2, IDL and LDL was examined using a stable isotope tracer technique and kinetic analysis.

Results: Total cholesterol (TC), VLDL-C and LDL-C were decreased by 41%, 56% and 40% during therapy with AT. Both TC and VLDL-C were lower during FE (18 and 57%). Triglycerides were lowered by AT and FE (65 and 76%). AT, but not FE decreased apolipoprotein B plasma concentrations. AT accelerated dilipidation of VLDL1 and induced trends towards faster elimination of VLDL2, IDL and LDL and lower rates of total apo B secretion. Fenofibrate had no effect on apolipoprotein B secretion. It similarly accelerated VLDL1 dilipidation and direct catabolism. However, a delay in LDL catabolism was observed despite a shift from small dense to larger LDL particles.

Conclusions: While AT favorably affects the metabolism of all apo B containing lipoproteins, the beneficial effects of FE are confined to the VLDL density range.

MoP53:W6
Short term effect of atorvastatin on plasma levels of MCP-1, ICAM-1 and C-reactive protein in patients with coronary artery disease

A. Figueredo1, L. Reinares1, A. Rueda1, J.C. Pontes, A. Rodrigue1, M. Ruiz-Yagle1, C. Diaz1, C. Puyayo2, G. Hernandez3, A. Fernandez-Cruz1, R. Patino1. 1 Hospital Clinico San Carlos Madrid; 2 Parke-Davis, Spain

Objectives: The reason why HMG Co-A reductase inhibitors reduce cardiovascular mortality in patients with atherosclerosis, is partly unknown. An attractive possibility is that, together with the lipid-lowering effect, their anti-inflammatory properties might contribute to this clinical benefit.

Methods: We have measured plasma levels the inflammatory markers ICAM-1, MCP-1 and C-Reactive Protein (CRP) before and after six weeks of Atorvastatin treatment (10 mg/day), in 40 hyperlipidemic patients with stable coronary artery disease and 16 control subjects.

Results: (median range)

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Controls</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/l)</td>
<td>0 (0-3.1)</td>
<td>0.9 (0.65-1.6)*</td>
</tr>
<tr>
<td>MCP-1 (µg/ml)</td>
<td>156 (130-203)</td>
<td>217 (182-275)*</td>
</tr>
<tr>
<td>ICAM-1 (ng/ml)</td>
<td>264 (214-329)</td>
<td>285 (263-322)*</td>
</tr>
</tbody>
</table>

*p < 0.05 vs controls. Mann-Whitney U test. *p<0.01 vs patients-baseline. Wilcoxon test

There were no significant correlation between lipid levels and none of the measured proteins.

Conclusions: Atorvastatin significantly reduced plasma concentrations of MCP-1 and CTP after six weeks of treatment. Since MCP-1 is probably the major chemotactant for monocyte recruitment during atherogenesis, this effect may explain part of Atorvastatin’s therapeutic action.
MoP54:W6  Analysis of factors that influence the C-reactive protein (CRP) lowering effect of atorvastatin and bezafibrate in mixed dyslipidemia (the ATOMIX Study)

R. Aristegui1, 2, J.A. Gómez-Gerique1, C. Diaz2, X. Masramon1, J.M. So2, G. Hernandez2. 1For the ATOMIX Study Group; 2Central Laboratory UNILABS, Madrid; *I = V Department, PARKE-DAVIS, Barcelona, Spain

Lipid-lowering agents decrease CRP values in hypercholesterolemic patients. The influence of Cardiovascular (CV) risk factors on this effect has not been evaluated.

Objectives: To evaluate the effect of individual cardiovascular risk factors on C-reactive protein lowering by Atorvastatin or Bezafibrate in patients with mixed dyslipidemia.

Methods: Atorvastin is a 1-year, double-blind study that evaluated the efficacy and safety of Atorvastatin (10 to 40 mg/day) versus Bezafibrate (400 mg/day) in patients with mixed dyslipidemia. One hundred and three patients were selected to measure their CRP serum levels at baseline and after one year of treatment (analyzed by immunonephelometry). A non-parametric multivariate regression analysis of the influence of lipid-lowering treatment, sex, initial CRP levels and CV risk factors (diabetes, postmenopausal, familial history of CV disease, hypertension, men older than 45, obesity, personal history of CV disease, smoking and baseline HDL-C level) on CRP lowering by Atorvastatin or Bezafibrate has been used.

Results: Multivariate analysis showed that CRP changes significantly correlated with baseline CRP levels (beta = -0.816; p < 0.0001), presence of CV disease (beta = 1.150; p < 0.005), smoking (beta = 0.831; p < 0.05) and treatment with Atorvastatin (beta = -0.83; p < 0.05).

Conclusions: Atorvastatin decreased significantly more than Bezafibrate the CRP levels of patients with mixed dyslipidemia. In addition, high baseline CRP levels, absence of CV disease and smoking are associated with higher reductions of CRP.

MoP55:W6  Evidence of a relationship between the increase of HDL cholesterol and the decrease of serum triglycerides and LDL cholesterol after simvastatin

A. Branchi1, A.M. Fiorenza2, A. Torri3, F. Muzio3, A. Revelli1, D. Sommariva2. 1Department of Internal Medicine, University of Milan, Maggiore Hospital IRCCS, Milan; 2Department of Internal Medicine, G. Salvini Hospital, Garbagnate Milanese, Italy

Objective: The mechanism by which simvastatin increases HDL-C in hypercholesterolemic patients is not clear. The purpose of the present study was to investigate whether the increasing effect of the drug on HDL-C was related to its hypotriglyceridemic activity.

Methods: The study was carried out on 408 patients treated with simvastatin 10 mg for 2 months. Total cholesterol and serum triglycerides were measured with current enzymatic methods and HDL-C was determined after phosphotungstate precipitation. LDL-C was calculated following Friedewald.

Results: On average, HDL-C significantly increased by 4% after simvastatin, the increase was however seen in only 220 patients (+20%; in 201 patients HDL-C did not increase (~11%). The patients in whom HDL-C increased had baseline HDL-C lower (49 ± 0.9 mg/dl) and LDL-C (228 ± 3.4 mg/dl) and serum triglycerides (178 ± 5.0 mg/dl) higher than patients in whom HDL-C did not increase (56 ± 1.0, 220 ± 3.1 and 163 ± 5.1 mg/dl, respectively). Multiple regression analysis with ∆ HDL-C as dependent variable showed that ∆ LDL-C (partial F = 10.845), baseline serum triglycerides (partial F = 18.541), ∆ triglycerides (partial F = 27.522) and baseline HDL-C (partial F = 66.832) gave an independent contribution to the changes in HDL-C level.

Conclusions: Results of the present study show that the increase in HDL-C after simvastatin is dependent on baseline HDL-C level and on the changes the drug determines on LDL-C and, in particular, on serum triglycerides. This might suggest that the increasing effect of simvastatin on HDL-C is due to a reduction of the heterexchange of cholesterol between HDL and apo B containing lipoproteins because of the diminution of their serum concentration by effect of the drug.

MoP56:W6  The effects of atorvastatin and fenofibrate on the levels of malonyldialdehydrate, homocysteine and the size of LDL particles in combined hyperlipidemia

V. Melenovsky, J. Malík, D. Wichterle, J. Simék, R. Cska, J. Skrha, A. Parizkova, R. Poledeń. General University Hospital, Institute for Clinical, Experimental Medicine, Prague, Czech Republic

Objectives: Atorvastatin is a new treatment option in patients with combined hyperlipidemia, alternative to fibrates. The effects of both drugs on lipid parameters, size of LDL particles and plasma levels of total homocysteine (tcHcy) and malonyldialdehydrate (MDA) has never been compared in this diagnosis. Therefore a single blinded, randomised, crossover study has been planned – Fenofibrate vs. Atorvastatin Trial (FAT).

Methods: 29 middle aged non-smokers with combined hyperlipidemia, otherwise healthy, were randomised to atorvastatin (A) 10 mg o.d. or micronized fenofibrate (F) 200 mg o.d. The treatment was crossed over after 10 weeks. Fasting blood samples were drawn at baseline, in the middle and at the end of the trial. tcHcy was determined by HPLC, MDA was measured as TBARS by fluorometric assay. Size of LDL particles was determined by gel filtration after LDL ultracentrifugation.

Results: expressed as differences of mean values against baseline. Paired t-test was used for comparison, p < 0.05 was considered significant.

<table>
<thead>
<tr>
<th></th>
<th>after F-brate</th>
<th>after A-brate</th>
<th>p for F vs A</th>
</tr>
</thead>
<tbody>
<tr>
<td>cholesterol (mmol/L)</td>
<td>-12.03±5</td>
<td>-22.89±4</td>
<td>**</td>
</tr>
<tr>
<td>triglycerides (mmol/L)</td>
<td>-49.73±4</td>
<td>-52.19±4</td>
<td>**</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>+0.12±4</td>
<td>+0.03±4</td>
<td>**</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>-7.55±5</td>
<td>-33.57±5</td>
<td>**</td>
</tr>
<tr>
<td>LDL size (nm)</td>
<td>+3.13±3</td>
<td>-22.76±3</td>
<td>**</td>
</tr>
<tr>
<td>tcHcy (µmol/L)</td>
<td>+36.59±5</td>
<td>-0.72±5</td>
<td>**</td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>-21.52±4</td>
<td>-23.64±5</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.005; ***p < 0.0005

Conclusion: F was significantly more efficient than A in reduction of triglycerides. A was significantly more efficient then F in reduction of total cholesterol and LDL-C. Both F and A reduced MDA and increased size of LDL particles, but only F significantly increased tcHcy levels. Differences in the effects of F and A on MDA, tcHcy and size of LDL particles were not significant.

MoP57:W6  Effect of atorvastatin 10 mg and of simvastatin 20 mg on serum triglyceride level

A. Branchi1, A.M. Fiorenza2, A. Torri3, F. Muzio3, A. Revelli1, C. Berra2, D. Sommariva1. 1Department of Internal Medicine, University of Milan, Maggiore Hospital IRCCS, Milan; 2Department of Internal Medicine, G. Salvini Hospital, Garbagnate Milanese, Italy

Objective: Some reports suggest that atorvastatin is more powerful than other statins in decreasing serum triglyceride (TG) level in hyperlipidemic patients. This study compares the hypotriglyceridemic activity of atorvastatin and of simvastatin given at the doses that elicit the same hypocholesterolemic effect.

Methods: 200 hypercholesterolemic patients on stable low fat low cholesterol diet, 100 treated with atorvastatin 10 mg a day and 100 with simvastatin 20 mg a day.

Results: The 2 groups of patients had similar mean baseline LDL-C and HDL-C. Serum TG were higher in atorvastatin than in simvastatin group, the difference was however non statistically significant. After 2 months of therapy, the decrease of serum TG in atorvastatin treated group was significantly greater than in simvastatin group. LDL-C and HDL-C similarly changed in both groups. The decrease of serum TG was correlated with the baseline serum TG levels (r = 0.66 in atorvastatin and r = 0.48 in simvastatin patients). Multiple regression analysis with the change in serum TG as dependent variable and baseline serum TG and treatments as independent variables showed that baseline serum TG, but not the treatments, was significantly associated with the changes in serum TG.

<table>
<thead>
<tr>
<th></th>
<th>Atorvastatin 10 mg</th>
<th>Simvastatin 20 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline %</td>
<td>change %</td>
</tr>
<tr>
<td>Serum triglycerides mg/dl</td>
<td>193 ± 8.8</td>
<td>22 ± 2.6</td>
</tr>
<tr>
<td>LDL-C mg/dl</td>
<td>229 ± 5.6</td>
<td>-33 ± 1.7</td>
</tr>
<tr>
<td>HDL-C mg/dl</td>
<td>50 ± 1.2</td>
<td>5 ± 2</td>
</tr>
</tbody>
</table>

Conclusions: The hypotriglyceridemic activity of atorvastatin 10 mg and of simvastatin 20 mg is similar and is dependent on the pretreatment serum TG level.
Cohort study of 132 patients on statin-niacin combination therapy for dyslipidemia

W.L. Duvall, M.A. Blazing, S. Saxena, J.R. Gayton. Duke University Medical Center, Durham, NC, USA

Objective: To determine the safety and efficacy of statin-niacin combination therapy in a referral lipid clinic population.

Methods: All patients started on statin-niacin combination therapy in the Duke Lipid Clinic from November 1992 through May 1999 were monitored for tolerability, safety, and efficacy. Niacin formulations were immediate-release (71%), extended-release (28%), and slow-release (1%). Lipid diagnoses included combined hyperlipidemia (40%), familial hypercholesterolemia (21%), dysbetalipoproteinemia (5%), and others.

Results: Statin-niacin therapy was tolerated by 77% of patients. In those with baseline lipid profile values off all lipid medication (n = 37), statin-niacin therapy reduced LDL-C 31% and increased HDLC 29% (p = 0.002, both comparisons). In this group, mean niacin dose was 1180 mg/day; most common statin dose was pravastatin 40 mg. At niacin doses ≥ 1000 mg/day (mean 1480) added to a constant statin regimen (n = 29), HDLC increased 20% (p < 0.001), and total cholesterol, triglycerides, and total/HDL cholesterol ratio decreased significantly. Even at niacin doses < 1000 mg daily (mean 580, n = 23), HDLC increased 13% (p < 0.05), and total/HDL cholesterol ratio decreased significantly. In patients with atherosclerotic disease (n = 91), LDLC ≥ 100 mg/dl was achieved in only 35%, but total/HDL cholesterol ratio ≥ 4.5 was achieved in 62%. Side effects leading to dose limitation or cessation included upper gastrointestinal complaints in 14 of 132 patients, fatigue or myalgia in 7, and skin rash in 7. One patient developed a generalized weakness lasting 3 months. No acute, respiratory adverse effects were minor.

Conclusions: Combined statin and niacin can provide significant improvements in dyslipidemia with acceptable tolerability and safety.

Effects of simvastatin and atorvastatin on total and fractionated HDL cholesterol

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Objective: To measure specific HDL cholesterol (HDL-C) subfractions by NMR spectroscopy to better understand previously reported larger increases in total HDL-C with simvastatin (S) compared to atorvastatin (A).

Methods: Retrospective analysis of a previously conducted multicenter, randomized, open-label, parallel, 12 week clinical trial with S=40 or 80 mg/day, and A=20 or 40 mg/day [Ann J Cardiol. 1999; 83:1476-7]. Samples analyzed using NMR spectroscopy (LipoMed) providing total and 5 size-increasing subfractions, i.e., H1 (smallest) to H5 (largest).

Results: As shown in differences ± SE and % change from baseline.

Conclusions: Both doses of S increase HDL-C more than A and specifically S-80 increases large HDL more than A-40. Large HDL-C fractions (i.e., H3 + H4 + H5) are thought to be more protective against atherosclerosis than small HDL-C fractions.

Effect of itavastatin on lipid metabolism in NIDDM patients with hyperlipidemia

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Objectives: To assess the effect of itavastatin, which is a novel HMG-CoA reductase inhibitor, on the metabolism of lipid in 28 non-insulin-dependent diabetes mellitus (NIDDM) patients with hyperlipidemia.

Methods: Itavastatin was administered for 8 weeks to NIDDM patients with hyperlipidemia, and Low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and remnant like particles cholesterol (RLP-C) were determined before and after administration. Moreover, the LDL peak particle diameter was determined, using polycrylamide gel electrophoresis before and after administration.

Results: As the administration of itavastatin, LDL-C, TC, TG, and RLP-C, showed significant decreases, respectively, and a significant increase was observed in HDL-C.

Conclusions: Itavastatin lowers lipid and lipoprotein levels in NIDDM patients with hyperlipidemia.

Effect of atorvastatin and bezafibrate on C-reactive protein in mixed dyslipidemia (the ATOMIX Study)

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C-reactive protein (CRP) levels increase in people with severe Cardiovascular disease (CAD). HMG CoA reductase inhibitors (statins) decrease CRP values in hypercholesterolemic patients. However, the effect of lipid lowering agents on CRP levels in patients with mixed dyslipidemia has not been addressed.

Objective: To determine the efficacy of two hypolipidemic drugs on serum CRP levels of patients with mixed dyslipidemia.

Methods: We evaluated CRP serum levels at baseline and after 6 months in 103 participants in the ATOMIX Study (Atorvastatin vs Bezafibrate in Mixed Dyslipidemia). Following a 6-week placebo period, patients were randomly (double-blind) assigned to 400 mg of Bezafibrate or 10 mg of Atorvastatin increasing to 20 or 40 mg after 8 and 16 weeks depending on LDL-C values and following European Atherosclerosis Society recommendations. Total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol were evaluated. CRP was determined by immunonephelometry (Hoechst Behring).

Results: After 6 months of treatment, CRP levels were significantly reduced by 32% (p = 0.0001) in the Atorvastatin group whereas only a slight modification was seen with Bezafibrate (−3%; p = 0.53). The effect of Atorvastatin was not dose dependent. No correlation was found between changes in CRP levels and changes in total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride levels.

Conclusions: Atorvastatin but not Bezafibrate, decreased serum concentration of CRP in patients with mixed dyslipidemia and this effect was not dose dependent and not related to changes in lipid levels. These results suggest an additional antiatherogenic effect of atorvastatin.
The LDL particle diameter was increased by 0.732 ± 1.188 nm from 26.363 ± 1.127 nm (pre-administration) to 27.095 ± 1.360 nm (post-administration).

Conclusions: It was shown that ivatavastatin has an improving effect on the abnormalities of lipid metabolism in NIDDM patients.

MoP63:W6 Baseline lipid and lipoprotein concentrations in the participants of the prospective study of pravastatin in the elderly at risk (prospers)  
A. Gaw, C.J. Packard. For the PROSPER Executive. Royal Infirmary, Glasgow, Scotland.

Objectives: PROSPER is a randomized controlled trial designed to test the hypothesis that pravastatin will reduce cardiovascular and cerebrovascular events in high risk elderly subjects. Subjects were included if their total cholesterol levels were between 4.0-9.0 mmol/L and their fasting triglyceride levels were ≤ 6.0 mmol/L. We present the preliminary analysis of the baseline data from the study cohort.

Methods: 5804 men and women aged 70 to 82 years were randomized between Feb'98 and April 99. Pre-randomization, two fasting blood samples were collected and analyzed in the CDC-standardized laboratory in Glasgow. Lipids were measured using standard enzymatic assays and VLDL, LDL & HDL-C were quantified using the beta-quanti protocols of the LRC. No subject was receiving lipid lowering therapy at baseline.

Results: The mean values of the two baseline profiles are shown in the table, compared with the baseline lipid values of other large statin trials.

<table>
<thead>
<tr>
<th>Study</th>
<th>TC</th>
<th>Trig</th>
<th>HDL</th>
<th>VLDL</th>
<th>LDL (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROSPER</td>
<td>5.7</td>
<td>1.6</td>
<td>1.3</td>
<td>0.6</td>
<td>3.8</td>
</tr>
<tr>
<td>4S</td>
<td>6.8</td>
<td>1.5</td>
<td>1.2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>WOSCOPS</td>
<td>7.0</td>
<td>1.8</td>
<td>1.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LIPID</td>
<td>5.6</td>
<td>1.6</td>
<td>0.9</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CARE</td>
<td>5.4</td>
<td>1.8</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ACPACS</td>
<td>5.7</td>
<td>1.8</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Conclusions: Although elderly and at high risk of vascular disease (57% subjects had a history of vascular disease, with the remaining being at high risk of a first event) the PROSPER cohort have a mean lipid profile indistinguishable from most middle aged populations studied to date.

MoP64:W6 Atorvastatin and plasma homocysteine levels in subjects with familial hypercholesterolemia (FH)  
A. Bertolotto, L. Pucci, S. Bandinelli, D. Lucchese, R. Navalesi, G. Penno. Endocrinology and Metabolism, Pisa, Italy.

Objectives: Adverse vascular effects induced by homocysteine, involve endothelial injury, smooth-muscle cells proliferation and LDL oxidation. Positive correlation between levels of homocysteine and LDL-Ch was found. To evaluate the effects of atorvastatin on homocysteine levels in 22 subjects with FH.

Methods: Subjects (14 M, 8 F), non-diabetic, non-obese (25 ± 3 kg/m²), normotensive (129/81 ± 11/7 mmHg), discontinued lipid lowering therapy for at least 6 weeks. Lipids, lipoproteins, and homocysteine [by enzyme immunoassay (EIA), Axis Biochemicals ASA, Oslo, and monoparticulate enzyme immunoassay (MEIA), IMx Homocysteine, Abbott Laboratories, Illinois] were measured at baseline, 1-month (atorvastatin 20 mg), 6-month and 1-year (40 mg). Twenty-two healthy subjects acted as controls (ANOVA for repeated measures).

Results: Baseline homocysteine levels were higher in FH than in controls (EIA: 13.0 ± 3.6 vs 10.1 ± 3.9, p = 0.013; MEIA: 11.9 ± 3.7 vs 10.1 ± 2.8 μmol/L, p = 0.03). Atorvastatin reduced LDL-Ch by 45% (from 301 ± 43 to 157 ± 36 mg/dL) with lowest at 1-month, 20 mg (180 ± 37 mg/dL, p = 0.0001). Homocysteine did not change at 1-month, (EIA: 13.1 ± 3.4; MEIA: 12.6 ± 3.9 μmol/L), while was reduced at 6-month (EIA: 10.9 ± 2.9; MEIA: 10.0 ± 3.0 μmol/L, data confirmed at 1-year: EIA: 10.1 ± 2.7, p = 0.0001; MEIA: 10.4 ± 3.0 μmol/L, p = 0.019). Change in homocysteine was related to baseline homocysteine (r = 0.65, p = 0.001), but not to reduction in LDL (r = 0.21, p = 0.34).

Conclusions: Independently of LDL-lowering, atorvastatin reduces homocysteine in FH. Mechanisms of homocysteine lowering and its role in enhancing desirable effects of statins are unknown now. Improvement of endothelial function may be assumed as a link.

MoP65:W6 Efficacy and safety of the extended-release formulation of fluvastatin  
J. McKenney1, C. Ballantyne2, B. Trippe3, S. Manfreda4. 1National Clinical Research, Inc., Richmond, Virginia; 2Baylor College of Medicine, Houston, Texas; 3Drug Research & Analysis Corporation, Montgomery, Alabama; 4Novartis, East Hanover, New Jersey, USA.

Objective: To determine the lipid-lowering efficacy and the safety of the new extended-release (ER) formulation of fluvastatin 80 and 160 mg/day (Lescol XL®) in patients with hypercholesterolaemia 1a/IIb.

Methods: Following a 4-week placebo/dietary run-in phase, 123 patients with low-density lipoprotein cholesterol (LDL-C) ≥ 160 mg/dL and triglycerides ≥ 400 mg/dL were randomised (1:1:1) to fluvastatin 40 mg immediate-release (IR) formulation, or 80 mg or 160 mg (2 x 80 mg) ER formulation, for 6 weeks. All dosages were administered once daily (qam).

Results: The results showed a linear dose-response relationship in LDL-C reduction. Over 60% of patients in the 160 mg ER group achieved a ≥40% reduction in LDL-C (compared with 33% and 10% of patients in the 80 mg ER and 40 mg IR groups, respectively). Other lipid parameters were also dose ordered (see table). All dosages were well tolerated.

Least squares mean (SE) % change from baseline at last assessment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fluvastatin ER</th>
<th>Fluvastatin IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>160 mg (n = 40)</td>
<td>80 mg (n = 40)</td>
<td>40 mg (n = 40)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>–29.2 (1.5)</td>
<td>–25.5 (1.5)</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>–40.7 (2.0)</td>
<td>–30.7 (2.0)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>7.7 (2.3)</td>
<td>7.0 (2.3)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>–18.0 (4.4)</td>
<td>–13.3 (4.5)</td>
</tr>
</tbody>
</table>

* p = 0.05 vs fluvastatin 80 mg ER; †p < 0.05 and ‡p < 0.001 vs fluvastatin 40 mg IR. SE, standard error; HDL, high-density lipoprotein.

Conclusions: The fluvastatin ER formulation is an effective and well tolerated once-daily treatment for primary hypercholesterolaemia. The long-term safety of the ER 160 mg dosage requires further investigation.

MoP66:W6 HDL and triglyceride response with statins differ in their determinants and between drugs  
A. Wierzbicki, P. Lumby, G. Chik, M. Crook. St. Thomas' Hospital, London, UK.

Objective: Variability in response of LDL-cholesterol to statin therapy is well documented. However, less is known about the variation in triglyceride and HDL response.

Methods: This study investigated the factors affecting triglyceride and HDL response to simvastatin or atorvastatin therapy in 150 patients with severe hyperlipidaemia (LDL = 7.04 ± 2.06 mmol/L) and coronary artery disease (51%) using a cross-over trial format.

Results: The HDL response to statin therapy ranged from a fall of 77% to a rise of 15%. In multiple regression analysis (r² = 0.885; P < 0.0001) only drug treatment with simvastatin (β = 0.077; p = 0.05), starting LDL saturation index (β = 0.129; P = 0.0008), starting HDL saturation index (β = 0.107; p = 0.0079), and changes in LDL saturation index (β = 0.183; p < 0.0001) and changes in HDL saturation index (β = 0.301; p < 0.0001) and apolipoprotein B (β = 0.351; p < 0.0001) or change in LDL (β = 0.406; p < 0.0001) correlated with a rise in HDL. Triglyceride response with both statins ranged from +0.15 mmol/L to –0.55 mmol/L. In multiple regression analysis the correlation was weaker than for HDL (r² = 0.545; P = 0.001) and only HDL saturation index (β = 0.578; p = 0.01) and change in apolipoprotein B (β = 1.09; p = 0.001) were the significant factors in determining the response.

Conclusions: This suggests that statins have differential effects on HDL and triglyceride metabolism and that, in contrast to triglyceride reduction, individual drugs can differ in their effects on HDL response to therapy.

MoP67:W6 Hyperfibrinogenemia induced by statins correlates with drug therapy and cardiovascular risk factors  
A. Wierzbicki, P. Lumby, G. Chik, M. Crook. St. Thomas’ Hospital, London, UK.

Objective: Fibrinogen is an independent cardiovascular risk factor. Hyperfibrinogenemia secondary to statin drug therapy is a controversial and inconsistent finding.

Methods: This study investigated the factors predisposing to hyperfibrinogenemia induced by simvastatin and atorvastatin in 130 patients with severe hyperlipidaemia (LDL = 7.04 ± 2.06 mmol/L) and coronary artery disease (51%) using a cross-over trial format.
**Results:** Hyperbrinogenemia, measured by turbidimetry, after 12 weeks therapy was principally dependent in multiple regression analysis ($r^2 = 0.72$; $P < 0.001$) on drug therapy with atorvastatin ($\beta = 0.238$; $P < 0.001$), baseline fibrinogen ($\beta = -0.12$; $P = 0.001$), and initial concentrations ($\beta = 0.12$; $P = 0.002$) of apolipoprotein B concentrations and weakly with male sex and initial lipoprotein (a) concentration. It was independent of drug concentration, triglyceride, HDL and liver transaminases.

**Conclusions:** This study suggests that statin-induced hyperbrinogenemia is associated with changes in factors related to atherosclerotic plaque stability, rather than features of the insulin resistance syndrome or disturbances in liver function. Thus, it may have different prognostic implications to pre-treatment hyperbrinogenemia.

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**MoP68:W6 A HMG-CoA reductase inhibitor retards progression of atherosclerosis and regressed atherosclerosis in the rabbit aorta through a non-mediated system**


**Objective:** We determined the role of Simvastatin: HMG-CoA reductase inhibitor on the progression of atherosclerosis and the regression of atherosclerosis following removal of dietary cholesterol.

**Methods:** Exp. I – 32 male rabbits fed a 0.5% cholesterol diet for 8 weeks were divided into four groups and treated for 8 weeks (Group I: sacrifice. Group II: 0.5% cholesterol diet. Group III: 0.5% cholesterol diet plus simvastatin (5 mg/kg).) Atherosclerosis and vascular responses were determined. Exp. II – Male rabbits fed a 0.5% cholesterol diet for 8 weeks were divided into three groups. A1, hypercholesterolemic; A2, fed a regular diet for 12 additional weeks; A3, fed a regular diet with simvastatin (5 mg/kg/day). Same evaluations were done except Exp. I.

**Results:** Exp. I – Simvastatin treatment decreased the intimal thickening of aortae in Group III compared to those in Group I and II. The abolished thickening in the aortae in Group II was restored by simvastatin, and simvastatin treatment increased atherosclerotic plaques. Exp. II – Simvastatin treatment in A3 did not affect serum lipid levels. However, it decreased the atherosclerotic area and decreased esterified cholesterol concentrations. Tone-related basal NO release was larger in A3 than in A2. eNOS mRNA increased in A1 as compared with normal aorta and decreased in A2, however, it did not decrease in A3.

**Conclusion:** Exp. I; Exp. II: This is the first report of a decrease in eNOS mRNA in atherosclerosis after removal of dietary cholesterol and a reversal of it by a HMG-CoA reductase inhibitor, which may contribute regression of atherosclerosis.

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**MoP69:W6 Factors determining inter-individual variability of response to statin therapy**

R.P. Naoumovska1, F.H. O'Neil2, M. Bourbon1, C.K.Y. Neuwirth1, D.D. Patel1, B. Knight1, M. Axelsson2, G.R. Thompson1, Hammersmith Hospital, London, UK; Karolinska Hospital, Stockholm, Sweden

**Objectives:** Inter-individual variability in the extent of change in low-density lipoprotein cholesterol (LDL-C) during treatment with statins is well documented but poorly understood. This study investigates potential metabolic and genetic determinants of statin responsiveness.

**Methods:** 19 patients with heterozygous familial hypercholesterolaemia (FH) were sequentially treated with placebo, atorvastatin 10 mg/d, bile acid sequestrant (BAS) and the two combined, each for 4 weeks. Levels of LDL-C, malonlic acid (MVA, an index of cholesterol synthesis), 7a-OH-4-cholesten-3-one (7a-OH, an index of bile acid synthesis) and LDL receptor and HMG-CoA reductase mRNA levels (from mononuclear leukocytes, using real-time PCR) were determined after each treatment period.

**Results:** Atorvastatin reduced LDL-cholesterol by a mean of 32.5%. Above average responders (âLDL-C, -39.5%) had higher basal MVA levels (5.20 ng/mL ± 0.30 ng/mL) and a greater decrease in MVA on statin than below average responders (âLDL-C, -23.6%, p < 0.003; basal MVA 3.95 ng/mL ± 0.30 ng/mL, p < 0.01). Fewer patients had an apo E4 allele in the group compared with the poor responders (11% vs 6%, p < 0.05). There were no baseline differences between the two groups in 3-hydroxy-3-methylglutaryl CoA reductase or LDL receptor mRNA but the latter increased in the greater responders on combination therapy (p < 0.05). Prior or concomitant treatment with BAS did not enhance response to atorvastatin in poor responders, nor were severe FH mutations commoner in this group than in good responders.

**Conclusions:** Poor responders to statins have a low basal rate of cholesterol synthesis which may be secondary to a genetically determined (e.g. apo E4)

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**MoP70:W6 Studies on the metabolic fate of itavastatin, a new inhibitor of HMG-CoA reductase in vitro metabolism**

H. Fujino1, I. Yamada2, S. Shimada1, H. Masumoto2, M. Yoneda1, Tokyo Research Laboratory, Kowa Co., Ltd., Tokyo; 2 Central Research Institute, Nissan Chemical Industries, Ltd., Chiba, Japan

**Objective:** Itavastatin is a very potent competitive inhibitor of HMG-CoA reductase with a good absorption. Also, high bioavailability (about 80%) was observed in laboratory animal species except monkey. To evaluate the species difference and P450 isozymes in itavastatin metabolism, the in vitro metabolism was investigated.

**Results:** A relatively large amount of M-13 (8-hydroxy itavastatin) was observed in monkey microsome, but not in other animal species. The kinetic study of itavastatin metabolism suggested that M-13 was formed with relatively low intrinsic clearance on hepatic microsome. Based on the metabolism by microsome and hepatocyte of human and several animals, it was concluded that β-oxidation was major metabolic pathway in rat. On the other hand, lactonization and glucuronidation were major pathways in human and monkey. Studies on the recombinant human P450 microsome indicated that CYP2C9 was principally responsible for the hydroxylation of itavastatin, with some involvement of CYP2C8. In addition, no inhibitory effect on CYP mediated metabolism was detected in the tolbutamide 4-hydroxylation (CYP2C9) in the presence of itavastatin.

**Conclusions:** Itavastatin is scarcely metabolized in the liver, and may be highly unlikely encountered the P450 mediated drug-drug interaction in clinical practice.

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**MoP71:W6 Effect of itavastatin on postprandial triglyceride levels and lymph chylomicron in rats**

Taro Aoki1, Yasunobu Yoshinaka2, Hideco Suzuki1, Taro Tamaki1, Fumiya Sato1, Masaki Kitahara1, Yasushi Sato1, Tokyo Research Laboratories, Kowa Company Ltd., Tokyo; 2 Shizouka Research Station of Biological Science, Nissan Chemical Industries, Ltd., Shizuoka; Second Department of Internal Medicine, Chiba University, Chiba, Japan

**Objective:** Itavastatin (IV) is a potent HMG-CoA reductase inhibitor (statin) with prolonged action, due to the contribution of enterohepatic circulation of unmethylated IV differing from atorvastatin (AV). The effect of IV and AV on postprandial plasma triglyceride (TG) levels was examined on a hypothesis that prolonged inhibition of intestinal HMG-CoA reductase may result in decreased secretion of chylomicron (CM).

**Methods:** 1) Immediately after po administration of the statins to fasted rats, 20 m/kg of 8% fat (Clinical, Eisai) was orally given, Plasma TG levels were measured every 2 h up to 12 h. 2) Rats were cannulated into the thoracic duct and the stomach, and after standing overnight in Bollman cages to stabilize lymph flow, IV and then 8% fat was injected into the stomach. Lymph was collected every 1 h until 8 h, and CM was separated with ultrafiltration to measure lipid and protein contents.

**Results:** In control, TG levels started to increase at 4 h and reached the maximum at 6 h. The TG elevation was attributed to the increase in CM, CM remnant and VLDL. IV significantly lowered the 6 h TG levels and 4–12 h AUC of TG levels at 1 and 0.5 mg/kg, respectively. AV significantly reduced the AUC at 4 mg/kg showing less effect than IV in reference to the inhibitory activity on liver sterol synthesis (EDSO: AV 0.24 vs IV 0.13 mg/kg). 2) Though not significantly, IV (1 mg/kg) decreased CM-TG and phospholipids (PL) in 2nd (1–2) through 5th (4–5) h lymph. TG, PL and total cholesterol (TC)/phospholipid ratios in 4th and 5th-h lymph CM (combined) was changed by -32, -21 and +19%, respectively, with IV.

**Conclusions:** Single dose of IV lowered postprandial TG levels significantly at 0.5–1 mg/kg (4–8 times the EDSO on liver sterol synthesis) in rats. The effect may be attributable to the change in lymph CM caused by intestinal action of IV.
MoP72:W6 Hypolipidemic effect of itraavastatin and other statins in guinea pigs
Hideo Suzuki1, Hiroyuki Yamazaki2, Taro Aoki1, Taro Tanaka1, Fumiyasu Sato1, Masaki Kitahara1, Yasushi Saito1. 1Tokyo Research Laboratories, Kowa Company Ltd., Tokyo; 2Scribner Research Station of Nissan Chemical Industries, Ltd., Salamina; 3Second Department of Internal Medicine, Chiba University, Chiba, Japan

Objective: Itraavastatin (IV) inhibited liver sterol synthesis potently and long-lastingly, and extensively lowered plasma lipids by increasing hepatic LDL receptor and decreasing VLDL secretion in guinea pigs (Atherosclerosis 1999; 146: 259). We examined hypolipidemic effect of various statins in guinea pigs to gain further insight into the statin action.

Methods: 1) After 2 week administration of IV, simvastatin (SV), pravastatin (PV), fluvastatin (FV), cerivastatin (CV) and atorvastatin (AV) to guinea pigs at various doses not affecting body weight, plasma and liver lipids were measured. 2) Liver endoplasmic reticulum (ER) was isolated and measured for lipid content and activity of ER proteins participating in lipid metabolism (IV 3 and SV 30 mg/kg).

Result: 1) Each statins except CV dose-dependently lowered TC, among them IV showed the most potent effect. Only IV and AV significantly lowered TG. A significant reduction of liver cholesterol content (attributed to cholesteryl ester (CE) reduction) was observed for IV and AV. Liver TG content tended to increase with SV, PV and FV, and there was no such a tendency with IV and AV. 2) Though not significantly, TG content in ER and activity of DGAat of ER, the TG synthesis enzyme, increased with SV, and less changes were observed for IV. Activity of MTP, which is necessary for both apoB particle formation at rough ER and TG droplet production for smooth ER, increased by SV but not by IV, thus showed difference between IV and SV.

Conclusions: IV showed the strongest TG-lowering effect among the statins tested in guinea pigs. Only IV and AV, the long-acting statins, significantly lowered TG. It is suggested that statins with prolonged action lower TG by decreasing CE supply necessary for VLDL formation and by subsiding an increasing tendency of TG production at ER after statin action.

MoP73:W6 Fluvastatin: Extended-release vs immediate-release formulation
M. Mancini1, A. G. Olsson2, B. Palacios3, P. Pauciligo1. 1University Federico II, Napoli, Italy; 2University Hospital, Linköping, Sweden; 3Novartis AG, Basel, Switzerland

Objective: To compare the efficacy and safety of fluvastatin 80 mg extended-release (ER) once-daily (qpm) with that of fluvastatin 40 mg marketed immediate-release (IR) formulation once-daily (qpm) and twice-daily (bid) in patients with primary hypercholesterolaemia Ila/IIb.

Methods: After a 4-week placebo/dietary run-in phase, 695 patients with low-density lipoprotein cholesterol (LDL-C) ≥ 160 mg/dl and triglycerides ≤ 400 mg/dl were randomised (2:1:1) to fluvastatin ER 80 mg qpm (n = 346), IR 40 mg qpm (n = 174) or IR 40 mg bid (n = 175) for 24 weeks.

Results: After 24 weeks, 55% and 37% of the ER 80 mg qpm group, compared with 25% and 10% in the IR 40 mg qpm group, achieved LDL-C reductions of ≥ 35% and ≥ 40%. Reduction in LDL-C with ER 80 mg qpm (33.7%) was equivalent to IR 40 mg bid (33.5%) but significantly (p = 0.001) superior to IR 40 mg qpm (24.4%). These results were confirmed by the last- assessment data (see table) which also showed a dose-ordered response in the secondary variables. The incidence of adverse events and elevations in liver and muscle enzymes was comparable in the three groups.

Least squares mean (SE) % change from baseline to last assessment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fluvastatin ER</th>
<th>Fluvastatin IR 40 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL cholesterol</td>
<td>−32.6% (0.9)*</td>
<td>−24.3% (1.2)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>−22.0% (0.7)*</td>
<td>−15.9% (0.9)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>8.1% (1.0)</td>
<td>6.2% (1.2)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−12.3% (1.9)</td>
<td>−8.2% (2.4)</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>−25.2% (0.9)*</td>
<td>−18.8% (1.1)</td>
</tr>
</tbody>
</table>

*p < 0.001 vs IR 40 mg qpm; HDL = high-density lipoprotein; SE = standard error

Conclusions: Fluvastatin ER 80 mg qpm is therapeutically superior to, and as safe as, IR 40 mg qpm as a starting dose in patients with primary hypercholesterolaemia.

MoP74:W6 Effect of immediate start with statins after acute myocardial infarction (AMI) on the reoccurrence of cardiovascular events during the following year
A.T.W. Nøkle, L. Steffensen, A. Nordby, J.B. Hansen. Department of Medicine, Institute of Clinical Medicine, University of Tromsø, Tromsø, Norway

Objective: To register the frequency of statin prescription during the initial hospitalization for AMI and how it affected the reoccurrence of coronary events (angina, MI and death) the following year.

Methods: A retrospective study among patients of both sexes with AMI, age below 70 and who survived the initial hospitalization at the University Hospital of Tromsø during 1995–1998.

Results: 482 patients with AMI, 76% men, mean age 57.4 (range 33–70 yrs) were included in the study. The percentage of patients at discharge treated with antithrombotic drugs (Warfarin and ASA) and b-blockers remained stable during the four years period and accounted for 95% and 83%, respectively. Statin treatment was started in 55% of the patients within 12 weeks after the AMI. Increasing total cholesterol and decreasing age were significant predictors for statin prescription. The proportion of patients with total cholesterol above 5.0 mmol/l who started statin treatment during hospitalization increased gradually from 41% in 1995 to 88% in 1998 (p < 0.001). Reoccurrence of coronary events decreased in the same period from 32% in 1995 to 21% in 1998 (p < 0.05). Coronary events during the following year were predicted by a previous history of cardiovascular disease and pathological exercise-test.

Conclusions: The increase in statin use after AMI during 1995–98 was accompanied by a decrease in the reoccurrence of coronary events. This observation may indicate a favourable effect of early treatment with statins after AMI.

MoP75:W6 Efficacy of atorvastatin compared with fenofibrate in patients with combined hyperlipidemia
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A 52-week open-label, randomized, parallel group multicenter study was conducted to compare the efficacy and safety of atorvastatin versus micronized fenofibrate in patients with combined hyperlipidemia. Following a 4-week wash-out period and a 6-week placebo period, 106 patients [baseline LDL-cholesterol (LDL-C) ≥ 135 and <250 mg/dl and triglycerides (TG) ≥ 150 and <500 mg/dl] were randomized to receive either atorvastatin 10 mg or micronized fenofibrate 200 mg. After 6 weeks of treatment, the dose of atorvastatin was doubled for 18 patients with non-normalized LDL-C or TG values. A response to treatment was defined as TG < 200 mg/dl and LDL-C < 175 mg/dl (low risk), 155 mg/dl (moderate risk) and 135 mg/dl (high risk). Efficacy and safety were evaluated after 24 weeks (W 42) and 52 weeks (W 52) of treatment (table 1).

Table 1: Mean percentage changes after 24 weeks (W 24) and 52 weeks (W 52) of treatment (ITT Popula-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Atorvastatin</th>
<th>Fenofibrate</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C W 24</td>
<td>−35.7 ± 10.7</td>
<td>−12.7 ± 18.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>W 52</td>
<td>−35.7 ± 10.5</td>
<td>−17.4 ± 18.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG W 24</td>
<td>−25.1 ± 23.7</td>
<td>−39.4 ± 24.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>W 52</td>
<td>−33.2 ± 26.1</td>
<td>−41.2 ± 27.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

There were higher percentages of responders after 24 weeks with atorvastatin (67.3%) than with fenofibrate (22.2%) (p < 0.001). These results were confirmed at week 52. The overall safety profile of patients was similar in the two treatment groups. In conclusion, atorvastatin was more effective than fenofibrate to normalize simultaneously LDL cholesterol and triglycerides in patients with combined hyperlipidemia.

MoP76:W6 Atorvastatin 20 mg/day in the treatment of patients with familial hypercholesterolaemia: Effect on lipids and endothelial dysfunction
A. Suskovic, T. Balakhroneva, O. Pogorelova, M. Tsvengova, O. Atkov, V. Titov, V. Kukharchuk. Cardiology Research Complex, 121552, Moscow, Russia

Objective: 1) To investigate lipid-lowering efficacy, safety and tolerability of atorvastatin 20 mg/day (A-20) in the treatment of patients (pts) with familial hypercholesterolaemia (FH). 2) To ascertain if treatment with A-20 affects on endothelial-dependent vasodilatation (EDV) in FH pts.

Methods: Eleven FH pts aged 54.0 ± 4.7 (1 m, 10 F) with baseline level of low-density lipoprotein cholesterol (LDL-C) > 7 mmol/l were given A-20 for 3 months. Lipids and liver function tests were taken at baseline, after 1 and 3 months of therapy. Endothelial function was determined at baseline and after 3 months of therapy using high resolution ultrasound of brachial artery (D. Celermajer et al, Lancet 1992: 340: 1111–15).

Results: Lipid-lowering results are given below (mean values, mmol/l):

<table>
<thead>
<tr>
<th></th>
<th>TG 200 (X)</th>
<th>TG 200-249 (Y)</th>
<th>TG &gt; 250 (Z)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base line</td>
<td>%Δ from baseline</td>
<td>Base line</td>
</tr>
<tr>
<td>TC</td>
<td>175 ± 14</td>
<td>--15 ± 23</td>
<td>219 ± 14</td>
</tr>
<tr>
<td>LDL-C</td>
<td>80 ± 14</td>
<td>--28 ± 14</td>
<td>90 ± 14</td>
</tr>
<tr>
<td>HDL-C</td>
<td>44 ± 14</td>
<td>12 ± 31</td>
<td>47 ± 11</td>
</tr>
</tbody>
</table>

p < 0.01; ns = not significant.

Conclusion: The effects of pravastatin treatment on plasma TG were greater in those subjects with higher baseline levels. There were no differences in the reduction of TC levels between the groups. Patients with TG ≥ 250 had greater increments in HDL-C.

MoP79:W6

Lipoprotein abnormalities in hyperlipidemic patients before and after hypolipidemic treatment

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Objective: Increases in serum liver enzymes have been commonly found in patients receiving statins and fibrates. However, fatty liver, frequently observed in these patients often leads to fluctuation of liver enzymes confounding monitoring. We undertook the present study to assess liver function abnormalities before and after hypolipidemic treatment in patients followed-up in our clinic.

Methods: A total of 263 patients with various types of dyslipidemia were studied.

Results: On admission, abnormalities of liver enzymes were observed in 24 patients (9.1%) (mainly minor increases in SGPT and γGT). These patients were mostly overweight with a mean BMI of 28 kg/m² and had significant hyperlipidemia. On their first visit, 6–8 weeks after drug administration, 25 patients (9.5%) had minor increases in serum liver enzymes and specifically in SGPT in 16 patients and γGT in 15 patients. On their last visit, on average 15 months later 24 patients (9.1%) had also minor increases in serum liver enzymes (less than twice the upper normal limits). It should be mentioned that most patients with derangements of liver function on treatment and the majority of patients with increased liver function enzymes before treatment experienced a trend towards full normalization of these values during follow-up.

Conclusion: Minor transient liver function abnormalities are noticed in patients receiving hypolipidemic drugs. However, even prior to treatment some overweight patients exhibit insignificant increases in liver enzymes.

MoP80:W6

The effect of atorvastatin lipid-lowering therapy in smoker and nonsmoker patient with hyperlipoproteinemia


We were following up risk factors for coronary heart disease, within the framework of the National Program for Cardiovascular Disease Prevention, and we found that all these factors were highly present. In our population 35.5% are smokers.

Objective: We wanted to evaluate is the efficacy of atorvastatin lipid-lowering therapy similar in smokers and nonsmokers patient with hyperlipoproteinemia.

Methods: In this prospective, one year study 94 outpatient, aged between 40 and 70, 45% women and 55% men, 37 smokers and 63% nonsmokers, with hyperlipoproteinemia were evaluated. They received atorvastatin 10 mg once daily. Lipid parameters were measured before and after the treatment.

Results: Mean baseline lipid levels (mmol/l) were: TC 8.1; TG 2.5; LDL 6.2; HDL 0.9; VLDL 1.4; LDL/HDL ratio 8.1; After the atorvastatin therapy mean % reduction in lipid parameters were: in nonsmokers lowered TC by 31%; TG by 36%; LDL by 41%; VLDL by 44%; LDL/HDL ratio by 57%; TC/HDL ratio by 47%; and in smokers group lowered TC by 28%; TG by 28%; LDL by 32%; VLDL by 30%; LDL/HDL ratio by 32% and TC/HDL ratio by 42%.

Conclusion: The results demonstrate that atorvastatin lipid-lowering therapy is more effective in nonsmokers then in smokers.
Long-term effects of NK-104 (Itavastatin), a new HMG-CoA reductase inhibitor, in patients with heterozygous familial hypercholesterolemia

H. Mabuchi, Hokuriku FH Study Group; Second Department of Internal Medicine, Kanazawa University, Japan.

The efficacy and safety of itavastatin, a new HMG-CoA reductase inhibitor, in the long-term treatment for 52 to 104 weeks, were examined in 36 patients (male/female = 17/19, mean age = 51.5 ± 12.1 years) with heterozygous familial hypercholesterolemia. After the observation period for 4 weeks or longer, 2 mg/day of itavastatin was administered for 8 weeks, and changed to 4 mg/day afterward. As a result, significant decreases by 30.7% from the initial value of 343.8 ± 62.4 mg in TC were observed at week 8. During the treatment with 4 mg/day, significant decreases by 36.6% from the initial value were also observed at week 16, and the dosage-escalation effects were observed as further decreases by 8.1% from the TC values at week 8. Significant decreases level such as 30.6 to 37.0% in the TC values were observed after 16 weeks treatment. Similarly, the LDL-C value of 260.0 ± 60.3 mg/dl decreased by 41.7% at week 8, and 49.5% at week 16. These values showed further decreases by 11.9%, at week of 16, with that at week 8, and continuously stabilized effects by increasing dosage were observed, similarly to the change in TC value.

As for the safety, adverse reactions were observed in 4 cases (11.1%), and were divided into 2 cases (5.6%) of subjective and objective symptoms, and 2 cases (5.6%) of abnormalities in laboratory tests. The above results show that thoroughly improving effects of itavastatin on TC and LDL-C which are regarded as the essential parameters for the treatment of patients with heterozygous familial hypercholesterolemia, were stable and persistent in a long treatment.

Clinical evaluation of NK-104 (Itavastatin) in long-term treatment of patients with hyperlipidemia

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The long-term administration clinical trial of NK-104 (itavastatin) was performed in 317 subjects of nationwide multicenters. As the initial dose, 2 mg/day was set up for initial 8-week treatment, and by taking the TC values at week 4 into considerations, 1.2, and 4 mg/day were set as doses after week 8 till week 52, were p.o. administered once a day after supper till week 52. The evaluation at week 52 showed -28% in TC change rate, -39% in LDL-C change rate, 6.1 mg/dl increase in HDL-C, and -31% of TG change rate in patients with TG level equal to or higher than 150 mg/dl. In the comparison by stratification, FH patients also showed the linearity similar to that of non-FH patients.

The adverse drug reactions of which relationship with itavastatin could not be denied occurred in 7% of the cases with symptom/finding onset and 18% of cases with an onset of abnormality in laboratory tests, but no increase in the incidence rates was observed throughout the administration period. No case caused a myopathic symptom accompanying CK increase or disturbances of renal/hepatic function, and no serious adverse event attributable to itavastatin was observed. Based on the results, the persistent improving effect in serum lipid and safety of NK-104 were confirmed in long-term administration.

Lipid-altering efficacy and safety of simvastatin in women and elderly patients with hypercholesterolemia

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Objective: Hypercholesterolemic women and elderly patients have a substantial risk of coronary heart disease. We report the combined efficacy and safety results for simvastatin (S) in these patients from 4 large studies.

Methods: All 4 studies had similar randomized, multicenter, controlled, double-blind, parallel-group designs. Following a 4 week diet/placebo run-in, a total of 1936 patients were randomized to S 40 or 80 mg for 36–48 weeks.

Results: The lipid-altering efficacy of S 40 and 80 mg was comparable between men and women and between elderly (≥65 years) and non-elderly patients. Moreover, there was no notable difference in the incidence of myopathy and rhabdomyolysis in liver aminotransferases between male and female patients or the elderly or non-elderly, for the 40 and 80 mg groups.

Conclusions: S provided substantial LDL-C and TG reductions in all patient groups reviewed. S 40 and 80 mg had similar efficacy and safety profiles in women as in men and in elderly as in non-elderly patients.

Metal-catalyzed oxidation of apolipoprotein B-100 forms 5-hydroxy-2-amino valeric acid as a specific marker of oxidative attack on arginine and proline residues

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A modification of apolipoprotein (apo) B-100 structure by oxidation of its amino acid residue side chains is supposed to be an important mechanism involved in atherogenesis. There is difficulty in quantifying this type of modification because of a lack of specific assays. We developed a methodology based on the oxidation of protein arginine and proline residues by a metal-catalyzed oxidation system (containing 25 mM ascorbate and 0.1 mM Fe(III)) to 5-hydroxy-5-amino valeric acid (HAVA). We determined HAVA by using derivatization to N(O)-ethoxycarbonyl ethyl esters and gas chromatography-mass spectrometry (selected ion monitoring) technique in purified preparations of polyarginine, polyproline, and different apoB-100 preparations in nonoxidized and oxidized states. Femtomole levels of HAVA can be reproducibly measured in this manner. Results demonstrate that proline and arginine residues of polyamino acids and apoB-100, resp., are reactive toward oxygen radicals in vitro and ex vivo. The formation of HAVA correlates well with the amount of proline and/or arginine present in the polyamino acids as well as in apoB-100. For apoB-100 HAVA formed after 40 hours of oxidation was approximately 25% of its total proline plus arginine amount. Furthermore, HAVA level correlates well with the measurement of carbonyl group formation (r = 0.981, p = 0.0009) used as a generally accepted but nonspecific index of protein oxidation. Thus, HAVA as a specific marker of oxidized arginine and proline residues could prove to be a useful assay for studying apoB-100 damage by oxidative attack.

Oxidative stress and apolipoprotein C-III in binary system predicts the levels of risk for atherosclerosis in normolipidemic and hypertriglycerideremic subjects

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Objective: Oxidative stress (OS) is an important risk factor for coronary artery disease (CAD). However, the OS levels critical for the initiation of atherosclerosis is not known. The purpose of this study was to determine the range of critical OS levels and to examine their predictive power for CAD in combination with lipoprotein variables in normolipidemic (N) and hypertriglycerideremic (HTG) subjects.

Methods: Using the ratios of molecular species of cholesterol esters (CE) containing C20/C16 (R1) and C18/C16 (R2) fatty acids as indexes of OS in plasma (Lee, Atherosclerosis 146, 221, 1999), the OS levels were measured in 120 N and 68 HTG subjects including 12 subjects with CAD, lipid and apolipoprotein profiles were determined in all subjects. Results of these measurements were analyzed statistically using a binary-mixing system with OS on the x-axis and lipoprotein variables on the y-axis.

Results: Among lipoprotein variables only apoC-III provided a complete separation of N and CAD. A combination of OS and apoC-III resulted in a map showing 3 regions differing in risk for CAD. An area of lowest risk was present in the N group with high R1 (>0.55) and R2 (<5.0) and low apoC-III (<12.5 mg/dl). The area of highest risk was located in the HTG + CAD group with low R1 (<0.55) and R2 (<5.0) and high apoC-III (>15 mg/dl). HTG
subjects with no CAD overlapped with 30% of N subjects characterized by the same low R1 and R2 values and low apoC-III (<12.5 mg/dL) and representing an area of moderate risk.

Conclusion: This study showed that OS levels critical for initiating CAD are expressed by low R1 (<0.55) and R2 (<5.0) and that high OS in combination with high levels of apoC-III may be predictive of CAD risk in N and HTG subjects.

MoP5:W29 Inhibition of low-density lipoprotein (LDL) lipid peroxidation by policosanol, a new cholesterol-lowering agent
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Policosanol is a mixture of high molecular weight aliphatic alcohols isolated and purified from sugar cane wax with cholesterol-lowering effects demonstrated in healthy volunteers, patients with type II hypercholesterolemia and patients with dyslipidemia associated to non-insulin dependent diabetes mellitus. Policosanol acts by inhibiting cholesterol biosynthesis and increasing LDL-C processing. Because oxidation of LDL plays a crucial role in the pathogenesis of atherosclerosis and the resistance of LDL to in vitro oxidation has been possibly correlated with the extent of atherosclerosis, we investigated the effects of policosanol on LDL oxidation in a double-blind, placebo-controlled study conducted in 69 healthy volunteers. We tested the susceptibility of LDL to lipid peroxidation in a cell-free system, the incubation of copper ions and in a more physiological system, macrophage-mediated oxidation. Policosanol administered within its dosage for lowering cholesterol (5 and 10 mg/day), significantly increased lag phase (up to 1.5-fold), decreased the rate of conjugated diene generation and significantly inhibited macrophage-mediated lipid peroxidation (up to 37%). We further studied the effects of policosanol in other double-blind, randomized, placebo-controlled trials undertaken in 20 patients with type II hypercholesterolemia at high atherosclerotic risk. Even when these patients showed increased susceptibility of LDL to lipid peroxidation, policosanol administered at its starting dose (5 mg/day) significantly prolonged lag phase of diene generation curve (13.9%) when compared to placebo. Also, policosanol tended to decrease diene propagation rate, but such reduction (11.3%) did not reach statistical significance compared with placebo. In conclusion, this study demonstrated that policosanol in addition to its cholesterol-lowering effects has other properties that enables it to reduce the potential of LDL to undergo lipid peroxidation. Such effect can be considered of promising value in the management of atherosclerosis since the reduction of both, plasma LDL concentration and its susceptibility to oxidation may reduce the atherosclerotic risk.

MoP6:W29 In vivo complex formation of modified alpha-antitrypsin with LDL
S. Mashiba1, Y. Wada1, M. Takeya1, A. Sugiyama2, T. Hamakubo2, T. Kodama1, K. Uchida1, 1Hagakuo Co. Ltd., Kyoto; 2Department of Molecular Biology and Medicine, RCST, University of Tokyo; 3Department of Pathology, University of Kumamoto, Japan

Objective: Inactivated form of alpha-1-antitrypsin (AT) and LDL co-exist in gel permeation chromatography, and to confirm the association of modified AT with LDL, monoclonal antibody against oxidized by chloramine T and inactivated AT was established.

Methods and Results: From several antibodies obtained, an antibody which did not react neither with native AT, trypsin or elastase-AT complex, but did with chloramine T treated AT and oxidized LDL-AT complex was selected and named as OxAT-4. Characterization of OxAT-4 was confirmed by binding assay with various types of aldehyde modified AT competing ligands. Presence of modified AT within whole LDL fraction was shown by immunoprecipitation using OxAT-4. LDL with modified AT (AT-LDL) was purified using affinity chromatography with OxAT-4 coated carrier. Western blotting using anti apolipoprotein antibody and OxAT-4 confirmed the colocalization of apo-B and modified AT in this purified fraction of LDL. By immunohistochemistry, localization of OxAT-4 antigen in atherosclerotic core was observed, but not completely identical that of macrophage. Degradation assay indicated the significant increase of uptake of AT-LDL by mouse peritoneal resident macrophage.

Conclusion: Complex formation with modified AT and LDL in plasma was strongly suggested. Immunohistochemistry revealed the possibility that after the production by monocyte/macrophage, some degree of AT may be changed into AT-LDL in arterial wall, degraded there, and may contribute to atherogenesis.

MoP7:W29 Induction of gene expression by phenolic antioxidants, BO-653 and probucol and BHQ in human endothelial cells
W. Takabe1, C. Matak1, Y. Wada1, M. Ishii1, Y. Okutani2, O. Cynshi1, N. Noguchi1, H. Abrutani1, T. Hamakubo1, T. Kodama1, E. Nik1. 1RCST, University of Tokyo; 2Daichich pharmaceutical Co., Ltd Tokyo; Chugai pharmaceutical Co., Ltd, Shizuoka, Japan

Objective: The effects of anti-atherogenic antioxidants on the gene expression in human endothelial cells were investigated using DNA chip.

Methods: Human umbilical vein endothelial cells (HUVEC) were grown to confluent state to which 2,3-dihydroxy-5-hydroxy-2,2dipentyl-4,6-ditert-butyl-

benzo-furan (BO-653), probucol and butylated hydroquinone (BHQ) were added as a DMSO solution. After 6 hr treatment, the cells were scraped and the mRNA was recovered. The expression of 6,416 genes was analyzed using a set of oligonucleotide array (DNA chip Affymetrix).

**Results:** Among 6,416 genes, 21 genes including genes encoding mitochondrial proteins and proteins related to oxidative stress response were induced more than 3 folds by BO-653, probucol and BHQ. A gene of cytochrome P-450 IA1 isozyme which is one of drug-metabolizing phase 1 enzymes was expressed only by BHQ treatment. For other genes, BO-653 showed a similar induction pattern to BHQ rather than probucol.

**Methods:** Four subfractions of VLDL (A, B, C & D; where A denotes the largest and most buoyant) were isolated by sequential ultracentrifugation requiring a total preparation time of less than 3.5 hours, compared to 18 hours reported by other procedures. Each subfraction was assessed for: lipid composition, peroxidation (HPOs: a marker of in vivo peroxidation) and conjugated diene production (a marker of in vitro peroxidation).

**Results:** As the subfractions decreased in size and increased in density (A → D) the percent triglyceride decreased 86% → 62% while cholesterol increased 4% → 58%. With increasing density of the subfraction the distribution of fatty acid changed: SFA decreased 39.3% → 37.1%, MUFAs remained unchanged 38.4% → 37.6% and PUFA increased 23.2% → 27.0%. HPOs were not different between the subfractions 7.4% → 6.4 nmol/mg protein. Lag time resulted demonstrated that the subfractions became more susceptible to oxidation (142 → 98 min; p < 0.05) as they decreased in size and increased in density. **Conclusions:** Four VLDL subfractions (A → D) were isolated by sequential density gradient ultracentrifugation requiring 1/5th of the time of previously reported procedures. For the first time we have shown how, like LDL, VLDL subfractions become more susceptible to oxidation with decreasing size and increasing density. This method may be applied to patient groups for the detection of abnormalities within their VLDL subfractions that may not be detectable when examining whole VLDL.

**MoP9.W29** Measurement of paraoxonase (PON) concentrations in coronary heart disease (CHD) subjects by sandwich ELISA

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Serum paraoxonase (PON) is associated with high density lipoproteins, and has been shown to prevent the peroxidation of low density lipoprotein phospholipids. We have developed a sensitive sandwich ELISA, using specific monoclonal antibodies against PON to measure serum PON concentration.

We have measured serum PON in healthy Japanese subjects (n = 87) and CHD patients (n = 35) diagnosed by angiography. Serum PON concentrations in healthy subjects at 120 polymorphism were 69.5 ± 10.3 (QQR), 63.0 ± 11.0 (QRR), 52.8 ± 10.8 (RRR) µg/mL, and in CHD patients were 56.8 ± 18.2 (QQR), 56.7 ± 15.5 (QRR), 46.9 ± 9.44 (RRR) µg/mL. Serum PON concentrations in CHD patients were lower than those in healthy subjects, especially in RR (P < 0.001). Both paraoxonase specific activity and arylesterase specific activity in CHD patients were also lower, especially in RR (P < 0.001).

These results suggested that PON in CHD patients must be inactivated due to being exposed to high oxidative stress. The measurement of PON concentration can be useful tool for the detection of oxidative stress and the progression of atherosclerosis.

**MoP10.W29** Rapid isolation of VLDL subfractions: Assessment of composition and susceptibility to oxidation

J. McEnery, E.R. Trimble, I.S. Young, Department of Clinical Biochemistry, The Queen’s University of Belfast, Belfast, UK

**Objective:** To establish a procedure for the subfractionation of VLDL by rapid ultracentrifugation.

**Methods:** Four subfractions of VLDL (A, B, C & D; where A denotes the largest and most buoyant) were isolated by sequential ultracentrifugation requiring a total preparation time of less than 3.5 hours, compared to 18 hours reported by other procedures. Each subfraction was assessed for: lipid composition, peroxidation (HPOs: a marker of in vivo peroxidation) and conjugated diene production (a marker of in vitro peroxidation).

**Results:** As the subfractions decreased in size and increased in density (A → D) the percent triglyceride decreased 86% → 62% while cholesterol increased 4% → 58%. With increasing density of the subfraction the distribution of fatty acid changed: SFA decreased 39.3% → 37.1%, MUFAs remained unchanged 38.4% → 37.6% and PUFA increased 23.2% → 27.0%. HPOs were not different between the subfractions 7.4% → 6.4 nmol/mg protein. Lag time results demonstrated that the subfractions became more susceptible to oxidation (142 → 98 min; p < 0.05) as they decreased in size and increased in density. **Conclusions:** Four VLDL subfractions (A → D) were isolated by sequential density gradient ultracentrifugation requiring 1/5th of the time of previously reported procedures. For the first time we have shown how, like LDL, VLDL subfractions become more susceptible to oxidation with decreasing size and increasing density. This method may be applied to patient groups for the detection of abnormalities within their VLDL subfractions that may not be detectable when examining whole VLDL.

**MoP11.W29** Evidence of ATP and glucose depleted areas within the atherosclerotic plaque in vivo

M. Levin, M. Evaldsson, O. Wiklund, G. Bondjers, T. Björnholm. The Wallenberg Laboratory for Cardiovascular Research, Göteborg, Sweden

According to the Anoxemia theory of atherosclerosis, an imbalance between the demand for and supply of oxygen and nutrients in the arterial wall is a key factor in the development and progression of atherosclerotic lesions. However, the energymetabolic situation of the arterial wall in vivo is largely unknown.

At our lab the presence of hypoxic areas at depth in the atherosclerotic plaque in vivo has been demonstrated in lesions > 4-500 µm thick.

**Objective:** The aim of the present study was to determine local concentrations of ATP (adenosine triphosphate), glucose and lactate within the atherosclerotic arterial wall in vivo at high spatial resolution.

By get a reflection of the in vivo situation, aortas from rabbits (n = 9) with experimental atherosclerosis were snap-frozen in situ in the anesthetized animal and consecutive cryosections were used for the different analyses.

**Results:** In plaques exceeding a certain thickness, ATP (>450 µm) and glucose depleted (>5-600 µm) areas were demonstrated in the central parts of the plaque. Lactate concentrations were homogenous in the plaque.

**Conclusions:** ATP and glucose depleted areas were demonstrated at depth in the atherosclerotic plaque. We believe that this is a result of an insufficient diffusion of glucose and oxygen due to the thickness of the lesion, maybe in combination with an increased local metabolic demand with the plaque.

These results lend support to the Anoxemia theory of atherosclerosis.

**MoP12.W29** Modification of high density lipoproteins: Effects on Ox-LDL cytotoxicity and on cholesterol efflux

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Elevated plasma levels of high density lipoproteins (HDL) are believed to be antiatherogenic. Among the mechanisms by which HDL might exert their effects, attention has been paid to the role of HDL in reverse cholesterol transport. Furthermore, HDL may be antiatherogenic by preventing the cytotoxicity of oxidized low density lipoproteins (ox-LDL). HDL also undergo modifications that may affect their biological activities. Lipoygenases (LOX) belong to a family of enzymes which may play a role in atherogenesis because of their ability to oxidize lipoproteins. In this study we investigated the effect of lipoygenase-mediated modification of HDL (LOX-HDL) on their ability to prevent the cytotoxicity of Ox-LDL and on cholesterol removal from cells and aimed at relating these effects to changes in apo AI structure. Ox-LDL are cytotoxic to endothelial cells; this effect could be reverted by the presence of increasing concentrations of HDL (70.8% ± 2.3 % of control in the presence of Ox-LDL versus 141.3± ± 2.43 in the presence of Ox-LDL + HDL 400 µg protein/ml). When LOX-HDL were added to cells incubated with Ox-LDL, the improvement of cell viability was lower than in presence of native HDL.
(83.3% ± 0.8% of control), suggesting that the oxidative modification impairs their antiatherogenic role. We also investigated the ability of LOX-HDL to stimulate cholesterol efflux from cultured cells. Compared with native HDL, LOX-HDL showed a reduced ability to stimulate cholesterol efflux from preloaded macrophages (at 200 µg protein/ml: 83% of control with LOX-HDL). Changes in apo A1 conformation were evaluated by using a panel of murine monoclonal antibodies. The oxidative modification of HDL (both Cu²⁺- or LOX-oxidated) dramatically increased the expression of epitope for monoclonals mAb8, mAb9, mAb11 and mAb19, while decreased that for mAb5, suggesting profound changes of apo A1 structure that may affect its biological properties.

**MoP15:W29** Oxidized LDL triggers the lysis of human macrophages concurrently to but independently of the induction of apoptosis by depleting intracellular ATP

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In previous studies, we showed that programmed cell death in mature human macrophages induced by oxidized LDL (OxLDL) involves oxidized thiol on apoB100 and requires CD36. Here we tested the hypothesis that OxLDL concurrently triggers apoptosis and cell lysis in human macrophages. We found that compounds inhibiting OxLDL-induced apoptosis did not prevent cell lysis. Apoptosis was detected with the monoclonal antibody apo2.7 directed at the mitochondrial antigen 7A2. Cell lysis was assessed as the release of radiolabel from [³H]adenine-loaded macrophages. Our studies with an oxidation-sensitive fluorescent probe suggest that OxLDL stimulated peroxide formation in macrophages. Furthermore, the radical scavenger Trolox inhibited OxLDL-induced cell lysis but did not prevent apoptosis. Depletion of cellular glutathione (GSH) with diethylmaleate and inhibition of GSH synthesis with buthionine sulfoximine dramatically increased the cell lytic effect of OxLDL. Conversely, loading macrophages with ascorbic acid and concurrently increasing cellular GSH with L-oxothiazolidine-4-carboxylic acid reduced the susceptibility of macrophages to cell lysis. Finally, incubation with OxLDL resulted in the total depletion of ATP from macrophages. This loss of ATP was prevented by Trolox but not by dehydroascorbic acid, an inhibitor of OxLDL-induced apoptosis.

We conclude that in addition to the induction of apoptosis, OxLDL triggers the loss of membrane integrity in human macrophages. This concurrent but independent process appears to involve the initiation of radical-mediated membrane (lipid) oxidation, possibly by direct diffusion of lipid-centered radicals from OxLDL to the macrophage membrane. The expansion of the oxidative reactions into the cell interior results in the depletion of cellular ATP which in turn leads to the loss of membrane integrity.

**MoP16:W29** Fibrillar C-terminal fragment of alpha-1-antitrypsin activates human monocytes via oxidative mechanisms

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**Objective:** Monocytes express a high level of anti-inflammatory cytokines. Prototypical inactivation of AAT with subsequent generation of cleaved fibrilligenic form of AAT, C-terminal fragment (C-36) may amplify the inflammatory response. Fibrillar C-terminal fragment is suggested to be mediated via a common oxidative stress mechanism. We undertook to determine whether fibrillar C-36 effects also involve this common pathway.

**Methods:** Monocytes were isolated from buffy coats by the Ficol-Hypaque procedure. Chemotaxic protein-1 (MCP-1) expression was assayed by immunobossay, caspase activity determined by the spectrophotometry. Super-oxide produced from the NADPH oxidase was monitored by the superoxide dismutase-inhibitable rate of cytochrome c reduction. Mitochondrial membrane potential was measured from the intracellular distribution of dye, JC-1. Measurement of malonaldehyde and 4-hydroxy-alkens was used as an indicator of lipid peroxidation.

**Results:** Monocytes stimulated with C-36 fibrils (10 µM) for 1 h showed elevation in MCP-1 by 36 times, induced NADPH oxidase activity by 6.5-fold, increased lipid peroxidation by 25-fold, alteration in mitochondria membrane potential and increased caspase-3 activity by 24%. Treatment of monocytes with C-36 fibrils for 24 h resulted in increased cystolic cathepsin D activation (by 97%). Native AAT only showed concentration and time dependent stimulatory effects on MCP-1 protein expression, and these appear to be independent of oxidative stress.

**Conclusion:** Multiple activities of AAT identify it as a critical agent in orchestrating the inflammatory response in diverse processes including atherogenesis.
MoP17:W29  Platelet activation by oxygen free radicals in hypercholesterolemic patients

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Objective: Previous studies provide evidence that platelets, in analogy to other circulating blood cells, are able to produce oxygen free radicals (OFR), like super oxide anion (O$_2^-$). The present study was performed to evaluate a possible production of OFR from platelets of 25 hypercholesterolemic patients, with no history of coronary artery disease, hypertension, diabetes and without any hypolipidaemic therapy. Normal subjects acted as controls.

Methods: O$_2^-$ was determined by chemiluminescence using a Bio-Orbit 1251. Studies on platelet aggregation were performed following the Born method. The stimulating effect of OFR on platelet aggregation was studied with proper "scavengers" able to inhibit OFR production leading to a decrease in platelet aggregation.

Results:

<table>
<thead>
<tr>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ter-Cholesterol (mg/dl)</td>
<td>266.15</td>
</tr>
<tr>
<td>DL-Cholesterol (mg/dl)</td>
<td>184.55</td>
</tr>
<tr>
<td>O$_2^-$ production nmoles/3 x 10 cel/mm</td>
<td>2.11</td>
</tr>
</tbody>
</table>

Conclusions: Data of the present study provide evidence of significantly (P < 0.02) higher platelets production of OFR (O$_2^-$) in hypercholesterolemic patients, able to affect platelet activation by stimulating the arachidonic acid metabolism. Such as OFR production can play a significant role in the platelet hyperaggregability observed in hypercholesterolemia and may represent an important marker of atherosclerosis progression.

MoP18:W29  Paraoxonase (PON1) & coronary heart disease (CHD)

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Objective: To determine the relative importance of the PON1 genetic polymorphisms compared to PON1 quality (activity and concentration) in CHD

Methods: PON1 activity with paraoxon as substrate, concentration by ELISA and the PON1-55 and -192 polymorphisms by PCR and restriction digestion plus serum lipid and lipoprotein concentrations were determined in 427 subjects with angiographically proven CHD and 282 healthy controls.

Results: Subjects with CHD has higher serum triglycerides and lower total cholesterol and apo A1 then controls (all P < 0.05). However, there were no differences in HDL-C or apo-B between the populations. Serum PON1 activity was in the CHD was 50% of that found in controls (122.8 (3.3-802.8) vs 214.6 (26.3-620.8) U/L, P<0.001) and the PON1 concentration was also significantly lower (71.6 (11.4-489.3) vs 89.1 (16.8-527.4) g/L, P < 0.001) in the CHD population. Both were reduced independently of PON1 genotype. However, there were no differences in the PON1-55 and -192 gene distributions between the 2 populations and no effect of the polymorphisms on serum lipids or lipoproteins.

Conclusions: These results indicate that the importance of PON1 in hydrolysing lipid-peroxides, derangement of the quality of the enzyme is more important than genotype.

MoP19:W29  The influence of different doses n-3 fatty acids (n-3 FA) with and without supplementation of vitamin E (vit E) on the levels of reactive oxygen species (ROS) in circulating human leukocytes

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Objective: To investigate the degree of peroxidation assessed as levels of different intracellular ROS in circulating leukocytes, after treatment with low and high doses of n-3 FA with and without supplementation of vit E.

Methods: The effects were studied in non-diabetic men (40–73 yrs), with serum total cholesterol concentration 6–9 mmol/L and triglycerides ≥ 1.5 mmol/L, and without any medication. They were randomised to n-3 FA 1.0 g/d (group 1), n-3 FA 6.0 g/d (group 2) or n-3 FA 6.0 g/d combined with vit E 400 mg/d (group 3). Fasting citrated whole blood was collected before and after 6 weeks intervention. Applying flow cytometric techniques, the basal levels of ROS as well as the levels obtained in the absence and presence of phorbol 12-myristate 13-acetate (PMA, 100 ng/mL) for 90 min with or without measured in circulating monocytes (MO) and granulocytes (GR). The mean fluorescent intensity (MFI) in 10$^4$ leukocytes of the fluorochromes dihydroethidium (DHE, 5 μM), dihydrorhodamine 123 (DHR, 5 μM) and dichlorofluorescin-diacetate (DCF, 5 μM), reflecting mainly the levels of superoxide anion (O$_2^-$), peroxynitrite (ONOO$^-$) and hydrogen peroxide (H$_2$O$_2$), respectively, was recorded. When evaluating the PMA-stimulated samples, a stimulation index was calculated in which the autofluorescence of the cells and the procedure-related influences, were taken into consideration.

Results: After 6 weeks intervention there were no significant changes in the intracellular leukocyte levels of ROS in group 1. In group 2 a significant increase was observed in the basal levels of ONOO$^-$ in GR (p = 0.011), whereas no changes were found after in vitro PMA-stimulation. In group 3 no changes were observed in the basal condition, but a significant decrease could be demonstrated in the PMA-stimulated level of O$_2^-$ in MO (p = 0.001). With the letter variable the difference in changes between the groups was also statistically significant (p = 0.017).

Conclusion: Although small changes were noted regarding intracellular ROS levels in circulating leukocytes, no fundamental influence on the degree of peroxidation could be demonstrated subsequent to 6 weeks supplementation with low and high doses of n-3 FA with or without vit E.

MoP20:W29  Increased leukocyte levels of reactive oxygen species (ROS) in populations at risk for atherosclerotic disease

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Objective: To investigate intracellular ROS levels in circulating leukocytes in populations at risk for atherosclerosis as compared to healthy individuals, with the purpose to estimate the potential usefulness of leukocyte ROS as markers for the disease.

Methods: The reference group (ref-group) consisted of 6 individuals (aged 35–65 yrs, 50% women) with normal serum total cholesterol levels, using no medication. The risk populations consisted of 27 non-diabetic men (aged 40–73 yrs), with un-treated hypercholesterolemia and without any medication, and 12 individuals (aged 39–56 yrs, 42% women) with well-controlled insulin-dependent diabetes mellitus. Citrated whole blood was collected at fasting condition. Applying flow cytometric techniques, the basal levels and the phorbol 12-myristate 13-acetate (PMA, 100 ng/mL) stimulated production of ROS, were measured in circulating monocytes (MO) and granulocytes (GR). The mean fluorescent intensity (MFI) in 10$^4$ leukocytes of the fluorochromes dihydroethidium (DHE, 5 μM), dihydrorhodamine 123 (DHR, 5 μM) and dichlorofluorescin-diacetate (DCF, 5 μM), reflecting mainly the levels of i.e. superoxide anion (O$_2^-$), peroxynitrite (ONOO$^-$) and hydrogen peroxide (H$_2$O$_2$), respectively, was recorded.

Results: As no significant differences in leukocyte ROS were observed between the two risk populations, these data are grouped together (n = 39). Except for H$_2$O$_2$ levels in MO, the basal levels of all types of ROS in both MO and GR were significantly higher in the risk population as compared to the ref-group (p < 0.01). In PMA-stimulated whole blood there were significantly increased levels of O$_2^-$ in MO (p < 0.0001) and of H$_2$O$_2$ in GR (p < 0.05) in the risk population compared to the ref-group.

Conclusion: Increased levels of different ROS could be demonstrated in circulating leukocytes from a population at risk for atherosclerotic disease compared to healthy controls, which may indicate a higher degree for oxidative reactions.

MoP21:W29  Hemolysis increases atherogenesis despite induction of hemeoxygense in cholesterol-fed rabbits

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Objective: To explore if intravascular hemolysis that can induce heme-mediated oxidation of apoB lipoproteins in the arterial intima may contribute to atherogenicity.

Methods and Results: We used a model of increased intravascular hemolysis (IVH) caused by intravenous phenylhydrazine administration in rabbits with and without diet-induced moderate hypercholesterolemia (HC). Evaluating the antioxidative status of plasma, free radical generation, indicated that at the end of the treatment period this was compromised by the IVH. After
10 weeks the animals with the combined HC + IVH showed 30-40% more of the inner aortic surface covered with lesions than the animals with only HC. The animals with only IVH or the controls with normal cholesterol showed no lesions. Western blots showed that herin oxidase (HO-1) expression in aorta and other tissues was markedly increased by phenylhydrazine and it was correlated with the extent of IVH. However this enzyme, that is the main agent for heme conversion to bilirubin and biliverdin appeared not to protect the animals from the atherogenicity of hypercholesterolemia.

Conclusions: The data suggest that the increased oxidative stress associated with IVH and its associated heme formation potentiates the atherogenicity of the hypercholesterolemia. Induction of HO-1 appear not to be sufficient to counteract this condition.

MoP22:W29 The expression of human ATP-binding cassette transporter 1 mRNA increased by oxidized LDL.

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Objectives: The aim of this study is to evaluate the regulation of human ATP-binding cassette transporter 1 (ABC1) mRNA expression by oxidized LDL.

Methods: Human monocytic THP-1 cells were cultured and differentiated into macrophage with PMA. We incubated them with LDL, oxidized LDL, or Trogilitazine in various conditions. Total RNA was obtained by AGIC method. Quantitative RT-PCR and Northern blot analysis for ABC 1 mRNA were performed.

Results: Stimulation of LDL and oxidized LDL increased the expression of ABC 1 mRNA in dose and time dependent manners. The stimulation of oxidized LDL is stronger than that of LDL for the expression of ABC 1 mRNA. Trogilitazine which is thought to be an agonist for PPARγ did not increase the expression of ABC 1 mRNA.

Conclusion: These results indicate that oxidized LDL and LDL induced the expression of ABC 1 mRNA of cultured macrophage. This might relate the regulation of intracellular LDL cholesterol and blood HDL cholesterol levels.

MoP23:W29 An oxidized derivative of cholesterol increases the release of soluble vascular cell adhesion molecule-1 from human umbilical vein endothelial cells in culture.

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Oxidative modification of low density lipoprotein (LDL) is regarded as one of early features of atherogenesis. We have examined the effect of oxysterols, a group of bioactive oxidized lipids in modified LDL, on the expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human umbilical vein endothelial cells (HUVECs). Treatment of HUVECs with 7-ketocholesterol, a product of cholesterol autoxidation, resulted in an increased release of soluble VCAM-1 into the medium of the cells. At 0.125 μM, 7-ketocholesterol increased soluble VCAM-1 levels by 100%. 7-Keto-cholesterol did not enhance the expression of mRNA for VCAM-1. Other molecular species of oxysterols such as 7β-hydroxy- or 25-hydroxycholesterol had no effect on soluble VCAM-1 levels as well as on VCAM-1 mRNA. Western blot analysis revealed that the soluble VCAM-1 in the conditioned medium, as well as in the control medium, had a molecular size of 100 kDa. Stimulation of HUVECs pretreated with TNF-α, which induces the expression of VCAM-1, further increased the levels of soluble VCAM-1 in the culture medium. Again, 7-ketocholesterol did not affect the VCAM-1 mRNA levels. These results suggest that certain molecular species of oxysterols may release VCAM-1, probably by shedding, from the endothelium and may regulate the interactions between blood cells and the vascular wall.

MoP24:W29 Protective role of phospholipase A2 in processes of LDL modification.

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Objective: Phospholipase A2 modified LDL rich in lysophosphatidylcholine (LPC) have received much attention. The changes caused by LPC accumulation in them, on structure and function of LDL was studied.

Methods: Lipid-protein particles (pl-LDL) were obtained by treatment of LDL with phospholipase A2 from bee venom.

Results: Half of phospholipids in pl-LDL was changed to LPC, the composition of other lipids and protein structure were unaffected. Three MBAs against different apo B epitopes were used to test immunoreactivity of pl-LDL. MBAs 4C11 interacting with apo B epitope (residue 2377-2658) placed near receptor site, showed significant decrease immunoreactivity. In contrast, increase in 4C11 binding was demonstrated to be function of oxidation extent of LDL. Thus changing of half PC to LPC modified apo B translocation in lipoprotein globule in opposite manner than oxidative modification did it. Two fold increase of pl-LDL affinity to immobilized LDL-receptor was shown in contrast to LDL. pl-LDL as well as LPC abolished homon derived Ca2+ elevation in platelets and platelet aggregation induced by PAF, ADP and thrombine. The effect persisted in Ca free medium, indicating that pl-LDL and LPC did not abolish modification of intracellular stores with above mentioned indicators. Neither LPC no pl-LDL suppressed platelet aggregation. Inhibited effect depended of LPC concentration and platelet incubation time with pl-LDL or LPC. Half maximum effective LPC concentrations were identical for itself LPC and phi-LDL and were 2-4 μM.

Conclusion: It was concluded that LPC incorporation in LDL can act as a metabolite reconstituting LDL properties changed by their oxidation. So, phospholipase A2 can be supposed to play a protective role in the processes of LDL modification.

MoP25:W29 Oral vitamin B12 lowers homocysteine in patients with ESRD more effectively in the homoyogous T/T MTHFR 677 patients.

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Objective: The efficacy of vitamin therapy with B12 and folic acid in addition to a daily multivitamin diet containing 1 mg/day of folic acid was studied in 82 end-stage renal disease (ESRD) patients with homocysteine (hcys) levels >16 μMol. Before enrolment all patients were receiving a multivitamin daily, containing 1 mg of folate and 6 μg of B12. All patients then received 1 mg of oral vitamin B12 daily for four weeks, followed by random assignment to 0, 5, or 15 mg of folic acid daily. MTHFR 677 C→T genotype was measured and the effect of vitamin therapy on hcys levels was examined within the genotypes.

Results: The distribution of the MTHFR 677 genotype was 52%, 37% and 12% and the mean homocysteine at base line was 21.19 (4.88) 22.38 (4.69) and 29.84 (12.30) μMol/L for the C/C, C/T and T/T genotypes respectively. Patients with the T/T genotype had significantly elevated baseline hcys levels compared to either the C/C and T/C patients (P = 0.008). B12 therapy reduced the hcys levels by 2.77 (3.20), 3.46 (3.2) and 9.12 (8.35) in the MTHFR C/C, C/T and T/T patients respectively (P = 0.001 T/T vs C/T, C/C). Folate supplementation with 5 mg/15 mg did not further reduce homocysteine level in any of the genotypes (p = 0.35). Serum B12 and RBC folate levels were not different between groups at baseline or after folate supplementation except that RBC folate levels were significantly elevated in the T/T patients after four weeks of B12 supplementation. Atherosclerosis was present in 29% of the patients and did not differ between the MTHFR 677 genotypes.

Conclusion: Patients with the homoyogous T/T MTHFR mutation responded to B12 therapy significantly better than those with the C/C or T/T genotypes. Extra supplementation with 5 mg or 15 mg of folic acid did not lower homocysteine levels significantly in any of the MTHFR 677 genotypes compared to the placebo group.

MoP26:W29 Oxidized low density lipoprotein induces reactive oxygen species in endothelial cells: Effect on the intracellular nitric oxide availability.

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Objective: The impaired endothelium-dependent relaxation, the earliest alteration in hypertension, is largely due to a reduction in nitric oxide (NO) activity. Oxidized low density lipoprotein (oxLDL) has been demonstrated to increase reactive oxygen species (ROS) in endothelial cells. Since ROS may reduce NO aim of this study is to evaluate the effect of oxLDL on intracellular ROS formation and NO availability in endothelial cells.

Methods: Bovine aortic endothelial cells (BAECs) were incubated with native LDL and 5 μM Cu2+-oxLDL for 5 min. 2’-7’-dichlorofluorescin
MoP27:W29  Carnitines inhibits oxygen free radical release by endothelial cell and leukocytes
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1Istituto di Clinica Medica; 2Istituto di Clinica Ostetrica e Ginecologica, Rome, Italy

Objective: To demonstrate the role of carnitines in cellular release of oxygen free radical (OFR).

Methods: We analysed if carnitines influence OFR release by endothelial cells and human monocytes. Human vascular endothelial cell (HUVEC) were incubated 10 minutes at 37°C with scarlet concentrations (10-50 𝜇M) propionyl-carnitine and then stimulated with 4 ng/ml LPS. To assess OFR release by leukocytes human blood mixed with Na-citrate (ratio 9:1) was taken from healthy volunteers and stimulated with 1 𝜇M FMLP, a specific leukocyte activator, in presence of scarlet (10-50 𝜇M) acetyl-carnitine concentration. O2 and OH• release by HUVEC and monocyte respectively was measured as we previously described (Circulation 1997, 95: 885–891).

Results: Composed to control propionyl-carnitine reduced dose-dependently O2 release by endothelial cell; percentage decrease was 4.5%, 54% and 82% with 10, 25, and 50 𝜇M respectively; compared to control, OH• release by leukocytes was reduced by 23.8%, 32.9%, and 34.9% with 10, 25 and 50 𝜇M acetyl-carnitine respectively.

Conclusions: This study shows for the first time that cellular release of OFR may be modulated by carnitines and suggests to investigate the relationship between intracellular carnitines and OFR in conditions characterized by enhanced oxidative stress.

MoP28:W20  Pro- and anti-oxidative activities of human monocytes: Effect of concentration of oxidatively modified low density lipoprotein
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1Wallenberg Laboratory; 2Phagocyte Research Laboratory, Göteborg, Sweden; 3Heart Research Institute, Sydney, Australia

Objective: Oxidatively modified low density lipoprotein (oxLDL) is believed to play a major role in the development of the atherosclerotic plaque. This study was designed to investigate if human monocytes, obtained from peripheral blood, secrete reactive oxygen species (ROS) in response to oxLDL.

Methods: The production of extracellular and intracellular ROS was detected as isoalomin/luminol-enhanced chemiluminescence (CL) in 5·10⁵ cells, in the presence of native LDL, acetylated LDL (aLDL) or oxLDL.

Results: The results show that oxLDL itself gave rise to a high CL signal. No increased chemiluminescence was seen when LDL or acLDL was analyzed. Addition of monocytes decreases the CL response of oxLDL at concentrations >50 𝜇g/mL. This suggests that the cells activate a defense system against oxidative stress in response to the ROS production in oxLDL. On the contrary, adding low amounts of oxLDL (<50 𝜇g/mL) to monocytes the production of ROS increased, indicating an pro-oxidant activity provided by the cells.

Conclusions: This study shows both anti- and pro-oxidative effects of human monocytes, and may improve our understanding of the complexity and sometimes contradictory results from in vitro studies using modified LDL.

MoP29:W29  Oxidized low density lipoprotein (OxLDL) induces an increase in nuclear Ca²⁺ in cultured but not freshly isolated smooth muscle cells (VSMC)
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Objectives: OxLDL and Ca²⁺ are thought to be important in atherogenesis. Although it is known that oxLDL increases cell Ca²⁺, it is not clear if this is a response only observed in atherosclerotic conditions. Therefore, we examined cellular distribution of Ca²⁺ in response to oxLDL in freshly isolated control VSMC and VSMC maintained in a cultured environment where they are thought to have a pre-atherosclerotic phenotype.

Methods: SMC were obtained from aorta or portal vein by explant (only 1st passage cells) or enzymatic dissociation (freshly isolated). Cells were loaded with Indo-2 or fluo-3 to measure [Ca²⁺]i and exposed to minimally oxLDL (100 𝜇g/ml). Ratiometric imaging in a confocal microscope (UV laser) was used to examine intracellular Ca²⁺.

Results: Cultured VSMC exhibited an immediate rise in [Ca²⁺], after exposure to oxLDL. Surprisingly, this was primarily localized to the nucleus. This was observed irrespective of the Ca²⁺ indicator dye used. Conversely, in freshly isolated VSMC, oxLDL did not induce an increase in cellular Ca²⁺.

Conclusions: OxLDL induces a Ca²⁺ signaling response that may be important for gene expression in VSMC. This action may be limited to VSMC with an altered phenotype similar to that observed in atherosclerosis.

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MoP30:W29  The formation of probucol and alpha-tocopherol radicals modulates the formation of lipid peroxides, influence of ubiquinol-10
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Objective: It has been shown that the oxidative stress status and the formation of oxidative modified low-density lipoprotein (LDL) plays an important role in pathogenesis of atherosclerosis. In this connection, the aim of our study is to investigate the influence of lipid peroxides and ubiquinol-10 on the production of probucol and alpha-tocopherol phenoxyl radicals in model systems.

Methods: These model systems contained investigated antioxidants and LDL or the sodium dodecylsulfate micelles. The formation of s-tocopherol and probucol phenoxyl radical was detected after the addition of hemin and H2O2 or arachidonic acid hydroperoxides (LOOH) in those model system.

Results: The intensity of the probucol radical EPR signal increased in the presence H2O2 or LOOH. However, the addition of LOOH to this model system containing the sodium dodecylsulfate micelles decreased the concentration of tocopherol radicals. Besides, it has been found that ubiquinol-10 stimulates probucol radical formation during the LDL oxidation.

Conclusions: Thus, probucol is an effective quencher for free radical intermediates formed as a result of interaction of heroin and H2O2 or LOOH while ubiquinol-10 is the mediator of this process. However, in model system containing LOOH, the oxidation of alpha-tocopherol did not result in the formation of free radical products.

MoP31:W29  Nuclear accumulation of cell cycle proteins in response to oxidized low density lipoprotein
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Objective: To compare the capacity of oxidized low density lipoprotein (oxLDL) to induce nuclear accumulation of cell cycle proteins during cellular proliferation to that of fetal bovine serum (FBS).

Methods: Cultured human fibroblasts seeded onto glass coverslips were serum starved for five days before being treated with either 1) serum-free media and 0, 10 or 50 𝜇g/ml oxLDL or 2) 5% FBS and 0, 10 or 50 𝜇g/ml oxLDL for 24 or 48 hours. Cells were then fixed and stained with antibodies to cell cycle proteins Cdk 4, Cyclin D1, Cdk 2, Cyclin A, Cdk 2, Cyclin B1 and proliferating cell nuclear antigen (PCNA). Nuclear fluorescence was quantified by analysis of confocal micrographs with ImageJ software.

Results: Incubating serum starved fibroblasts with serum-free media and oxLDL resulted in significant increases in nuclear levels of all cell cycle proteins examined. Levels of Cdk 4, Cyclin D1, Cdk 2, Cyclin A, Cyclin B1 and PCNA in oxLDL-treated cells surpassed those in cells treated with 5% FBS alone. However in cells treated with 5% FBS in combination with...
Oxidized phospholipids activate PPARα in a phospholipase A2-dependent manner

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The peroxisome proliferator-activated receptor α (PPARα) is a transcription factor belonging to the PPAR subfamily of nuclear receptors. Fatty acids and eicosanoids are natural PPARα ligands. Here, we show using transient transfection assays that oxidized (oxLDL) but not native low-density lipoproteins (LDL) dose-dependently activate PPARα in endothelial cells without affecting PPARα protein expression. Fractionation of oxLDL lipids followed by transactivation experiments demonstrated that the oxidized phospholipid component in oxLDL is responsible for PPARα activation. Furthermore, using specific inhibitors, it is shown that oxLDL-mediated PPARα activation requires phospholipase A2 activity. Finally, we found that, similarly as the synthetic PPARα ligand Wy-14643, oxLDL induced expression of the fatty acid transport protein-1 (FATP-1) in human primary endothelial cells. Our findings define a novel group of PPARα activators and provide a molecular basis for certain effects of these biologically active phospholipids on gene transcription.

Atravastatin and simvastatin interfere with NF-κB pathway activation elicited by reactive oxygen species in vascular smooth muscle cells and monocytes through a decrease in IκBα phosphorylation

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Objective: To investigate the effect of lipophilic HMG-CoA reductase inhibitors, torcetrapib (Atv) and simvastatin (Sv) on NF-κB activation pathway induced by O2− and H2O2 in rat vascular smooth muscle cells (VSMC) and human monocytes.

Methods: NF-κB binding activity of nuclear extracts was assayed by electrophoretic mobility shift assay (EMSA). IκBα, p-IκBα and IκK-α levels were measured by western blot in whole cellular extracts.

Results: O2− induced an increase of NF-κB activity from 15 min (6.7 ± 3 fold; p < 0.01) and 30 min (2.3 ± 2; p < 0.05) and diminished until 120 min in cultured VSMC. Pretreatment for 1 h with Atv and Sv 10−5 M and 10−4 M diminished NF-κB activity in around 52% (p < 0.05) and 43% (p < 0.05), respectively. Similar results were obtained in mononuclear cells (THP-1). H2O2 also increased NF-κB activity in VSMC at 90 min (2.3 ± 1; p < 0.05). Atv and Sv (10−5 M) pretreatment reduced NF-κB activity induced by H2O2 around 28% (1.6 ± 1; NS) and 21% (1.8 ± 0.7; NS) respectively. Cell incubation with O2− caused a disappear in the cytosolic IκBα levels at 60 min that reappeared at 120 min. Atv and Sv (10−5 M) prevented the decrease of IκBα levels maintaining them at control levels. Phosphorylated IκBα (p-IκBα) levels increased from 15 min with a maximum between 30 and 60 min and decreased at 90 min. Atv and Sv (10−5 M) decreased p-IκBα levels at 60 min. IκK-α levels were maintained as control ones along the time of stimulation with O2− and were slightly reduced by Atv and Sv (10−5 M) preincubation.

Conclusion: These data suggest that some statins could interfere with the NF-κB signaling pathway activation induced by reactive oxygen species. These drugs could therefore play an important role in preventing the oxidative stress implicated in the initiation and progression of atherosclerosis.

NO and peroxinitrite in hypercholesterolemia


Objective: In this work we determined the nitrate and nitrotyrosine concentrations of blood plasma to evaluate the NO and peroxinitrite production in human hypercholesterolemia.

Methods: Plasma samples were obtained from hypercholesterolemic (HC) (n = 18) and normolipidemic subjects (N) (n = 12) after centrifugation in EDTA-coated tubes. Nitrate blood plasma concentration was determined by chemiluminescence elicted by reaction of *NO with ozone, after nitrate reduction with VCl3, in the *NO analyzer (NO2TM200, Sievers, Corp.). Nitrotyrosine concentration was determined by a new chemiluminescence competitive ELISA developed in our laboratory with a polyclonal antibody. Apo B concentration was determined by nephelometric immunoassays (Dade Behring).

Results: The levels of plasma nitrate, nitrotyrosine, apo B, total cholesterol and LDL-cholesterol were significantly higher in HC than in N subjects. The concentrations of total cholesterol, apo B and LDL-cholesterol were positively correlated with nitrate and nitrotyrosine concentrations in blood plasma.

Conclusions: These results suggest that 1) there is an increase of *NO production in hypercholesterolemia; 2) a higher *NO inactivation seems to occur by its reaction with superoxide anion to form peroxinitrite; 3) the increase of nitrate concentration in blood plasma may result from peroxinitrite degradation, and 4) cholesterol, mainly that associated to LDL, may be related to the modulation of *NO concentration in blood plasma.

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Lipoprotein substractions, antioxidant content and activation of platelet adhesion


Introduction: Platelet activation contribute to thrombosis and arteriosclerosis. Antioxidative lipoprotein profile (ALP) characterized by small, more susceptible to oxidation, dense LDL- and triglyceride rich lipoproteins, have been established as the risk factor of coronary artery disease. Since lipoproteins were found to mediate platelet activation, the aim of the study was to investigate the influence of lipoprotein particle distribution profile and antioxidant content on the platelet adhesion ex vivo.

Methods: Human platelets and lipoproteins were isolated from plasma of healthy volunteers. Adhesion of human washed blood platelets to different subpopulations of native lipoproteins immobilized in microtiter wells was examined. The total number of adhered platelets was measured as the platelet acid phosphatase activity. The LDL and HDL particle distribution profile, α tocopherol and β carotene content; susceptibility to ex vivo oxidation and reactive lysine amino groups (R-Lys) in the lipoproteins were tested.

Results: Platelet adhesion was activated by all lipoproteins tested. However VLDL and small dense LDL were the most potent activators. Degree of adhesion correlated with LDL and HDL particle density, oxidative susceptibility and R-Lys, α tocopherol, β carotene contents.

Conclusion: Lipoprotein particle distribution strongly influences platelet adhesion. VLDL and antioxidant-poor LDL and HDL subpopulations are the most potent factors in such platelet activation.
protein as determined by Western blot and was correlated with production of F2-isoprostanes. In addition, pretreatment of RAW 264 cell with HCY increased in dose-dependent manner the ability of this cells to uptake of OX-LDL. All these effects were inhibited by superoxide dismutase-SOD (1000 U/ml) We also demonstrate that macrophages isolated from mice following intraperitoneal injection of HCY showed increased (6-fold) expression of CD36.

Conclusion: These results indicate that HCY alters the function of macrophages by reacting directly with cells and causing oxidative stress and upregulating expression of CD36, a receptor for oxidized LDL.

MoP37:W29  Oxidative stress in cholesterol fed rabbits

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Objective: We measured plasma and tissue levels of phosphatidylcholine hydroperoxide (PCOOH) of rabbits fed with cholesterol by chemiluminescence-high performance liquid chromatography (CL-HPLC).

Methods: Male Japanese White rabbits (body weight = 2 kg) were divided into two groups, (1) fed 100 g/day of standard diet (control group, n = 5), and (2) fed the same quantity of diet supplemented with 1% cholesterol (Ch group, n = 5) for 8 weeks. PCOOH concentration of plasma and each tissue, including kidney, liver, and aorta, was measured with CL-HPLC as previously reported. Atherosclerotic lesions were histologically estimated.

Results: Plasma PCOOH levels were greatly increased by cholesterol feeding. This level of Ch group was 5-7 times higher than that of control group. PCOOH was accumulated in aorta and other tissues. Atherosclerotic lesion was markedly progressed in Ch group as previously reported. Elevated plasma PCOOH induced atherosclerosis. These results indicated that hypercholesterolemia by dietary cholesterol induce peroxidation of phospholipids in vivo, and the increased plasma PCOOH is one of the important initiators and landmark of atherosclerosis.

Conclusions: We concluded that assessing the plasma PCOOH levels was very useful to understanding the mechanisms of atherosclerosis and other pathological events involve to lipid peroxide.

MoP38:W29  Opposite atherogenic effects of oxidised LDL on apolipoprotein A1 and B synthesis in HEPG2 cells

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Objective: Oxidised LDL are recognised to be a key element in atherosclerosis. Although the liver is central in the lipoprotein metabolism and secretes major apoproteins (ApoAI and ApoB), the effects of oxidised LDL on this organ have not been studied so far. ApoAI is an active component of the antiatherogenic HDL-mediated reverse cholesterol transport. Conversely, ApoB concentration in the plasma is positively correlated with coronary risk. In this study, we investigated the influence of modified LDL on both ApoAI and B synthesis by HepG2 cells.

Results: Our in vitro results showed that even though cell viability was not altered, treatment with oxidised LDL lead to a decrease in ApoAI secretion and to an increase in ApoB secretion evaluated by radiolabeled leucine incorporation and specific immunoprecipitation. Parallel pulse-chase studies and Northern blot analysis showed that oxidised LDL impaired ApoB degradation but not ApoAI whereas only ApoAI mRNA were decreased. These results confirm that ApoAI is transcriptionally regulated whereas post-transcription controls ApoB secretion. Furthermore, modified LDL resulted in an increase in acetate incorporation in both triglycerides and cholesterol esters therefore indicating that a stimulation of lipid biosynthesis is involved in the oxidised LDL-induced increase in ApoB secretion.

Conclusion: Our data suggest that oxidised LDL-induced increase in ApoB and decrease in ApoAI secretion may contribute to the high LDL and low HDL-cholesterol found in atherosclerotic patients.

MoP39:W29  Treatment with natural anti-oxidants from vitis vinifera decreases lipid peroxidation in non insulin-dependent diabetics

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Objective: To test the effect of a highly standardized extract of vitis vinifera (VV) seeds, vs placebo, on lipid peroxidation in vivo in insulin-dependent diabetics mellitus (NIDDM), smoking subjects. Urinary excretion of 8-epi-PGF2α was used as an index of lipid peroxidation.

Methods: A double-blind, cross-over parallel trial was performed enrolling a total of 24 NIDDM subjects. All subjects were also smokers. After 4 weeks of wash-out (maintaining the anti-diabetic therapy and/or the ipoglycemic controlled diet), patients were randomly assigned to one of the two study arms. Subjects received 500 mg/day of the standardized extract of VV, complexed with soybean lecithin (1:3, w/w) according to a patented process, or lecithin alone. Treatments lasted 4 weeks, followed by 4 weeks of wash-out and cross-over.

Overnight urines were collected at enrollment, and at the end of each 4 week period. Urinary 8-epi-PGF2α was measured by a specific ELA after sequential solid phase extraction on reverse- and normal-phase cartridges. Results were expressed as mean ± SD of pg/mg creatinine.

Results: 18 subjects completed the study. Basal values of 8-epi-PGF2α urinary excretion averaged 195 ± 101. Under placebo, excretion of 8-epi-PGF2α was 203 ± 85, and decreased to 173 ± 62 under treatment with VV extract (p < 0.05, Wilcoxon Signed Rank test, paired data analysis).

Conclusions: As previously shown, NIDDM patients showed elevated urinary levels of 8-epi-PGF2α, a marker of lipid peroxidation. Treatment with an extract of VV seeds provided significant antioxidant protection.

P:W37 OTHER TOPICS

MoP1:W37  Glutathione peroxidase activity and blood lipids in women with diabetes mellitus and myocardial infarction

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Objective: The aim of our study was to investigate the relationship between the glutathione peroxidase activity (GPx) and blood lipids in women with diabetes mellitus (DM) and myocardial infarction (MI).

Subjects and Methods: Erythrocytic GPx activity, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and blood glucose were determined in three groups of subjects: the control group comprised 127 apparently healthy women, the group with DM included 49 patients with regulated glyceria (6.5 ± 1.9 mmol/L), and the group with MI consisted of 29 women. GPx activity was determined using Randox kit, the other blood parameters by standard biochemical methods and LDL-C was calculated using the Friedewald formula.

Results: The results obtained showed statistically significant decrease (p < 0.005) of GPx activity only in the group of patients with DM, when compared to controls (26.8 ± 8 vs. 30.9 ± 8.8 U/g Hb). There was no significant correlation between GPx and serum cholesterol (TC, LDL-C and HDL-C) in the both group of patients. The values of GPx and TG were in pure negative correlation (r = −0.142) only in diabetics. A significant negative correlation (r = −0.524) between GPx activity and blood glucose was found in the DM group.

Conclusions: The reduced activity of GPx, which was in a significant negative correlation with blood glucose, may increase the risk for atherosclerosis even in patients with regulated diabetes mellitus.

MoP2:W37  Superoxide dismutase and glutathione peroxidase activity in hypertensive women

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Objective: The purpose of this study was to assess superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity in relation to blood level of LDL-cholesterol (LDL-C) in hypertensive women.

Methods: The SOD and GPx activities were measured by Ransod and Ransel test respectively, both produced by Randox, LDL-C was calculated by the Friedwald equation. According to the obtained values of LDL-C, the examined hypertensive women were divided into three groups: first group (I), was composed of 22 women, LDL-C < 3.32 mmol/L; second group (II), included 19 women, LDL-C 3.32-4.1 mmol/L and the third group (III), consisted of 22 women, LDL-C > 4.1 mmol/L. 98 healthy women were taken as a control.

Results: The activity of SOD was significantly decreased in women of the II (965 ± 202 U/gHb), p < 0.005 and the III group (933 ± 127 U/gHb), p < 0.001 in comparison with the control (1136 ± 270 U/gHb). The GPx activity was statistically significant decreased in all three groups: I (28.1 ± 5.2 U/gHb), p < 0.005; II (27.0 ± 4.4 U/gHb), p < 0.001 and III (27.1 ± 4.0 U/gHb), p < 0.001 v.s. control (33.6 ± 9.3 U/gHb).

Conclusion: The results have shown that there is an interaction between LDL-C and antioxidant enzymes activity and suggested that marked decrease of these enzymes in hypertensive women may increase the risk of atherosclerosis as well as it may imply on the incidence of coronary heart disease.

MoP3:W37  Antioxidant enzymes in women with dyslipidemia
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Objective: The aim of our study was to investigate the relationship between serum cholesterol level and red blood cell activity of antioxidant enzymes, glutathione peroxidase (GPx) and superoxide dismutase (SOD), in women with dyslipidemia.

Subjects and Methods: SOD and GPx, as well as, standard sera parameters of dyslipidemia (total cholesterol, TC, high-density lipoprotein cholesterol, HDL-C, low-density lipoprotein cholesterol, LDL-C, and triglycerides, TG), were determined in a total number of 184 female subjects. SOD and GPx activities were determined using Randox kits, TC, HDL-C and TG were assayed by classical techniques, and LDL-C was calculated. The control group included 98 healthy women with TC < 5.17 mmol/L and HDL-C > 0.9 mmol/L. According to the values of TC and HDL-C, the rest of the subjects (n = 86) were divided in three dyslipidemia groups: a. TC < 5.17 mmol/L and HDL-C < 0.9 mmol/L (n = 20), b. TC 5.17-6.2 mmol/L and HDL-C < 0.9 mmol/L (n = 22) and c. TC > 6.2 mmol/L (n = 44). TG were less than 2.3 mmol/L in all groups.

Results: The obtained values showed a statistically significant decrease of SOD and GPx activities (p < 0.05 and p < 0.025) in group b (1050 ± 155 and 27.9 ± 11, U/gHb) and in group c (1043 ± 192 and 28.4 ± 7.4, U/gHb), when compared to the controls (1132 ± 270 and 33.6 ± 9.3, U/gHb). The investigated correlation between cholesterol (TC, HDL-C and LDL-C) and antioxidant enzymes was very pure in all groups.

Conclusions: Our results suggest that decreased antioxidant protection may increase the risk of atherosclerosis in patients with dyslipidemia and that increased intake of antioxidants may have a role in preventing coronary heart disease.

MoP6:W37  Pseudohypertiglyceridemia in 3-year-old boy
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A high serum glycerol level, characteristic of glycerol kinase deficiency, is the cause of a false high concentration of serum triglycerides as determined by a standard method in which glycerol is evaluated after glyceride hydrolysis. This pseudohypertiglyceridemia is not confirmed in lipoprotein fractions analyzed by electrophoresis and precipitation/ultracentrifuge methods.

Here we present a 3-year-old boy with pseudohypertiglyceridemia due to glycerol kinase deficiency. On the first day of life he showed respiratory and circulatory failure, muscular hypotonia, substantial metabolic acidosis and hypertiglyceridemia. In the preliminary lipids/lipoproteins profile examination a discrepancy between serum TG concentration and VLDL lipoprotein fraction was seen. This indicated a high serum glycerol level as the cause of a false serum TG rise. It was confirmed by serum glycerol determination with a specific enzymatic method, with a high signal of glycerol in the urine GC-MS profile and low glycerol kinase activity in leukocytes.

All suspected hypertiglyceridemias should be verified by serum lipoprotein fraction analysis and determination of glycerol in the serum and urine.

MoP7:W37  Enhancement of chylomicron-like emulsion lipolysis in patients with heart failure
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Objective: Evaluate the chylomicron metabolism in patients with heart failure.

Methods: We studied 14 patients with heart failure (HF) and coronary artery disease (CAD), 10 patients with HF without CAD, 56 patients without HF but with CAD and 34 subjects without HF or CAD. A chylomicron-like emulsion labeled with 3H-Trilin (TO) and 14C-Cholesterol Oleate (CO) was injected intravenously in bolus after a 12-hour fast and blood samples were collected in predetermined intervals during 60 minutes to determine the fractional clearance rate (FCR) of the labels.
**MoP8:W37** Preheparin serum lipoprotein lipase mass is negatively related to coronary atherosclerosis

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**Objective:** In preheparin serum, there exists lipoprotein lipase (LPL) mass with little activity. The clinical significance of this preheparin serum LPL mass (preheparin LPL mass) is unclear. We evaluated the correlation between preheparin LPL mass and the severity of coronary atherosclerosis by comparing with other risk factors such as age, smoking, family history, hyper tension, hyperuricemia, diabetes mellitus, total cholesterol, triglyceride, high density lipoprotein cholesterol and body mass index.

**Method:** The subjects were 70 men undergoing coronary angiographic examination, who were suspected of coronary artery diseases. Significant narrowing was defined as ≥75%. Preheparin LPL mass was measured using specific monoclonal antibody with ELISA (Daiichi Kagaku Ltd).

**Results:** Preheparin LPL mass was negatively related to the number of stenotic lesions and also with the extent of coronary disease. Multivariate analysis showed that preheparin LPL mass had the highest t-value (−2.53, −2.3) for the number of stenotic lesions and for the extent of the stenotic lesion among the risk factors listed above.

**Conclusion:** Those results suggest that low preheparin LPL mass may be deeply involved in the progression of coronary atherosclerosis.

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**MoP9:W37** Platelet volume and coronary heart disease

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**Purpose:** We have studied the medium platelet volume (MPV) in a group of patients with symptomatic and angiographically confirmed coronary heart disease (CHD), comparing them with a healthy control group.

**Methods:** 50 patients and 50 controls were included. Both groups had similar medium age and male/female proportion. Every CHD diagnosis was made after cardiac angiography, and every patient had clear symptoms of ischemic heart disease. Patients with diabetes mellitus, hypertension, obesity, stroke, pericardic artheropathy, hepatopathies, pulmonary diseases, recent bleeding, nicotine, alcohol intake ≥80 grams per day, increment of erythrocyte medium volume or any other haemathologic alteration were excluded.

Subjects in the control group were healthy, and had no symptoms of ischemic heart disease.

In both groups, blood cells count and MPV were measured through Coulter system.

**Results:** The medium MPV of the group with patients with CHD was 10.07 ± 1.12 femtoliters (fl), while in the control group was 8.38 ± 0.64 fl (p < 0.01). Considering a cutting point of 9.5 fl, the positive predictive value was 0.98, with a sensibility of 0.8 and a specificity of 0.94

**Conclusions:** Patients with clinically evident coronary heart disease, have an increase in the medium platelet volume. We can consider a MPV ≥ 9.5 fl as a good positive predictive value for CHD, with a high specificity and a good sensibility.
MoP2.H1 Ramipril induces regression of the atherosclerotic lesions in apo E deficient mice
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Objective: We have recently shown that the ACE inhibitor Ramipril significantly reduced the progression of atherosclerosis in the apolipoprotein (apo) E deficient (E) mice. Therefore the aim of the present study was to examine the effect of ramipril on the regression of atherosclerosis in this animal model.

Methods: Four-month old E mice in which significant atherosclerosis with fatty streaks and advanced fibroproliferative lesions were present were treated with either Ramipril (5 mg/kg/d) in their drinking water or placebo for a period of 2 months. The 2 groups were compared to four-month old E mice.

Results: Ramipril treatment significantly reduced the atherosclerotic lesion area in E mice at the age of 6 months, compared to placebo-treated E mice at the age of 6 and even 4 months by 42% and 28%, respectively. In search for a possible mechanism for this effect, we observed that Ramipril inhibited Ox-LDL uptake by mouse peritoneal macrophages (MPM) derived from E mice at the age of 6 months, compared to placebo-treated E mice at the age of 6 and 4 months by 42% and 15%, respectively. This effect was associated with a parallelized significant reduction in the expression of CD36 mRNA by 47% and 18%, respectively.

Conclusions: Ramipril not only inhibited the progression but also induced regression of atherosclerosis in E mice. This could be possibly explained by reduced Ox-LDL uptake via the macrophage CD36 receptor. These results may be of great importance especially in view of the clinical studies showing a beneficial effect of Ramipril that reduced morbidity and mortality in patients with coronary artery disease or multiple risk factors.

MoP3.H1 Fatty streak development in mammomatous aorta depends on both high fat/cholesterol diet and endothelial injury
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Objective: To investigate the interaction between high fat/cholesterol diet and endothelial injury on fatty streak development in mammomatous aorta.

Methods: Adult male mongrel monkeys were fed a control diet (7% fat, 0.05% cholesterol, n = 6) or a high fat/cholesterol diet (HFD diet, 15.5% fat, 0.5% cholesterol, n = 15) for 10 months. A further 13 adult male mongrel monkeys were fed the HFD diet for 3 months after which surgical catheterisation of the aorta was performed using an embolectomy catheter inserted through the femoral artery. The inflated balloon was passed 3 times along the length of the aorta, the artery was tied and the incision sutured. After surgery, 9 animals continued on the HFD diet and 4 returned to the control diet for a further 7 months. At the end of the intervention the aorta was removed and stained with Oil Red-O to detect fatty deposits. Blood samples were collected monthly for plasma total cholesterol (TC) analysis.

Results: There were no fatty deposits in the aorta of animals fed the control diet with or without surgery when mean TC levels over the last 7 months were 5.33 ± 0.38 mM and 4.89 ± 0.23 mM respectively. Of the animals fed the HFD diet alone, 10 responded with a sustained increase in TC (18.03 ± 4.26 mM, range 7.78 to 54.13 mM) over the last 7 months of the intervention. Of these only 5 developed negligible fatty deposits in the aorta (<0.3% total area). In 6 of 9 surgically treated animals fed HFD diet TC reached 30.50 ± 7.90 mM (range 6.88 to 56.62 mM), which was associated with fatty streaks covering 21.3 ± 6.4% of total area. The remaining 3 animals had TC levels of 5.66 ± 0.30 mM and showed no evidence of fatty deposits. In animals subjected to both HFD diet and surgery there was a significant correlation between TC and fatty streaks (r = 0.859, p = 0.003).

Conclusion: In mammomatous monkeys that respond to HFC diet with a significant increase in TC it was necessary to superimpose endothelial injury to induce fatty streak development in the aorta.

MoP4.H1 Application of a rabbit ear chamber to continuous intravital-microscopic study on arterogenesis in vivo
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Objective: To elucidate microcirculatory changes of behavior and structure of cellular elements during experimental arterogenesis in the rabbit.

Methods: Adult male rabbits whose ear lobes had a transparent round-table chamber (rabbit ear chamber, REC) for intravital-microscopy were subjected repeatedly to different arterogenic interventions. The microcirculatory events were observed throughout the experiment under conscious conditions in the same animals.

Results: Although various characteristic microcirculatory changes were noticed during the intervention of cholesterol feeding, tobacco smoke inhalation, artificial renal hypertension and others, quite striking features of them were increase in intravascular adhesiveness of leukocytes and swelling of lipid-laden macrophages and their transformation into foamy cells in the connective tissue in the REC during cholesterol feeding. These changes advanced in accordance with progressing hyperlipidemia and aortic accumulation of cholesterol and were modified appreciably by the other interventions including lipid lowering treatments.

Conclusions: The continuous intravital-microscopy using the REC during the initial stage of experimental arterogenesis appears to be feasible for testing preventive measures against atherosclerotic disorder.

MoP5.H1 Effect of atorvastatin treatment on structure and ultrastructure in the aorta of dyslipidemic rabbits
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Objective: To evaluate the effect of atorvastatin treatment on structural and ultrastructural changes in aorta from rabbits with dyslipidemia.

Methods: Morphometric and ultrastructural analyses were performed in aorta from rabbits fed or not with a diet containing 0.5% cholesterol + 14% coconut oil for 14 weeks and treated or not with atorvastatin (2.5 mg/kg/day).

Results: Rabbits fed the dyslipidemic diet presented higher plasma cholesterol and triglyceride concentrations as compared with controls. This was associated with intima-media thickening and, consequently, aortic stenosis (29 ± 3% since vessel cross-sectional area did not change. Accumulations of foam cells, smooth muscle cells and collagen mainly accounted for the intima thickening, while media thickening was a consequence of collagen accumulation. Endothelial integrity was disrupted in the lesion areas and the endothelial cells presented numerous alterations (lipid deposit, retilcum dilatation, shape change etc) in dyslipidemic rabbits. No differences were observed in the number of elastic layers in the media between both groups, although dyslipidemic rabbits presented numerous ruptures and showed larger thickness variability than control ones. Smooth muscle cell size was similar in both groups although dyslipidemic rabbits presented a gradual outward pattern of synthetic phenotype modification. Atorvastatin treatment attenuated plasma lipids in dyslipidemic rabbits (p < 0.05) which were nevertheless higher than those of controls. In addition, atorvastatin treatment reduced lesion area and consequently increased aortic lumen in dyslipidemic rabbits. It did not modify media thickening although it reduced its variability. Likewise, it prevented the majority of the ultrastructural changes observed in endothelial and smooth muscle cells.

Conclusions: Chronic atorvastatin treatment exerts a protective role in vascular structure and ultrastructure even in the presence of high cholesterol and triglyceride plasma levels.

MoP6.H1 Development of a lipid-rich, unstable plaque in rabbits, monitored by histology and intravascular ultrasound
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Objective: Aim of the study was the generation of a rabbit model of atherosclerosis, with morphological characteristics similar to human unstable atheromatous plaques, and the evaluation of the reliability of intravascular ultrasound (IVUS) technology to study, in vivo, the development of atherosclerotic lesions in this model.

Methods: New Zealand White rabbits underwent perivascular electrical injury at both common carotid arteries, together with a 1.5% cholesterol diet. At different time points, rabbits were sacrificed and carotids analyzed by histology. Repeated IVUS evaluations were performed in the course of lesion development.

Results: After injury, the lesioned arterial segment undergoes progressive changes: from diffuse cellular mortality, to macrophage infiltration in the media, up to final migration of macrophages to the neointima, resulting in eccentric, macrophage and lipid-rich lesions. At IVUS, lesions clearly resem-
ble those described as "unstable plaques" in the clinical setting. Quantitative and morphometric analyses of plaques show a significant correlation between histological and IVUS measurements at each time point analyzed.

**Conclusions:** A rabbit model of vascular injury in the common carotid arteries was generated, that results in the formation of atherosclerotic lipid-rich unstable plaques. IVUS methodology is a reliable approach to characterize, qualitatively and quantitatively, atherosclerotic unstable plaques in rabbits and to directly evaluate the efficacy of a number of locally or generally delivered therapeutic agents.

**MoP7.1**

**Treatment of endogenous hypercholesterolemic rabbits with CI-1027, a novel lipid regulator, results in significantly increased HDL-C and decreased LDL-C**


**Purpose:** CI-1027 raises HDL in a variety of rat models. The purpose of this study was to study the effects of CI-1027 in the endogenously hypercholesterolemic (EH) rabbit, a model in which lipidprotein metabolism more closely resembles that of man.

**Methods:** Rabbits maintained on a casein-rich cholesterol free diet become hypercholesterolemic. In this study, rabbits were fed a casein-rich diet for a period of 8 weeks to establish a hypercholesterolemic state prior to the initiation of drug therapy. The lipoprotein profile at this time was characterized by LDL-C > 200 mg/dl and HDL-C < 25 mg/dl. Rabbits were then allocated (based on total plasma cholesterol) into 4 groups (n = 12/group): casein diet alone, or the casein diet containing CI-1027 to deliver doses of 10, 30, or 100 mg/kg/day. This treatment period lasted for an additional 8 weeks.

**Results:** Following as little as two weeks of treatment, HDL-C increased by 24% and 64% (30 and 100 mg/kg/day doses, respectively) as compared to non-treated controls. CI-1027 either reduced LDL-C or attenuated the increase in LDL-C in all dose groups. Additionally, in both the 30 and 100 mg/kg/day dose groups, the combined HDL-C increase and LDL-C decrease resulted in a greater than 40% decrease in their respective LDL/HDL ratios.

**Conclusion:** CI-1027 treatment resulted in significant HDL elevation in an animal with lipoprotein metabolism similar to that of man. The additional effects on LDL-C and Lp(a) lowering (reported previously), as well as improvements in the LDL/HDL ratio, demonstrate that CI-1027 may be a novel treatment for a variety of dyslipidemias.

**MoP8.1**

**The ACAT inhibitor, avasimibe, reduces aortic cholesterol content in apoE knock-out mice, independent of its plasma hypcholesterolemic effects**


**Objective:** ApoE knockout mice develop atherosclerosis in the absence of dietary cholesterol, and are one of the most extensively studied models of atherosclerosis. The purpose of this study was to determine whether treatment of apoE knockout mice with the ACAT inhibitor, avasimibe, results in decreased aortic cholesterol accumulation independent of its plasma cholesterol effects.

**Methods:** ApoE knockout mice (Jackson Laboratories) were maintained on chow diet alone, chow + β-sitosterol (2%) or chow + avasimibe (100 mg/kg) for a total of 16 weeks. The β-sitosterol group was included as a control to obtain cholesterol lowering similar to the avasimibe-treated animals. Lipids and lipoprotein parameters were measured monthly throughout the study. Aortic cholesterol content (free cholesterol [FC] and cholesterol ester [CE]) were measured by HPLC following Folch extraction.

**Results:** Mice maintained on diet alone had an average plasma cholesterol of 538 ± 68 mg/dl, whereas mice treated with β-sitosterol or avasimibe had reduced cholesterol levels (317 ± 65 mg/dl and 276 ± 12 mg/dl, respectively). The decrease in plasma cholesterol was due to decreases in VLDL and LDL; HDL-C was unchanged. The aortic CE content of avasimibe-treated mice was significantly reduced (greater than 70%) as compared to both non-treated controls and mice fed β-sitosterol. The aortic FC content was also significantly reduced in avasimibe-treated animals as compared to both the control and β-sitosterol-treated animals.

**Conclusions:** This experiment provides additional support for a direct anti-atherosclerotic effect of the ACAT inhibitor, avasimibe, independent of its effect on plasma cholesterol levels.

**MoP9.1**

**The new thromboxane receptor antagonist, S18886, but not aspirin inhibits atherogenesis in apo E deficient mice**

A.J. Cayatte, Y. Du, J. Oliver-Krasinski, G. Lavielle, T.J. Verbeuren, R.A. Cohen. Boston University Medical Center, Vascular Biology Unit, Evans Biomedical Research Center, Boston, MA, USA

Atherosclerosis involves a complex array of factors including leukocyte adhesion and platelet vasoactive factors. Aspirin decreases the production of platelet thromboxane (TXA2) whose actions can also be effectively antagonized by blocking TXA2 receptors. The purpose of this study was to determine the role of platelet-derived TXA2 in atherosclerotic lesion development by comparing the effect of aspirin and the TXA2 receptor antagonist, S18886. The effect of 11 weeks treatment with aspirin (20 mg/kg/day) or S18886 (5 mg/kg/day), on aortic root atherosclerotic lesions, serum levels of intercellular adhesion molecule-1 (ICAM-1), and the TXA2 metabolite, TXB2, was determined in Apo E deficient mice at 21 weeks of age. Treatment did not affect body weight or serum cholesterol levels. Aspirin, but not S18886, significantly decreased serum TXB2 levels indicating the efficacy of aspirin in preventing platelet synthesis of TXA2. S18886, but not aspirin, significantly decreased aortic root lesions and serum ICAM-1 levels.

<table>
<thead>
<tr>
<th>Lesion area</th>
<th>TXB2</th>
<th>sICAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.240 ± 0.023</td>
<td>57.4 ± 2.7</td>
</tr>
<tr>
<td>AsA</td>
<td>0.259 ± 0.026</td>
<td>74.2 ± 4.7</td>
</tr>
<tr>
<td>S18886</td>
<td>0.186 ± 0.051*</td>
<td>42.1 ± 7.6</td>
</tr>
</tbody>
</table>

*P < 0.05

These results indicate that inhibition of TXA2 synthesis with aspirin has no significant effect on atherogenesis or adhesion molecule expression. The results with S18886 further suggest that blockade of TXA2 receptors inhibits atherosclerosis by a mechanism independent of platelet-derived TXA2, perhaps by preventing the expression of adhesion molecules stimulated by an unidentified eicosanoid.

**MoP10.1**

**Effect of genetic background and diet on plasma fibrinogen in mice. Possible relation with susceptibility for atherosclerosis**

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To clarify the effect of genetic factors, diets and their interactions on plasma fibrinogen concentrations, we examined plasma fibrinogen levels in four strains of mice, which differ in their susceptibility to cholesterol-induced atherosclerosis. The mice were fed normal breeding chow and four different semi-synthetic diets for different time periods. When maintained on basal diet, two strains (129J and C3H/HeJ) exhibited a significantly higher plasma fibrinogen concentration (2.1, and 1.9 mg/ml) than C57BL/6J and BALB/C strains (1.4 mg/ml). After 1 week an increase in plasma fibrinogen of approximately 40% was induced by all semi-synthetic diets in C57BL/6J strain. A similar rapid response was observed in BALB/C mice only when put on the diet containing high cholesterol, high saturated fat and 0.5% cholate (N diet). After a period of 8 weeks an increase in plasma fibrinogen of ~50-50% was observed in all strains on semi-synthetic diets as compared to basal diet. In contrast to plasma fibrinogen, no increase was observed in the fibrinogen Aα, Bβ- and γ-chain mRNA levels in the liver on the same diets. The fibrinogen mRNA levels even decreased by ~20-50% in all strains on the N diet. To determine whether the acute phase response is responsible for the observed increase of plasma fibrinogen in the above-mentioned strains, we analysed two established acute phase markers: i) haptoglobin (positive, in plasma and liver) ii) mouse major urinary protein (negative, only in liver). No acute phase response was observed in all strains after being maintained on the saturated fat, unsaturated fat, and without fat diets at various times. However, the N diet leads to a rapid and strong acute phase response in C57BL/6J and BALB/C mice and a much slower and weaker response in 129J and C3H/HeJ mice.

This study indicates that: -- genetic background determines the plasma fibrinogen levels on basal diet; -- plasma fibrinogen levels alter by diet, and the extent of the changes due to diet depends on the genetic background; -- the increase of fibrinogen in plasma due to the diets is independent of transcription and acute phase response; -- the diet-induced increase of fibrinogen was very fast in the very high atherosclerosis-susceptible strain C57BL/6J and very slow in the very high atherosclerosis-resistant strain C3H/HeJ. It remains possible that high plasma fibrinogen levels play a causal role in the development of atherosclerotic plaques in humans.
MoP11:H1 Expression of human apolipoprotein (apo) A-II in apoE-deficient mice reproduces the effects of a major familial combined hyperlipidemia gene
J. C. Escobar-Gil, J. Julve-Gil, A. Marzal-Casacuberta, J. Ordóñez-Llanos, F. González-Sastre, F. Blanco-Vaca, Servei de Bioquímica de l’Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

**Objective:** We have shown a dose-related pro-atherogenic effect of human (h) apoA-II in transgenic mice when they were fed an atherogenic diet (J. Lipid Res 1998; 39:457–62). Because the pro-inflammatory and hepatotoxic effects of the atherogenic diet, we crossed hapoA-II transgenic mice with apoE-deficient mice (apoE−/−) to assess hapoA-II effects on atherosclerosis susceptibility when they are fed a chow diet.

**Methods:** Mice were used for studies at 12–13 weeks of age. Plasma lipid levels and aortic lesion size were quantified as previously described. Lipolytic activities against exogenous substrates were measured in post-heparin plasmas using a [3H]triolis oleate emulsion. In vivo clearance of the autologous radiolabeled VLDL was also determined. Triglyceride (TG) production rates were measured after an i.v. injection of Triton WR-1339.

**Results:** The lesion areas of apoE−/− mice overexpressing hapoA-II (72 mg/dl) (apoE−/−, hapoA-II) were ≈3.0-fold higher compared with apoE−/− mice. Lesion increase was accompanied by a significantly increase in plasma TG (24-fold) and cholesterol (2.5-fold) levels as a result of a substantial age-dependent increase of apoB-containing lipoproteins. The results of subsequent experiments demonstrated that apoE−/−, hapoA-II mice had similar age-related features of familial combined hyperlipidemia (FCHL): reduced HDL cholesterol, normal lipolytic activities and in vivo clearance of radiolabeled VLDL, and a significantly increased TG production.

**Conclusion:** These results demonstrate that apoE−/−, hapoA-II mice fulfilled the criteria for being considered an animal model of FCHL.

MoP12:H1 Long term vitamin E feeding attenuates aortic atherosclerosis independent of plasma lipid levels in heterozygous LDL-receptor deficient mice
O.L. Volger1, J. van der Boom1, W. van Duyvenvoorde1, K. van Wijk2, J. Mathor2, R. Leenen3, A.J.C. Roodenburg2, L.M. Havekes1, H.M.G. Princen4, G. Goubouis Lab. TNO-PG, Leiden; 1 Unilever Research Vlaardingen, The Netherlands

**Objective:** The anti-atherosclerotic effects of vitamin E feeding was assessed in female heterozygous low density lipoprotein (LDL) receptor deficient mice. These mice were used since they have a human like lipoprotein distribution with LDL as major fraction.

**Methods:** The mice received for 58 weeks a control diet with a basal vitamin E content of 0.0035% (w/w) (N = 9) or a diet supplemented with vitamin E to a content of 0.07% (w/w) (N = 11).

**Results:** Vitamin E feeding (i) led to a two-fold increase in plasma vitamin E levels, but had no effects on plasma lipids and ex vivo CUSOX induced LDL/VLDL oxidation and (ii) caused a 30% decrease (P = 0.009) of the atherosclerotic lesion area in the aortic root, although the severity of the atherosclerotic lesions was not diminished (P = 0.114).

**Conclusions:** Long term vitamin E feeding attenuates the lesion size, but not the severity of atherosclerosis, without affecting plasma lipid levels in heterozygous LDL-receptor deficient mice.

MoP13:H1 Altered expression of multiple genes in the aorta of apolipoprotein-E deficient mice with atherosclerosis: Determination by hybridization of arrayed cDNA clones
Y.-J. Geng, K. Kil, S.W. Casscells, J.T. Willerson. Dept. Internal Medicine, University of Texas, Houston, USA

**Objective:** Mice deficient in expression of apolipoprotein-E (apoE) develop hyperlipidemia and intimal lesions in aorta, highly resembling those seen in human atherosclerosis. This study was aimed at determining whether there is alteration in vascular gene expression during the development of atherosclerosis in apoE-null mice.

**Methods:** 12–15 month old apoE-null but wild type (C57BL/6J) control mice fed normal chow showed advanced atherosclerotic lesions or atheroma in aorta as determined by H&E and oil-red O staining. A high throughput cDNA array method was employed to simultaneously determine expression of more than 600 genes in the aortic segments with or without atherosclerosis. The cDNA clones including genes involved in oncogenesis, cell cycle, stress response, signaling, apoptosis, transcription, adhesion, cytokoskeleton and matrix were arrayed on a nylon membrane (Clontech). mRNA was prepared from total RNA isolated from the aorta of apoE-null and wild type mice, converted into cDNA by reverse transcription, and labeled with 32P for probing the membrane.

**Results:** Expression of multiple genes was altered by 50% in apoE-null mice (n = 25), compared to the wild type controls (n = 15). The major genes altered included those participating in regulation of lipid metabolism (e.g., apolipoprotein E, DNA repair (p21), apoptosis (fas and bcl2 families), and inflammation (MCP)). Housekeeping genes (e.g., beta-actin and ribosomal proteins) showed little difference between apoE-null and wild type aortas.

**Conclusions:** Atherosclerosis caused by apoE deficiency is associated with a marked alteration in expression of genes, in particular those important for vascular cell survival and inflammation. Further characterization of the altered genes may shed new insight into the molecular mechanism(s) underlying the development of atherosclerosis in this experimental model.

MoP14:H1 Paradoxical increase of plaque growth and collagen deposition in atherosclerotic mice lacking plasmagrin activator inhibitor-1 (PAI-1)
A. Lutten1, J.-M. Herbert1, L. Moones1, P. Tipping3, D. Collen1, F. Lupu4, P. Carmeliet1, 1 Center for Transgene Technology and Gene Therapy, VIB, KU Leuven, Belgium, 2 Haemobiology Department, Sanofi Toulouse, France, 3 Center for Inflammatory Diseases, Monash Medical Center, Victoria, Australia, 4 Vascular Biology Laboratory, Thrombosis Research Institute, London, UK

**Objective:** Epidemiologic studies indicate that high plasma levels of plasmagrin activator inhibitor-1 (PAI-1) are a risk factor for progression of coronary artery disease and reinforcement. However, the mechanism of action of PAI-1 locally secreted inside plaques and its causal role in plaque progression has not been conclusively studied.

**Methods and Results:** Mice deficient in PAI-1 (PAI−/−) were intercrossed with atherosclerosis-prone apolipoprotein E deficient mice (apoE−/−) and fed a cholesterol-rich diet for prolonged periods. As in humans, PAI-1 plasma levels were significantly higher in atherosclerotic apoE−/− mice. Unexpectedly, atherosclerotic plaques were consistently larger and contained more collagen in apoE−/−PAI−/− than in apoE−/− mice. Plaque fibrosis in apoE−/−PAI−/− mice appeared to result from increased levels of active transforming growth factor-β (TGF-β) that can be generated from its latent form by plasmin. However, collagen in apoE−/−PAI−/− plaques appeared disorganized and immature, most likely as a result of increased plasmin-mediated proteolytic breakdown by the accumulation of macrophages. Plasmin levels in apoE−/−PAI−/− plaques were higher than in apoE−/− plaques.

**Conclusions:** Contrary to our expectations, the well-known atherosclerosis risk factor PAI-1 inversely correlated with plaque growth and collagen deposition. Moreover, the collagen, although more extensively deposited, failed to become organised in apoE−/−PAI−/− plaques, thereby weakening – instead of strengthening- the plaques.
Monday June 26, 2000: Read by Title Abstracts

**T:W1 DIABETES**

**MoT1:W1**  
**Leptin and cardiovascular risk factors among overweight diabetics**  
L. Koeva, D. Dimitrov, T. Rousseva.  
Department of Endocrinology and Gastroenterology;  
Department of Radiology Medical University Varna, Bulgaria

**Objective:** Overweight is thought to be an independent cardiovascular risk factor, especially among diabetic subjects. The possible relation of leptin – a hormone that regulates body weight, with cardiovascular risk factors, is still controversial.

**Methods:** We examined the relation of leptin, measured with RIA kit, and the different components of the metabolic (X) syndrome in 70 diabetic patients (45 women and 25 men).

**Results:** Serum leptin levels significantly correlated with body mass index (BMI) (r = 0.58 – women; r = 0.76 – men), cholesterol (r = 0.38 – women; r = 0.31 – men), total triglycerides (r = 0.76 – women; r = 0.79 – men) LDL (r = 0.24; women; r = 0.37; men), LDL/HDL ratio (r = 0.23; women; r = 0.36 men), non-HDL cholesterol (r = 0.26; women; r = 0.27 men), fasting plasma glucose (r = 0.79 women; r = 0.79 men) and systolic blood pressure (r = 0.47 men).

**Conclusions:** Leptin may be associated with several cardiovascular risk factors related to the metabolic syndrome.

**MoT2:W1**  
**Clinical trial of plant tallen-ester induced cholesterol-lowering in NIDDM-patients**  
P. van der Vleuten, K. Bouter. Bosch Medencentrum, Den Bosch, the Netherlands

**Objective:** To assess the potential of using plant tallen-ester enriched products in a clinical setting.

**Methods:** Hypercholesterolemia has been correlated with NIDDM. Since spreads enriched with plant tallen-ester have proven to be effective in a regular population, this trial has taken it one step further, into the clinical possibilities with NIDDM-patients.

We started with a population of 29 patients. These patients were instructed to use the spread merely instead of their regular product. From the initial 29 patients 3 could afterwards not eat the criteria to be entered in the trial (those being; an HbA1c < 9 for the last year, no history of retinopathy or other cholesterol-compromising diseases and an age below 75). The data of 4 others could also not be used due to extensive business trips. The population had an average total cholesterol of 5.19 mmol/l (sd = 0.88), LDL of 3.10 mmol/l (sd = 0.92), HDL 1.02 mmol/l (sd = 0.25), triglycerides of 2.38 mmol/l (sd = 1.43), HbA1c of 7.31 (sd = 1.19), an average age of 62.4 years (sd = 10.4) BMI of 27.4 (sd = 7.13). 10 patients used insulin. Only 4 patients stated to be concerned with their cholesterol-level. The patients returned after 23 days (sd ± 7).

**Results:** Tot. cho. had (a = 0.011) dropped 8.0% to 4.77 mmol/l (sd ± 0.90) and LDL had dropped 5.3% to 2.89 mmol/l (sd ± 1.01) but this was not statistically significant. Patients responded very positive.

**Conclusion:** There are strong signs that these products may be useful in preventing Hypercholesterolemia. Most interesting was the use of these products by elderly because they reported to virtually have no problem in keeping the diet. However more research is needed.

**MoT3:W1**  
**Characteristics of diabetic patients with early coronary disease**  
Department of Cardiology;  
Hospital de Cabueñes (Gijon);  
Hospitcal Central de Asturias (Oviedo), Spain

**Purpose:** To determine the characteristics of patients under 50 years of age with coronary disease and diabetics.

**Methods:** Consecutively, 227 male patients (pts) before 50 years of age (mean age 42 ± 4 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease were prospectively studied. Smoking habits, arterial hypertension, diabetes and dyslipemia were determined during the acute phase by means of a questionnaire, physical examen and fasting analysis. Two groups were established according to whether the patients were diabetics or nor. In order to determine new coronary events, a mean follow-up of 32 ± 13 months was carried out.

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>Diabetes (21)</th>
<th>Without diabetes (206)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart failure</td>
<td>3 (14%)</td>
<td>8 (4%)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

No differences were found in the prevalence of arterial hypertension, smoking habits or dyslipemia in either group. No differences were seen during follow-up in the presence of mortality, angina, myocardial infarction, heart failure and the need for coronary revascularisation.

**Conclusions:** Patients with clinical onset of coronary heart disease before 50 years and with diabetes present a greater frequency of heart failure during follow-up than non-diabetics patients.

**MoT4:W1**  
**Prevalence of non insulin dependent diabetes mellitus during 1976–1997 in Iran**  
Isfahan Cardiovascular Research Center Isfahan, Iran

Diabetes is one of vast non-communicable disease in the world. The change in life style is the main causes of the prevalence of diabetes. Unfortunately, precise and comprehensive studies are not performed on the prevalence of type II diabetes of national level in Iran most done researches are emphasized in certain population or after in one city population. On the other hand, diagnostic procedures for diabetes also are different in these studies. In this article, an efforts is done with a review on part studies from 1976 to 1997 to inform generally on the prevalence of this disease in different cities in Iran in order to make health policies and preventive and care performances. By comparing the prevalence of this disease in men and women, it is apparent that there is more prevalence of diabetes in women than men in a certain area.

On the other hand prevalence rate was observed when the estimation was based on 2 hct than fasting blood suger. It seems that determining the high risk groups for this disease has great importance for appropriate actions for diabetes prevention.

**MoT5:W1**  
**Preventative effect of 'PROBUCOL' on micro-albuminuria in type II diabetic patients**  
K. Amano1, M. Kasuga2. The P-HIT study group;  
kakageawa National Hospital, Kakogawa;  
kobe University School of Medicine, Kobe, Japan

**Objective:** To examine the antioxidant effect of 'PROBUCOL' on the reduction of micro-albuminuria in type II diabetic patients.

**Methods:** 52 type II diabetic patients were randomly divided into two groups ('PROBUCOL' vs 'PRAVASTATIN'), who had the same background (varation of HbA1c < 1%, blood pressure < 140/90, 200 < l-chol < 280, w/o any drugs which might have the effect for micro-albuminuria, including ACE-I). Every 6 months for 3 years, micro-albuminuria for 24 hours of two groups were measured as AER (albumin excretion ratio, between 15 and 200 µg/min).

**Results:** AER of 'PROBUCOL' group (Group A) showed 32.8 (0 mo), 28.0 (6 mo, P = 0.6), 22.6 (12 mo, P = 0.15), 11.0 (18 mo, P = 0.05), 5.2 (24 mo, P < 0.006). On the other hand, AER of 'PRAVASTATIN' group (Group B) showed 29.3 (0 mo), 62.5 (6 mo, P = 0.13), 35.3 (12 mo, P < 0.89), 52.6 (18 mo, P < 0.27), 12.6 (24 mo, P = 0.68).

**Conclusions:** These data indicated that 'PROBUCOL' had the antioxidant effect of it on the reduction of micro-albuminuria in type II diabetic patients rather than the suppressive effect of cholesterol of it.
Relevance of genetic predisposition and lifestyle factors in the pathogenesis of type 2 diabetes mellitus and cardiovascular complications

M. Kruisbock,1 E.E. Blaak,2 E.J.M. Feskens2 R. van Dam2 J.M. Dekker3 R.J. Heine1 T.W.A. de Bruin1 H. Jansen3 H. Molhuizen2 G. Nijpels3 C. Stehouwer5 steering group project: 1UM/AM, Maastricht; 2RIVM, Bilthoven; 3VU/AMC, Amsterdam; 4EUR, Rotterdam, The Netherlands

Worldwide, about 100 million people suffer from type 2 diabetes mellitus (DM) today. The risk on cardiovascular diseases (CVD) in type 2 DM is increased to about twice as high as that in the nondiabetic population. To determine the contribution of lifestyle and genetic variability in lipid metabolism to the development of type 2 DM and cardiovascular complications, a joint framework of two cohorts is being conducted in the Netherlands, the Hoorn Study and the MORGEN-project in Maastricht. Approximately 9000 participants will be invited from the cohort of the Hoorn study. From the MORGEN-project, 5000 participants aged between 40 and 70 years with a number of risk factors for type 2 DM will be invited for an oral glucose tolerance test (OGTT). From this group 250 subjects with newly diagnosed type 2 DM, 250 subjects with impaired glucose tolerance will be invited for subsequent studies. In both cohorts the next measurements will take place: OGTT, anthropometry, non-invasive measurements of vascular function and prevalent vascular disease, and microalbuminuria. Also, questionnaires will be taken to determine some lifestyle factors like physical activity, dietary intake and smoking habits. Further, plasma samples will be drawn for determination of insulin, triglycerides, total-, HDL- and LDL-cholesterol, NEFA, HbA1c, LDL-size and oxidability, apo A-1, apo B and homocysteine. HLP, LPCAT and CETP polymorphisms and apo CIII and E genotypes will be determined using genomic DNA isolated from white blood cells. All subjects will be followed with respect to CVD morbidity and mortality and will be invited for a follow-up examination three years later.

Apollipoprotein (a) phenotype and retinopathy in diabetic patients

T. Suzuki, K. Okazaki, S. Sato, H. Nakano, K. Ohta, S. Moteri. Nippon Medical School, Tokyo, Japan

Objective: The aim of this study was to clarify the relationship between apolipoprotein(a) phenotype and diabetic retinopathy.

Subjects and Method: Serum Lp(a) concentration and apolipoprotein(a) phenotype were analyzed in 115 diabetic patients. Apolipoprotein(a) phenotype were classified into 7 subtypes (F, B, S1, S2, S3, S4, O (Null)) by the method SDS electrophoresis with Western blotting. Patients were defined as high molecular group with S3, S4, O (Null) and as low molecular group with F, B, S1, S2. Diabetic retinopathy is classified to 4 groups: non-retinopathy group (R0), simple retinopathy group (R1), pre-proliferative retinopathy group (R2) and proliferative retinopathy group (R3).

Result: Apolipoprotein(a) phenotypes in patients with diabetic retinopathy in high group was distributed as follows: R0 61.6%, R1 8.9%, R2 21.1%, R3 8.9%. Those in the diabetic patients without retinopathy in low group was distributed as follows: R0 46.7%, R1 26.7%, R2 13.3%, R3 13.3%. The frequency of diabetic retinopathy in low molecular group was significantly higher than that of diabetic retinopathy in high molecular group. On multiple logistic regression analysis, duration of diabetes, low molecular group is an independent risk factor for diabetic retinopathy.

Conclusion: These results provide a significant e retinopathy of a contribution of low molecular Lp(a) to the increased risk for the diabetic retinopathy.

Urinary excretion of N-acetyl-β-D-glucosaminidase is related to diabetic macroangiopathy in elderly type 2 diabetes mellitus

K. Oba, K. Okazaki, S. Sato, T. Suzuki, H. Nakano, S. Moteri. Nippon Medical School, Tokyo, Japan

Objective: To investigate whether urinary excretion of N-acetyl-β-D-glucosaminidase (NAG) is related to diabetic macroangiopathy, we compared the levels of urinary NAG between ischemic heart disease (IHD) group and non-IHD group in elderly patients (≥60 years old) with type 2 diabetes mellitus.

Methods: The elderly patients (n = 88) without clinical proteinuria were enrolled in the present study. The subjects were divided into 2 groups, IHD group (n = 23) and non-IHD group (n = 75), according to findings of ischemic changes on electrocardiogram. Urinary NAG activity was measured spectrophotometrically with N-acetyl-β-D-glucosaminide as substrate (NAG test Shionogi). Ratios of urinary NAG (NAG index) and albumin (Albunin index) to urinary creatinine were calculated from random urine samples collected on two or more separate occasions within three months.

Results: Mean age and duration of diabetes were significantly higher in IHD group than in non-IHD group. The level of urinary NAG index in IHD group was 11.5 ± 6.5 (mean ± SD) higher than that of non-IHD group (7.8 ± 3.4). There was no significance in the levels of urinary Albumin index and HbAlc between two groups. Urinary NAG index was identified as independent risk factor for IHD by multivariate logistic regression analysis.

Conclusion: A elevation of urinary NAG excretion in elderly diabetic patients is a clinical proteinuria may predict future development of diabetic macroangiopathy.

Plasma triglyceride levels decrease in NIDDM subjects when consuming high fat, low carbohydrate intakes

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Objective: To examine the effects of an increase in fat and decrease in carbohydrate intake on plasma triglyceride levels and VLDL triglyceride synthesis in normal weight subjects.

Methods: Patients treated for NIDDM and control subjects were examined after 3 dietary treatment periods of 3 months each. Control subjects were gender and age-matched (+2 yrs) to this group of NIDDM individuals. Treatment periods 1 and 3 required consumption of a high CHO (>50%) and low fat (<30% energy) diet. Treatment period 2 required increase in monounsaturated fat intake of up to 10%. The energy content of treatment periods were 1, 2 and 3. During the last week of each treatment period, a 7-day food record was collected. During the last day of this dietary record D20 was consumed to measure VLDL TG synthesis over a 24 hr. period. Fasting blood samples were collected for measurement of blood lipids, glycemic control and deuterium incorporation into the VLDL TG.

Results: Results indicate a reduction in plasma TG level for subjects achieving the high fat, low CHO intake in comparison to the treatment period providing a high carbohydrate low fat intake.

Conclusion: Increasing the fat level with monounsaturated fatty acid, in NIDDM subjects consuming a high CHO, low fat diet reduces plasma TG levels.

Preheparin lipoprotein lipase mass might be reflecting the amount of produced lipoprotein lipase in the whole body

K. Koida1 T. Oyama1 T. Murano2 H. Watanabe2 Y. Miyashita1 M. Totuka1 K. Shirai1 1Internal Medicine 2Clinical Laboratory Medicine, Sakura Hospital, Toho university, Chiba, Japan

Objective: The role and clinical significance of lipoprotein lipase mass in serum before heparin injection (preheparin LPL mass) was studied.

Subjects and Method: Subjects were 58 hyperlipidemic patients and 422 normal lipid group. Type 2 diabetes mellitus patients were 58, LPL enhancers, triglyceride and bezafibrate were administered to 31 type 2 DM and 40 hypertriglyceridemia respectively. Preheparin LPL mass was measured using specific monoclonal antibody with ELISA (Daichi Kagaku Ltd).

Results: In hyperlipidemias, preheparin LPL mass in IV and IIb were significantly lower than that of control. In type 2 diabetes mellitus, preheparin LPL mass was significantly lower than non diabetic controls. HbA1c was negatively correlated with preheparin LPL mass. An insulin-sensitizer tritigatione administration to type 2 diabetes mellitus patients enhanced preheparin LPL mass and HDL-cholesterol, whereas HbA1c and triglyceride decreased. Bezaflbrate administration to type IIb or IV decreased triglyceride and increased HDL-cholesterol and preheparin LPL mass.

Conclusion: These results suggested that preheparin LPL mass can be an indicator of the produced amount and LPL in whole body.
MoT11:W1  Postprandial increase in atherogenic remnant-lipoprotein in type 2 diabetes mellitus with fasting normolipidemia
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Objective: An abnormal postprandial increase in remnant-lipoprotein is recently noticed to be an important risk factor for atherosclerosis in type 2 diabetes mellitus (DM) with fasting normolipidemia. The ratio of insulin content in peripheral vessel to that in portal one may be higher in being treated with insulin than with sulfonylurea (SU). The purpose of this study is to clarify if the postprandial increase in remnant-lipoprotein is associated with the difference between insulin-action to peripheral adipose tissue and that to liver by the diabetic treatments.

Methods: A physiological test-meal was given to 8 insulin-treated inpatients and 9 SU-treated ones of type 2 DM with fasting normolipidemia. Triglyceride and cholesterol of remnant-like lipoprotein (RLP-TG and -C) in serum before and 1, 2, 4 hours after giving the test-meal were measured by using immunoaffinity-gels.

Results: Fasting and postprandial (1, 2 h) levels of RLP-TG and the area under the curve of this (AUC-RLP-TG) were significantly higher in SU-treated group than in insulin-treated group. There was no significant difference in fasting and postprandial levels of RLP-C between the two groups. Fasting serum TG level was significantly higher in SU-treated group than in insulin-treated group. Serum total cholesterol, HDL-C and fasting plasma glucose levels were almost the same between the two groups.

Conclusions: These results indicate that an abnormal postprandial increase in chylomicron-remnant shown as the change of RLP-TG might be associated with the difference between insulin-action to the two organs by the diabetic treatments in type 2 DM patients with fasting normolipidemia.

MoT12:W1  7-ketocholesterol-induced apoptosis of vascular smooth muscle cells enhanced under diabetic condition
Y. Miyahata1, T. Oyama1, M. Totsuka2, H. Watanabe2, K. Shin2.
1Department of Internal Medicine, 2Clinical Laboratory Medicine, Sakakibara Hospital, School of Medicine, Toho University, Chiba, Japan

Objective: We reported that oxysterols induce apoptosis in vascular smooth muscle cells (VSMCs). In this time, oxysterol sensitivity of VSMCs under diabetic condition and this mechanism were studied.

Method: VSMCs of OLETF and LETO rat, and 7-ketocholesterol (7-keto) as oxysterols were used. The cell proliferation was evaluated by the outgrowth ratio and the cell number. The amount of fragmented DNA were measured by ELISA. c-myc expression was analyzed by Western blotting.

Results: In OLETF’s VSMCs, the outgrowth ratio and an increase in cell numbers were higher than in LETO’s VSMCs. By the addition of 7-keto (100 μM), the fragmented DNA of OLETF’s VSMCs increased significantly compared with LETO’s one. The expression of c-myc was enhanced with the addition of 7-keto (100 μM). The expression of c-myc in OLETF’s VSMCs was much higher than that of LETO’s.

Conclusion: These results suggested that in VSMCs under diabetic condition, the ability of proliferation raised and 7-keto-induced apoptosis enhanced. The excess c-myc expression might be involved in 7-ketocholesterol-induced apoptosis.

MoT13:W1  Structural and antioxidant properties of serum albumin altered by glycation and oxidation
E. Bourdon, N. Loreau, D. Blanche.1 INSERM U498, Biochimie des lipoprotéines; 2Université de Bourgogne, 71, bd Jeanne d’Arc, Dijon, France

Objectives: Reduced levels of serum albumin, the most abundant protein in the plasma, are consistently associated with an increased mortality risk. Various biological properties evidenced by direct effects of the albumin molecule may explain its antiatherogenic effects. The present work investigated whether in vitro glycation or oxidation would affect the antioxidant properties of bovine serum albumin (BSA).

Methods: Glycation was performed by long term incubations (60 days) of BSA with increasing concentrations of glucose at 37°C. Minimally oxidised BSA was obtained after controlled incubations of dialysed BSA samples with an azo-type generator of free radicals. Albumin specifically modified on sulphur containing residues was also studied.

Results: Native BSA presented antioxidant activities by significantly inhibiting copper-mediated LDL oxidation and free radical-induced hemolysis.

MoT14:W1  Lipid lowering therapy is advisable in more than half type 2 diabetic patients in fairly good metabolic control
A. Torri1, A. Branci, 2C. Berri1, E. Dalla Valle1, D. Sommariva1.
1Department of Internal Medicine, G. Salvini Hospital, Carimate Milanese, 2Department of Internal Medicine, University of Milan, Maggiore Hospital IRCCS, Milan, Italy

Objective: According to the recent guidelines of the American Diabetes Association (ADA), lipid lowering drug therapy should be initiated when LDL cholesterol (LDL-C) level remains >100 mg/dl in type 2 diabetes with cardiovascular disease (CVD) after optimization of diabetic therapy and behavioral intervention aimed to control dyslipidemia and obesity. LDL-C > 130 mg/dl is the initiation level of drug therapy in subjects without CVD.

Methods: The CVD risk profile was evaluated in 624 type 2 diabetic patients (296 females, 328 males). Their ages ranged from 30 to 75 years (mean 59 ± 4.0 years). Serum triglycerides (TG) were higher than 400 mg/dl in 24 patients and in them LDL-C (Friedewald equation) was not calculated.

Results: 542 of 624 patients were in fairly good metabolic conditions. Of them, 74 (14%) had CVD. Of CVD patients, 55 (74%) had LDL-C > 100 mg/dl and of non CVD patients, 240 (51%) had LDL-C > 130 mg/dl. Of the remaining 228 patients, 27 had serum TG > 400 mg/dl and 92 had other risk factors such as low HDL-C, hypertension and smoking. Of the 82 patients in bad glycemic control, 8 (10%) had CVD (5 with LDL-C > 100 mg/dl) and 74 did not have CVD (55% with LDL-C > 130 and 3% with TG > 400 mg/dl).

Conclusions: 54% of diabetic patients in fairly good diabetic control need cholesterol lowering therapy. Since ADA recommendations include high TG levels as a target for intervention, further 22 (4%) patients must be treated with lipid lowering agent. According to some authorities, the initiation level of drug therapy is LDL-C > 100 mg/dl when other coronary risk factors are present. This should lead to 409 (75%) the number of diabetic patients in fairly good diabetic control requiring a lipid lowering therapy.

TW2 THROMBOSIS AND FIBRINOLYSIS

MoT11:W1  Plasmin generation reduced in hyperterglyceridemia, but enhanced in low HDL cholesterol
Akito Kawaguchi, Hiwasato Kato, Akira Yamamoto. National Cardiovascular Center, Osaka, Japan

Impaired fibrinolysis frequently associated with hyperterglyceridemia (HTG) accompanied by low HDL cholesterol (HDL-C) plays a central role in onset of coronary events. We assessed the independent contribution of HTG and low HDL-C to plasmin generation as the final response of fibrinolytic system.

Methods: 161 patients with coronary artery disease (CAD) were studied on serum lipids, plasmin/alpha-2 plasmin inhibitor complex (PIC) as a marker of plasmin generation, PAI-1 and tPA antigens as regulators of fibrinolytic system. In addition, post-heparin plasma was obtained to estimate endothelial free-TFPI as an anti-thrombotic property of vessel wall. To evaluate the independent significance of TG and HDL-C, the patients were divided into four categories (2 x 2 factorial groups) according to the presence or not of HTG (>130 mg/dl) and/or low HDL-C (<35 mg/dl) and compared the fibrinolytic profile by 2-way ANOVA and a partial correlation coefficient analysis.

Results: PAI-1 is proportional to tPA levels, and both PAI-1 and tPA are negatively correlated to PIC. The patients with HTG showed increased tPA and PAI-1, but reduced PIC. On the other hand, the patients with low HDL-C showed increased PIC independent tPA level and increased heparin-releasable free TFPI.
Conclusion: Plasmin generation is reduced in the patients with HTG, although TG is positively correlated with PAI-1 and tPA. Increased iPA level proportional to PAI-1 is interpreted as a compensatory augmentation for inhibitory effect of plasmin conversion by PAI-1. On the contrary, the patients with low HDLc have enhanced plasmin generation independent of iPA and increased free-TFF1. Enhanced fibrinolytic activity and anti-thrombotic property in low HDLc suggests compensatory responses against atherothrombotic milieu derived from decreased vascular protective function by HDL particle.

MoT2-W2 Relationship between hemostasis and hyperhomocysteinemia induced by methionine loading in vitamin B6 and folate deficiency
S.-J. Chang, C.Y. Liao, Y.-C. Li. Department of Biology, National Cheng Kung University, Tainan, Taiwan, ROC

Background: There is increasing evidence that hyperhomocysteinemia (HHcy) is an independent risk factor for thrombosis. The mechanism that increased tendency of thrombosis associated with HHcy is not clear. Imbalance of hemostatic system may contribute to thrombosis.

Objective: To measure plasma concentration of homocysteine (Hcy) and investigate the relationship between Hcy level and clotting factors in vitamin B6 and folate deficient rats loaded with methionine (Met).

Methods: Sprague-Dawley rats fed with control (CD), vitamin B6 deficient (B6D), folate deficient (FD) or vitamin B6 plus folate deficient (B6FD) diet were injected with ethyl thiolate (0.9%) or Met (0.1 g/kg body weight) and blood sample was collected 2 hours thereafter. Plasma Hcy concentration was quantified by HPLC after derivatization with SBD-F. Clotting factors including antithrombin III (AT III) protein C (PC) (plasminogen (PLG) and plasma concentration of heparin were determined by a chromogenic assay with a chromogenic kinetic system device.

Results: Plasma Hcy concentrations of B6D FD and B6FD rats were significantly higher than that of CD rats (B6D = 25.17 ± 4.38 μM, FD = 24.96 ± 4.08 μM, B6FD = 33.33 ± 1.33 μM, CD = 3.41 ± 0.23 μM, p < 0.01). Plasma Hcy concentrations were negatively correlated to AT III (r = 0.09, p < 0.05) PC (r = 0.09, p < 0.05) and PLG levels (r = 0.38, p < 0.05). However, no correlation between plasma Hcy concentration and heparin level was found.

Conclusions: We suggest that HHcy induced by Met alters plasma concentration of clotting factors and may contribute to the imbalance of hemostasis in vitamin B6 and folate deficient status.

MoT3-W2 Increased prevalence of haemostatic abnormalities and osteoarthritis (OA) in a young cohort of patients with coronary heart disease (CHD) and hyperlipidemia
D.M. Cologhun1, E.A. Chernis1, G.V.L. Nielsen1, P. Dubois1, H.K. Outerbridge2, D. Battistutta1, 1Greenhouses Hospital; 2Core Research; 3QUT, Brisbane, Australia

Objective: To investigate associations between CHD and OA.

Method: Patients (pts) with mixed hyperlipidemia (MH) and CHD were compared with a frequency and age matched control group (C) with no clinical signs of OA and a group of pts with X-ray confirmed OA but without clinical CHD. 20 pts, mean age 47 years (22–59) with MH and CHD were enrolled. These were compared with 23 OA pts and 29 volunteer controls. Presence or absence of OA in the MH/CHD group was based on X-rays of the hips and knees read by two blinded researchers. Fasting bloods were analyzed for haemostatic factors. Ordinal logistic regression modelling assessed associations after adjustment for residual age and sex effects.

Results: 13 of 20 CHD pts were X-ray positive for OA.

<table>
<thead>
<tr>
<th>Test</th>
<th>C</th>
<th>OA</th>
<th>CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>24.6</td>
<td>27.8</td>
<td>29**</td>
</tr>
<tr>
<td>HDL</td>
<td>121</td>
<td>126</td>
<td>122**</td>
</tr>
<tr>
<td>HDLc</td>
<td>145</td>
<td>167*</td>
<td>205*</td>
</tr>
<tr>
<td>TC</td>
<td>77</td>
<td>83***</td>
<td>83*</td>
</tr>
<tr>
<td>CHOL</td>
<td>4.9</td>
<td>5.75*</td>
<td>8.20***</td>
</tr>
<tr>
<td>HOMOC</td>
<td>3.25</td>
<td>3.73</td>
<td>5.36*</td>
</tr>
<tr>
<td>TG</td>
<td>0.93</td>
<td>1.51***</td>
<td>4.37***</td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.01; ***p < 0.001

Conclusions: OA in the MH/CHD group is significantly more prevalent than expected. A strong association exists between hyperlipidemia and haemostatic risk factors in CHD pts. These haemostatic risk factors are also associated with OA in non-CHD pts.

MoT4-W2 Thrombogenic risk factors in patients with thrombangiitis obliterans
M. Brodman, W. Renner, G. Stark, M. Winkler, E. Pabst, C. Hofmann, H. Köppel, E. Pilger. Division of Angiology, Department of Internal Medicine, University Hospital Graz, Austria

Objective: Thrombangiitis obliterans (TAO) is a segmental inflammatory occlusive disease which primarily affects small or medium sized arteries and veins in the extremities of young adults who have a history of heavy smoking. As it is a fact in histological findings in arteries of patients with TAO that the segmental inflammatory process in the vessel wall is accompanied by a thrombotic occlusion in the arteries, we saw the purpose of our study to evaluate if prothrombotic risk factors can be found in patients suffering from TAO at a higher level than in a control group lacking deep venous thrombosis or arterial disease.

Methods: 28 patients (17 male and 11 female) with a history of TAO were enrolled in our study. All patients were evaluated for the following thrombogenic risk factors: antithrombin III, protein C, protein S, cardilipin antibodies, lupus anticoagulants, factor V Leiden (F5 1691A), Prothrombin 20210 A (F2 20210A) and Factor XIII Val34Leu (F13 1007T).

Results: F2 20210A alleles were more frequent but not statistically significant among patients than among controls (7.1% versus 3.4%). None of the patients had a homozygous status of F2 20210A mutation.

F5 1691A alleles tended to be less frequent among patients than among controls (3.6% versus 9.4%), but did not reach significance. None of the patients had a homozygous status of F5 1691A mutation.

F13 1007T alleles were equally frequent for the heterozygous status among patients and controls (39.6% versus 38.3%) but showed a trend of being more frequent in the homozygous status in the patients group than in the control group. (10.8% versus 7.1%) The difference was not statistically significant.

None of the evaluated patients showed either a deficiency of antithrombin III, protein C and S, or elevated cardilipin antibodies IgM and IgG or a lupus anticoagulant.

Conclusions: Out of the presented data it is possible to conclude that prothrombotic risk factors are not involved in the pathogenesis of TAO.

MoT5-W2 Effects of atorvastatin on fibrinolytic system in patients with familial hypercholesterolemia
L. Paccio, S. Bandinelli, D. Lucchesi, F. Caricato, R. Navalevi, A. Bertolotto, G. Penno. Endocrinology and Metabolism, Pisa, Italy

Objective: Fibrinolysis is mainly regulated by a balance between tPA and PAI-1. Decreased fibrinolysis is linked with increased PAI-1 and tPA antigen levels, the last reflecting mainly tPA/PAI-1 complexes. We aimed to evaluate the effects of atorvastatin on plasma tPA and PAI-1 antigen levels.

Methods: Twenty-two non-diabetic patients with familial hypercholesterolemia (FH; 14 M, 8 F; 49 ± 7.8 years-old; BMI 25.2 ± 3.2 kg/m²) discontinued their lipid lowering drugs for at least 6 weeks. At baseline, after 1 month (atorvastatin 20 mg/day) and 2 months (40 mg/day), fasting lipids, tPA, PAI-1 Ag, tPA-1 Ag (TintelEtt, PAI and TintElyze PAI-1; Biopool, Sweden) were measured. Twenty-two healthy subjects acted as controls. ANOVA for repeated measures was used to study the effects on lipids, tPA and PAI-1 Ag levels.

Results: Atorvastatin reduced total-cholesterol by 39% (373 ± 44 mg/dl at baseline vs 250 ± 30 and 229 ± 30 mg/dl after 4 and 24 weeks, respectively, p < 0.0001); LDL by 46% (296 ± 45 vs 181 ± 33 and 159 ± 33 mg/dl, p < 0.0001); Apo-B by 39% (220 ± 30 vs 155 ± 25 and 134 ± 26 mg/dl, p < 0.0001) and triglycerides by 23% (136 ± 58 vs 104 ± 45 and 101 ± 45 mg/dl, p < 0.0005). No changes in HDL (50 ± 11, 49 ± 11 and 50 ± 11 mg/dl) and apo-A1 (141 ± 19, 140 ± 26 and 146 ± 27 mg/dl) were observed. Baseline tPA and PAI-1 Ag levels were higher in FH than in controls (tPA 9.4 ± 4.5 vs 7.3 ± 2.8 mg/nl, p = 0.019; PAI-1 28.9 ± 15.4 vs 19.4 ± 14.6 mg/nl, p = 0.032). tPA and PAI-1 levels did not change after 4 weeks of therapy (tPA 10.9 ± 5 mg/nl; PAI-1 25.9 ± 16.1 mg/nl) while significantly reduced themselves after 24 weeks (tPA 7.7 ± 3.7 mg/nl, p = 0.05; PAW 19.6 ± 8.6 mg/nl, p = 0.037).

Conclusions: Atorvastatin, an highly effective drug in reducing LDL in FH, seems to have a promising role in regulating fibrinolysis.
T:W3 IMAGING OF ATHEROSCLEROSIS

MoT1:W3 Multimodal imaging of human atherosclerosis and its complications
A. Ansari. Department of Cardiovascular Medicine, Abbott Northwestern Hospital, Minneapolis, Minnesota, USA

In order to systematically study atherosclerosis (Ath), there is need of imaging modalities which are reproducible and amenable to serial longitudinal studies at reasonable cost. The various imaging modalities currently in vogue to image Ath are divided into three broad categories: 1) Noninvasive, e.g. ultrasound (US), magnetic resonance imaging (MRI), magnetic resonance angiography (MRA), computerized axial tomography (CAT), and electron beam computed tomography (EBCT); 2) Semi-invasive, e.g. transesophageal ultrasound (TEE); and 3) Invasive, e.g. contrast angiography and endovascular ultrasound. Invasive modalities are not well suited for longitudinal and epidemiological studies because of high costs, inherent complications, and radiation exposure, whereas noninvasive modalities are best suited for that purpose but at present are not cost-effective except for US. Imaging of Ath and its complications (thrombus, ulceration, dissection, and aneurysmal dilatation) by various modalities is illustrated with clinical correlation, and the need to develop a low-cost but high-quality noninvasive modality to study the progression and regression is emphasized.

MoT2:W3 Ischemic heart disease and cerebrovascular disorders in patients with high HDL blood level and with low HDL, normal cholesterol and triglycerides
B. Lipovetsky. Institute of the Human Brain, St.-Petersburg, Russia

Objective: presentation of unconventional evidence obtained from the patients with ischemic heart disease (IHD) or cerebrovascular disorders (CVD) who showed a high HDL blood level or low HDL blood without the increased cholesterol (C) and triglycerides (TG).

Methods: Blood lipids were determined in the biochemical department of the Institute for Experimental Medicine by the semiautomatic standardized method. 25 patients under 65 with IHD or CVD were examined: 10 patients with HDL-C blood level > 60 mg/dl (group 1), 15 patients with HDL-C < 36, normal total C (TC) and TG (group 2). The atherogenic index (AI) was calculated as a ratio between the sum of VLDL-C and LDL-C to HDL-C. None of the patients (PS) had suffered from arterial hypertension of diabetes mellitus.

Results: The blood lipids (mg/dl) for both groups of patients are presented in the table.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PS</th>
<th>age</th>
<th>TC</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>57 ± 5</td>
<td>286 ± 23</td>
<td>185 ± 22</td>
<td>72 ± 3.0</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>49 ± 3</td>
<td>198 ± 50</td>
<td>142 ± 10</td>
<td>29 ± 1.5</td>
<td>6 ± 0.3</td>
</tr>
</tbody>
</table>

TG blood level of both groups was <150.

Group 1 (4 men, 6 women) included 8 PS with IHD (2 PS suffered myocardial infarction (MI)) and 2 PS with CVD (one of them survived both MI and a stroke). Group 2 (14 men, 1 woman) consisted of 8 PS who had had MI and 7 PS a stroke.

Conclusion: It has been established that the high HDL-C blood level are do occur in the PS with MI or CVD. This suggests that the HDL is characterized by some structural and functional defect. What concerns the PS with low HDL blood level without hyperlipidemia they ordinarily suffered from IHD as well as from CVD.

MoT3:W3 Evaluation of aortic atherosclerosis by transesophageal echocardiography
P. Exzedes1, C. Corrim1, L.M. Oliveira1, M. Carrageta1. 1 Hospital Garcia de Orta, Servico de Cardiologia, Almada, Portugal

Objective: to image the thoracic aorta by transesophageal echocardiography (TEE) and study the atherosclerosis (morphology and extent of atheroma plaques) and atherosclerosis (stiffness) and secondarily correlate them with serum lipid levels (cholesterol, HDL, LDL and triglycerides).

Methods: we studied 29 patients (pts) who underwent TEE electively (male 18 pts, age 57.8 ± 14.6). The parameters to evaluate were: the stiffness coefficient = (vAIAvAIPADiastr)/(Dyso/Dmni), the morphology, location and extent of atheroma plaques. The systolic distension (Dyso) was the difference between the maximal and the minimal dimensions (Dmni) of the aortic diameter measured by M mode. The lesions were classified in 4 degrees (0–3): 0-normal intima, 1-intimal thickening, 2-atheroma, 3-complicated lesion. Five aortic segments were studied: arch, D1-D4 (descending aorta at 5 cm intervals from the first 25 cm distal of the incisors line). We calculated the individual score

\[ S = \frac{1}{3} \times (angA/180 + 2 \times angB/180 + 3 \times angC/180, angA, represents the angles occupied by the lesions and n (1–3) the severeness of atherosclerosis of each lesion.

The total atherosclerosis index (TAI) was Scscore

\[ TAI = 0.82 \pm 0.74, \text{ stiffness coefficient mean (SC)} = 9.56 \pm 15.72. \]

There were no significant correlations between the lipid levels and the TAI or SC. The only significant positive correlations were: TAI vs age (r = 0.62, p = 0.000) and SC and diastolic blood pressure (BP) (r = 0.42, p = 0.000).

Conclusions: The best visualized segments belong to the descending aorta (25 to 40 cm from the incisors). In this group of patients the lipid levels didn’t seem to be a preponderant factor in aortic atherosclerosis. The most important factors were age for atherosclerosis and BP for sclerosis.

MoT4:W3 Age-related changes in lipid composition of platelets' membranes as a risk factor of development and progression of atherosclerosis in elderly people
O.V. Korkushko, K.G. Sarkisov, Y.Yu. Lishchevskaya, T.J. Gorbach. Institute of Gerontology, AMS of Ukraine, Kiev; Kharkov Medical University, Kharkov, Ukraine

Objective: Some prerequisites for progression of atherosclerosis are known to exist in an aging organism. As it has been shown by us earlier in another study, an increase in platelets' aggregation activity in elderly people is one of leading risk factors of this process. The membrane mechanism from our point of view is one of primary mechanisms of platelets' homeostasis destabilization. The aim of present investigation was to study the changes in platelets' aggregation activity as well as the relationship between the platelets' functional state and lipid composition of their membrane in aging.

Methods: Toward this end in view a group of practically healthy subjects aged 60–70 and a group of their younger counterparts aged 20–29 were studied using the methods of aggregometry and thin-layer liquid chromatography for assessment of platelets' functional state and the lipid composition of platelets' membrane, respectively.

Results: The results obtained showed an age-related increase in platelets' aggregation activity as evidenced by the decrease in latent period of adrenaline-induced aggregation with augmentation in rate and intensity of the process. The lipid composition of platelets' membrane in people of higher age group experienced also pronounced changes which included a significant increase in cholesterol, triglycerides, lysosphatidylcholines, and phosphatidylinositolsides with a decrease in total phospholipids and phosphatidylethanolamines. The results of correlation analysis testify to the relationship of an increase in platelets' aggregation activity to changes in lipid composition of their membranes.

Conclusions: The changes in lipid composition of the platelets' membrane result in an increase of platelets' aggregation activity and therefore they are one of the risk factors and a cause of frequent development of atherosclerosis, thrombosis, and thromboembolic complications.

MoT5:W3 Elevated homocysteine levels in patients qualified for coronary bypass surgery
H. Bukowska, M. Brykczynski, S.Wiechowski, K. Chelstowski, M. Naruszewska. Pomeranian Medical University, Szczecin, Poland

Objective: To study the frequency of elevated homocysteine levels in patients qualified for surgical treatment of ischemic heart disease.

Methods: 155 patients aged 35–77 years with angiographic diagnosis of coronary heart disease were qualified for bypass surgery. Serum levels of homocysteine (HCY), fibrinogen (FB), lipoprotein (a) (LP(a)), triglycerides (TG), total (TCH), LDL (LDL-CH) and HDL cholesterol (HDL-CH) were measured preoperatively.

Results: The following mean values were obtained: HCY: 15.5 ± 5.6 \( \mu \text{mol/l} \); FB: 311 ± 71 mg/dl; LP(a): 36 ± 37 mg/dl; TG: 154 ± 70 mg/dl; TCH: 215 ± 41 mg/dl; LDL-CH: 143 ± 40 mg/dl; HDL-CH: 37 ± 8. Homocysteine levels exceeded normal limit of 14 \( \mu \text{mol/l} \) in 87 patients (56.2%). A positive correlation was noted between homocysteine and LDL cholesterol concentrations.
Conclusion: High incidence of moderately elevated homocysteine levels and correlation of homocysteine with LDL cholesterol seem to reflect marked consumption of animal fat and possibly a high methionine content, with a simultaneous shortage of food containing folic acid, vitamins B6 and B12 necessary for the catabolism of homocysteine.

T:W4 INFECTIONS, CHD, AND ATHEROSCLEROSIS

MoT1:W4 Chlamydia pneumoniae and cytomegalovirus-positive immune complexes and acute phase proteins in stroke
G. Gromadzka, B. Tarnacka, A. Czlonkowska. Institute of Psychiatry and Neurology; Sobieskiej 1/9, 02-957 Warsaw, Poland

Objective: The inflammatory processes concomitant by acute phase proteins (A-PP) elevation can initiate or amplify the development of atherosclerotic lesions leading to acute vascular incidents. A strong association between chronic infections and atherosclerosis has been observed. Cellular and humoral immune reactions are important link between infection, inflammation and vascular lesions but the exact mechanism remains unclear.

Material and Methods: We investigated: the concentration of circulating immune complexes (CIC) (precipitation method), the Chlamydia pneumoniae and CMV- specificity of CIC (ELISA method), and the levels of A-PP: CRP and fibrinogen (Fb) (nephelometric method, Claus clotting assay, respectively) in the sera of ischemic stroke patients (at the 1st day after stroke onset) and healthy age-matched control group.

Results: The mean serum concentration of CIC was significantly higher in patients’ than in control group (0.191 vs. 0.098). The prevalence of positive anti-CMV IgG titers was found in CIC of 69.4% patients and 11.3% controls. Cep antigen was detected in CIC of 52.2% patients and 27.1% controls. The mean serum level of CRP was significantly higher in patients compared with controls (7.70 mg/L, 2.41 mg/L, respectively). The Fb levels were also significantly higher in patients’ than in control group (341 mg/L vs. 291 mg/L).

Conclusions: Observed in our study Cep and CMV positive CIC could maintain the development of vascular lesions by endothelial injury, complement system pathway activation, atherosclerotic plaque destabilization. Acute phase proteins which concentrations were parallel with increased levels of Cep and CMV-positive CIC could maintain atherogenesis, too. Different mechanisms are possible as elucidation of inflammation – atherosclerosis – acute vascular events pathway. Further investigations are needed.

MoT2:W4 Severe multivessel coronary disease in young patients with HIV infection following protease inhibitors therapy

Objectives: We describe two young patients with HIV infection and prolonged antiretroviral treatment including protease inhibitors (PI), which developed severe multivessel coronary heart disease demonstrated clinically and through coronary angiography.

Case Reports: Two young men, aged 33 and 34, diagnosed of HIV infection ten years ago and following antiretroviral therapy with a mean duration of thirty months, developed myocardial infarction (one as an inferior as the other as a large anterior infarction). Cardiac catheterization demonstrated severe three vessel coronary lesions and systolic dysfunction (in one case with ejection fraction lower than twenty per cent). Both showed low viral loads and smoking habit was the only known coronary risk factor. During the treatment with PI, both patients showed increased levels of total serum cholesterol as well as LDL fraction.

Conclusions: Patients with HIV infection seem to have a higher risk for coronary disease adjusted to their age. PI could play an important role rising lipid serum levels or perhaps by an unknown mechanism. Survival prognosis of these patients has improved with antiretroviral treatment, but in some cases, as the ones we present in this report, could be marked by coronary heart disease instead of their infectious disease.

It is prior to design prospective studies to establish the need of a diagnosis and therapeutic approaches in these patients.

T:W5 GROWTH FACTORS, CYTOKINES, AND ATHEROSCLEROSIS

MoT1:W5 Growth factor influence on the vascular wall neoangiogenesis
I. Mikadze. Laboratory of Vascular Transplantation, Research Institute of Surgery, Tbilisi, Georgia

Objectives: To analyze the role of endothelial cell growth factor in the vessel wall organization.

Materials: Three group of experiments were performed: 24 mongrel dogs endarterectomy taken from carotid artery and endothelial cell growth factor was elaborated; 71 grafting of abdominal aorta PTFE prosthesis elaborated by endothelial cell growth factor in dogs; vessel wall (vein, aorta) application on the external surface of prosthesis after abdominal aorta grafting in dogs, 21 experiments. In the first two groups concentration of endothelial cell growth factor consisted 0.05–0.0002 mg/cm² vessel surface.

Results: Tissue ingrowth of substitution’s wall had more neointimal hyperplasia than endarterectomy vessel wall. It was noted that applied vessel’s hyperplasia on the substitution wall was more in the using part of aorta versus vein application. In the both event the same endothelial cells migration on the luminal surface was noted. After endothelial cell growth factor elaborated of vascular prosthesis in concentration 0.0002 mg/cm² did not have hyperplasia of neointimal cells.

Conclusions: High concentration of growth factor develops of neointimal hyperplasia. Over concentration of growth factor is not leading in the miointimal hyperplasia reasons.

MoT2:W5 Homocysteine stimulates monocyte chemotaxis towards endothelial cells
F. Sung. Y. Siow, K. O. The University of Hong Kong, Hong Kong SAR, China

Objectives: To study the effect of homocysteine on MCP-1 mRNA levels and MCP-1 protein secretion by endothelial cells and subsequent monocyte chemotaxis towards endothelial cells.

Methods: The effect of homocysteine on MCP-1 mRNA levels and MCP-1 protein secretion by endothelial cells were investigated by RT-PCR analysis and ELISA respectively. Monocyte chemotaxis towards conditioned medium from endothelial cell culture was studied by 48-well chemotaxis chamber.

Results: Homocysteine increased MCP-1 mRNA levels and protein secretion by endothelial cells. Monocyte chemotaxis towards conditioned medium collected from homocysteine-treated endothelial cells was higher than those from untreated endothelial cells.

Conclusions: Homocysteine stimulates MCP-1 expression by endothelial cells and subsequent monocyte migration towards endothelial cells. This may partly explain the mechanism underlying the association between hyperhomo- cysteinemia patients and atherosclerosis.

MoT3:W5 Soluble intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in dyslipidemic patients
G. Nowicka, A. Jarosz, M. Kozłowska-Wojciechowska, National Food and Nutrition Institute, Warsaw, Poland

Cell adhesion molecules are likely to play an important role in the pathogenesis of atherosclerosis. Elevated levels of ICAM-1 in men with coronary heart disease have been reported and elevated ICAM-1 appeared as a myocardial infarction risk factor. In the present study plasma concentrations of soluble forms of ICAM-1 and VCAM-1, and levels of fibrinogen, tPA and PAI-1 were measured in the group of 161 dyslipidemic patients: 89 women (25 with documented CHD) and 72 men (26 with documented CHD). Among women with documented CHD significantly elevated levels of ICAM-1 and VCAM-1 were found, while among men with CHD only VCAM-1 levels were significantly higher as compared to patient group without CHD symptoms. Men with documented CHD had also significantly elevated tPA and PAI-1 levels. No differences in serum cholesterol, LDL-cholesterol and triglycerides between studied patient groups were observed. However, both men and women with the history of CHD had significantly enhanced serum apo B levels, in men also lower apo A1 levels and in women higher frequency of elevated Lp(a) were found. Our results indicate that non-traditional risk markers can

be helpful in risk assessment in dyslipidemic patients, however further studies are needed to develop proper diagnostic strategy.

**MoT4:W5**
Polymorphisms at the promoter region of the CTLA-4 gene are associated with Wegener’s granulomatosis in a Swedish population
R. Giscombe, XiongBiao Wang, Ann Kari Lefvert. Immunological Research Unit CMM, Karolinska Institutet, Stockholm, Sweden

**Objective:** To analyse genetic associations to the CTLA-4 gene in WG

**Methods:** The CTLA-4 gene polymorphism at the promoter region position −318 (CT) and in exon 1 at position +49 (A/G) was determined by PCR based methods. We examined 52 Swedish WG patients and 122 ethnically matched healthy controls.

**Results:** The genotype C/C decreased (69% vs. 86%) and that of C/T increased (31% vs 14%) in patients with Wegener’s granulomatosis when compared with the controls (p = 0.0344). There were no significant differences in the axmon 1 gene polymorphism between the patients and controls.

**Conclusions:** The CT polymorphism in the promoter region of CTLA-4 gene is associated with WG whereas the A/G polymorphism in exon 1 is not.

**MoT5:W5**
Production of specific antibodies to the long platelet-derived growth factor (PDGF) AA isoform
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1Wallenberg Laboratory, Sahlgrenska Universitystjukhuset, Göteborg, Sweden; 2Dept. of Cellular Biology, Univ. of Barcelona, Barcelona, Spain; 3UPR9021 du CNRS, I.B.M.C., Strasbourg, France

**Introduction:** Atherosclerotic process leads to narrowing of the lumen partly due to accumulation of glycosaminoglycans (GAGs) and smooth muscle cells (SMC) in the arterial wall. PDGF, a well recognized mitogen for SMC, is a dimer consisting of A and/or B chains, both present as long and short isoforms. The long dimers (AA and BB) bind with high affinity to GAGs. This kind of interaction may explain the modification of proliferation of SMC during atherosclerosis.

**Objective:** To produce specific antibodies to the AA4 in order to estimate the amount of AA4 in atherosclerotic tissue.

**Methods and Results:** P112- (GGPREGSGKRRRKLX), corresponding to the basic extension of AA4, was used for rabbit immunization. Serum was passed through an affinity column with bound P112. Antibodies (anti P112) were eluted with 4.5 M MgCl2. Experiments in Surface Plasmon Resonance Analysis (with AA4 bound to the sensor chip and the buffer perfused with anti P112 in the absence and presence of different PDGF isoforms) showed that anti P112 had highest affinity towards the AA4, low affinity towards the BB and no affinity to the short isoforms. Similar results were obtained with dot blots. When the binding of PDGF-isoforms to SMC in culture was investigated, only AA4 was recognized by anti P112, although the binding of other isoforms was confirmed by commercially available antibodies.

**Conclusions:** These results showed that anti P112 antibodies are highly specific for PDGF-AA4 and will be further used in immunohistochemical experiments with atherosclerotic tissue.

**MoT6:W5**
Urokinase plasminogen activator in injured adventitia increases the number of myofibroblasts and augments neovessellititis growth
O. Plekhanova, V. V. Parfyonova, V. Stepanova, A. Bobik, V. Tkachuk, 
1Cardiology Research Center, Moscow, Russia; 2Baker Medical Research Institute, Melbourne, Australia

**Objective:** Adventitial fibroblasts play a critical role in restenosis, but the mechanisms regulating their differentiation and proliferation are not known. We assessed how elevations in urokinase plasminogen activator (uPA) and its proteolytic activity affect fibroblast differentiation and proliferation after injury of the rat carotid adventitia.

**Methods:** Aortic injury was inflicted surgically, and the effects of perivascular application of recombinant uPA and proteolytically inactive r-uPA (HQQ) associated 96 hours later. Differentiation of fibroblasts to myofibroblasts was assessed from the expression of alpha smooth muscle actin, proliferation by the expression of PCNA and development of the neovessellititis by quantitative morphometry.

**Results:** After the injury the frequency of alpha actin positive adventitial cells increased from 6.8 ± 1.47% in uninjured artery to 24.9 ± 6.8% (P < 0.05), after the r-uPA their frequency was 55.7 ± 10.4% (P < 0.05), while after proteolytically inactive r-uPA (HQQ) adventitial fibroblasts averaged 30 ± 8.7% (P > 0.05, from control). Recombinant uPA increased the frequency of PCNA positive cells from 11.9 ± 1% in control to 51.2 ± 6.1% (P < 0.05) and also increased the size of the neovessellititis by 207%, while uPA (HQQ) was ineffective.

**Conclusions:** We conclude that uPA, which is expressed by adventitial fibroblasts after injury can augment adventitial cell accumulation and adventitial growth early after injury to the rat carotid artery, by mechanisms involving proteolysis.

**MoT1:W6**
Efficacy and safety of a new HMG-CoA reductase inhibitor, NK-104, in patients with hypertriglyceridemia – Randomized double-blind, cross-over placebo controlled study
J. Sasakli, Y. Ikeda, K. Yamamoto, M. Ageta, K. Akakawa, 1Department of Internal Medicine, School of Medicine, Fukuoka University, Fukuoka, Japan

**Objective:** To assess the lipid-lowering effect of NK-104 (tiavastatin, a new 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor) on levels of serum triglyceride (TG) and other lipoproteins in patients with hypertriglyceridemia and safety.

**Methods:** 47 subjects of hyperlipemic patients showing serum total cholesterol (TC) of 220 mg/dl or more and TG of 150 mg/dl or more were divided into 2 groups and each received either NK-104 2 mg or placebo for first 8 weeks, and then cross-over was done for second 8 weeks. Various tests including the serum lipid were performed and the serum lipids were compared between both groups by ANOVA (analysis of variance).

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>TG (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>384.5</td>
<td>278.0</td>
<td>178.5</td>
</tr>
<tr>
<td>NK-104 [N]</td>
<td>242.8 (−20.9%)</td>
<td>203.7 (−26.1%)</td>
<td>166.2 (−35.6%)</td>
</tr>
<tr>
<td>Placebo [P]</td>
<td>362.2 (−1.0%)</td>
<td>274.3 (−0.7%)</td>
<td>183.8 (4.9%)</td>
</tr>
</tbody>
</table>

a) difference between [N] and [P], \*p = 0.01; \**p = 0.001

Significant differences were observed between both groups in the change rates of TG, TC, and LDL-C and the change in HDL-C. In addition, significant differences were also observed between both groups in apolipoprotein (apo) A-I, apo A-II, apo B, apo C-II, apo C-III, apo E, VLDL-TG, and LDL-C. No difference was observed in the LPL and HTGL activity.

There was no difference between both groups in the incidence rates of adverse reactions including laboratory abnormalities.

**MoT2:W6**
Risk evaluation and stroke prevention in the elderly – Cervastatin trial (RESPECT)
K. W. Lauterbach, T. Binnen, U. Harnischmacher, D. Ludwig, P. Hanrath, W. Krone, W. Lehmann, D. Leys, K.-L. Neuhaus, E. Windler, Univ. Cologne; RWTH Aachen; City Hospital Kassel; Univ Hamburg, Germany; Hospitat Roger Salengro, Lille, France

**Background:** The ‘Risk Evaluation and Stroke Prevention in the Elderly – Cervastatin Trial&rsquo; (RESPECT) will be the first study to evaluate whether cervastatin should also be used for primary prevention of stroke in patients who are at risk of stroke but do not have coronary heart disease. RESPECT will also examine the effect of cervastatin on cognitive decline, osteoporosis, colon cancer and kidney function.

**Study Population:** 10,000 65–80-year-old out-patients without known coronary artery or cerebrovascular disease, but with an increased risk of stroke: LDL-C 130–199 mg/dl; systolic blood pressure (BP) > 160 mmHg or diastolic BP > 95 mmHg; or receiving antihypertensive therapy.

**Methods:** RESPECT is a primary care, multi-center, randomized, double-blind, placebo-controlled, parallel-group comparison study using cervastatin (0.4/0.8 mg once daily) vs placebo. A 4-week placebo run-in phase will be followed by an 8-week treat-to-target phase (cervastatin 0.4 mg vs placebo). If a target of 120 mg/dl LDL-C is not achieved after 4 weeks, serum dose will be increased to 0.8 mg cervastatin. The minimum planned treatment time is 4 years per patient, until 548 strokes have been observed.
Primary Evaluation Parameters: First stroke event (ischemic stroke; primary intra cerebral hemorrhage; stroke unknown whether of ischemic or hemorrhagic origin). First primary cardiac event (myocardial infarction; heart failure requiring hospitalization; cardiovascular death).

Secondary Evaluation Parameters: Death for any reason; angina pectoris event; CABB/PTCA, invasive diagnostic/therapy of peripheral arterial occlusive disease; adenomatous colon poly/colorectal carcinoma event; bone fracture; development of proteinuria; change in cognitive function and further events. Cost-benefit and safety analyses will also be undertaken.

MoT3:W6 Efficacy and safety of cerivastatin 0.8 mg versus pravastatin 40 mg for treatment of dyslipidemia

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Aim: To compare efficacy and safety of cerivastatin (C) 0.8 mg/day with pravastatin (P) 40 mg/day in patients with primary hypercholesterolemia.

Methods: This was a multinational, randomized, double-blind, double-dummy, parallel group study. On entry, all patients had LDL-C ≥ 4.9 mmol/l (≥190 mg/dl) and triglycerides ≤ 3.95 mmol (≤350 mg/dl). Patients were randomized to receive C or P for 12 wks, after a 6-wk single-blind placebo run-in. Patients underwent an automated titration event (the double-blind phase: C 0.2 mg/d or P 10 mg/d for the 1st 4 wks, C 0.4 mg/d or P 20 mg/d for the 2nd 4 wks, and C 0.8 mg/d or P 40 mg/d for the 3rd 4 wks (final period).

Results: The mean % change in LDL-C from baseline to the end of the final period (the primary efficacy parameter) was: −38.9% in the intention-to-treat (ITT) cerivastatin group (n = 81) and −33.2% in the ITT P group (n = 83) (p = 0.0024). The advantage of C over P for reducing LDL-C was confirmed in the per-protocol population: −39.1% vs −33.3% (p = 0.0029). In all, 57% (n = 46) of the C group had a LDL-C decrease >40% from baseline, compared with 29% (n = 24) in the P group. Total cholesterol also decreased significantly more in the C group compared with P: −29.6% vs −25.2% (ITT) (p = 0.0026). A total of 171 patients was included in the safety analysis; 86 in the C group and 85 in the P group. Both C and P were well tolerated; 18.6% (n = 16) of patients receiving C and 16.5% (n = 14) receiving P had adverse events considered to be possibly or probably related to drug therapy. Two patients from the C group and 1 from the P group withdrew due to such adverse events.

Conclusions: Cerivastatin titrated to 0.8 mg/d reduced LDL-C and total cholesterol levels significantly more than pravastatin titrated to 40 mg/d, in patients with primary hypercholesterolemia. Cerivastatin and pravastatin were both well tolerated, with comparable safety profiles.

MoT4:W6 Clinical evaluations of pleiotropic effects of pravastatin

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Objective: Evidence from in vitro and in vivo studies indicates that benefit from cholesterol-lowering treatment with statins is derived from not only reducing LDL-cholesterol but also additional pleiotropic effects. However, it is still uncertain to what extent these pleiotropic effects can be detected in clinical practice. To evaluate the pleiotropic effects of pravastatin, we investigated the changes in new risk markers (homocysteine, CRP, NO3, tissue factor (TF), matrix metalloproteinase (MMP) –3, s-ICAM-1) during clinical administration.

Methods: We measured and analyzed serum lipid levels and markers above before and 4 or 8 weeks after administration of 10 mg daily pravastatin administration in 11 hypercholesterolemic patients.

Results: Total and LDL cholesterol decreased significantly (17% and 26%). Homocysteine tended to decrease (6.9 ± 1.3 to 6.3 ± 1.4 mmol/l, p = 0.077). CRP decreased, but not significantly (606 ± 1123 to 264 ± 179 ng/ml, p = 0.321). Interestingly, CRP rather increased in 3 out of 11 patients, whose % LDL decreases were mild (8, 14, 25%). Basal total and HDL cholesterol level correlated with % CRP decreases. NO3, TF, MMP-3, s-ICAM-1 didn’t change significantly. But, % TF changes correlated with changes in % homocysteine (p = 0.029), % TG changes correlated with % NO3 changes (p = 0.017).

Conclusions: In clinical pravastatin administration, serum homocysteine level tended to decrease, and CRP decreased in the patients whose LDL cholesterol reduced moderately (over 25%). These results should be confirmed by further experiments.

MoT5:W6 Use of simvastatin treatment in patients with combined hyperlipidemia in clinical practice

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Objective: To describe and understand current care of simvastatin-treated patients with combined hyperlipidemia in routine clinical practice.

Methods: In a six-month prospective observational study, data (N = 230) on demographics, simvastatin dosage, cardiac risk factors, and lipid profile were collected from August 1997 to December 1998 at 20 sites across the United States.

Results: Overall, mean percent reduction in total cholesterol levels was 27% (P < 0.001), low-density lipoprotein cholesterol (LDL-C) was 35% (P < 0.001), and triglycerides was 28% (P < 0.001). Among those patients with low baseline high-density lipoprotein cholesterol (HDL-C) values (<35 mg/dl, N = 49), there was a 17% increase in HDL-C (P ≤ 0.001) and 35% of these patients achieved National Cholesterol Education Program HDL-C goal (i.e., ≥55 mg/dl). Coronary heart disease (CHD) patients were started on significantly higher doses (mean = 15.1 mg) compared to non-CHD patients (mean = 11.5 mg) (P < 0.001). Overall, 74% of patients achieved LDL-C goal (52% on starting dose, 22% after one titration). Among those patients who were not at goal and had a follow-up lipid profile result available, only one patient (2%) was at the maximum dose (80 mg) and 69% were on 20 mg or less. Approximately 63% of patients with CHD, 80% of patients with ≥2 risk factors, and 91% of patients with <2 risk factors achieved LDL-C goal.

Conclusions: Multiple factors contribute to LDL-C goal achievement in a usual care setting. A significant opportunity exists to increase the number of patients who achieve LDL-C goal by appropriate dose titration and/or starting patients on a higher initial dose of simvastatin.

MoT6:W6 Lipid lowering treatment in early coronary disease

A. Batalla1, G. I. Cubero2, J. J. R. Reguero3, S. Hevia4, E. Merino4, J. C. Sammarini5, M. Sieres1, A. Cortina1. 1Department of Cardiology; 2Hospital de Cabueñas (Gijón); Hospital Central de Asturias Oviedo, Spain

Purpose: To determine the lipid lowering treatment employed in males under 50 years of age with coronary disease.

Methods: Consecutively, 227 male patients (pts) before 50 years of age (mean age 42 ± 4 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease were prospectively studied. The lipid lowering treatment employed was determined at discharge. After a mean follow-up of 32 ± 13 months the lipid lowering treatment used in 215 patients was recorded (13 patients died during the follow-up). For statistical analysis the Chi-Square test was applied.

Results:

<table>
<thead>
<tr>
<th></th>
<th>Acute phase (227)</th>
<th>Follow-up (215)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid lowering drugs</td>
<td>84 (77%)</td>
<td>110 (51%)</td>
</tr>
<tr>
<td>Statins</td>
<td>47 (27%)</td>
<td>75 (35%)</td>
</tr>
<tr>
<td>Fibates</td>
<td>37 (17%)</td>
<td>19 (9%)</td>
</tr>
<tr>
<td>Resins</td>
<td>2 (1%)</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>Combinations</td>
<td>8 (3%)</td>
<td>13 (6%)</td>
</tr>
</tbody>
</table>

Conclusions: In the follow-up of males under 50 years of age with coronary disease, a significant increase was seen in the use of lipid lowering drugs due to a increase in the use of statins.

MoT7:W6 Fluvatatin extended-release formulation is effective in treating primary hypercholesterolemia

W. Ingull1, A. D. Marais2, R. Aronson3, S. Manfreda4. 1Baylor College of Medicine, Houston, Texas; 2Novartis, East Hanover, New Jersey, USA; 3University of Cape Town, Observatory, South Africa; 4Lifestyle Metabolism Center, Toronto, Ontario, Canada

Objectives: To assess the efficacy and safety of the new extended-release (ER) formulation of fluvatatin with that of the immediate-release (IR) formulation in patients with primary hypercholesterolemia 1a/IIa.

Methods: After a 4-week dietary/placebo phase, 442 patients with low-density lipoprotein cholesterol (LDL-C) ≥ 160 mg/dl and triglycerides ≤ 400 mg/dl, were randomised to fluvatatin ER 80 mg once daily (qmn) (n = 141), IR 40 mg qmn (n = 146) or IR 40 mg twice daily (bid) (n = 155) for 24 weeks.

Results: At 24 weeks the least squares mean percent LDL-C reduction with
ER 80 mg qpm was 10.3 points greater than with IR 40 mg qpm, (33.3% vs 23.2%, p < 0.001) and comparable with IR 40 mg bid (31.4%). Reductions of ≥ 35% in LDL-C were seen in 51% of the ER 80 mg qpm group (44% for IR 40 mg qpm, 48% for IR 40 mg bid). The evaluations at last assessment supported these data (see table). All dosages were well tolerated. The incidence of liver enzyme elevations in the ER 80 mg qpm and IR 40 mg qpm groups was lower than in the IR 40 mg bid group.

**Least squares mean (SE) % change from baseline to last assessment**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fluvastatin ER</th>
<th>Fluvastatin IR 40 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 mg qpm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>-33.3 (1.4)*</td>
<td>-22.3 (1.4)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>10.8 (1.4)*</td>
<td>4.6 (1.0)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-10.0 (2.7)</td>
<td>-11.7 (2.6)</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>11.2 (1.1)*</td>
<td>5.9 (1.1)</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>-23.3 (1.3)*</td>
<td>-16.4 (1.3)</td>
</tr>
</tbody>
</table>

**HDL = high-density lipoprotein; SE = standard error; *p < 0.001 vs 40 mg qpm; †p < 0.01 and ‡p < 0.05 vs 40 mg bid.**

**Conclusions:** The oncedaily dosage of fluvastatin ER 80 mg is more effective than, and as safe as, oncedaily IR 40 mg in lowering LDL-C levels in patients with primary hypercholesterolemia.

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**MoT8:W6**

Atorvastatin and short courses of plasmapheresis in the treatment of patients with homozygous familial hypercholesterolaemia

A. Sussekew, L. Kotova, A. Elishay, M. Tvorogova, V. Kukharchuk.

*Cardiology Research Complex, 121552, Moscow, Russia*

**Objectives:** To access the lipid lowering efficacy, safety and tolerability of atorvastatin 20–40 mg/day (A-20 and A-40) and short courses of plasmapheresis (P) in the treatment of patients (pts) with homozygous familial hypercholesterolaemia (HFMH).

**Methods:** Two female pts with HFMH (KN aged 13 years, CN – 25 years) were treated with combination of short courses of P (#3 weekly/bi-monthly) and atorvastatin 20–40 mg/day. Lipid-lowering efficacy was accessed as percent change in lipids over 15 and 14 months subsequently, clinical efficacy in CN pt having ischemic heart disease – as % changes in rate pressure product (RPP) at – 1 mm of ST depression using ECG exercise test over the treatment period.

**Results:** Lipid results (absolute values, mmol/l) are given below.

**MoT9:W6**

Atorvastatin for severe treatment refractory hypercholesterolaemia

F. Heller¹, O. Descamps², B. Boland².

*¹Centre Hosp. Jolimont-Lobbes; ²University Hospital Saint Luc Brussels, Belgium*

**Objective:** The aim of this multicenter, open label, compassionate use study was to assess the efficacy and safety of atorvastatin up to 80 mg in patients with severe hypercholesterolaemia refractory to conventional therapy.

**Methods:** Overall, 62 patients with persisting hypercholesterolaemia (low-density lipoprotein cholesterol (LDL-C) > 200 mg/dl) despite receiving maximally tolerated lipid-lowering therapy were included. After an optional wash-out period of two weeks, patients were enrolled in an initial 8 weeks dose-finding phase, during which an optimal dosage regimen was elaborated for each patient. Atorvastatin dosage could be increased every 4 weeks if needed. Fifty-seven patients completed this 8-weeks period. If response to atorvastatin was inadequate, patients could enter a long-term extension phase.

**Results:** Mean LDL-C on previous lipid-lowering therapy was 277 mg/dl. At baseline (after an optional two weeks wash-out) lipid levels were as follows: LDL-C 339 mg/dl, triglycerides (TG) 201 mg/dl, high-density lipoprotein cholesterol (HDL-C) 51 mg/dl. At week 8, LDL-C and TG decreased to respectively 188 and 134 mg/dl; HDL-C increased to 53 mg/dl. Mean LDL-C reductions from baseline in the 20, 40 and 80 mg groups were 46% (n = 9), 41% (n = 32) and 49% (n = 16). The corresponding reductions in TG were 18%, 20% and 38%. HDL-C levels increased by resp. 0.2%, 5% and 9%. Overall treatment was well tolerated.

**Conclusion:** In patients with treatment refractory hypercholesterolaemia atorvastatin results in significant additional LDL-C lowering (72% compared to previous maximally tolerated therapy) and is well tolerated.

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**MoT10:W6**

Global coronary heart disease risk changes under combined drug treatment of metabolic syndrome patients

N. Perova, M. Mamedov, O. Komatova, V. Metelskaya. *National Research Center for Preventive Medicine, Moscow, Russia*

**Objective:** to study the influence of short-term combined therapy with lipid-lowering and hypotensive drugs on CHD global risk in patients with metabolic syndrome components clustering.

**Methods:** 45 patients aged 40–65 were included into 8-wks trial. They were randomized into 3 parallel groups receiving the following drug combinations: (I) simvastatin (MSD, USA) plus atenolol (Norton, UK), (II) simvastatin plus indapamide (Servier, France); (III) etofibrate (Merz, Germany) plus indapamide. Lipids were measured enzymatically, apoproteins by immunonephelometry. Global CHD risk was calculated according to 8-yrs model "PROCOM STUDY". Inclusion criteria: BMI > 25 kg/m², waist circumference > 94 cm for males and > 80 cm for females, total C > 6.5 mmol/l and/or TG > 2.3 mmol/l, SBP 140–180 and/or DBP 90–105 mm Hg.

**Results:** CHD global risk changes

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Simvastatin 10 mg + atenolol 50–100 mg</td>
<td>40.0 ± 4.8</td>
<td>11.8 ± 2.3*</td>
</tr>
<tr>
<td>II. Simvastatin 10 mg + indapamide 2.5 mg</td>
<td>43.7 ± 6.2</td>
<td>18.6 ± 4.5*</td>
</tr>
<tr>
<td>III. Etofibrate 500 mg + indapamide 2.5 mg</td>
<td>30.3 ± 4.9</td>
<td>26.7 ± 6.2</td>
</tr>
</tbody>
</table>

The decrease of the global CHD risk (under treatment with combination 1) was related to the significant fall of LDL-C by – 37%, TG – 22%, apo B/AI ratio – 25% and HDL C raise – 14%. SBP and DBP decreased by 16% and 13%, respectively; fasting glucose/insulin ratio increased by 43%. The combination II resulted in marked HDL C elevation-24%, whereas LDL C, apo B, SBP and DBP decreased less than under combination I. Under treatment of combination III there were no changes in LDL C and HDL C; however TG level decreased by 24%; SBP and DBP – by 21% and 13%, respectively.

**Conclusion:** While all three therapies had similar hypotensive effect, the differences in CHD global risk changes in metabolic syndrome patients were predominantly associated with character and degree of lipid spectrum alterations.

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**MoT11:W6**

Low dose pravastatin therapy alters endothelium generated mediators and thrombotic tendency

L. Tokgozoglu¹, N. Koylan², I. Soydan¹, N. Domanic², R. Ener², E. Atalay¹, M. Kayikcioglu¹, ¹Hacettepe; ²Istanbul; ³Ege Universities, Turkey

To evaluate the pleotropic effects of pravastatin, 80 hypercholesterolemic patients (27 male, age 53 ± 9) were treated with 10–20 mg pravastatin for 12 weeks after a placebo run in period of 6 weeks. The effects of pravastatin on thrombosis, endothelium generated mediators and free radicals was evaluated. After treatment, total cholesterol (TC), LDL and TC/HDL decreased significantly whereas HDL and triglycerides were altered nonsignificantly (p = 0.0001). However, factor 7 and plasminogen levels decreased and superoxide dismutase (SOD) increased significantly (p = 0.03, 0.08, 0.02). Furthermore, treatment...
there was a significant correlation between the decrease in F7 and plasminogen (p = 0.003) and TC/HDL (p = 0.04). The increase in SOD was correlated to the decrease in tPA levels (p = 0.001). The changes in these parameters were similar in patients taking 10 or 20 mg pravastatin. We conclude that low dose pravastatin significantly alters the endothelium and the predisposition to thrombus before some of its beneficial effects on lipids are evident.

**Methods:** We have studied a population from the Healthy Heart Program Lipid Clinic (LC). This retrospective study categorised 663 patients into one of three groups following two consecutive visits (termed visits A and B) to the LC: (I) - Presenting to LC with no drug treatment (TX) at visits A and B, (II) - Lipid lowering TX at both visits A and B, and (III) - Lipid lowering TX at visit A which is changed to atorvastatin and on atorvastatin at visit B. Lipid and lipoprotein profile data were compared from visit A and visit B.

**Results:** Serum TC, LDL-C, and TG of groups II and III were significantly reduced at visit B whereas HDL-C was not significantly changed; the lipid profile for group I did not change significantly (See Table).

<table>
<thead>
<tr>
<th>Group</th>
<th>Δ TC (%)</th>
<th>Δ LDL-C (%)</th>
<th>Δ HDL-C (%)</th>
<th>Δ TG (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>-0.96 ± 0.083</td>
<td>-0.047 ± 0.048</td>
<td>0.0089 ± 0.0101</td>
<td>-0.138 ± 0.152</td>
</tr>
<tr>
<td>II</td>
<td>-0.185 ± 0.051*</td>
<td>-0.015 ± 0.043*</td>
<td>-0.0042 ± 0.0089*</td>
<td>-0.223 ± 0.0897*</td>
</tr>
<tr>
<td>III</td>
<td>-1.151 ± 0.120*</td>
<td>-0.079 ± 0.103*</td>
<td>-0.0032 ± 0.0107</td>
<td>-0.644 ± 0.235*</td>
</tr>
</tbody>
</table>

*p ≤ 0.05, intra-group analysis II & III; *p < 0.001, inter-group BC analysis

Comparison of the relative change (RC) in serum lipids between groups II & III showed that atorvastatin treatment led to a significantly higher reduction in TC and LDL-C, and Lp(a).

**Conclusions:** Atorvastatin was more effective in lowering serum TC and LDL-C compared to other lipid lowering therapies.

**Lipid lowering with pravastatin in acute unstable angina:**

Effect on heart rate variability

N.A. Vaulin, I.S. Yavelov, O.V. Averkov, N.A. Graitsiansky. *Center for Atherosclerosis, IPCM, Moscow, Russia*

**Background:** We have previously reported that pravastatin (P) in patients with acute unstable angina (UA) led to rapid lowering of cholesterol (CH) levels. But little is known about changes of clinical parameters have not been presented.

**Objective:** To assess effect of rapid lipid lowering in acute UA with P on heart rate variability (HRV) – parameter which has been supposed to have some prognostic value in acute coronary syndromes.

**Material and Methods:** 39 patients aged 35-65 years hospitalized due to UA were randomized to open P (n = 20) or no P (n = 20) within 24 hours after index attack of pain. Dose of P depended on LDL CH level and was 40-80 mg/day. Total (TP); 0.003-0.40 Hz), very low (VLF; 0.003-0.04 Hz), low (LF); 0.04-0.15 Hz) and high (HF; 0.15-0.40 Hz) frequency powers were evaluated during 24-hour Holter ECG monitoring on days 1 and 7. Presence and duration of ischemia were also registered.

**Results:** Mean decrease in 23% for total and in 33% for LDL CH by day 7 was not associated with reduction of number and duration of ischemia episodes while spectral HRV parameters tended to be higher in P group.

**Conclusion:** Rapid lowering of total CH and LDL CH by P in acute UA was associated with higher VLF power and overall tendency to better HRV.

**Comparison of the efficacy and safety of atorvastatin and fenofibrate in the treatment of mixed hyperlipidemia**

M. Tuncer, A. Comlekci, S. Akar, S. Yeliş, Dokuz Eylül University School of Medicine, 1. Dept of Internal Medicine; 2. Endocrinology, Izmir, Turkey

**Objective:** We have compared the efficacy and safety of atorvastatin and fenofibrate, both of which claim potency in mixed hyperlipidemia (MHL).

**Methods:** After a 6-week baseline period with diet, 117 patients with MHL were randomized to either atorvastatin 10 mg/day (N = 56) or fenofibrate 200 mg/day (N = 61) for 12 weeks. Patients having baseline triglyceride (TG) levels higher than 800 mg/dl were assigned to fenofibrate group. Serum lipid profiles (total cholesterol, HDL, LDL, and TG) and CPK, AST and ALT were measured at weeks 0, 6, and 12. The two groups were age, sex and BMI-matched. Baseline serum lipids were similar with the exception of TG which was significantly higher in fenofibrate group.

**Results:** Both drugs significantly decreased the LDL and TGs (p < 0.003)

**Replacement of usual lipid lowering treatment by atorvastatin: Effect on plasma lipid levels**

J.F. Bowden, P.H. Pritchard, J.J. Frohlich. *Healty Heart Program, St. Paul’s Hospital, UBC, Vancouver, BC, Canada*

**Objective:** To investigate the efficacy of atorvastatin versus other lipid lowering treatment regimens.
and increased the HDL (significant in fenofibrate group; p = 0.000) at week 12. No biochemical toxicity was documented but there were 17 drop-outs by not showing up (atorvastatin: 4, fenofibrate: 13, p = 0.037) and 3 by severe GI side effects (atorvastatin: 1, fenofibrate: 2).

**Conclusion:** Both atorvastatin and fenofibrate are effective in MHL but atorvastatin may be better accepted and tolerated by the patients.

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**T:H1 ANIMAL MODELS IN Atherosclerosis**

**MoT1:H1** Spontaneous myocardial infarction of WHHL rabbits

M. Shiomi, T. Ito, Kobe University School of Medicine, Kobe, Japan

**Objective:** WHHL rabbits, a LDL receptor-deficient animal model, suffered from spontaneous myocardial infarction (MI) by selective breeding. We examined MI of WHHL rabbits histopathologically.

**Methods:** The hearts of WHHL rabbits died suddenly were immersion-fixed with Bouin’s. The hearts were embedded in paraffin. Sections were cut serially at 100 or 50 µm intervals. Each section was immunohistochemically stained with 1A4, a muscle actin-specific monoclonal antibody, and RAM-11, a rabbit macrophage-specific monoclonal antibody, and conventionally stained with H & E, Elastic van Giesen, and Azan-Mallory stains. The areas of coronary plaque and myocardial lesion were measured with a color image analyzer.

**Results:** Myocardial lesions were mainly observed at the lateral wall and posterior wall of the right ventricle, the apical and apex. Many lesions consisted of dissolution of the muscle cells with replacement of fibrosis. Around the fibrosis region, infiltration of lymphocyte, and disappearance of muscle cells were observed. The lesions were located circumferentially at the subendocardial region or transmurally at the posterior wall. The myocardial lesions were correlated to the severe coronary lesions (over 90% narrowing). In these lesions, a large amount of macrophages were observed in the superficial layer and atheromatous core. The content of macrophages and extracellular lipid deposits were markedly increased in the lesions of WHHL rabbits suffered from MI compared to the conventional WHHL rabbits.

**Conclusion:** Our observation suggests that occlusion due to macrophage infiltration at the superficial layers of the plaque is one of the causes of MI.

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**MoT2:H1** Effects of lecithin on calcification by vesicles isolated from aortas of cholesterol-fed rabbits

H.T. Hsu, O. Tawfik, F. Sun, University of Kansas Medical Center, Kansas City, USA

**Objective:** Advanced vascular calcification in atherosclerosis weakens arterial walls, thereby imposing a serious rupturing effect. Morphological and in vitro calcification studies indicate that membrane vesicles of aortas may have a role in calcification. This study of the role of carbohydrates on calcification was initiated by a previous observation that carbohydrate binding concanavalin (Con A) stimulates calcification without affecting ATPase.

**Methods:** Atherosclerosis was induced in rabbits by a diet supplemented with 0.5% cholesterol and 2% peanut oil for 4 months. Calcifiable vesicles were then isolated from aortas by crude collagenase digestion. Calcification was assessed by 4Ca deposition, Fourier transform-infrared spectroscopy and alizarin stain for mineral. Various lecithins with different sugar-binding specificity were tested for their effects on calcification.

**Results:** Lecithin that bind specifically to glucosides, mannosides, or galactosides such as Con A and Abrus precatorius agglutinin enhance calcification by two-fold. However, lecithins including wheat germ agglutinin or Helix pomatia agglutinin that specifically bind acetyl glucosides or acetyl galactosides, respectively had no effect. Only glucosides and galactosides can reverse the stimulation. The stimulation was reversible since a short exposure of vesicles to Con A followed by removal of the lectin from calcifying media failed to sustain stimulation.

**Conclusion:** Putative vesicle-associatesable glucoside or galactoside but not their acetyl derivatives may play a role in vesicle-mediated calcification.

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**MoT3:H1** Rabbit strain affects patterns of aortic permeability and lipid deposition

T. Staughton, S. Barnes, P. Weinberg, Animal & Microbial Sciences, University of Reading, Reading, UK

**Introduction:** Fatty streaks occur downstream of branch sites in immature human aortas but upstream of them at later ages. Comparable age-related distributions occur in rabbits on a normal diet. These distributions may reflect patterns of arterial permeability, which is elevated downstream of branches in young rabbits but upstream in old ones. In young cholesterol-fed rabbits, lesions occur downstream, but they are seen upstream or downstream in adult ones.

**Objective:** To determine why the lesion distribution in adult cholesterol-fed rabbits varies between trials.

**Methods:** Hypercholesterolemia was induced in mature male New Zealand White rabbits by using various combinations of the different base diets, cholesterol levels, dietary durations and rabbit ages employed in earlier trials. Lesion patterns around intercostal branch ostia were determined by applying a frequency mapping technique to aortas that had been stained for lipid. Transport patterns were determined in rabbits fed a normal diet. Rhodamine-labelled albumin was administered iv and allowed to circulate for 10 min before aortas were fixed. Its uptake was measured by quantitative fluorescence microscopy of sections through branches.

**Results and Conclusions:** Lesion patterns varied greatly between animals but no consistent effect of any of the dietary variables or of the age of the mature rabbits was found. Retrospectively, however, it was noticed that the pattern depended on the supplier from which the rabbits were obtained. This variable could also account for the different patterns seen in previous trials. Furthermore, it was found to determine the age at which transport around branch ostia switched from the downstream to the upstream pattern; this age varied from c. 6 months to >3 years in the different strains. When rabbit strain was taken into account, there was a consistent correlation between transport and lesion patterns, supporting a causal relation.

**This study was supported by the BHF**

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**MoT4:H1** Increased production of very low density lipoproteins in transgenic mice overexpressing human apolipoprotein A-II: A mouse model of familial combined hyperlipidemia

J. Juve-Gil, J.C. Escolà-Gil, Á. Marzal-Casacuberta, J. Ordóñez-Llanos, F. González-Sastre, F. Blanco-Vaca. Servei de Bioquímica, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

Familial combined hyperlipidemia (FCHL) is characterized by overproduction of very low density lipoproteins (VLDL). Transgenic mice that overexpress human apolipoprotein (apo) A-II (line 11.1) developed a pronounced hypertriglyceridemia (9-fold), moderate hypercholesterolemia (1.5-2-fold), increased plasma free fatty acid levels (1.8-fold) and susceptibility to develop atherosclerosis upon feeding high fat, high cholesterol (HF/HC) diet. Hypertriglyceridemia may result from delayed or increased VLDL production, or both. Lipolysis of autologous VLDL from 11.1 transgenic mice by lipoprotein lipase contained in postheparin plasma was not different from control mice (6.8 ± 0.7 vs 5.2 ± 0.4 mmol oleate released/mL-min). Furthermore, the fractional catabolic rate of the radiolabeled VLDL triglycerides were similar in 11.1 transgenic mice and in control mice (1.2 ± 0.1 vs 1.10 ± 0.02 mmol/g lipids/24 h/d) (pool 6). The hepatic VLDL production rate was measured after injection of Triton WR1339. The VLDL production rate in 11.1 (1 h: 1.96 mmol/L, P < 0.01; 2 h: 4.23 mmol/L, P < 0.01) was 2.6-fold that of control mice (1 h: 0.78 mmol/L; 2 h: 1.70 mmol/L). We conclude that the hypertriglyceridemia observed in 11.1 transgenic mice on a HF/HC diet is due to enhanced hepatic triglyceride synthesis and secretion rather than a disturbed lipolysis. Therefore these mice constitute an useful model in which to study the molecular mechanisms leading to FCHL.

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**MoT5:H1** Dietary vegetable-wood or vegetable-wood-derived stanol esters reduce atherothrombotic lesion size and severity in APOE3-Leiden transgenic mice

O.L. Volger1, J. van der Boom1, E.C.M. de Wit1, W. van Duyvenvoorde1, G. Hemmen1, J. Plas2, L.M. Havekes1, H.L.G. Princen1, R.P. Mensink2 1Ghauvis Lab. TNO-PG. Leiden; 2Department of Human Biology, Maastricht University, Maastricht, The Netherlands

**Objective:** The hypocholesterolemic and anti-atherosclerotic effects of vegetable- and wood-based dietary stanol esters were compared in female APOE3-Leiden transgenic mice.

**Methods:** The mice (N = 10 per group) received for 38 weeks a control diet or diets containing 1.0% (w/w) stanols derived from either vegetables (sitostanol (S) 65.7%, campesterol (C) 30.1%), wood (S 87.6%, C 12.4%) or a mixture of both (S 72%, C 28.4%). The dietary stanols were provided as stanol esters.

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Results: Vegetable (−46%), wood (−42%) and vegetable/wood (−51%) stanol esters decreased the plasma cholesterol levels (P < 0.0001) by reducing the cholesteryl ester (CE) contents in plasma very low density (VLDL), intermediate density (IDL) and low density-lipoprotein (LDL). Stanol ester feeding did not change plasma triglyceride levels. Dietary stanol esters reduced atherosclerotic lesion area (P < 0.0001) by 91 ± 13% (vegetable), 97 ± 4% (wood) and 78 ± 34% (vegetable/wood). Stanol ester feeding attenuated lesion severity (P < 0.0001) from regular intimal fatty streaks (type 2 lesions) in controls to individual intimal foam cells (<type 1 lesions) in the treatment groups.

Conclusions: Feeding of stanol esters dramatically reduced the amount and severity of atherosclerosis, independent of its source (S/C content), by decreasing VLDL−, IDL− and LDL-cholesterol in APOE*3-Leiden transgenic mice.
Tuesday June 27, 2000: Workshop Abstracts

W:7 FATTY ACIDS: THE LINK BETWEEN INSULIN RESISTANCE AND DYSLIPIDAE

TuW1:7 Pathogenesis of fatty acid induced atherogenic dyslipoproteinemia
Allan D. Sniderman. McGill University, Montreal, Quebec, Canada

Hypertriglyceridemic hyperapoB is the most common atherogenic dyslipoproteinemia. It has four major features: hypertriglyceridemia; increased numbers of small dense LDL particles; low HDL cholesterol; and delayed postprandial triglyceride (TG) clearance. Inappropriate diversion of fatty acids (FA) to the liver due to reduced FA trapping by adipose tissue appear to be the most common pathogenetic mechanism underlying hypertriglyceridemic hyperapoB and the objective of this presentation will be to explicate what is known of the molecular mechanisms responsible for this phenomenon.

Our attention has focused on the regulation of the rate at which FA can be taken up by adipocytes and converted to TG. That effort has led to the description of the Acylation Stimulating Protein (ASP) pathway which regulates the rate adipocyte TG synthesis and influences FA release. The proportion of FA which are released from chylomicrons that enter the nearby adipocytes is variable and is determined by the ability of the adipocyte to incorporate these newly released FA into TG. Diminish FA trapping by adipocytes and necessarily, FA delivery to the liver will increase and so will hepatic apoB secretion and plasma apoB.

We have recently completed an initial series of experiments in ASP knock-out mice. The results support an important physiologic role for the ASP pathway but indicate that the phenotypic consequences of dysfunction of the ASP pathway are critically modulated by gender and insulin sensitivity. These studies point to a model of linked, but distinguishable, steps in peripheral clearance of TG rich lipoproteins and their interaction will have to be appreciated if the pathogenesis of familial combined hyperlipidemia is to be explicated.

TuW2:7 Insulin resistance and postprandial lipid metabolism
D.W. Erkelens. Utrecht, Netherlands

Insulin resistance is at the origin of the syndrome X, encompassing among many risk factors hypertriglyceridemia. It may however well be that the second is operative in enhancing the first or that both are the consequence of one causative mechanism.

Artificial hypertriglyceridemia, with free fatty acid (FFA) increase, strongly reduces insulin mediated glucose uptake during clamping. Postprandial suppression of free fatty acid levels is reduced or even reversed in both insulin resistant type 2 DM patients and familial combined hyperlipidemia (FCH) patients. Growth hormone deficiency is among others characterized by a delayed postprandial clearance of triglyceride rich remnant particles. Spontaneous daily triglyceride profiles (day-trip’s) show even in normal males a substantial dependence on insulin resistance (assessed as HOMA ratio).

These and other data suggest that insulin resistance and disturbed postprandial lipid metabolism are closely linked, mutually dependant and possibly caused by insufficient suppression of free fatty acid flux from lipolysis.

TuW3:7 Fatty acids and insulin resistance: A genetic and physiological perspective
Timothy J. Atkinson. MRC Clinical Sciences Centre and Imperial College School of Medicine, Hammersmith Hospital, London, UK

Glucose and fatty acids are major cellular energy substrates. In many states of insulin resistance, excess availability of fatty acids co-exists with decreased insulin-mediated glucose uptake and utilisation. We have studied the genetics of glucose and fatty acid metabolism in adipocytes from the spontaneously hypertensive rat (SHR). In this model, defects in adipocyte fatty acid metabolism and insulin action associate with insulin resistance and dyslipidaemia at the whole body level. In a genome-wide linkage scan, a major SHR quantitative trait locus (QTL) for defective insulin action shared coincident peak linkage to the telomere of rat chromosome 4 with defective fatty acid metabolism.

Previously mapped SHR QTLS for dyslipidaemia and hypertension also map to the same chromosome 4 locus. Using cDNA microarrays and radiation hybrid mapping, we identified aective SHR gene, Cld3, that resides at the peak of linkage to these QTLS. Cld3 is a transmembrane transporter of long-chain fatty acids and receptor for oxidised low density lipoproteins, and is proposed to play a key part in foam cell formation. Cld3 is directly induced by PPARγ, the target of the thiazolidinediones rosiglitazone and pioglitazone, and transgenic mice display marked abnormalities of lipid and carbohydrate metabolism. These observations indicate that Cld3 plays a key role in regulation of cellular and whole body metabolism of carbohydrates and lipids.

TuW4:7 Fatty acid binding proteins in different human adipose tissue depots: Relationships to serum insulin concentrations
R.M. Fisher1, P. Eriksson1, J. Hoffstedt2, A. Hamsten1, P. Arner1, 1King Gustaf V Research Institute; 2Department of Medicine, Huddinge Hospital, Karolinska Institute, Stockholm, Sweden

Objective: To investigate the fatty acid binding proteins (FABPs) adipocyte lipid binding protein (ALBP) and epididymal fatty acid binding protein (EFABP) expressed in different human adipose tissue depots.

Methods: Omental (om) and subcutaneous (sc) adipose tissue samples were obtained from 19 obese individuals (10 female; 9 male, age 41.1 ± 2.4 years, BMI 42.8 ± 1.2 kg/m²). ALBP and EFABP RNA levels were quantified by northern blot analysis (expressed relative to the ribosomal 18S subunit). ALBP protein levels by Western blot analysis (expressed relative to actin).

Results: In sc compared to om adipose tissue, ALBP RNA and protein levels were 37% (P < 0.02) and 11% (not significant) higher respectively. There were no significant differences in EFABP RNA levels. The ALBP:EFABP RNA ratio was 23% higher in sc compared to om adipose tissue (P = 0.03). ALBP:EFABP RNA ratios were inversely related to serum insulin concentrations in both sc and om adipose tissue (r = –0.618 and r = –0.577 respectively, both P ≤ 0.02). ALBP protein and serum insulin levels were inversely correlated in om (r = –0.475, P < 0.04), but not in sc adipose tissue.

Conclusions: Differences in ALBP and EFABP expression in sc and om adipose tissue might be related to the metabolic differences observed between these two depots. Adipose tissue FABPs may be important in man in the link between obesity and insulin resistance.

TuW5:7 Changes in matrix proteoglycans induced by fatty acids in hepatic cells: effects on lipoprotein binding
U. Olsson1, A.-C. Egeti2, M. Rodriguez Lee1, G. Bondjers1, G. Camejo1, 1Wallenberg laboratory, Göteborg, Åstråkenes; 2Malmö, Sweden

Objective: The dyslipidemia of insulin resistance and type 2 diabetes is characterized by elevated circulating non-esterified fatty acids (NEFA) and lipoprotein remnants. Microvascular and macrovascular complications of type 2 diabetes are characterized by changes in extracellular matrix proteoglycans (PG). Excess exposure to NEFA in vitro alters the amount and composition of extracellular PG in endothelial cells and arterial smooth muscle cells (1, 2). In liver extracellular PG contribute to the uptake of triglyceride (TG)-rich lipoprotein remnants (3). We explored if NEFA can also alter the extracellular PG of hepatic cells and if this could change their affinity for remnant lipoproteins, a hypothetical mechanism that could contribute to the dyslipidemia of insulin resistance and type 2 diabetes.

Methods: Cultured HepG2 cells and livers from obese rats were used as in vitro and in vivo models to study PG synthesis and binding of lipoproteins.

Results: HepG2 cells cultured in medium with 300 μM albumin-bound linoleic acid increased markedly their PG secretion. The glycosaminoglycans of the secreted proteoglycans where enriched in chondroitin sulfate proteoglycans (CS) at the expense of heparan sulfate. Livers of obese Zucker fa/fa rats that are insulin resistant and have high circulating levels of NEFA and TG-rich remnants showed also an increased expression of CS-proteoglycans when compared to lean littersmates. The changed proteoglycan composition decreased the affinity of remnant βVLDL particles to PG isolated from HepG2 cells and Zucker obese rat livers.

Conclusions: Elevated fatty acid levels modulate PG in hepatic cells. If present in vivo, this could affect the clearance rate of remnant particles in insulin resistance and type 2 diabetes and contribute to its dyslipidemia.

References

TuW6-7 Adipose tissue insulin resistance in familial combined hyperlipidemia (FCH), but not type 2 diabetes mellitus (DM2)
C.J.H. van der Kallen, F.G. Bouwman, R.W.J. van de Hult, W.D. Boeckx, T.W.A. de Bruin. Lab. Molecular Metabolism and Endocrinology, Maastricht University, Maastricht, The Netherlands

Objective: To test in both DM2 and FCH the hypothesis that in both DM2 and FCH insulin induced suppression of hormone sensitive lipase (HSL) activity is reduced as consequence of insulin resistance.

Methods: Subcutaneous adipose tissue biopsies were obtained from healthy controls (C, n = 11), DM2 (n = 12) and FCH (n = 10) subjects. Immediately following isolation, mature adipocytes were incubated with isopenaline or insulin for 2 hours. Both glycerol and free fatty acids (FFA) levels were measured in the incubation media.

Results: Isopenaline stimulated the release of glycerol as well as FFA in all groups, with the highest release in DM2 (p < 0.05 vs C and FCH). Insulin decreased FFA release in C and DM2, but not in FCH, indicating impaired insulin sensitivity towards fractional FFA-reesterification in the adipocytes.

Data in table represent mmol/40,000 cells/h (mean ± sd).

<table>
<thead>
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<td>17.1 ± 10.8</td>
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</table>

Conclusions: In DM2 maximum lipolysis activity is high, probably due to larger adipocytes (data not shown). In FCH adipocytes, FFA release did not change under the influence of insulin. This suggests that in FCH the FFA metabolism is disturbed. This may be due to impaired acylation, or TG synthesis, or oxidation, eventually resulting to a higher FFA flux to the liver, contributing to the hyperlipidemia.

TuW7-7 Spectrum of nuclear lamin A/C mutations and metabolic phenotypes in familial partial lipodystrophy
Robert A. Hengele, Carol M. Anderson, Jian Wang, Herian Cao. The John P. Roberts Research Institute, London, Canada

We were the first to report that Dunnigan-type familial partial lipodystrophy (FPLD) with insulin resistance, diabetes, hyperlipidemia, hypertension and early atherosclerosis results from a mutation, namely R482Q, in LMNA, the gene that encodes nuclear lamins A and C. We have since identified three novel and extremely rare missense mutations in LMNA, namely V440M, R482W and R584H. Examination of the clinical and biochemical phenotypes in carriers of mutant LMNA revealed that hyperinsulinemia and perturbations in plasma lipids preceded the development of plasma glucose abnormalities and hypertension. Our findings indicate that: 1) a spectrum of LMNA mutations underlies FPLD; 2) aberrant lamin A, and not lamin C, underlies FPLD, since R584H occurs within LMNA sequence that is specific for lamin A; 3) compound heterozygosity for mutant LMNA is associated with a relatively more severe FPLD phenotype, but not with complete lipodystrophy; and 4) environmental factors appeared to be partially related to the variation in phenotype severity in LMNA mutation carriers. Thus, rare mutations in a nuclear structural protein are associated with markedly abnormal qualitative and quantitative metabolic phenotypes. The precise mechanism by which mutant LMNA causes fat-wasting in specific anatomical sites is unknown. It is also not clear whether the metabolic disturbances in FPLD are a direct result of deficient or defective intracellular function due to the mutant LMNA or are merely secondary to the abnormal distribution of adipose tissue. In either event, our genetic analysis has implicated an etiologic role for aberrant nuclear lamin A in FPLD. These naturally occurring LMNA mutations will need to be evaluated in vitro molecular and cellular analyses in order to understand why specific cell types and tissues are selectively affected.

W8 GENE THERAPY AND OTHER NEW TREATMENTS

TuW1:8 VEGF gene transfer in the treatment of coronary heart disease and peripheral vascular disease
S. Yli-Herttuala, A.I. Virtanen Institute and Department of Medicine, University of Kuopio, Kuopio, Finland

Vascular gene therapy is a new area where only a few preliminary results from human trials are available (1). Most of the clinical trials are centered around therapeutic angiogenesis, treatment of restenosis, arterial cytoprotection or a combination of these effects. Intravascular adenoviral gene transfer in human peripheral arteries in the leg has been proved feasible with infusion-perfusion catheters (2). Detectable transgene expression was achieved in a maximum of 5% of arterial cells. Beneficial effects have been reported after intramuscular VEGF gene transfer into the muscle or artery of an ischaemic limb or myocardium (3–6). Several gene therapy trials with various types of VEGF are currently ongoing (1). Results are expected within 1-2 years.

Based on current information, gene therapy in the cardiovascular system seems to be safe and well tolerated, although edema has been seen in legs treated with intramuscular VEGF gene therapy and hypertension has been reported in some patients. Even though gene therapy has shown promising results in some areas of cardiovascular diseases, further developments in gene transfer vectors, gene delivery techniques and identification of effective treatment genes will be required before the full therapeutic potential of gene therapy can be assessed.

References

TuW2:8 Gene therapy for dyslipidemias
Lawrence Chan. Departments of Molecular & Cellular Biology and Medicine Baylor College of Medicine, Houston, Texas 77030, USA

Elevation of atherogenic plasma lipoproteins is a major risk factor for atherosclerosis development. Somatic gene therapy is a novel experimental approach for the treatment of hyperlipoproteinemia and dyslipidemia. Successful gene therapy requires the availability of safe and efficient gene delivery vectors. Although both viral and non-viral vectors are highly efficient in delivering transgenes to the liver, they exhibit substantial toxicity and have been associated with significant morbidity and mortality in clinical trials. A helper-dependent adenoviral vector (HD-Ad) deleted of all viral protein genes was developed at Baylor College of Medicine, and an efficient production system for this vector was developed by Dr. Frank Graham at McMaster University. In collaboration with Dr. Arthur Beaudet in the Department of Molecular & Human Genetics at Baylor, we used HD-Ad to deliver lipid-lowering genes to the liver of mouse and nonhuman primate models of hyperlipidemia. A single injection of HD-Ad for LDL receptor, VLDL receptor, apoA-I or apoE produced long-term (6 months to >1 yr) hepatic transgene expression with negligible toxicity, reversed dyslipidemia and prevented atherosclerosis development. The data support the feasibility and safety of using HD-Ad vectors for the treatment of lipid disorders in clinical trials.

TuW3:8 Gene therapy for proliferative vascular disease
K. Walsh. Division of Cardiovascular Research, St. Elizabeth’s Medical Center, and Program in Cell, Molecular, and Developmental Biology, Sackler School of Biomedical Sciences, Tufts University, Boston, MA, USA

I will provide an overview of the gene targeted genes and delivery systems that we are evaluating for the therapy of proliferative vascular disorders. The promise of gene therapy for post-angioplasty restenosis is that one has the opportunity to deliver genetic material to the site of balloon inflation at the time of intervention. The delivery of genetic material to these sites creates a depot for recombinant protein expression, thereby avoiding limitations imposed on other therapies by the short repetition periods experienced with small molecules and macromolecules delivered to the vessel wall. The altered expression of genes within the targeted cells will, in theory, alter the course...
of the wound healing process to minimize reocclusion of the vessel. I will
discuss treatment strategies that have been shown to be efficacious in
limiting post-angioplasty restenosis through evaluation with in vivo model systems.
Possible strategies that we have investigated utilize genes encoding factors
that are either cytotoxic (Fas ligand and hammerhead ribozyme to Bcl-2) or
cytostatic (Rb and p21). We have also investigated the therapeutic utility of
a transcriptional regulator of integrin expression that was isolated from smooth
muscle cells (Gax). I will also briefly discuss critical issues of delivery with
devices and gene control that must be considered for successful application of
this therapy.

TuW4.8 VEGF-C adenovirus gene transfer reduces intima formation
in rabbits
Mikko O. Hiltnen¹, Marja Lahtinen¹, Mikko P. Turunen¹, Michael Jeltsch²,
Juha Hartikainen¹, Tuomas T. Rissanen¹, Johanna Laukkanen¹, Mari Niemi¹,
Marja Koistila¹, Tomi P. Häkkänen¹, Antti Kivelä²,1, Berndt Enholm²,
Hanna Manniakoski¹, Anna-Mari Turunen¹, Kari Altala², Seppo Yli-
Hertuala³. ¹A.I. Virtanen Institute, University of Kuopio, Kuopio;
²Molecular Cancer Biology Laboratory, Haartman Institute, University of
Helsinki, Helsinki, Finland

Background: Gene transfer may provide new possibilities for the treat-
ment of postangioplasty restenosis. In this study we analyzed the effects of
adenovirus-mediated VEGF-C gene transfer on neointima formation after
endothelial denudation in rabbits. For comparison, a second group was treated
with VEGF-A adenovirus and a third group with lacZ adenovirus.

Methods and Results: Aortas of cholesterol-fed New Zealand White
rabbits were balloon denuded and gene transfer was performed three days
later. Animals were sacrificed 2 and 4 weeks after the gene transfer and
intima/media ratio (I/M), histology and cell proliferation were analyzed. Two
weeks after the gene transfer I/M in the lacZ-transfected control group was
0.57 ± 0.04. VEGF-C gene transfer reduced I/M to 0.38 ± 0.02 (P < 0.05 vs.
lacZ group). I/M in VEGF-A treated animals was 0.49 ± 0.17 (ns). Expression
of VEGF receptors 1, 2 and 3 were detected in the vessel wall by using
immunocytochemistry and in situ hybridization.

Conclusions: VEGF-C adenovirus gene transfer is effective in reducing
intimal thickening. VEGF-C may be useful for the treatment of postangioplasty
restenosis and vessel wall thickening after vascular manipulations.

TuW5.8 Feasibility of gene transfer through bone marrow cells using
lentiviral vector
S. Jovinge¹, L. Arhpé², L. Brånén³, P.K. Shah⁴, T. Rajavashist⁴.
¹Atheroscl. Res. Center, Cedars-Sinai MC, UCLA, Los Angeles, CA; ²UCLA
CVRI, Los Angeles, CA, USA; ³Dep of Med, Univ. Hospital MAS, University of
Land, Malmö, Sweden

Objective: To establish a feasible model for gene transfer for vascular disease
using bone-marrow cell transduction.

Methods: We investigated the hypothesis that lentiviral vectors transduce
bone-marrow cells (BMC) which, when injected to totally body irradiated
(TBI) C57Bl/6 mice, result in successful engraftment. A shuttle expression
plasmid encoding the enhanced green fluorescent protein (EGFP) under the
cytomegalo virus major immediate early promoter/enhancer (CMV) was pack-
eged into lentiviral particles. For comparison, a CD11b controlled expression
system was used.

Results: Transduced bone-marrow cells showed EGFP expression on flow
cytometry. EGFP expression was also detected in peripheral blood cells (PBC).
The EGFP expression in PBC was detected up to six months after bone marrow
transplantation. To increase the specificity of gene-transfer to the vessel wall,
the same vector-system was used substituting the CMV-promoter with the
monocyte/macrophage specific CD11b promoter. The relative strength of this
promoter was similar to the CMV-promoter in flow-cytometry based assay
systems. This later system was used in bone marrow transplantation of apo
E deficient mice and EGFP-expression in the atherosclerotic plaques was
established demonstrated.

Conclusion: We have demonstrated the successful transduction of murine
bone marrow cells using lentiviral vectors encoding EGFP. Furthermore,
transplantation of bone-marrow transduced with lentiviral vectors driven by
CD11b promoter, we have also demonstrated successful EGFP expression
in atherosclerotic plaques in apo E deficient mice. These findings thus
suggest the feasibility of bone marrow selective gene transfer with lentiviral
vectors targeting the vasculature.

TuW8.8 Therapeutic angiogenesis induced by HGF: Potential gene
therapy for Ischemic diseases
Motokuni Aoki¹, Ryuchi Morishitus², Yoshiaki Taniyama³, Keita Yamasaki³,
Yasuhiro Kaneda¹, Toshio Oghara¹. ¹Department of Geriatric Medicine;
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Japan

Objectives: The feasibility of a novel therapeutic strategy using angiogenic
growth factors by expediting collateral artery development has recently entered
the realm of treatment of ischemic diseases. In USA, human gene therapy for
angina and ASO has already begun and it gives a surprising effect. We already
reported that HGF has a powerful effect on the proliferation of endothelial
cells in vitro. In this study, we hypothesized the transfection of HGF gene into
ischemic hindlimbs and infarcted hearts could induce angiogenesis, potentially
restoring blood flow.

Methods & Results: Human HGF gene or control vector were transected
into ischemic limbs and myocardium by HVJ-liposome method. Although the
concentration of endogenous HGF in ischemic hindlimbs and infarcted hearts
were significantly decreased, this transfection showed a marked increase in
rat immunoreactive HGF, accompanied by the over-expression of human
HGF in the myocardium transfected with HGF gene, a significant increase in
PCNA-positive endothelial cells and the number of vessels could be observed
at 14 days after transfection. Angiogenic activity was also confirmed by the
activation of a transcription factor, etc, which is essential for angiogenesis,
assessed by immunohistochemistry and electrophoretic mobility shift assay.
Also, the complemented HGF by the transfection into the ischemic hindlimbs
showed a significant increase in the number of vessels, resulting in a significant
increase in blood flow assessed by Laser Doppler Image.

Conclusion: The constant production of the local HGF will be considered
as an innovative therapeutic angiogenesis strategy for ischemic diseases.

TuW7.8 Optimisation of in vivo arterial transfection
E. O'Brien, X. Ma, C. Glover, H. Miller. Ottawa Heart Institute, Canada

Objective: Gene therapy for the treatment of vascular disease is limited by
transfection efficiency and/or undesired biological responses (e.g., with viral
vectors). The purpose of this study was to determine an efficient method of
delivering liposome/DNA complexes into balloon-injured rabbit iliac arteries
using a delivery catheter.

Methods: Cationic liposomes were made from a 1:1 (wt/wt) mixture of
DOTAP and DOPE. The plasmid pcMV-AP containing the human placenta
alkaline phosphatase (AP) reporter gene was used as a marker gene for these
experiments. Prior to initiating the in vivo experiments, the optimal ratio of
liposome to DNA complex, as well as the persistence of transgene expression
were determined in vitro using cultured vascular SMCs. The liposome/DNA
complex was then delivered under pressure using a Dispatch catheter to rabbit
iliac arteries that were balloon injured 5 days prior gene delivery. Transfection
efficiency was defined as the percentage of transfected cells/total cells per
high power field.

Results: The optimal ratio of liposome to DNA was 8:1 (wt/wt). AP
expression in transfected SMCs persisted for 28 days, although the percentage
of transfected cells declined with time (e.g., at 24 hours: 27.3% ± 2.9%; at
28 days: 4.0% ± 0.1%). The peak transfection efficiency in cultured smooth
muscle cells was seen at 24 hours post-transfection. As well, smooth muscle
cell proliferation in vitro enhanced the transfection efficiency (e.g., 12.6 fold
higher than quiescent cells). In vivo experiments were performed on 9 balloon
injured rabbit iliac arteries, with half of the arteries receiving the pcMV-AP
plasmid and the other half receiving the liposome only (no plasmid). Low
levels of transfection were observed in arteries harvested 1 day post delivery.
However, 67 arteries harvested 3 days post-delivery had multiple regions of
focal transgene expression involving all 3 arterial layers. No gene expression
was found in the uninjured aorta.

Conclusion: Liposome mediated gene transfection to all vessel layers can
successfully be performed in vivo using local delivery, and may provide an
ideal means of targeting vascular disease processes.
W:9 GEOGRAPHIC EPIDEMIOLOGY OFATHEROSCLEROSIS

TuW1.9 CHD risk factors in Indians: A global comparison
K.S. Reddy. All India Institute of Medical Sciences, New Delhi, India
As the engines of health transition gather pace, the epidemic of coronary heart disease (CHD) is accelerating in India, with rise in CHD burdens reported especially in urban settings. Excess mortality due to CHD reported in migrant Indians in several countries, is also a potent of increased risk for Indians. Studies in migrants have been unable to explain the excess risk on the basis of conventional risk factors. High frequency of the metabolic syndrome, elevated lipoprotein 'a' levels and plasma homocysteine have been incriminated in migrant studies. Comparative studies of urban and rural populations in India and migrant-nonmigrant comparisons, however, reveal a gradient of CHD risk best explained by rising levels of conventional risk factors (body mass index, plasma cholesterol and blood pressure). For any level of total cholesterol, Indians appear to have a higher total cholesterol to HDL cholesterol ratio and for any level of LDL cholesterol, the small dense LDL fraction appears to be higher. Thus the lipid Pool is more atherogenic at each level of cholesterol, indicating the need for different guidelines for dyslipidemia. Increments of body mass index even within the 'normal' range, are associated with a marked rise in CHD risk factors, in urban-rural comparisons. The migrant studies, comparing different gene pools in a similar environment, identify the non-conventional risk factors as explanatory. The urban-rural and migrant-nonmigrant comparisons, contrasting the same gene pool in different environments, identify the conventional risk factors as explanatory. The excess CHD risk of Indians seems to be related to a confluence of both sets of risk factors and warrants investigation and intervention at both levels.

TuW2.9 Globalisation and coronary heart disease
Objective: To explore the relationship between globalisation and the emerging epidemics of coronary heart disease (CHD) in developing countries.
Methods: The first part of this presentation analyses the critical elements of the modern phase of globalisation and their theoretical impacts on the occurrence of CHD. The second part describes recent trends in CHD in developing countries and assesses the actual impact of globalisation on the epidemics.
Results: The core of modern globalisation is economic interconnectedness and the associated policy regimes. The two facilitating domains are technological, especially of information and communication technologies, and cultural. The specific health risks of globalisation include: the spread of smoking-caused diseases as the tobacco industry rapidly globalises its marketing and promotion strategies; the diseases of dietary excesses, as food production and food processing becomes intensified and as urban consumer preferences are shaped by globally promoted images; the diverse public health consequences of the proliferation of private car ownership; and the resulting rise of obesity. These risks are likely to exacerbate the effects of ageing on the population risk for CHD. The burden of CHD is clearly increasing in developing countries. The age specific effects of the globalisation of risk on CHD rates are not yet so obvious, although the available evidence is limited.
Conclusion: The prevention and control of the emerging CHD epidemics will require a global policy response, not just national initiatives. Sustainable surveillance systems are required to monitor the epidemics and the effects of the prevention policies.

TuW3.9 Males with mild and severe coronary atherosclerosis in five European populations over a 25 year period
N.H. Sterbny 1, V.S. Zhdanov 1, A.M. Vikher 1, J. Duskova 1, 1Malmo University Hospital, Malmo, Sweden; 2Russian Cardiology Complex, Moscow, Russia; 3Charles University, Prague, Czech Republic
Objective: To study atherosclerotic (Aht) changes over 25 years in subjects belonging to groups with mild or low atherosclerosis (LAt h) and severe or high atherosclerosis (HAt h).
Methods: Aht in the coronary arteries was studied on autopsy material during the early 1960’s (1st study) and the late 1980’s (2nd study) in males, 20–59 years of age, from Malmo, Sweden; Prague, the then Czechoslovakia; and Riga, Tallinn and Yalta, the then Soviet Union. During the 1st study 3597 and during the 2nd study 3456 cases were included. The HAt h group included subjects who had died from manifestations of Aht, hypertensives and atherothrombectomies excluded. The LAt h group included mainly subjects who had died from violence or suicide.
Results: The number in the HAt h group was 911 and 1218, resp. in the 1st and 2nd study, in the LAt h group 1146 and 665, resp. The proportion of males belonging to the HAt h group increased in all populations except in Prague. The proportion of males belonging to the LAt h group decreased in Riga, Tallinn and Yalta but showed no change in Malmo and Prague. In the HAt h group Aht of coronary arteries expressed as extent of raised lesions, was of significantly greater severity in the 2nd study in Riga, Tallinn and Yalta but significantly less severe in Malmo; in Prague no difference was observed. The same pattern was found in the LAt h group: Aht decreased in Malmo, did not change in Prague but increased in Riga, Tallinn and Yalta.
Conclusion: Differences in the development of Aht in males of five European populations over a 25 year period were expressed in changing proportions of subjects with mild and severe Aht as well as changing level of Aht in these groups.

TuW4.9 The second nation-wide study of atherosclerosis in infants, children and young adults in Japan
C. Yutani. Japanese Pathological Study Group of Atherosclerosis in Youth; Department of Pathology, National Cardiovascular Center, Osaka, Japan
Objective: This paper reports the results of the second nation-wide cooperative study of atherosclerosis in young Japanese with ages ranging from 1 month to 339 years, who were autopsied between 1991 and 1995 in 67 hospitals in Japan.
Methods: Atherosclerotic lesions in 1066 aortas and 974 coronary arteries from 1253 autopsied patients were classified into fatty streaks, fibrous plaques and complicated lesions and were then quantified with the help of a computerized method. The definition and the method of the quantification of the atherosclerotic lesions most identical to those of the previous study which was performed 13 years ago and the results of the current study were compared with those of the previous study.
Results: Atherosclerosis of aorta, determined by surface involvement (SI) of atherosclerotic lesions and atherosclerotic index (AI), increased with age in both sexes of the former and the current studies and their tendency for the progression of the extent of atherosclerotic lesions appeared to be similar. Among the three segments of the aorta, the percent intimal surface involved with all lesions was greatest in the abdominal aorta for every age except less than 1 year and the proportion of raised lesions (the sum of the fibrous plaque and complicated lesion) to total lesions was greatest in the abdominal aorta. Fatty streaks preceded the other lesions and accounted for the largest portion of the lesions. Fibrous plaque and complicated lesions developed in the later decades of life. In the aortic segments no significant changes were detected between the two nation-wide studies. In the coronary arteries, the mean values of SI and AI in the males of the current study were significantly greater than those in the male of the former studies and in the female of the both studies in the 3rd and 4th decades.
Correlation of some risk factors with SI and AI of aorta and coronary arteries were analyzed. Age, serum total cholesterol, blood pressure, body mass index and heart weight were significantly correlated with SI and AI of aorta and coronary arteries.
Conclusion: Serum total cholesterol appeared to be more strongly correlated with the extent of fatty streaks than was systolic blood pressure and vice versa with that of fibrous plaques.

TuW5.9 A pathologic survey of atherosclerotic lesions in chinese youth
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Objective: In order to provide justification about prevention of atherosclerosis, a pathologic survey was conducted to study the pathogenesis and prevalence of premature atherosclerosis in Chinese youth.
Methods: One-hundred and fifty-seven aortae and ninety-one hearts of autopsy were collected from Chinese youth who died of accident, with age ranging from 15 to 39 years. Both macroscopic and microscopic examinations were taken. Primary antibodies such as CD31, CD45RO, CD3, CD68, α-smooth muscle specific actin, and desmin were employed in the immunohistotechnical technique.
Results: Types I, II lesion was found in all of the aortae, and most of coronary arteries. The prevalence of type III lesion was 34% in aortae, 27% in coronary arteries, and significant higher in smokers. Only one case with familial hypercholesterolemia showed severe coronary artery atherosclerotic lesions. Cubic structure change of endothelial cells, CD68-positive macrophage-derived foam cells and activated T lymphocytes (CD45RO and CD3 positive) underlying endothelial cells and predominant desmin negative smooth muscle cells were found in atherosclerotic lesions.

Conclusions: Early atherosclerotic lesions are common within arterial intimina of Chinese youth without smoking is an important risk factor. Endothelial dysfunction, macrophage and T lymphocyte activation, and smooth muscle cell dedifferentiation are the major pathologic findings in early atherosclerosis.

Hypercholesterolaemia as a risk factor for coronary heart disease in the Asia-Pacific region: The ASPAC study
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Objective: To determine the prevalence of hypercholesterolaemia and rates of dietary advice and drug treatment in CHD patients in more than 180 randomly selected hospitals in the Asia-Pacific region.

Methods: Medical records were reviewed over 6 months follow-up among 4,112 patients admitted with myocardial infarction or unstable angina. Hypercholesterolaemia was defined as documented history, blood level ≥ 5.5 mmol/L or use of lipid-lowering therapy at any time.

Results: Cholesterol measurement rates after CHD ranged from 42% (Thailand) to 99% (Japan) of patients. Mean cholesterol levels in Asian countries ranged from 4.9 to 5.9 mmol/L (190 to 229 mg/dL) with 33% (Taiwan) to 63% (Malaysia) of measured patients having blood levels of ≥5.5 mmol/L compared with 54% in Australia and 72% in New Zealand. Formal dietary advice appeared to be given or documented in most countries. From 16% to 52% of hypercholesterolaemic patients received drug therapy, with HMG CoA reductase inhibitors most common. Follow-up measures were infrequent, but with 74% of elevated levels failing to fall below 5.5 mmol/L. Rates of use of cholesterol-lowering drugs correlated strongly with Gross National Product per capita.

Conclusions: The prevalence of hypercholesterolaemia in CHD varies more than two-fold in the region. Measurement and treatment rates also vary widely. A "treatment gap" exists between the recent evidence of benefit from cholesterol lowering treatment and our recent practice patterns. A coordinated approach to cholesterol management is needed.

W:10 TRANSPANTATION Atherosclerosis

Renal transplantation arteriosclerosis is a cell-mediated intimal immune response
Hai-Lu Zhao, Hong-Fen Li, Li-Bi You, Julian A.J.H. Critchley.

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Objective: To study the pathogenesis of renal transplantation arteriosclerosis.

Methods: One-hundred and two human renal allografts were removed surgically due to function failure, examined pathologically. Cases with arteriosclerosis were studied using immunohistochemical technique. Antibodies specifically for T lymphocytes, B lymphocytes, macrophages, endothelial cells, smooth muscle cells, cytomembranous growth factor (CMGF), Bcl-2 protein, transforming growth factor (TGF-β) were employed.

Results: Thirty-eight renal allografts developed arteriosclerosis, which accounted for 93% of the failed allografts survived for more than 1 year. In CMF-infected renal transplants, the incidence of definite arteriosclerosis with intimal extensive lymphocytes and macrophage/fom cell infiltration is 82%, compared with 23% of those without CMF viral inclusions (p < 0.001). Immunostains showed that more than 80% of the intimal intimated inflammatory cells were activated T lymphocytes. The other infiltrates beneath endothelial cells were mainly macrophages and lipid-loaded foam cells. Endothelial cells appeared degeneration and proliferation changes, mitosis was occasionally found, α-Actin-positive smooth muscle cells predominated over desmin-positive ones. Aberrant bcl-2 and TGF-β were also observed.

Conclusions: Renal transplantation arteriosclerosis is indicated the major limit for long-term renal allograft survival. It may be resulted from T lymphocyte-mediated arterial intimal immune injury with subsequent smooth muscle proliferation.

Expression of thrombospordin-1 (TSP-1) in human cardiac allografts
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Expression of endogenous angiogenic growth factors is significantly elevated while angiogenesis is not present in cardiac allografts. We hypothesize that other factors expressed in allografts may alter vascular response to angiogenic growth factors. TSP-1 is a matrix glycoprotein that inhibits angiogenesis and facilitates the proliferation and migration of smooth muscle cells (SMC) by growth factors.

Methods: Quantitative RT-PCR and immunohistochemistry were used to analyze expression of TSP-1 and its receptors in endomyocardial biopsies from human cardiac allografts and normal human hearts. In vitro experiments were used to investigate regulation of TSP-1 by cytokines and allostimulation.

Results: Expression of TSP-1 mRNA was significantly increased in human cardiac allografts compared to normal control. TSP/GAPDH ratio was 1.26 ± 0.21 in cardiac allografts vs. 0.26 ± 0.03 (p = 0.005) in normal hearts. Persistent elevation of TSP-1 was strongly associated with the severity of CAV. CD36 and CD47 were also elevated in allografts. Immunohistochemistry demonstrated intense expression of TSP-1 in cardiac allografts, predominantly in intimal SMC from arteries with severe CAV. In vitro experiments demonstrated that TSP-1 was induced in mixed lymphocyte cultures. IL-1 beta, IFN-gamma, and TNF alpha strongly induced TSP-1 expression in SMC.

Conclusions: Expression of TSP-1 and its receptors is significantly increased in human cardiac allografts and is associated with the severity of CAV. Cytokines and allostimulation regulate TSP-1 expression in SMC and T cells. Augmented levels of TSP-1 and its receptors in human cardiac allografts may alter vascular response to angiogenic growth factors by inhibiting angiogenesis and promoting SMC proliferation characteristic of CAV.

Transplant vascular disease: Potential sites of intervention
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Three separate target sites have so far been discovered to present fibrinoid displaia in chronic rejection after the process has become autonomous of the triggering event. Interference with receptors, particularly with their early signaling events, regulating smooth muscle cell migration and replication and targeting to two separate sets of vasculo-protective gene products: somatostatin receptor subtypes 1, 4 and estrogen receptor beta. For rational drug design, aiming specifically to agonize or antagonize a given receptor, ligand or enzyme, the following four modalities exist. Gene therapy, monoclonal antibodies, chimeric or humanized, non-degradable peptides based on D- rather than L- amino acids and peptidomimetics, which are organic compounds lacking the peptide bond. Only the last approach will generate credible orally available drug candidates. This approach will need reliable modeling of the target receptor, combinatorial chemistry and high throughput screens using cell lines permanently overexpressing the desired genes. In regard to receptor protein tyrosine kinases, the crystal structure of the phosphorylation sites of at least EGF, PDGF, FGF and IGF-1 receptors are known and they are sufficiently different to generate receptor-specific drugs. In regard to 7-transmembrane G-protein coupled receptors, the modeling of SST and subtypes will be a particularly demanding task, as the receptor cannot be crystallized. In regard to estrogen receptor subtypes, the ligand-binding domains have already been crystallized. Indications for these drugs would not be limited to transplantation but will also include other forms of fibroepithelial vasoclaspatics, such as complications of bypass surgery, PTCA procedures, autoimmune and diabetic vasculopathies and possibly variations of the common form of atherosclerosis.

Endothelial cell changes in microvessels of organ allografts
Z. Jurevka, H.J. Knieriem, V. Minkova

Department of Pathology, Med. Faculty Sofia, Bulgaria; Department of Pathology, Bethesda Hospital, Dussburg, Germany

Objective of the study was to evaluate the micro- and ultrastructure of microvessels in long term heart- and kidney allografts.

Methods: Histologic and electronmicroscopic investigations were performed on: a) endomyocardial biopsies from 16 long-term heart-transplant
recipients, 2 of whom died 13, resp. 15 months after transplantation on cardiac failure; b) renal biopsies from 12 long-term kidney-transplant recipients, 2 of them died 11, resp. 25 months after transplantation on renal failure.

Results: In all of the 4 deceased patients consecutive biopsies from the organ allograft revealed during the entire post-transplant period only transitional signs of mild cellular rejection. By light microscopy some swelling or proliferation of microvessel endothelial cells were observed. Electronmicroscopy demonstrated however severe endothelial alterations in capillaries, arterioles and even venules, manifested by prominent swelling of endothelial cells with loss of subcellular organelles and severe narrowing of the vessel lumina. At autopsy the pattern of severe graft vasculopathy could be detected throughout the allograft.

Conclusion: Our findings in organ allograft biopsies suggest that microvascular endothelial damage could represent a marker for evolving transplant vasculopathy and chronic vascular rejection.

TuW5:10 Lipoprotein(a) inhibits proliferation of human umbilical venous endothelial cells (HUVEC) in vitro
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Objective: We have previously shown that Lipoprotein(a) (Lp[a]) is a risk factor for chronic renal transplant rejection. Histologically, the affected vessels show significant intima proliferation. To investigate if Lp(a) is a growth factor for endothelial cells we have studied the effect of Lp(a) on the proliferation of human umbilical venous endothelial cells (HUVEC) in a cell culture model.

Methods: HUVECs were activated by a 24 h-incubation with interferon y (IFN-y). Afterwards, the cells were incubated with different concentrations of Lp(a) or low density lipoproteins (LDL) for another 24 h in the presence of IFNy. The proliferation of the cells was measured by the uptake of bromodesoxyuridine (BrdU) of the cells as an expression for DNA-synthesis.

Results: While LDL-incubation stimulated the proliferation of endothelial cells in vitro, the incubation of HUVECs with Lp(a) inhibited proliferation. This effect was significant and dependent on the concentration (10-200 g/ml) and time of incubation (4-48 h). The activation of the cells with IFN-y had no influence on these effects.

Conclusions: Against our expectations, Lp(a) seems to have an inhibitory effect on proliferation of HUVECs. To study this effect more closely further experiments are necessary.

TuW1:11 The role of vascular smooth muscle cell apoptosis in atherosclerosis and restenosis
Martin R. Bennett, Addenbrooke’s Centre for Clinical Investigation, Level 6, Box 110, Addenbrooke’s Hospital, Cambridge CB2 2QQ, UK

The orthodox view holds that vascular smooth muscle cell (VSMC) proliferation is a major contributor to disease states such as atherosclerosis or restenosis after angioplasty, arguing that deregulated cell proliferation and phenotypic differences in VSMCs in these processes generate the disease. As a consequence, major efforts have been made to inhibit VSMC proliferation, which have been unsuccessful in inhibiting clinical events in either disease. More recently, it has been recognised that VSMCs and their products, extracellular matrix and collagen, comprise the major structural components of the atherosclerotic plaque, and a reduction in cell numbers, either by inhibition of cell proliferation or increased apoptosis may be detrimental. VSMC accumulation in atherosclerosis is now viewed as a repair process, and failure of repair may lead to plaque rupture. In fact, plaque VSMCs from advanced human plaques show poor proliferation, early senescence and increased apoptosis, and are thus a ‘senescent’ phenotype, incapable of effective repair. Plaque VSMCs show intrinsic defects in both mitogenic and survival signalling, and activation of cell cycle machinery in plaque VSMCs induces apoptosis, not cell proliferation. Thus, although plaque VSMCs are surrounded by mitogens, they cannot repair a damaged plaque effectively. In addition, plaque VSMCs show increased sensitivity to agents that induce DNA damage and p53 activation, such as free radicals and nitric oxide.

In restenosis after angioplasty, the major determinant of restenosis is the extent of negative remodelling of the vessel, not the degree of neointimal accumulation. However, both VSMC proliferation and apoptosis regulate vessel caliber in remodelling, so that cell cycle inhibition may inhibit negative remodelling. In contrast to VSMCs from primary plaques, VSMCs from human restenosis lesions show increased cell proliferation and delayed senescence, but retain high rates of apoptosis, and the sensitivity to DNA-damage induced apoptosis. This appears to be a true ‘repair’ phenotype. Examination of cell cycle machinery reveals stable differences in expression of cyclins, cdk, and cdk inhibitors that underlie this difference. Such differences may account for the failure of conventional anti-proliferatives to inhibit neointimal accumulation in restenosis.

TuW2:11 Inhibition of angioplasty restenosis by vascular brachytherapy: Mechanisms of action and role of the adventitia
Josiah N. Wilcox. Emory University, Atlanta, GA, USA

Post-angioplasty restenosis is a major problem confronting cardiology today. While most studies have focused on neointimal development after angioplasty, recent data indicates that negative vascular remodeling, seen as constriction of the external elastic lamina, may contribute to lumen loss. Intravascular brachytherapy has been shown to be effective in blocking post-angioplasty restenosis and vascular remodeling in experimental animal models. Early results from clinical trials suggest that restenosis rates with brachytherapy are <15%. Previously we have described the proliferation of myofibroblasts in the adventitia surrounding porcine coronary arteries after angioplasty. Tracing studies indicate that these cells migrate from the adventitia and contribute to the cellular mass of the neointima. Experimental studies from our laboratory suggest that adventitial myofibroblasts may be one of the most important targets of brachytherapy. Radiation treatment of porcine coronary arteries after angioplasty reduces proliferation of adventitial myofibroblasts, inhibits the expression of PDGF by these cells, prevents formation of a myofibroblast scar at the angioplasty site and improves vascular remodeling. One of the mechanisms by which radiation appears to work is by increasing the expression of p21 in adventitial cells. These studies support the hypothesis that adventitial myofibroblasts contribute to vascular remodeling associated with angioplasty and emphasize the potential of radiation therapy in the control of restenosis.

TuW3:11 Construction and characterization of an HBGAM/FGF-1 chimera for vascular tissue engineering
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1Department of Surgery; 2Cell Biology, Mayoowd, IL; 3Department of Tissue Biology, Holland Laboratories of the American Red Cross, Rockville, MD, USA

Objective: Cardiovascular tissue engineering approaches to vessel wall restoration have focused on the potent but relatively non-specific and heparin-dependent mesenchymal cell mitochond FG-1. We constructed an heparin-binding growth-associated molecule (HBGAM/FGF-1) chimera by linking full length human HBGAM to the amino-terminus of human FGF-1b (211-154) and tested it’s activities on SMCs and ECs.

Methods: SMCs and ECs proliferations in response to the HBGAM/FGF-1 chimera and FGF-1 were measured by 3H-thymidine incorporation.

Results: In the presence of heparin the HBGAM/FGF-1 chimera stimulated less SMC proliferation (P < 0.00001) at 0.15 pmol versus 0.05 pmol. By contrast the chimera retained full stimulating activity on EC proliferation with an EDSO of 0.03 pmol for both the cells. Unlike the wild type protein, the chimera possessed heparin-independent activity and less synergistic response by the addition of heparin with no synergy at concentrations > 0.3 pmol. In the absence of heparin the chimera induced dose-dependent EC and SMC proliferation at 0.03 pmol compared to the wild-type FGF-1 which stimulated minimal DNA synthesis at 3.0 pmol concentrations.

Conclusions: The HBGAM/FGF-1 chimera displays significantly greater land uniquely heparin-independent mitogenic activity for both cell types and in the presence of heparin a significantly greater EC specificity.

This chimeric construct may provide a novel approach to engineering endothelialized surfaces without the concurrent fibroelastic reaction elicited by wild type FGF-1.
**TuW4:11**  
**Relationship between monocye chemoattractant protein-1 and restenosis after coronary angioplasty**

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**Objective:** Inflammation appears to play a pivotal role in the development of restenosis after coronary angioplasty (PTCA). Activation of leukocytes to areas of vessel injury is an important factor in this inflammatory response. The monocye chemoattractant protein-1 (MCP-1) is a specific chemoattractant of leucocytes and can modulate other functions of these cells e.g., generation of reactive oxygen species such as superoxide anion (O2•−). The purpose of our study is to investigate the role of this chemokin on the restenosis.

**Methods:** We measured circulating levels of MCP-1 and vitamin C before and after PTCA in 50 patients (30 M; 20 F; aged 62 ± 5 yr) who underwent PTCA and who had repeat angiograms at 6-month follow-up. Restenosis occurred in 14 (28%) patients. Levels of MCP-1 were measured by ELISA assay (values as pg/ml) and vitamin C was measured by a spectrophotometric assay (values as µmol/L).

**Results:** As shown in table, there were no differences before PTCA between the two groups. However, after PTCA, patients with restenosis showed significantly elevated levels of MCP-1 and significantly reduced plasma concentration of vitamin C.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre-PTCA 1 d post</th>
<th>5 d post</th>
<th>15 d post</th>
<th>180 d post</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1 restenosis</td>
<td>480 ± 42</td>
<td>816 ± 17*</td>
<td>755 ± 158*</td>
<td>712 ± 54*</td>
</tr>
<tr>
<td>no restenosis</td>
<td>470 ± 69</td>
<td>618 ± 102</td>
<td>594 ± 111</td>
<td>450 ± 72</td>
</tr>
<tr>
<td>VIT C restenosis</td>
<td>39 ± 3</td>
<td>23 ± 9*</td>
<td>23 ± 4</td>
<td>24 ± 4*</td>
</tr>
<tr>
<td>no restenosis</td>
<td>40 ± 2.4</td>
<td>35 ± 2</td>
<td>39 ± 3</td>
<td>40 ± 3</td>
</tr>
</tbody>
</table>

*P < 0.0001; **P = 0.007,  *P < 0.0001

Moreover, MCP-1 levels were significantly correlated (P < 0.0001) with monocyte activity, measured as O2•− production from healthy subjects monocytes incubated with serum of patients, both before and 24 hours after PTCA (ρ = 0.87 and p = 0.0528, respectively).

**Conclusion:** this study suggests that: a) MCP-1 may play a key role in the pathophysiology of restenosis after PTCA; b) this effect is mediated, at least in part, by an increased monocyte O2•− production and consequent circulating vitamin C consumption.

**TuW6:11**  
**Polysyline as a vehicle for extracellular matrix-targeted intravascular drug delivery, providing high accumulation and long-term retention within the vessel wall**


**Background:** Catheter-based delivery methods are currently being elaborated for local intravascular delivery of concentrated drugs, particularly for treatment of restenosis after coronary angioplasty. Short retention time of the delivered drugs in the vessel wall critically reduces the efficacy of this technique. We propose an approach of extracellular matrix (ECM)-directed drug targeting. The approach is based on a concept of a bifunctional drug consisting of an anti-restenotic effector moiety and an “affinity vehicle” capable of delivering and retaining the drug within the ECM of the vessel wall. The “affinity vehicle” should bind to an abundant component of the vessel wall, in order to provide a high concentration and ubiquitous distribution of the bound drug within the vessel wall.

**Objective:** As a first step in the elaboration of this approach, we studied polysyline as one of potential “affinity vehicles”, which might bind to negatively charged glycosaminoglycan components of the vascular ECM.

**Methods and Results:** Fluorescence-labelled poly-L-lysine was shown to bind abundantly to all layers of cross-sections of human vessels in a plasma environment. After delivery under pressure into a segment of a human umbilical artery, polysyline was concentrated through a luminal layer (50-100 µm) of the vessel wall, and was retained therein after 72 hours of perfusion without noticeable losses. Also after in vivo delivery into a segment of a rat carotid artery, polysyline was still present in the vessel wall after 72 hours, whereas control FITC-albumin was washed out in 1-2 hours. No major thrombotic or inflammatory complications were documented.

**Conclusions:** Polysyline can be considered as a potential “affinity vehicle” within the proposed approach of ECM-targeted local drug delivery. Testing of anti-restenotic drugs potentially usable in this approach is currently underway.

**W:12**  
**Diet and Bioactive Components of Food**

**TuW12:12**  
**A locus conferring resistance to diet-induced hypercholesterolemia and atherosclerosis on mouse chromosome 2**

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Dietary cholesterol is known to raise total and low density lipoprotein cholesterol concentrations in humans and experimental animals, but the response among individuals varies greatly. We identified a mouse strain, C57BL/6J (B6J), that is resistant to diet-induced hypercholesterolemia, in contrast to m phenotype seen in other common strains of mice including the closely related C57BL/6J (B6J) strain. Compared to B6J, B6By mice exhibit somewhat lower basal cholesterol levels on a chow diet, and show a relatively modest increase in absolute levels of total and LDL/LDL cholesterol in response to an atherogenic diet containing 15% fat, 1.25% cholesterol and 0.5% cholate. Correspondingly, B6By mice are also resistant to diet-induced aortic lesions, with less than 15% as many lesions as B6J. Food intake and cholesterol absorption are similar between B6By and B6J mice.

To investigate the gene(s) underlying the resistant B6By phenotype, we performed genetic crosses with the unrelated mouse strain, A/J. A genome-wide scan revealed a locus, designated Dietl, on chromosome 2 showing highly significant linkage (lod = 9.6) between B6By alleles and hypo-response to diet. Examination of known genes in this region suggested that this locus represents a novel gene affecting plasma lipids and atherosclerosis in response to diet.

We have now isolated this locus by constructing congenic strains in which the Dietl gene from B6By has been placed on other genetic backgrounds. Using these congenics, fine structure mapping of the gene has been initiated. We are also utilizing chimerae technologies to test for variations in gene expression to aid in the identification of candidate genes.
PPARs: Fatty acid-activated receptors controlling lipid metabolism and inflammation

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor family. The hypolipidemic fibrates and the antidiabetic glitazones are synthetic ligands for PPARα and PPARγ, respectively. Furthermore, fatty acids and eicosanoids are natural PPAR ligands. PPARs function as regulators of lipid and lipoprotein metabolism and glucose homeostasis and influence cellular proliferation, differentiation and apoptosis. PPARα is highly expressed in tissues such as liver, muscle, kidney and heart, where it stimulates the β-oxidative degradation of fatty acids. PPARα furthermore mediates the action of the hypolipidemic drugs of the fibrates class on plasma lipoprotein metabolism. PPARγ is predominately expressed in intestine and adipose tissue. PPARγ triggers adipocyte differentiation and promotes lipid storage. In addition, PPARs play a role in inflammation control. PPAR activators inhibit the activation of inflammatory response genes by negatively interfering with the NF-κB and AP-1 signalling pathways. PPAR activators exert these anti-inflammatory activities in different immunological and vascular wall cell types such as monocytes, endothelial, epithelial and smooth muscle cells in which PPARs are expressed. These findings indicate a modular role for PPARs in the control of lipid and glucose metabolism as well as in the inflammatory response with potential therapeutic applications in inflammation-related diseases, such as atherosclerosis.

Obesity and diabetes gene loci in genetically obese mice

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While obesity is an important risk factor for Type II Diabetes, background genetic factors substantially modify an individual’s risk for developing the disease. We sought to identify modifier alleles present within a murine model of insulin resistance developed by our laboratory – the BTBR × C57BL/6J F1 mouse. In lean F2 mice, these alleles result in impaired glucose tolerance and impaired glucose uptake into muscle and adipose tissue. We hypothesized that these alleles might also lead to severe diabetes in ob/ob animals. If obese BTBR and B6 mice differed in their susceptibility to diabetes, we could identify the loci responsible for the modification of the diabetes syndrome. We intercrossed the ob allele into the BTBR strain using marker-assisted backcrossing for 4 generations, and intercrossed to produce ob/ob mice. The Nf1 mice (BTBR.ob/ob) have markedly higher fasting levels of plasma glucose compared to B6-ob/ob at 10 weeks (females: 350 vs. 170 mg/dl, p < 10-7; males: 460 vs. 250 mg/dl, p < 10-4). BTBR.ob/ob mice also have significantly lower fasting plasma insulin levels at 8 and 10 weeks. We generated 250 F2 ob/ob mice. These F2 mice exhibit a 5-fold range in fasting plasma glucose at 10 weeks of age (150-750 mg/dl) and a 60-fold range in fasting plasma insulin (2-120 ng/ml). The F2 mice show great variability in pathophysiology of β-cells. We genotyped the F2 panel at 120 polymorphic markers and used composite interval mapping techniques to detect segregating QTL. We report two highly significant linkages to the plasma glucose trait. One of the two loci also shows highly significant linkage to the plasma insulin trait. Surprisingly, although all of the F2 animals were ob/ob, their body weight showed a large range – 40-75 g. We therefore mapped two gene loci that together control 30% of the variance in body weight. In conclusion, we have shown that alleles within the BTBR strain exacerbate the obesity/diabetes syndrome in ob/ob mice, and we have detected two such modifier loci with highly significant linkages. In addition, we have mapped two body weight loci that do not involve the leptin pathway.

Consumption of plant stanol esters increase LDL receptor expression in mononuclear cells from non-hypercholesterolemic subjects

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Introduction: Plant stanol esters lower serum LDL cholesterol by reducing intestinal cholesterol absorption. This causes a compensatory increase in cholesterol synthesis. Whether LDL receptor expression is also changed, is not known. We therefore decided to analyze effects of stanol ester consumption on LDL receptor expression on three subpopulations of human mononuclear cells.

Methods: 112 men and women consumed for a four week run-in period a low erucic acid rapeseed (LEAR) oil based margarine and shortening, followed by an 8 week test period of the same rapeseed oil based products enriched with wood (n = 34) or vegetable oil (n = 36) based plant sterol ester mixtures. Daily stanol intake was 3.8-4.0 g. A control group consumed no plant stanol esters (n = 42). LDL receptor expression was measured in a subset of 36 subjects (n = 12 of each group) by flow cytometry using FITC labeled MABs against the LDL receptor. Monocytes, T-and B-lymphocytes were identified by PE-conjugated MABs.

Results: Consumption of plant stanol esters significantly (P < 0.001) lowered LDL cholesterol with 13-15%. LDL receptor expression on monocytes increased by 50% (P = 0.011) and 13% (P = 0.039) in the wood based and the vegetable oil based group respectively. Compared to the control group, T-lymphocyte LDL receptor expression was 34% (P = 0.033) higher in the wood and 20% (P = 0.155) in the vegetable oil based group. Expression on B-lymphocytes was not affected.

Conclusion: Consumption of plant stanol esters induces a higher expression of the LDL receptor on the surface of mononuclear cells. Whether this is due to enhanced receptor synthesis and/or lower degradation remains to be established.

Role of sequestration in hepatic uptake of chylomicron remnants

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Objective: To visualize chylomicron remnant (CR) accumulation in the space of Disse (SD) and determine the role of the LDL receptor, the LDL receptor-related-protein (LRP), hepatic apoE, and HSPG in sequestration in the SD.

Methods: CR were labeled with the fluorescent dye, DiD (DiD-R), and perfused into isolated livers from LDL receptor-knockout (LRKO) mice, apoE-knockout (EKO) mice, apoE/LDLR double-knockout (DKO) mice and C57BL/6J (wildtype) mice in a single non-recirculating pass. Livers were processed for immunocytochemistry and confocal laser microscopy. Endothelial cells were labeled with FITC-conjugated anti-von Willebrand factor antibodies. Cellular periphery (F-actin fibers) were labeled with rhodamine-conjugated phallidin.

Results: In normal livers CR were rapidly internalized with little accumulation in SD. In LRKO livers, the capacity for remnant removal was reduced with substantial accumulation in the SD. To prove CR were not internalized by hepatocytes, endothelial, or Kupfer cells in LRKO, trypan blue was perfused through the liver to quench extracellular fluorescence. The majority of fluorescence in the LRKO animal was between the hepatocyte and the endothelial cells and was quenched. RAP (inhibitor of LRP) virtually eliminated DiD-remnant fluorescence in SD, suggesting the LRP was absolutely required for sequestration. In EKO livers, there was little accumulation of remnants in the SD and internalization into hepatocytes was normal. Accumulation of remnants in SD was significantly increased in DKO livers. Sodium heparin and fibroblast growth factor (ligands of HSPG) are being studied to evaluate the role of HSPG.

Conclusions: 1. There is sequestration of remnants in SD before endocytosis only in the absence of the LDL receptor. 2. Accumulation of remnants in the space of Disse is dependent on the LRP and does not require hepatic apoE. This technique will now allow evaluation of the role of HSPG in this process.

Possible role of SREBP1c in fish oil-mediated regulation of APOC-III gene expression

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Fish oil exert part of its effect by altering the expression of various genes involved in lipid metabolism. The goal of the present study was to assess the effect of fish oil on apoC-III metabolism. To this end, transgenic human apoC-III mice (hTgC-III) and wild type (WT) controls were fed either coconut oil (5%) or fish oil (5%) for 2 weeks. Blood plasma and apoC-III levels were lower in both the fish oil-fed WT and hTgC-III mice. The decrease in TG levels was associated with decrease in TG production rate and an increase in TG catabolic rate. The latter effect support the concept that lower levels of apoC-III are associated with improved clearance of TG. Fish oil treatment resulted in lowered levels of liver apoC-III mRNA in both strains suggesting that fish oil lowers apoC-III gene expression. Recently, fish oil has been shown to decrease at least the maturation and hence the transcriptional activity of the
transcription factor SREBP1c. In order to investigate if such decrease could explain the effects of fish oil on apo C-III and TG levels, coinfection assays in KK13 cells were conducted. We observed a specific and potent enhancement of the −1415/−24 fragment of the human apo C-III promoter activity by overexpressing the mature nuclear form of SREBP1c. Specific binding of SREBP on a putative E-Box located in position −87/−82 was confirmed in vitro by gel shift experiments using wild type or mutated oligonucleotide probes covering this fragment. In conclusion, since SREBP1c activated human apoC-III gene expression, and since fish oil reduces the nuclear SREBP1c level, SREBP1c could be involved in the decrease in plasma apoC-III and liver apoC-III mRNA levels observed with fish oil.

**TuW7:12** Long-chain N-3 fatty acids improve large artery elasticity in humans; DHA and EPA are equivalent

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**Objective:** To test the relative capacity of the 2 major fish oil n-3 fatty acids (EPA and DHA) to raise compliance (elasticity) of large arteries, since reduced compliance leads to systolic hypertension and possible coronary insufficiency.

**Methods:** 38 middle-aged dyslipidemic subjects were randomised into 3 groups to receive either placebo (PI) (14), 3 g EPA (12) or 3 g DHA (12) for 7 weeks in a double-blind parallel design trial. The groups were well matched for key variables. Outcome data: systemic arterial compliance (SAC) by non-invasive measurements of arterial pressure pulse waves and aortic flows; plasma lipoproteins; arterial pressures (BP). Comparisons by paired t-test, run-in versus end.

**Results:** 1. SAC improved with both fatty acids, run-in vs end: PI 0.150 and 0.150 units; EPA 0.149 and 0.202 (P < 0.005); DHA 0.147 and 0.186 (p = 0.012), Plasma TG and VLDL. TG fell significantly with EPA (28%) and DHA (31%); HDL C rose with DHA (10%), p = 0.002) but not EPA. LDL C and BP were not influenced. Plasma fatty acids: with EPA only EPA rose; with DHA both EPA and DHA rose; oleic fell.

**Conclusion:** Arterial compliance, a likely new risk factor for cardiovascular disease, was significantly improved when dyslipidemic subjects were given 3 g EPA or DHA.

**Lipoprotein profile improved**

**W:13 EXTRACELLULAR MATRIX**

**TuW1:13** Proteoglycans in atherosclerosis and restenosis

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Proteoglycans accumulate within atherosclerotic and restenotic lesions and contribute to increased tissue mass and altered vascular cell phenotypes. Furthermore, these molecules interact with lipoproteins to increase lipid retention throughout vascular lesions. Visceral, the major intestinal chondroitin sulfate proteoglycan (CSPG), increases as lesions progress but is rapidly degraded as lesions regress. Visceral is synthesized by arterial smooth muscle cells (ASM) as multiple mRNA spiked variants. Overexpression of visceral by cell mediated gene transfer alters ASM phenotype and influences extracellular matrix (ECM) composition in blood vessels subjected to experimental injury. Visceral interacts with hyaluronan to form complexes outside the cell, which are required for these cells to proliferate and migrate. Decorin is a small dermatan sulfate proteoglycan (DSPG) that influences vascular calcification and inhibits TGF-β1 activity when overexpressed by ASM transduced with decorin cDNA. Transfer of decorin overproducing ASMCs into injured arteries reduces intimal thickening and promotes collagen deposition. Decorin is synthesized also by endothelial cells during sprouting and tube formation in vitro and may, in part, regulate angiogenesis. Biglycan is another DSPG that accumulates in vascular lesions and interacts with lipoproteins. Interference of this interaction in diet induced atherosclerotic animal models blocks lesion formation. Heparan sulfate proteoglycans (HSPGs) are synthesized by ASMCs and endothelial cells and influence vascular cell adhesion, proliferation and migration. Removal of HSPG from injured arteries by heparinase treatment is effective in reducing the mitogenic response induced by bFGF in arterial smooth muscle cells. Collectively, these studies indicate multiple roles for specific proteoglycans in atherosclerosis and restenosis.

**TuW2:13** Expression of "proteoglycan-binding defective LDL" in transgenic mice

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Subendothelial retention of LDL through their interaction with proteoglycans has been proposed to be a key process in the pathogenesis of atherosclerosis. We have earlier shown that the substitution of the basic amino acids residues in Site B (residues 3359-3369) in apo-B100, the protein moiety of LDL, with neutral amino acids abolished both the LDL receptor-binding activity and the proteoglycan binding activities of the recombinant LDL. To test if the interaction between apo-B100 and proteoglycans is important for atherogenesis, we performed an extensive atherosclerosis study with five groups of transgenic mice expressing different forms of "proteoglycan-binding-defective LDL" or wild-type human LDL. The mice were fed a high-cholesterol diet for 20 weeks. To determine the extent of atherosclerosis, their aortas were stained with Sudan IV and analyzed by an en face procedure. The results showed that arteries of mice expressing "proteoglycan-binding-defective LDL" had less atherosclerotic lesions than mice expressing wild-type human LDL at equal plasma cholesterol concentrations. However, no differences could be seen after the mice were fed a high-cholesterol diet for 30 weeks. We also analyzed the retention of wild-type human LDL and "proteoglycan-binding defective LDL" in mouse and rabbit aortas ex vivo. The results showed that "proteoglycan-binding-defective LDL" are retained to a lesser extent in normal artery wall than normal recombinant LDL. In contrast, both LDL were retained to almost the same extent in artery wall with atherosclerotic lesions. These findings provide direct experimental evidence that interactions between apo-B100 and arterial proteoglycans are key in the initiation of experimental atherosclerotic lesions, and show that other mechanisms come into play as lesions progress.

**TuW3:13** Secretory sphingomyelinase and atherosclerosis

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**Background:** Our laboratory and others have accumulated a large body of evidence from in-vitro and cell-culture studies and from human & animal lesion analysis that secretory sphingomyelinase (S-SMase), a product of the acid SMase (ASM) gene secreted by macrophages (Mphs) and endothelial cells, is atherogenic. The proposed mechanisms include S-SMase-induced aggregation and matrix-retention of subendothelial lipoproteins, leading to Mph foam cell formation.

**Objective:** To determine if S-SMase is atherogenic using induced mutant mice.

**Methods:** Model #1: ApoE knockout (E0) mice with 0–2 copies of the ASM gene. Model #2: Mφ-targeted SMase transgenic (Tg) mice. Analysis: Proximal aortic cross-sectional lesion area at 25 weeks of age on a chow diet; and aortic LDL retention in vivo using a 111I-LDL17I-tyramine-cellobose-LDL assay.

**Results:** Model #1: ASM1 (heterozygous) mice secreted 30% of wild-type S-SMase and 65% of lysosomal SMase (L-SMase), and ASM0 mice had no S-SMase or L-SMase activity. The plasma cholesterol levels and lipoprotein profiles were very similar among the three different models. Aortic lesion areas (mm2) were 0.60 for E0/ASM2 (n = 7), 0.27 for E0/ASM1 (46% of E0/ASM2; n = 4; p < 0.002), and 0.22 for E0/ASM0 (37% of E0/ASM2; n = 12; p < 0.00002). This protective effect was observed in both genders and in both early and advanced lesions.

Model #2: Despite identical average plasma LDL concentrations, there was a 1.7–2.3-fold increase in LDL retention & degradation in oxidatively stressed artery of two lines of Mφ-SMase Tg mice vs. non-Tg mice. Preliminary data with a small # of mice revealed that the Tg mice had ~5-fold higher proximal aortic lesion area.

**Conclusion:** Smase promotes atherogenesis without altering plasma lipid levels, most likely by promoting the arterial retention and degradation of LDL.

**TuW4:13** Molecular basis for the association of group IIA phospholipase A2 and decorin in atherosclerotic lesions

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**Objective:** We recently reported that group IIA secretory non-pancreatic phospholipase A2 (sPLA2) is associated to collagen fibers in the extracellular matrix of human atherosclerotic plaques. In the present study we
explored if snpPLA2 may be associated to collagen fibers via interaction with decorin.

**Methods:** The distribution of snpPLA2 and decorin was studied in human atherosclerotic and non-atherosclerotic tissue by immunohistochemistry to compare their relative in vivo localization. In vitro binding experiments were performed to characterize the interaction of snpPLA2 and decorin.

**Results:** Decorin was detected within the snpPLA2-positive part of the intima close to the media in lesions. Electrophoretic mobility shift assay showed that snpPLA2 binds to decorin isolated from cell cultures of human fibroblasts. In addition, in a solid phase binding assay decorin enhanced the association of snpPLA2 to collagen type I and VI. Digestion of the GAG-moiety with chondroitinase ABC did not change the binding of snpPLA2 to decorin. Furthermore, snpPLA2 bound efficiently to a recombinant decorin core protein fragment B(1-548) (GAG545-Lys359). This binding was competed with soluble decorin and inhibited at NaCl-concentrations above 150 mM. The activity of snpPLA2 increased 2-3 folds in the presence of decorin or GAG-depleted decorin when using phosphorylcholine containing mixed micelles or low density lipoprotein as substrates.

**Conclusions:** The results show that snpPLA2 binds to the decorin protein core, and the interaction enhances snpPLA2-activity. As a consequence, this active extracellular enzyme may contribute to the pathogenesis of atherosclerosis by modifying lipoproteins and releasing inflammatory lipid mediators at places of lipoprotein retention in the arterial wall.

**TuW7:13 Oxidized LDL binds to macrophage-secreted extracellular matrix and is taken up by macrophages: An alternative approach to studies on lipoprotein cellular uptake**

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**Objective:** To analyze 1) whether macrophages can secrete an extracellular matrix (ECM) layer, 2) if Oxidized LDL (Ox-LDL) can bind to this macrophage-derived ECM layer and 3) whether binding of Ox-LDL to the macrophage-derived ECM leads to its uptake by macrophages.

**Results:** Macrophages were shown to produce an ECM layer as illustrated by electron-microscopic as well as optic-microscopic studies. Macrophage derived ECM could bind native LDL, as well as oxidized LDL (by 3 fold more than native LDL), in the presence of lipidoprotein lipase. The uptake of Ox-LDL by PMA-activated macrophages was found to be specific, dose- and time-dependent, and it was higher by 1.5 fold than the uptake of ECM-retained native LDL. Following labeling of the ECM glycosaminoglycans (GAGs) with ^35^S, the cellular uptake of ECM-retained Ox-LDL, as well as that of ECM-GAGs were obtained in parallel, illustrating that ECM-retained Ox-LDL is taken up by the macrophages together with the ECM-GAG which binds to the lipoprotein. These results were confirmed in vivo by using ECM layer from mouse peritoneal macrophages (MMP) that were harvested from the atherosclerotic apolipoprotein E deficient mice (E^(-/-). During mice aging (10-24 weeks) and development of atherosclerosis, the GAG content of their MMP-derived ECM increased by up to 52%, the ability of their MMP-derived ECM to bind Ox-LDL increased by up to 57%, and the uptake by J-774 A1 macrophages of Ox-LDL that was retained in MMP-derived ECM increased by up to 86%.

**Conclusions:** Thus, the present study demonstrated for the first time: (A) that macrophages can secrete an ECM layer; (B) that Ox-LDL can bind to this macrophage-derived ECM and (C) that binding of Ox-LDL to ECM can lead to its uptake by activated macrophages. This may represent a physiopathological phenomenon, leading to cholesterol and oestrogens accumulation in arterial wall macrophages, the hallmark of early atherosclerosis.

**W:14 PLAQUE INSTABILITY AND ACUTE CORONARY SYNDROMES**

**TuW1:14 Pathology of vulnerable plaques**

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Coronary atherosclerosis is by far the most frequent cause of ischemic heart disease, and plaque disruption with superimposed thrombosis is the main cause of the acute coronary syndromes of unstable angina, myocardial infarction, and sudden coronary death.

The risk of plaque rupture depends more on plaque vulnerability (plaque type) than on degree of stenosis (plaque size): lipid-rich and soft plaques are more vulnerable and prone to rupture than collagen-rich and hard plaques. Furthermore, they are highly thrombogenic after disruption.

There seem to be three major determinants of a plaque’s vulnerability to rupture: 1) size and consistency of the lipid-rich atheromatous core (the ‘gumel’); 2) thickness of the fibrous cap covering the core; and 3) ongoing inflammation and repair processes within the fibrous cap. Lipid accumulation, cap thinning, loss of smooth muscle cells (smc) and macrophage-related inflammation destabilize plaques, making them vulnerable to rupture. In contrast, smc-related healing and repair processes stabilize plaques, protecting them against disruption. Plaque size or stenosis severity tell nothing about a plaque’s vulnerability. Many vulnerable plaques are invisible angiographically due to their small size and compensated vascular remodeling.

The most feared consequence of plaque disruption is thrombotic occlusion of the artery. There are three major determinants of the thrombotic response...
to plaque rupture: 1) local thrombogenic substrate; 2) local flow disturbances; and 3) systemic thrombotic propensity.

To study the devastating consequences of atherosclerosis we have tried to develop an animal model of plaque rupture with superimposed thrombosis. Until now, we have failed to induce rupture of “vulnerable-looking” aortic root plaques in more than 200 middle-aged (>1 year old) apoE−/− mice exposed to extremely stressful stimuli.

**TuW2:14 Is there a mouse model of plaque rupture?**

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**Objective:** To determine the incidence of atherosclerotic plaque rupture at arterial branch points in apolipoprotein E knock-out (apoE−/−) mice.

**Methods:** Six male and five female apoE−/− mice were fed a diet supplemented with 21% lard and 0.15% cholesterol for up to 14 months. In animals that died, the principal branch points in the carotid arteries and aorta were removed and analysed. Sections were examined histologically, using haematoxylin and eosin. Miller’s elastin stain, or Martius Scarlet Blue, and by immunocytochemistry, using an antibody directed against a-smooth muscle actin to identify smooth muscle cells.

**Results:** Four of the male mice and four of the female mice died, after 46 ± 3 weeks of feeding (range 37 to 59 weeks). Lumenal thrombus associated with atherosclerotic plaque rupture was observed in three male and all four female mice. In six of these seven mice, an atherosclerotic plaque rupture was found where the brachiocephalic artery branches into the right common carotid and right subclavian arteries. The ruptures were characterised by fragmentation and loss of elastin in the fibrous caps of relatively small and lipid-rich plaques overlying large complex lesions, with intraplaque haemorrhage. Immunocytochemical analysis revealed loss of smooth muscle cells from ruptured caps.

**Conclusions:** These data suggest that long-term fat-feeding of apolipoprotein E knock-out mice is a useful and reproducible model of atherosclerotic plaque rupture, and that ruptures occur predominantly in the brachiocephalic artery.

**TuW3:14 Visualization of the vulnerable plaque**

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The composition of the atherosclerotic lesion rather than the degree of stenosis is currently considered to be the most important determinant for acute clinical events. Modalities capable of characterizing the atherosclerotic lesion may help to understand its natural history and detect lesions with high risk for acute events. Grossly, three histological features of the vulnerable plaque have been reported: size of the atheroma, thickness of the fibrous cap and inflammation. Imaging techniques are currently being deployed and under development to visualize these characteristics of the vulnerable coronary plaque. Most of these diagnostic modalities have the potential to locally detect one or more of the three histologically defined features of the vulnerable plaque. The highest resolutions are achieved by catheter based techniques like IVUS and Raman spectroscopy. Except for optical coherence tomography, however, the resolution of current techniques is still too limited to discriminate the thin fibrous cap. The non invasive imaging modalities like MPR suffer from inadequate resolutions but their unlimited penetration depth and their non invasive nature are advantages. Imaging techniques that visualize the plaque locally may provide new insight into the etiology of sudden progression of atherosclerotic disease or acute events. However, due to their local applicability, it is not expected that they will have prognostic properties for the development of acute clinical syndromes that often originate from non haemodynamically significant lesions. Therefore, systemic markers for inflammation may have more prognostic value for the identification of patients suffering from clinical events as a result of plaque rupture.

**TuW4:14 Increased serum MMP 9 concentrations in patients with angiographically assessed coronary artery disease**

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**Objectives:** Matrix metalloproteinases (MMPs) are upregulated in unstable atherosclerotic plaques. We examined whether MMP serum levels reflect the progression of coronary artery disease (CAD).

**Methods:** Serum matrix metalloproteinase-9 (MMP-9) concentrations were determined by ELISA in 61 patients (39 males, 22 females, mean age 56.5 years) who had narrowing in one or more coronary arteries assessed by coronary angiography. The control group consisted of 19 patients (9 males, 10 females, mean age 50.9 years), who had no pathological findings in coronary angiography.

**Results:** Serum MMP-9 concentrations tended to increase in the order: controls (32.2 ± 16.1 μg/L) > 1 or 2 vessel CAD (40.4 ± 25.1 μg/L) > 3 vessel CAD (57.3 ± 39.1 μg/L) (P = 0.011, ANOVA). In a logistic regression model adjusting for known CAD risk factors, serum MMP-9 was the strongest predictor of CAD (P = 0.013). In a 10 year follow-up, the serum MMP-9 concentration, taken before coronary bypass surgery, did not predict subsequent mortality from coronary events.

**Conclusions:** These results suggest that serum MMP-9 concentration is associated with severity of coronary narrowing and may have diagnostic value in evaluating the extent of CAD.

**TuW5:14 Lipoprotein-associated Phospholipase A2 (Lp PL-A2), an inflammatory marker and novel independent risk factor in the West of Scotland Coronary Prevention Study (WOSCOPS)**

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**Objective:** To determine the usefulness of chronic inflammation markers as predictors of risk in WOSCOPS.

**Methods:** A nested case-control study was designed from within the WOSCOPS cohort. 508 cases with CHD were age and smoking matched with 1160 controls. C Reactive Protein (CRP), Lp PL-A2 mass, fibrinogen (Fib) and white cell count (WCC) were measured at baseline or on frozen, stored samples. Relationship to risk was tested in Cox proportional hazards models. Risk ratios were estimated for quintiles (Q) of each variable.

**Results:** All four markers were strong predictors of risk in univariate analysis with about a 2 fold increase in risk comparing subjects in the highest vs lowest quintile. CRP correlated with Fib, WCC, body mass index, HDL and plasma triglyceride and in multivariate analysis its predictive capacity (like that of Fib and WCC) was much reduced. Lp PL-A2 was not correlated with other inflammatory markers and remained a strong independent risk factor in multivariate analysis. For Lp PL-A2, risks in Q 2–4 relative to Q1 were 1.26 (CI 0.83–1.92), 1.58 (1.04–2.4), 1.73 (1.16–2.60) and 1.66 (1.10–2.50) adjusting all other risk factors.

**Conclusions:** Chronic inflammation is a strong determinant of risk in asymptomatic, moderately hypercholesterolemic men. Lp PL-A2, an entity distinct from Group II secretory Lp-A2, is identified as a new, independent marker of CHD risk.

**W:15 GENETICS OF RISK FACTORS FOR CVD**

**TuW1:15 Genomic searches for genes that influence risk factors for CVD**

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**Objective:** To identify quantitative trait loci (QTLs) that influence CVD risk factors using genomic screens in families.

**Methods:** Our general approach is to perform genome-wide screens in large extended families that are not selected according to any particular disease. The genetic markers that are used for genomic searches are random microsatellite markers distributed throughout the human chromosomes at approximately 10 cM intervals. These markers are used for linkage analysis with variance component methods to identify chromosomal regions containing QTLs for CVD risk factors.

**Results:** We have conducted a genomic search in 480 family members from 10 large extended pedigrees of Mexican Americans in San Antonio. Our first published finding was the identification of a major QTL on chromosome 2 that influences serum levels of leptin hormone, an important risk factor for obesity. We have also identified QTLs on chromosomes 3 and 4 that influence LDL size class, an important CVD risk factor. In addition to lipid risk factors,
we are measuring levels of gene products involved in atherogenesis in the arterial wall. For example, we have found strong evidence for genetic control of serum levels of soluble P-selectin (h² = 0.70), and have detected major QTLs on chromosome 15 (LOD = 3.8) and chromosome 12 (LOD = 2.6).

**Conclusions:** Genomic searches in families are a powerful strategy to identify new QTLs that influence CVD risk factors.

**TuW2:15 Genetic factors and the response of CVD risk factors to regular exercise**

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**Objective:** To review the evidence for familial aggregation and the contribution of specific genes in the response of CVD risk factors to regular physical activity.

The 1996 USA Surgeon General’s Report on physical activity and health reviews several prospective studies concerning the relationship between levels of physical activity, CVD outcomes and CVD mortality rates. Collectively, these studies indicate that physically active adults are, on the average, less prone to CVD and have lower death rates from CVD than sedentary people. There are considerable individual differences in the changes observed in lipids and lipoproteins, blood pressure and other CVD risk factors as a result of exposure to exercise programs. A handful of twin studies and more recently the HERITAGE Family Study have shown that there is strong familial aggregation for the response pattern to regular exercise. The heritability for the changes in maximal oxygen uptake to a standardized exercise training program attained about 50 percent in HERITAGE. A genomic scan performed has revealed that several QTLs are linked to the responsiveness to regular exercise for cardiorespiratory endurance. However, thus far, candidate gene studies have evidenced only weak associations with the changes in CVD risk factors with regular exercise. The genetic dissection of the familial component of the response to regular exercise will be a complex undertaking requiring a variety of genomic and expression technologies.

**TuW3:15 Gene environment interaction in determining risk of ischemic heart disease**

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In understanding the impact of risk of thrombosis and Ischaemic Heart Disease (IHD), the modifying effects of different environmental factors experienced by individuals on the predisposition that they have inherited is particularly important. IHD risk has been determined in the second Northwick Park Heart Study, a twin candidate gene locus apolipoprotein (apo) CIII (C3238G [SstI]) and C-482T [insulin responsive element, IRE1]), apoAI (Thr347Ser), apoE (E2, E3, E4), apoB (C767T [XbaI]), Lipoprotein Lipase (Ser4847Stop), beta-fibrinogen (G-455A), factor VII (Arg353Gln), ACE (I/D), ENOS (intron 4) and stomelysin-1 (5A/6A). DNA was available on 2743 middle-aged men, free of IHD at baseline, recruited for prospective cardiovascular surveillance. Carriers of the apoAI gene Ser347 allele had a Relative Risk (RR) of 1.57 (95% CI: 1.07–2.21, p = 0.04) compared to Thr347 homozygous men, while stomelysin-1 5A/6A homozygous men had a RR of 1.91 (1.13–2.88, p = 0.02) compared with those with genotype 5A5A. Overall, men who were current smokers had a 2.20 (1.52–3.17, p = 0.0001) fold higher risk of IHD, and there was evidence for interaction between smoking and genotype in modulating risk at the stomelysin (p = 0.09), apoE (p = 0.02) and apoCIII (p = 0.002) loci. In particular, smoking was not associated with increased risk in the apoE4E3 group, but markedly augmented risk in apoE4 carriers (RR = 2.22 (1.33–3.72) in smokers compared with 0.58 (0.32–1.05) in non-smokers. Estimates of all of the genotype-associated IHD risk effects remained essentially unchanged after adjustment for the classical risk factors, suggesting that risk is not mediated through effects on plasma levels of measured lipids or clotting factors. Since men with the stomelysin genotype 5A5A and those who carry the apoE4 allele each represent 25% of the general population, this provides a strong argument for smoking avoidance in these individuals.

**TuW4:15 Linkage of blood pressure to a locus on chromosome 4 in Dutch dyslipidemic families**

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Genes contributing to the common forms of essential hypertension and blood pressure (BP) variation are largely unknown. This may result from the underlying genetic heterogeneity of this disorder. One approach to reduce such heterogeneity is to conduct gene finding efforts in families ascertained for common metabolic syndromes with associated hypertension. Familial combined hyperlipidemia (FCHL), a metabolic syndrome associated with insulin resistance, central obesity, and an increased frequency of hypertension, is such a disorder. Insulin resistance, defined by fasting insulin levels and more direct measures, is both associated with hypertension in cross-sectional studies and predictive in prospective studies. Furthermore, insulin resistance and FCHL are also accompanied by increased free fatty acid (FFA) levels. In the present study, we analyzed a 10 cM genome-wide scan in 18 Dutch FCHL pedigrees (N = 240) to search for genes contributing to BP in this metabolic syndrome. A multipoint genome scan of systolic (S) BP and diastolic (D) BP identified a region on chromosome 4 exhibiting a LOD score of 3.9 with SBP (peak marker D4S2639). Interestingly, FFA levels mapped to this region, with a LOD score of 2.4. Two-point linkage with markers under the peak also yielded evidence for linkage of SBP (P < 0.0008) and FFA (P < 0.009) to this locus, supporting the multipoint results. Alpha adducin, a gene involved in renal sodium handling, resides within this locus and has been associated with elevated blood pressure in both human populations and in animal models of hypertension. However, there is no evidence for an association between two intragenic polymorphisms within a-adducin and SBP in our Dutch population. In conclusion, this genome scan for BP has identified a chromosomal region harboring a potentially novel gene that contributes to hypertension associated with insulin resistant dyslipidemia.

**TuW5:15 Quantitative trait locus for vascular cell adhesion molecule-1 levels on chromosome 19**

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**Objective:** To detect, characterize and localize quantitative trait loci (QTLs) influencing serum vascular cellular adhesion molecule-1 (VCAM-1) levels in Mexican Americans from San Antonio, Texas.

**Methods:** We assayed serum levels of VCAM-1 in 471 Mexican Americans from 10 extended pedigrees using a commercially available ELISA kit. Initial statistical genetic analyses and a two-point, 20 cM whole genome linkage screen was accomplished using a maximum likelihood-based, variance decomposition approach. We followed these analyses with a multipoint, 15 cM, whole genome linkage screen.

**Results:** Our initial analyses showed that variation in VCAM-1 levels was influenced by additive genes (h² = 0.26), sex, diabetes, smoking, and menopause and provided suggestive evidence for QTLs on chromosomes 19 (LOD = 2.36) and 14 (LOD = 2.02). The 15 cM multipoint linkage screen yielded a maximum multipoint LOD score of 3.25 (p = 0.00012) on chromosome 19p. The 95% confidence interval localizes this QTL to 19p13.3 and subsumes a region containing excellent candidate loci for vascular biology, including loci for the mucosal addressin cell adhesion molecule-1 (MADCAM-1), the thrombomodulin A2 receptor (TM4A2R), and intercellular adhesion molecules-1 and-3 (ICAM-1 and ICAM-3).

**Conclusions:** A small-but-significant proportion of the phenotypic variance in normal serum levels of VCAM-1 due is the additive effects of genes. A QTL responsible for most of the additive genetic effect on VCAM-1 levels in this population is located on chromosome 19p13.3.

**TuW6:15 Acute coronary event risk-increasing polymorphisms of 5α-25 adrenergic receptor and serum paraoxonase genes: demonstration of a gene-gene interaction**

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**Objective:** We hypothesized that multiple gene polymorphisms which pre-
dispose to the same disease may have a synergistic rather than an additive effect.

Methods: We studied the associations between an \( \alpha_2 \)-adrenergic receptor polymorphism, a serum paraxonase polymorphism and the risk of developing an acute coronary event (ACE) in the 2682 men prospective KIIHD study. All men for whom both genotypic data were available and who were CHD-free at the baseline were included in the present analyses.

Results: Of the 1118 men, 48 (4.3%) developed an ACE during the follow-up. 16240 (6.7%) subjects were \( \alpha_2 \)-AR del/del homozygotes, 11/119 (9.2%) PON 54 Met/Met homozygotes and 4/25 (16%) that were homozygous for both developed an ACE. In multivariate Cox models adjusting for age, smoking, hypertension, blood lipids, obesity, diabetes, alcohol consumption, socioeconomic status and physical fitness, \( \alpha_2 \)-AR deletion homozygotes had 1.8-fold (95% CI 1.0 to 3.4, p = 0.045), PON 54 methionine homozygotes 2.4-fold (95% CI 1.2 to 4.9, p = 0.011) and homozygotes for both 5.0-fold (95% CI 1.8 to 14.2, p = 0.002) risk of developing an acute coronary event during the maximum 7.6 years of follow-up.

Conclusions: These data demonstrate the importance of simultaneous studying of multiple genes when assessing the effect of gene polymorphisms on cardiovascular diseases.

TuW2:16 HDL modifying agents: From small molecules to recombinant proteins for intervention in vascular disease

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Over the past three decades, the discovery and development of HDL modulating agents has been limited mostly to the "fraudulent fatty acid" class of drugs known as fibrates. More recently, with the discovery of specific nuclear receptors (e.g. PPARs), HDL receptors (e.g. SR1) and the ABC-1 gene casetse in the regulation of HDL metabolism, new opportunities have been presented to the worldwide pharmaceutical industry for discovering new, more effective drugs for elevating HDL, promoting reverse lipid transport and benefitting vascular disease. In addition to the focus on small molecule research, an unexploited area of research is the use of recombinant apo A-I proteins in phospholipid vesicles as "synthetic HDL particles" for the acute and chronic treatment of vascular disease complications (restenosis, unstable angina, ischemia, etc.). In addition to transgenic animal experiments involving knockout and overexpression of specific HDL-related genes, studies involving infusion of wild-type apo A-I, recombinant pro-apo A-I or variant forms (e.g. recombinant apo A-I Milano) in animal models of restenosis and atherosclerosis have confirmed the beneficial effects of this therapy for mobilizing cholesterol from arteries and/or reducing complications of vascular disease. Lastly, preliminary studies involving human subjects support the testing of different forms of apo A-I complexed to phospholipid as a new therapeutic and mobilizing and redistributing cholesterol, promoting its elimination through increased bile acid production and secretion, and potentially for treatment of atherosclerosis. A thorough review of the studies and their supporting data will be presented for this exciting new area of drug discovery research.

TuW3:16 HDL mutants and mimetics for the direct treatment of vascular disease

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Objective: The use of HDL or analogues for the treatment of vascular disease has come of age. ApoA-I liposomes have been shown to mobilize tissue cholesterol in patients with familial hypercholesterolemia (Eriksson et al, Circulation, 100, 594, 1999), by way of recruitment of cell cholesterol and formation of nascent small HDL (Nanjee et al, ATVB, 19, 979, 1999). Availability of the mutant apoA-I Milano in large amounts allows the direct evaluation of the potential therapeutic benefit of enhancing the reverse cholesterol transport system.

Methods: The recombinant apoA-I Milano (r apoA-IM) dimer, formulated with phospholipids, resembles the structure of native HDL. The product is a disk shaped complex of dipalmitoyl PC or oleoyl PC with r apoA-IM dimer; it is stable and well tolerated after intravenous injection.

Results: Preclinical data have been obtained in rabbit models of arterial restenosis and of neo-intimal proliferation induced by perivascular manipulation, and are supported by a direct antagonism toward aortic atherosclerosis in apoE KO mice (Sirtori et al, Atherosclerosis, 142, 29, 1999). The r apoA-IM/FL complex also improves endothelial dysfunction in apoE KO mice and exerts an antithrombotic effect in the ferrochrome lesioned abdominal aorta in rats. More recently, data have been obtained indicating that the r apoA-IM/PL complex, given intrapericardially to minipigs prior to PTCA, significantly inhibits intimal proliferation, giving a benefit in diameter stenosis of 52% compared to placebo. Finally, a single 1-2 h infusion of the r apoA-IM/PL complex has proved effective in a rabbit model of soft carotid plaque. Initial clinical evaluation of the product in volunteers is forthcoming.

TuW4:16 Bay 13-9592 (impitapide), an inhibitor of the Microsomal Triglyceride Transfer Protein (MTP), blocks secretion of Apo-B-lipoproteins


Objective: The aim was to identify inhibitors of lipoprotein secretion that are suitable as cholesterol and triglyceride lowering drugs.

Methods: In order to find such inhibitors we used the human hepatoma cell line HepG2, which shows differentiation-associated functions of parenchymal
liver cells such as secretion of ApoB-containing VLDL-like lipoproteins and of α-2-macroglobulin.

**Results:** BAY 13-9952 is an α-carboline derivative and was found to inhibit the secretion of apoB-associated particles from HepG2 cells with an IC_{50} value of 1.1 nM. Cell viability of HepG2 cells and general inhibition of secretory processes are not affected as indicated by the unchanged levels of α-2-macroglobulin secretion. MTP is a heterodimer composed of an unique 97-kDa subunit and the multifunctional protein disulfide isomerase (PDI). MTP is involved in the cotranslational translocation of lipids to nascent triglyceride-rich lipoproteins. A process specific for VLDL and chylomicron synthesis in hepatocytes and enterocytes, respectively. BAY 13-9952 potently inhibits the MTP-catalysed transport of lipids between synthetic small unilamellar vesicles. In this in vitro system, triglyceride transport is inhibited with an IC_{50} = 27 nM for partially purified MTP from porcine liver and with an IC_{50} = 10 nM for recombinant human MTP/PDI complex.

**Conclusion:** These studies demonstrate that BAY 13-9952 is a potent inhibitor of MTP with a potential use for the treatment of hyperlipidemias. Targeting this new mechanism may offer a new therapeutic principle for the treatment and prevention of CAD.

**TuW5:16** Probucol increases hepatic expression of High Density Lipoprotein (HDL) receptor, scavenger receptor class B type I (SR-BI), in vitro and in vivo. – enhancement of reverse cholesterol transport (RCT)


**Objective:** SR-BI is an established HDL receptor to mediate the selective uptake of HDL-lipids. The overexpression of SR-BI in the liver decreased plasma HDL-cholesterol levels and reduced atherosclerosis in murine models. Therefore, it is obvious that the enhancement of SR-BI-mediated pathway is one of the important therapeutic targets for atherosclerosis. Probucol is a potent hypolipidemic drug to reduce xanthoma formation in both human and rabbit low density lipoprotein receptor-deficient mutants and is unique in that this compound causes the reduction of plasma HDL-cholesterol levels. In the present study, we have examined the effect of probucol on the hepatic expression of SR-BI.

**Methods:** For in vivo and in vitro experiments, rabbit and human hepatoma cell lines, HepG2, were selected as experimental models, respectively.

**Results:** Probucol treatment decreased plasma HDL-cholesterol levels and induced smaller-sized HDL in rabbits. Hepatic expression of rabbit SR-BI mRNA expression was up-regulated by probucol-treatment. Next, we have examined the regulation of human SR-BI in HepG2 cells. By the addition of probucol into the media, both mRNA and protein levels of human SR-BI were increased in a dose-dependent manner with significantly increased uptake of Dil-labeled HDL-lipids.

**Conclusion:** These observations demonstrate that probucol up-regulated the hepatic expression of HDL receptor, SR-BI, in vitro and in vivo, suggesting that this compound increases the hepatic uptake of HDL-lipid as well as activates cholesteryl ester transfer as previously reported. In conclusion, probucol has a unique property to enhance the major protective system against atherosclerosis, RCT.

**TuW6:16** Rapid normalization of pre-established hypercholesterolemia in rhesus monkeys by the potent cholester absorption inhibitor ezetimibe (SCH58235)

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**Objective:** To determine if the cholesterol absorption inhibitor ezetimibe (SCH58235) reduces pre-established hypercholesterolemia (HC) and prevents diet induced HC in rhesus monkeys fed a "Western" diet.

**Methods:** The effect of ezetimibe (0.3, 1, 3, 10, 30 and 100 μg/kg/day, admixed in diet) on plasma lipids and lipoprotein composition was evaluated in rhesus monkeys fed a high fat/cholesterol Western diet containing 0.25% cholesterol and 22% fat for 3 weeks (n = 5/group). In one study, a crossover of the control and 100 μg/kg ezetimibe treatment groups was employed to study the duration of action of the drug and the time course of the reversal of pre-established HC.

**Results:** Rhesus monkeys fed a Western diet for 3 weeks results in an increase in plasma cholesterol (147 to 204 mg/dl) and LDL cholesterol (54 to 161 mg/dl). Ezetimibe at 3 μg/kg/day completely prevented these increases without significant changes in HDL or triglycerides (EDS = 0.5 μg/kg). In a related study, a single dose of an analog of ezetimibe was shown to decrease postprandial chylomicra cholesterol-esters by 69% without changes in other lipid components. In the crossover experiment, treating pre-established HC monkeys with 100 μg/kg ezetimibe reduced plasma cholesterol levels by 57% within 3 days of treatment and normalized plasma cholesterol within 10 days (294 to 148 mg/dl) and LDL (161–53 mg/dl). Despite a daily intake of 375 mg of dietary cholesterol, plasma cholesterol remained unchanged for 3 days after discontinuing ezetimibe treatment.

**Conclusion:** Ezetimibe rapidly reverses pre-established HC in rhesus monkeys predominantly through the normalization of LDL cholesterol. Removal of ezetimibe treatment results in a 3 day lag in plasma cholesterol rise due to its long duration of action. Ezetimibe will likely be effective in the treatment of hypercholesterolemia in humans.

**TuW7:16** The effect of metoprolol CR/XL on atherosclerosis

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**Objective:** To investigate whether metoprolol CR/XL treatment might affect subclinical atherosclerosis in subjects with hypercholesterolemia.

**Methods:** Subjects with primary hypercholesterolemia (total cholesterol ≥ 6.5 and LDL cholesterol ≥ 5.5 mmol/L) and also fulfilling the following ultrasound criteria: a maximum intima-media thickness (IMT) ≥ 1.0 mm and/or a measurable plaque in the far wall of the carotid artery were recruited to the present three-year prospective, randomised, placebo controlled, double-blind study. A total of 103 subjects started double blind treatment: 47 in the metoprolol CR/XL (100 mg) group and 56 in the placebo group. Twelve patients in each group withdrew from treatment. Results are reported for the remaining 79 subjects. Subclinical atherosclerosis was measured by B-mode ultrasound in the carotid artery each year during follow-up.

**Results:** Thirty-nine subjects in the placebo group and 33 subjects in the metoprolol CR/XL group were treated with statins with similar dose levels and total cholesterol was reduced from 9.4 to 6.0 mmol/L in the metoprolol CR/XL group and from 8.7 to 6.2 mmol/L in the placebo group. Furthermore, IMT of the common carotid artery in the metoprolol CR/XL group decreased from 0.905 ± 0.232 mm to 0.869 ± 0.169 mm and increased from 0.892 ± 0.177 mm to 0.922 ± 0.188 mm in the placebo group (p < 0.05 for difference in change between groups). The decrease in IMT in the metoprolol CR/XL group was obvious already after the first year of follow-up.

**Conclusion:** In subjects with hypercholesterolemia treated with lipid lowering drugs, the addition of 100 mg of metoprolol CR/XL seems to beneficially affect atherosclerosis development, as measured by IMT. This may reflect direct effects of metoprolol on arterial tissue.
Tuesday June 27, 2000: How-to Session Abstracts

**H:2 VASCULAR RECONSTRUCTION**

**TuH1:2**

**Graft endothelialization by in vitro lining**

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**Background:** Over the past 17 years, our group has developed and clinically applied an in-vitro endothelialization procedure whereby femoropopliteal ePTFE prostheses are confluently lined with cultured autologous endothelial cells prior to implantation. After successful non-human primate experiments in the 1980s, clinical trials were commenced in 1989.

**Patients and Methods:** Between June 1989 and December 1999 one-hundred and fifty patients received in-vitro endothelialized ePTFE grafts. In phase 1 of the study 24 patients received 27 endothelialized grafts and 16 patients 17 untreated grafts. In the subsequent phase 2 of the study endothelialization was offered to all patients who did not have a suitable saphenous vein available. Phase 2 commenced in June 1993 and included 126 patients receiving endothelialized ePTFE grafts (80 above knee and 36 below knee). In all 150 patients autologous endothelial cells were harvested from 4-5 cm segments of a subcutaneous vein (Phase 1: external jugular vein; phase 2: cephalic vein), grown to first passage mass cultures and confluently lined onto 6 mm ID ePTFE grafts precoated with fibrin glue. Patency assessment for Kaplan-Meier survivorship analyses was based on duplex sonography and angiography.

**Results:** Phase 1: Kaplan-Meier-Survivorship function revealed a primary patency rate at 9 years of 65% for the endothelialized group versus 16% for the control group (log-rank test p = 0.002, Wilcoxon test p = 0.003). Phase 2: The six-and-a-half year primary patency rate for phase 2 was 66.5% (63% for above-the-knee grafts and 78.2% for below-the-knee grafts).

**Conclusions:** Eleven years of clinical in vitro endothelialization provide strong evidence that autologous endothelial cell lining improves the patency of small diameter vascular grafts. The infection-free experience with 150 patients demonstrates that a cell culture-dependent procedure can be carried over into clinical routine.

**TuH2:2**

**Strategies for engineering endothelialization of blood contacting devices**

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Recent advances in cardiovascular tissue engineering have suggested strategies for in vitro assembly of cellular and extracellular components of the arterial wall. The long-term control of events occurring at the blood/device interface is likely critical to the clinical efficacy of these implants. Long term results will depend primarily upon resolution of three critical factors: 1) the persistence and viability of these biological structures, 2) acute and chronic regulation of cellular phenotypic characteristics and 3) control of immunologic barriers to allograft application. The endothelial cell is prominent in HLA antigen presentation and thus may negatively influence allograft success. The in vivo induction by the host of endothelialization may be stimulated via an induced directed angiogenesis resulting in trans-interstitial capillarization and surface endothelialization. Such host-derived endothelialization may obviate the immunologic hurdle to tissue engineered allografting. Strategies for inducing surface endothelialization may include the use of novel polymers capable of activating macrophages to produce endothelial cell chemotactant and mitogenic factors and the local delivery of either naturally occurring or recombinant "designer" angiogenic peptides.

**TuH3:2**

**Total tissue engineered vascular grafts**

Laura E. Niklason. Dept of Biomedical Engineering and Anesthesia, Duke University, Durham, North Carolina, USA

**Objective:** Develop a method for culturing arterial prostheses using a rapidly degradable polymer scaffold that is seeded with autologous vascular cells.

**Methods:** Vascular smooth muscle and endothelial cells from arterial specimens are expanded in culture. Tubular scaffolds consisting of polyglycolic acid (PGA) mesh are attached inside bioreactors, and vascular smooth muscle cells are seeded onto the PGA scaffolds. The bioreactors are then attached to a pulsatile perfusion system that provides radial distortion to the engineered vessels. Culture is maintained for 6–10 weeks, during which time the smooth muscle cells replicate and secrete extracellular matrix to form a confluent tissue, while the PGA matrix degrades. After 8 weeks, the lumina of the vessels are seeded with autologous endothelial cells, and pulsatile culture continues for 3–7 days.

**Results:** The rupture strengths of grafts cultured from bovine vascular cells are comparable to those of native human muscular arteries, and suture retention strengths are sufficient for arterial anastomosis. Smooth muscle cellular densities and collagen contents are comparable to native arteries, and the vessels contract to a variety of pharmacological stimuli. Smooth muscle cells stain positively for a range of markers of differentiation. In addition, the endothelial cell layer is adherent to the inner lumen and shows positive staining for several cell-specific markers. We have implanted autologous engineered arteries in a cohort of miniature swine and observed them to be functional for up to 4 weeks.

**Conclusions:** Implantable autologous arterial grafts may be cultured in vitro that display many functions of native arteries. Translation of these techniques to human cells remains the next hurdle in the work.

**H:3 IMPLEMENTATION OF PREVENTION PROGRAMMES**

**TuH1:3**

**Prevention of atherosclerosis: Do we practice what we know?**

G. De Backer. Dept of Public Health, Ghent University, Ghent, Belgium

Results from randomised controlled trials have shown that clinical events due to athero-thrombotic cardiovascular diseases can be prevented by lifestyle modifications and, when appropriate, by prolylactic use of certain drugs. That knowledge has increased and increased significantly. In recent years several surveys have been carried out in different countries to examine how that knowledge is implemented in clinical practice.

Recent analyses from the EUROASPIRE I survey have shown that only 33% of the patients receive lipid lowering drugs and in 36% of those receiving them total cholesterol was ≥5.5 mmol/l. If the treatment goals, given in the recommendations of European and other societies (1998) for total cholesterol (<5 mmol/l) and LDL cholesterol (<3 mmol/l) were applied, about 70% of those patients who were not using lipid lowering drugs and about 55% of those using such drugs would have needed more intensive action for cholesterol lowering.

Compared with results from other surveys, the overall picture is very similar although considerable variation exists across Europe. An integrated approach
TuH2.3  Shared care programs. Do they improve patient compliance?
J. de Velasco, J.A. Rodriguez, F. Ridocci. University General Hospital, Valencia, Spain

Objective: Action to improve secondary prevention (SC) in coronary heart disease (CHD) patients (pts) is needed. A large treatment gap exists between scientific evidence and the care that the pts recieve. Our objective was to try to bridge this gap.

Methods: A small task force was formed by the Cardiology Department to study the local practise of SC, to identify barriers and to look for solutions. We started a share care program with definition of goals and participation of primary care physicians. 306 CHD pts discharged from hospital during 1997 were included and followed for one year.

Results: More than 90% of the pts attended the hospital controls. 6 pts died and there were no drop-outs. The intervention of the program improved the level of all risk factors. Only 3.6% of the smokers, smoked one year later, 90% of hypertensives end 89% of diabetics were well controlled. The correction of dyslipemia was relevant but less successful. Despite of the increment of lipid-lowering drug prescription from 27% at discharge to 62% one year later, 33% of pts remined with a total chl, higher than 200 mg/dl (5.2 mmol/l) and 26% with a LDL-chol above to 130 (3.4 mmol).

Conclusion: A shared care program to control risk factors in CHD pts seems to be successful, even though more effort in dyslipemia control is needed, probably prescribing statins at higher doses.

TuH3.3  Quality assurance of secondary prevention of coronary artery disease
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Since 10 years health districts in Sweden (totally 79) have developed their own programs (quality standards) for secondary prevention of coronary artery disease based on national and international guidelines. These programs are the result from agreements between hospital and primary care physicians. In addition to quality standards, quality control has to be performed. For this purpose quality parameters have been identified and a computerised register for the whole of Sweden has been in work since January 1, 1998. It is owned in common by The Swedish Societies of Cardiology and Family Physicians.

Methods: Patients with acute myocardial infarction, coronary bypass surgery, and percutaneous coronary angioplasty are included. Patients are supplied with a booklet of the same type as have been used in Sweden for several years. It consists of short written information, tables and diagrams for monitoring of the risk factors over time and 7 report forms. These forms refer to the time of hospital leave and follow up at 3-6 months. 1, 2, 3, 4 and 5 years. Information to be filled in is about new main coronary events, patients' self-estimation of well being, different risk factors, and medication essential in secondary prevention.

Results: Since January 1, 1998 totally 51 districts of totally 79 have joined the program. Totally 8,212 report forms from hospital leave, 3,640 from 3-6 months, 1,117 from 1 year and 40 from 2 years of follow up have been sent in (figures from Feb 21, 2000). The results documented are generally excellent. However, the drop out rate is high. To reduce dropout rate 3 districts are engaged in collecting data on attitudes and obstacles expressed not only by all the patients enrolled in the program but also all physicians and nurses engaged.

Conclusion: Quality assurance of secondary prevention in patients with coronary heart disease needs engagement of both hospital and primary care physicians. Locally agreed programs (quality standards) have been successful and agreement exists on the principles of quality control. Those reporting show very good results. The reasons for high drop out rate is under investigation.

TuH4.3  Why can’t we do a better job? A systems approach to improving preventive cardiology services
T.A. Pearson. University of Rochester, Rochester, New York, USA

Numerous studies demonstrate agreement between recommended levels of preventive cardiology services and physicians’ opinions of their worth, but a large gap in the degree to which the services are actually performed. To address this paradox, the healthcare system might be dissected into the inpatient setting, post-discharge communication, the ambulatory care setting, and healthcare payors or professional bodies. The American College of Cardiology Evaluation of Preventive Therapeutics (ACEPT) Study collected data from 5,620 patients with coronary disease admitted to 53 U.S. hospitals to provide insights into ways that the “system” could be improved. Inpatient care often failed to evaluate risk factors, especially hyperlipidemia and diabetes, and infrequently initiated an intervention. Protocols to routinely measure lipids and glucose, checklists to verify attention to risk factors, and care plans to initiate interventions as part of the inpatient admission would alleviate these problems. Prevental risk factors and steps recommended to modify them are infrequently addressed in communications to primary care providers. A checklist of risk factors, interventions initiated, and work still required should be part of the postdischarge communication. Ambulatory care providers frequently did not initiate recommended interventions, or did not treat aggressively enough to attain goals. Primary care settings should be reorganized to assign preventive care to specific members of the health care team; referral to nurse case managers or cardiac rehabilitation programs is also worthwhile. Finally, healthcare systems and payors need to provide reimbursement and quality standards that encourage preventive services. Professional organizations can provide tools and education to assist in system reorganization. Physicians concur with the efficacy of preventive cardiology interventions but need the health care system to be modified to be more conducive to effectively implement well proven interventions.
Tuesday June 27, 2000: Poster Abstracts

P:W7  FATTY ACIDS: THE LINK BETWEEN INSULIN RESISTANCE AND DYSLIPIDAEMIA

Tup1:W7  Proatherogenic potentials of genetic deficiency of a major receptor for oxidized LDL, CD36. A possible candidate gene responsible for multiple risk factor clustering syndrome with insulin resistance

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Objectives: CD36 is one of the major receptors for oxidized LDL expressed in macrophages. It has been recognized that this molecule is widely expressed in human tissues and has multiple ligands, suggesting that CD36 has a variety of other functions than a receptor for oxidized LDL. Recently, studies with mice have raised possibilities that the rodent's homologue of this molecule might be a gene responsible for insulin resistance syndrome, though controversy still exists. The aim of this study is to know the clinical characteristics of human genetic CD36 deficiency, especially its atherogenicity.

Methods: In Study I, we have characterized the clinical profiles of 26 patients with this disorder. In Study II, we determined the frequency of CD36 deficiency in patients with coronary artery disease (CAD) and that in Japanese general population.

Results: In Study I, plasma triglyceride levels were higher in the patients, whereas HDL-cholesterol levels were lower in the patients than in controls. Fasting plasma glucose was elevated. Oral glucose tolerance test showed normal--impaired glucose tolerance with a delayed response of immunoreactive insulin in the patients. Hyperinsulinemic euglycemic clamp technique revealed mean whole body glucose uptake was decreased in the patients than in control subjects, suggesting CD36-deficient patients have insulin resistance. In Study II, the frequency of CD36 deficiency was 3-fold higher in the patients with CAD than in the general population.

Conclusions: These results showed human CD36 deficiency appears to be proatherogenic in association with insulin resistance and hyperlipidemia, suggesting this molecule may be a candidate responsible for multiple risk factor clustering syndrome with insulin resistance in humans.

Tup2:W7  Effect of atorvastatin on apolipoprotein B-100 kinetics in visceral obesity

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Objective: To examine the effect of an inhibitor of cholesterol synthesis on apolipoprotein B-100 (apoB) metabolism in visceral obese men with dyslipidaemia.

Methods: 7 viscero obese men (plasma triglycerides ≥ 1.5 mmol/L, cholesterol > 5.2 mmol/L, waist circumference > 100 cm and BMI > 29 kg/m²) were studied. Atorvastatin (40 mg/day) was given for 6 weeks. ApoB kinetics were measured using an intravenous bolus injection of 2H-Leucine (5 mg/kg), with subsequent isolation of plasma VLDL, IDL and LDL apoB fractions over 96 hours. The isotopic enrichment of the apoB-containing fractions was determined using gas-chromatography mass-spectrometry, Production rate (PR) and fractional catabolic rate (FCR) were derived by using a multi-compartmental model (SAAM-II).

Results: are presented in the table.

<table>
<thead>
<tr>
<th>ApoB, mg/L</th>
<th>Before</th>
<th>After</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>102.2 ± 35.2</td>
<td>75.8 ± 25.4</td>
<td>0.005</td>
</tr>
<tr>
<td>IDL</td>
<td>42.3 ± 4.4</td>
<td>29.7 ± 4.7</td>
<td>0.002</td>
</tr>
<tr>
<td>LDL</td>
<td>626.5 ± 104.8</td>
<td>341.4 ± 65.2</td>
<td>0.001</td>
</tr>
<tr>
<td>FCR, mg/kg/day</td>
<td>1.05 ± 0.58</td>
<td>0.52 ± 0.27</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Conclusions: The findings suggest that in obese men atorvastatin decreases the plasma concentration of all apoB-containing lipoprotein particles by increasing their clearance from plasma and not by decreasing their secretion or production. This may chiefly be due to upregulation of hepatic receptors as a consequence of inhibition of cholesterogenesis.

Tup3:W7  Chylomicron remnant metabolism in obese men with and without hypertriglyceridaemia

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Objective: To examine the metabolism of intestinal-derived lipoprotein remnants in viscero obese men with and without hypertriglyceridaemia.

Methods: 13 viscero obese men with plasma triglyceride (TG) ≥ 2.3 mmol/L and 12 men with TG < 2.3 mmol/L of similar age, BMI, waist-to-hip ratio and cholesterol level (waist circumference > 100 cm, BMI > 29 kg/m², plasma cholesterol < 6.5 mmol/L) were studied. Fasting remnant-like particle cholesterol (RLP-C) was measured by immuno-separation and apolipoprotein B-48 by enhanced chemiluminescent assays. Chylomicron remnant (CR) metabolism was also measured using intravenous bolus injection of chylomicron-remnant-like particles labelled with cholesterol 14C-olate, with subsequent measurement of 14CO2 in the breath over 10 hrs by isotope ratio mass spectrometry. The fractional clearance rate (FCR) of the CR-like particles was derived from the appearance of 14CO2 in breath using a multi-compartmental model (SAAM-II).

Results:

<table>
<thead>
<tr>
<th>Tg &lt; 2.3 mmol/L</th>
<th>Tg ≥ 2.3 mmol/L</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.63 ± 34</td>
<td>3.01 ± 0.78</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.66 ± 0.44</td>
<td>5.75 ± 0.41</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.95 ± 0.23</td>
<td>0.93 ± 0.17</td>
</tr>
<tr>
<td>RLP-C, mmol/L</td>
<td>0.58 ± 0.09</td>
<td>0.56 ± 0.27</td>
</tr>
<tr>
<td>Apo B-48, μg/mL</td>
<td>20.43 ± 4.89</td>
<td>28.80 ± 5.55</td>
</tr>
<tr>
<td>FCR of CR, pool/d</td>
<td>0.055 ± 0.043</td>
<td>0.072 ± 0.037</td>
</tr>
</tbody>
</table>

RLP-C was significantly higher (p < 0.001) and FCR of CR significantly lower (p = 0.006) in the obese groups compared with non-obese subjects (mean ± SD, plasma RLP-C 0.12 ± 0.01 mmol/L, FCR of CR 0.17 ± 0.13 pools/hr). Plasma TG level was strongly correlated with RLP-C (r = 0.843, p < 0.001) and apoB-48 (r = 0.625, p < 0.001).

Conclusions: Visceral obesity is generally associated with decreased CR clearance. Hypertriglyceridaemia in obese reflects a multiple-organ hypermetabolism. However, this is not related to impaired particle clearance as measured by the breath test, and may be due to an overproduction of intestinal-derived lipoprotein remnants.

Tup4:W7  Chylomicron remnant metabolism studied with a new stable isotope breath test in centrally obese subjects

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Objective: To test the hypothesis that chylomicron remnant (CR) metabolism is abnormal in normolipidaemic subjects (cholesterol ≤ 5.5 mmol/L, triglyceride < 2.0 mmol/L) with central obesity.

Methods: 22 centrally obese subjects (waist circumference > 94 cm for men, >80 cm for women and BMI > 27 kg/m²) and 23 non-obese subjects of similar age, sex and plasma lipid levels were studied. CR metabolism was measured using an intravenous bolus injection of CR-like particles labelled with cholesterol 14C-olate, with subsequent measurement of 14CO2 in the breath over 10 hours by isotope ratio mass spectrometry. The fractional clearance rate (FCR) of the CR-like particles was derived from the appearance of 14CO2 in breath using a multi-compartmental model (SAAM-II).

Results: are presented in the table. In a pooled analysis, the fractional clearance rate of CR was inversely correlated with BMI (r = 0.32, p =
0.012) and waist-hip ratio (r = -0.33, p = 0.012), but there were no significant associations with age, gender or plasma lipid levels.

Conclusions: The findings suggest that CR clearance is impaired in centrally obese subjects in the absence of overt hyperlipidaemia. This kinetic defect may contribute independently to the increased risk of cardiovascular disease in central obesity and requires further investigation.

TuP5:W7 Insulin action in young healthy adult offspring of parents with type 2 diabetes: Effect of vitamin E in a randomised double-blind placebo-controlled trial

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Objectives: Insulin resistance is implicated in the pathogenesis of diabetes and cardiovascular disease, and has been identified in offspring of parents with type 2 diabetes. Improvement of insulin action has been reported with antioxidant therapy, suggesting oxidative stress as a possible underlying mechanism. We aimed to determine the effect of antioxidant treatment on insulin action in young healthy adults with a parental history of type 2 diabetes.

Methods: Thirteen offspring (age 18–38 yrs, n = 11 M: 2 F, BMI < 30 kg/m²) completed a randomised, double-blind, crossover trial (12 weeks vitamin E 800 IU/day or placebo, 6 week washout). Insulin-stimulated glucose uptake was assessed by a euglycaemic hyperinsulinaemic clamp (1 μg/kg/min) at the end of each trial period.

Results: Vitamin E was well tolerated and compliance averaged 95% (serum vitamin E, active vs placebo: 50.8 ± 5.7 vs 23.7 ± 1.5 μmol/L, mean ± SEM, p = 0.0001). There was no difference between groups in BMI, blood pressure, fasting glucose, insulin or lipids. Exogenous glucose infusion rates (GIR) to maintain euglycaemia at steady state did not differ statistically between treatments (GIR, active vs placebo, 31.0 ± 3.0 vs 34.4 ± 2.6 μmol/kg/min, p > 0.5).

Conclusions: We have found no effect of pharmacological doses of vitamin E for 12 weeks on insulin sensitivity in healthy offspring of type 2 diabetic parents.

TuP6:W7 Insulin peptides and common carotid artery intima media thickness in 58-year-old men

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Objective: Several studies have indicated that proinsulin may be more proatherogenic than insulin. The aim was to examine the association between different insulin peptides and carotid artery intima thickness (IMT) as indicator of atherosclerosis.

Methods: 58-year-old men recruited from the general population (n = 391), free from cardiovascular disease and clinical diabetes were examined with ultrasound examination of common carotid artery IMT (bilaterally). Two-site immunoradiometric assays were used for measuring intact insulin and proinsulin. C-peptide was assessed by radioimmunoassay.

Results: IMT showed statistically significant correlations with systolic blood pressure (r = 0.36), BMI (r = 0.22), waist-hip ratio (r = 0.19), cholesterol (r = 0.11), triglycerides (r = 0.18), cigarette years (r = 0.22) insulin (r = 0.14), proinsulin (r = 0.17), C-peptide (r = 0.21). Only C-peptide and not proinsulin or insulin was independently associated with IMT. In a further stepwise multiple regression analysis 10% of the total variability in IMT was explained by systolic blood pressure (p < 0.001), cigarette years (p < 0.001), BMI (p = 0.002), and ApoB (p = 0.019). Insulin peptides showed no independent contributions to this variability.

Conclusion: C-peptide, as a measure of insulin secretion from the pancreas was independent of proinsulin and proinsulin, associated with common carotid artery IMT. However, C-peptide was not related to IMT independent of blood pressure, smoking, obesity and ApoB.

TuP7:W7 Interaction of serum apolipoproteins with scavenger receptor (SR)-BI

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Scavenger Receptor (SR)-BI is the first phylogenetically relevant receptor that binds high density lipoprotein (HDL) and promotes the uptake of cholesteryl ester (CE) from the core of HDL. In the current study, competitive binding experiments with the marine Y1-BST adenal cells were used to evaluate the apolipoprotein (apo) interaction with SR-BI. In competitive binding assays Y1-BST cells are incubated with 10 μg protein/ml of [125I]-HDL plus increasing amounts of competitor ligands. From these experiments the IC50 concentration of the competitor required to inhibit 50% binding of [125I]-HDL1 values of the various competitors are obtained. When presented as complexes with phospholipids, the exchangeable classes of apo examined (apo AI, AII, E2, E3, E4) all competed to the same extent for binding to SR-BI as measured by their IC50 values. Control experiments show that the major phospholipid on HDL, phosphatidylcholine, does not compete for binding with [125I]-HDL for SR-BI. To further examine the domain(s) of apo recognized by SR-BI variant forms of apo were generated. These include the cyanoen bromide cleavage of apo AI (N-terminal residues 1–86 and C-terminal residues 149–243) and peptides containing residues 1–43, 44–87 and 209–241 of apo AI. The results show that residues 1–43 of apo AI (which lacks a defined class A amphipathic helix) complexed with phospholipids is the only region of apo AI with a significant reduction in ability to compete with [125I]-HDL for binding to SR-BI. From these studies the structural motif of apo recognized by SR-BI seems to be the putative class A amphipathic helices present in these proteins. Studies with synthetic (non-apo sequence homology) class A peptides confirm this conclusion. From these findings the role of HDL's antiatherogenic properties will be better understood at the molecular level.

TuP8:W7 LDL-particle size and insulin resistance, measured with the hyperinsulimic clamp technique

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Objective: To evaluate the relationship between LDL particle size and insulin sensitivity.

Methods: 58-year-old men recruited from the general population (n = 103), free from cardiovascular disease, were examined in the morning after fasting overnight. Insulin sensitivity was determined by euglycaemic hyperinsulimic clamp as glucose infusion rate adjusted by fat free mass and LDL particle size by gradient gel electrophorosis.

Results: LDL particle size correlated significantly with glucose infusion rate (r = 0.33), serum triglycerides (r = −0.77), apolipoprotein B (r = −0.56), HDL cholesterol (r = 0.67), and insulin (r = −0.40). In a stepwise multiple regression with LDL particle size as dependent variable, triglycerides (r = 0.56) and HDL cholesterol explained 66% of the variability in LDL particle size. C-peptide had no independent effects on LDL particle size.

Conclusion: Small LDL particle size was associated with low insulin sensitivity, but this was not an independent association. Serum triglycerides and HDL cholesterol concentrations explain two thirds of the LDL particle size variation.

TuP9:W7 Plasma acylation stimulating protein and C3 in FCHL families

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A defect in adipose tissue triglyceride (TG) storage has been suggested as a pathogenetic mechanism behind familial combined hyperlipidaemia (FCHL). Aciylation stimulating protein (ASP) is a potent stimulator of TG synthesis in adipocytes. ASP is identical to cleavage product of complement C3a. The objective was to examine the role of plasma ASP and C3 in Finnish FCHL families.

Methods: Plasma ASP was available from 160 subjects (age 42 ± 12 years, M/F = 82/116) from 48 well-characterised FCHL families. Family members were divided in the hyper-TG group (H-TG) and normo-TG group (N-TG) using the serum TG’s age-sex specific 90th percentile as a cut-point. Normal lipoprotein spouses (n = 38) served as a control group. The
differences between groups were tested with two-way ANOVA (family number as a random factor). The FCOR program was used to assess familial correlations.

Results: Plasma ASP in the three groups (H-TG, N-TG and spouses) averaged 147 ± 54, 129 ± 51, and 116 ± 42 ng/mL, respectively (P = 0.043). Serum C3 concentrations in the three groups were 1.58 ± 0.38, 1.29 ± 0.33 and 1.16 ± 0.17 g/L, respectively (P < 0.001). Significant correlations (P < 0.05) were observed in siblings between C3 and TG, apo B, and HDL-cholesterol (negative correlation). For C3 there was a significant sibling-sibling correlation (P < 0.01). No familial correlations were observed for ASP.

Conclusions: FCHL patients representing hyper-TG phenotype have higher plasma ASP and C3 levels compared to their not affected relatives and normolipidemic spouses. While plasma ASP showed no evidence for familiality, these data suggest, however, that a common set of genes may contribute to the expression of TG, apo B, and HDL-cholesterol along with C3 in these families.

TuP10:W7 Using bioinformatics to identify novel SNPs in genes with relevance for insulin resistance

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Objective: To assemble a set of genes with relevance for insulin resistance and screen this for known and novel SNPs through web-based methods.

Methods: Genes of high relevance for physiological processes affected in insulin resistance were selected for the SNP screening. The selection was divided into 4 main categories: i) Adipogenesis & body weight regulation ii) Insulin & insulin-like function iii) Glucose uptake & utilisation & iv) Vascular function. Known SNPs were identified in databases: HGBASE (http://hgbase.interactiva.de/), OMIM (http://www.ncbi.nlm.nih.gov/OMIM/), HGMD (http://www.uwcm.ac.uk/uwcm/mg/hgmd.html) and dbSNP (http://www.ncbi.nlm.nih.gov/SNP/). To identify new SNPs, mRNA sequences from Genebank (http://www.ncbi.nlm.nih.gov/Genbank/index.html) were compared to dbEST (http://www.ncbi.nlm.nih.gov/BLAST/). Distinct differences between the Genebank sequence and EST sequences were categorised as potential SNPs.

Results: From approx. 120 genes, 60 potential SNPs were identified e.g. pos. 235 (g/t, Glu/Thr) and pos. 429 (c/g, Val/Val) in the IL-6 mRNA sequence. Neither one could be identified in DNA from 30 Swedish males after sequencing. 1 SNP in the sequence was found in databases. Several SNPs were found in genes after direct sequencing.

Conclusions: Several novel potential SNPs were identified using dbEST and a summary of known SNPs with relevance for the development of insulin resistance was created.

TuP11:W7 Obesity overrules the beneficial gender effects on triglyceride metabolism in non-obese subjects


Background: Postprandial lipemia is more efficient in females than in males. We evaluated whether gender differences in diurnal triglyceridemia are operative in obese subjects compared to lean volunteers.

Methods: Thirty-two obese (18 females and 14 males, BMI 31.3 ± 4.3 kg/m², fasting insulin 17.6 ± 13.1 mIU/L, fasting glucose 6.9 ± 2.7 mmol/L) and 106 lean subjects (54 females and 52 males, BMI 22.9 ± 2.3 kg/m², fasting insulin 8.2 ± 3.6 mIU/L, fasting glucose 5.1 ± 0.8 mmol/L) determined their diurnal capillary TG (TGc) profile on three different days. For each subject, mean absolute diurnal triglyceridemia (dTG-AUC) and mean incremental triglyceridemia (dTG-AUC) were calculated.

Results: TG-AUC and dTG-AUC in obese men (36.9 ± 13.7 and 8.2 ± 6.4 mmol/L, respectively) and obese women (34.5 ± 15.0 and 6.0 ± 4.5 mmol/L, respectively) were not significantly different. TG-AUC and dTG-AUC were lower in lean women than in lean men (16.5 ± 4.9 and 1.82 ± 3.5 mmol/L vs. 23.4 ± 6.5 and 6.9 ± 4.7 mmol/L). Since TG metabolism largely depends on fasting TG, diurnal triglyceridemia of a subgroup (6 obese men and 8 obese women) was compared to diurnal triglyceridemia of 14 non-obese controls, after matching for fasting TGc. Obese men had a similar dTG-AUC compared to non-obese men (6.1 ± 4.4 vs. 5.5 ± 4.1 mmol/L). Obese women showed significantly higher dTG-AUC compared to non-obese women (6.2 ± 4.7 vs. 1.0 ± 3.8 mmol/L; p < 0.05).

Conclusions: These results suggest that insulin resistance has a larger impact on diurnal triglyceridemia in obese women than in obese men.

TuP12:W7 Altered LDL-subfraction profile in individuals with normal glucose tolerance and insulin resistance

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Objective: A variety of studies have demonstrated a predominance of small, dense LDL as a risk factors that characterize the insulin resistance syndrome. This study addresses the question whether an altered LDL distribution pattern might already be observed in individuals with insulin resistance but still normal glucose tolerance.

Methods: The study population consisted of the offspring of patients with type 2 diabetes. All probands showed normal glucose tolerance. Individuals were divided according to their insulin sensitivity (ISI) in 50 insulin-sensitive and 49 insulin-resistant subjects. Both groups matched for age (29 years) and gender. Glucose tolerance was tested with a standard OGTT (WHO, 75 g Dextrose). Insulin sensitivity was assessed by the hyperinsulinemic euglycemic glucose clamp technique. LDL were separated into 6 subfractions by equilibration density ultracentrifugation.

Results: The medium dense LDL-3, LDL-4, and LDL-5 dominated the LDL subfraction profile in the insulin resistant group, these fractions being significantly higher than the respective fractions of the control group. Although the most dense LDL-6 fraction was higher in the resistant group, this difference was not of significance (see figure).

TuP13:W7 Triglyceride and VLDL-triglyceride responses to carbohydrate or fat is different in non-diabetic obese and lean women

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The goal of the present study was to study the influence of high fat or high carbohydrate meal on postprandial lipemia in lean and obese subjects. To this end, 11 lean and 8 non insulin-dependent obese women were given in a randomized order, either a carbohydrate meal (3.43 MJ, 166 g carbohydrates, 38 g proteins), or a fat meal (3.35 MJ, 70 g fat, 36 g proteins). Blood sampling was performed hourly during the eight hours following the meal. In both lean and obese triglyceride (TG) and VLDL-TG responses were higher after the fat meal (p < 0.0001) than after the carbohydrate meal. As expected, after the fat meal TG and VLDL-TG levels rose to reach a peak 3 hours postprandially. There was no evidence for any statically significant difference in TG and VLDL-TG response between lean and obese subjects. After the carbohydrate meal, TG and VLDL-TG rose significantly in obese, but not in lean subjects (p < 0.0001). TG and VLDL-TG peak was delayed to 5 hours after the carbohydrate meal in the obese subjects. In contrast, TG and VLDL-TG levels were not affected by carbohydrate meal in lean subjects. In lean subjects but not obese HDL-cholesterol decrease after the fatty meal. There was no evidence for any effect of carbohydrate meal on postprandial HDL-cholesterol levels in both lean or obese subjects. In conclusion, TG and VLDL-TG increase to a similar extend after a fatty meal in both obese and lean subjects. In contrast, carbohydrate meal increases TG and VLDL-TG in obese but not in lean subjects. This finding indicates that carbohydrate meal contributes to postprandial lipemia in obese subject indicating a potential unfavorable effect.
TuP14:W7 Effects of exercise-training and Gemfibrozil on HDL concentration and composition in abdominally obese men A. Pastoe1, J.P. Després1, 2, J. Bergeron1, M. Dumont1, M. Brochu3, I. Lemieux4, N. Alméras5, B. Lamarche6, D. Prud’homme1, 2, 3 Lipid Research Center; 4 Québec Heart Institute; 5 Division of Kinesiology, Laval University, Québec, Canada

Objective: To examine the combined effects of exercise-training and Gemfibrozil on HDL concentration and composition among abdominally obese men.

Methods: A double-blind randomized placebo-control study was conducted on 46 abdominally obese men (age: 45.8 ± 6.2 (SD) years; BMI: 31.3 ± 3.0 kg/m²). Men either received Gemfibrozil 600 mg b.i.d. or placebo for one year. After 26 weeks of treatment, all men participated in a 6-month exercise-training program which included 4 sessions per week of one hour exercise bouts performed at above 50% of VO2-max. HDL particle size was assessed by nondenaturing 4–30% polyacrylamide gradient gel electrophoresis.

Results: The one-year Gemfibrozil treatment, which included the 6-month exercise program, resulted in a significant increase in HDL-cholesterol (C) levels (p < 0.0002) which was explained by increased HDL-C (p < 0.03) without any significant modification in HDL particle size. On the other hand, exercise-trained men who received the placebo for a year showed a significant increase in HDL-C levels (p < 0.006) and in HDL particle size (p < 0.02) without any change in HDL-C concentration.

Conclusion: These results indicate that the increased HDL-C levels induced by fibrate therapy are not associated with an increased proportion of large HDL particles, the latter adaptation being, however, produced by endurance exercise-training.

TuP15:W7 Is the high triglyceride-low HDL cholesterol atherogenic dyslipidemia a small, dense LDL or HDL phenotype? I. Lemieux1, A. Pastoe1, M. Dumont2, M. Brochu3, N. Alméras4, J. Bergeron1, B. Lamarche6, D. Prud’homme1, 2, 3 Lipid Research Center, Québec Heart Institute; 4 CHU Sainte-Foy, University, Québec, Canada

Objective: As the small, dense LDL phenotype is associated with the high triglyceride (TG)-low HDL cholesterol (C) dyslipidemia, we examined the relationships of the above markers of the atherogenic dyslipidemia to HDL size assessed by 4–30% gradient gel electrophoresis (GGE).

Methods: Fasting plasma lipoprotein, lipid and insulin concentrations were assessed in a sample of 65 obese men (mean age: 45.5 ± 6.5 (SD) years and BMI: 31.5 ± 6.0 kg/m²). LDL particle size was measured using 2-16% nondenaturing polyacrylamide GGE.

Results: When tertiles of LDL or HDL particle sizes were compared, the subgroup of men with small LDL or HDL particles (first tertile) was characterized by elevated fasting TG levels and by reduced HDL-C and HDL-2C levels and by an increased C/HDL-C ratio compared to men in the top tertile for LDL and HDL sizes (p < 0.01). Finally, we observed a positive correlation between the LDL peak particle size and HDL particle size (r = 0.44, p < 0.0002).

Conclusion: Results of the present study indicate that synergetic reductions in both LDL and HDL particle sizes are associated with the high TG-low HDL-C dyslipidemia.

TuP16:W7 Abdominal obesity: the critical correlate of elevated plasma C-reactive protein levels associated with the features of the insulin resistance syndrome in men J.P. Després1, 2, A. Pastoe1, J. Bergeron1, I. Lemieux2, M. Dumont2, N. Alméras4, A. Nadeau1, D. Prud’homme1, 2, 3 Québec Heart Institute; 4 Diabetes Research Unit, Laval University, Sainte-Foy, Canada

Objective: The present study examined the relationships of plasma C-reactive protein (CRP) levels to the features of the insulin resistance syndrome.

Methods: We have examined the contribution of body composition assessed by hydrostatic weighing and of abdominal adipose tissue (AT) accumulation assessed by computed tomography (CT) to the variation in CRP levels associated with the atherogenic dyslipidemia of the insulin resistance syndrome in a sample of 168 men, aged 22 to 63 years, who were selected to cover a wide range of adiposity values (BMI from 21 to 41 kg/m²).

Results: Plasma CRP levels showed positive and significant correlations with total body fat mass (r = 0.42, p < 0.0001), waist girth (r = 0.38, p < 0.0001), as well as with abdominal subcutaneous and visceral AT accumulation measured by CT at L2±L3 and L4–L5 (0.27 ≤ r ≤ 0.34, p < 0.0005). Although CRP levels were positively correlated with plasma insulin levels measured fasting and following 75 g oral glucose load (p < 0.01), no significant correlations were found with plasma lipoprotein levels. Finally, comparison of body fatness, abdominal fat accumulation and of the features of the insulin resistance syndrome across quintiles of CRP levels revealed major differences in indices of abdominal AT accumulation between the lowest and top CRP quintiles, whereas no difference was found for variables of the plasma lipoprotein profile.

Conclusion: These results suggest that obesity and abdominal AT accumulation are the critical correlates of elevated CRP levels found in men with the atherogenic dyslipidemia of insulin resistance.

TuP17:W7 Conjugated linoleic acid (CLA) reduced abdominal visceral fat in obese men with the metabolic syndrome Ulf Risérus, Lars Berglund, Bengt Vessby. Clinical Nutrition Research Unit, Dept of Public Health and Caring Sciences Geriatrics, Uppsala University, Sweden

Objective: The metabolic effects of conjugated linoleic acid (CLA) have hitherto mainly been studied in animals. We investigated the short-term effect of CLA on abdominal visceral fat and related cardiovascular risk factors in obese men with signs of the metabolic syndrome.

Methods: 25 abdominally obese men (waist to hip ratio: 1.05 ± 0.05, body mass index: 32.0 ± 2.7 kg/m² (mean ± SD), 39-64 years old, participated in a double-blind randomised controlled trial for 4 weeks, receiving either 4.2 g CLA/day or placebo. The main endpoints were differences between the two groups in sagittal abdominal diameter (SAD), blood lipids, glucose, insulin and blood pressure.

Results: There was a significant decrease in SAD in the CLA group compared to placebo (P = 0.04, 95% CI: –1.12, –0.02), which corresponded to an estimated mean decrease in visceral fat by 4.4% (CT-based equations). Waist and waist to hip ratio was significantly decreased within the CLA group (P < 0.01). Other measurements of anthropometry, blood pressure or metabolism showed no significant differences between the groups.

Conclusions: These results suggest that CLA treatment for 4 weeks in men with metabolic disorders decreases SAD, indicating a loss of visceral fat, without significant effects on other cardiovascular risk factors. The possible clinical value of CLA in subjects with abdominal obesity needs to be further investigated in larger trials with longer duration.

TuP18:W7 The impact of dietary intervention on lipid metabolism and the development of insulin resistance in mice M. Muirling1, V.E.H. Dahlmanns1, M.C. Jong1, R.P. Mensink2, L.M. Havelkes1, 1TNO-Prevention and Health, Leiden; 2Dept. of Human Biology, Maastricht University, The Netherlands

Objective: To investigate the effects of dietary intervention on lipid metabolism and the development of insulin resistance.

Methods: To induce insulin resistance, male C57BL/6J mice were fed a high fat diet (HF; 46% of the energy is provided by corn oil) for a period of 20 weeks. After 20 weeks the C57BL/6J mice were divided into three groups: the first group was given a low fat diet (LF; 14.2% of energy provided by corn oil) for 12 weeks, the second group was put on energy restriction (ER; 75% of high fat diet) for 12 weeks, and the third group was maintained on an HF diet. Before and after the dietary intervention period glucose tolerance, insulin sensitivity, post prandial plasma lipid response, and hepatic VLDL-triglyceride (TG) production rates were measured.

Results: After 20 weeks on a HF diet, fasting plasma glucose and free fatty acid (FFA) in mice were similar as compared to the values at a regular chow diet (10.6 ± 0.6 vs 10.6 ± 1.0 mmol/L for glucose and 0.5 ± 0.1 vs 0.5 ± 0.1 mmol/L for FFA, respectively). Plasma levels of TG decreased after 20 weeks of HF feeding (0.2 ± 0.1 vs 0.3 ± 0.1 mmol/L), while plasma cholesterol levels were increased (3.4 ± 0.5 vs 1.8 ± 0.2 mmol/L). Furthermore, body-weight increased significantly from 22.7 ± 5.1 to 32.2 ± 7.7 g at 20 weeks of HF feeding. The insulin resistance state in the mice was evident after 20 weeks of HF feeding as oral glucose tolerance tests revealed significant levels of hyperglycemia and hyperinsulinemia after glucose administration. During the dietary intervention period, the body-weight of the LF- and LF-animals decreased to respectively 28.2 ± 3.5 and 25.9 ± 2.2 g. The body-weight of the HF animals increased further to 35.9 ± 11.7 g. Glucose and insulin tolerance tests revealed that at the end of the diet intervention, LF fed animals were
more glucose tolerant and more sensitive to the action of insulin as prior to the intervention period. In the ER- and HF group, glucose tolerance and insulin sensitivity didn’t show significant differences before and after the dietary intervention. In addition, at the end of the dietary intervention period, post prandial plasma lipid response and hepatic VLDL-TG production rates were similar between the three groups.

Conclusion: Insulin resistance, induced by high fat feeding, can be reversed by a low fat diet, rather than by energy restriction. These beneficial effects on a low fat diet appear to be independent of changes in lipid metabolism.

**TuP19:W7**

**Hyperglycemia, increased insulin sensitivity and decreased adiposity in human APOC1 transgenic mice**

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Objectives: Using hyperinsulinemie, euglycemic clamp tests, we previously found that in APOC1 transgenic mice the whole body insulin-mediated fatty acid uptake is decreased concomitant with an increased glucose uptake. To further explore these findings we investigated whether APOC1 overexpression can modulate the initiation and/ or development of obesity and insulin resistance.

Methods: APOC1 transgenic mice were either fed a high-fat (HF) diet (40% of the calories in the form of fat) for a period of 18 weeks, or crossbred on the obese ob/ob background.

Results: On both backgrounds of obesity, APOC1 transgenic mice exhibited a significant reduction in body weight and fat mass as compared to control mice. Furthermore, APOC1 transgenic mice developed severe hyperglycemia both after HF diet (230 vs 10.4 mmol/L) and on an ob/ob background (25.9 vs 17.9 mmol/L). Glucose and insulin tolerance tests revealed that on an ob/ob background, APOC1 overexpression leads to an increased glucose tolerance and an increased insulin sensitivity as compared to ob/ob only mice. Thus, the extreme hyperglycemia in APOC1 ob/ob mice was not accompanied by severe insulin resistance. Plasma insulin concentrations were similar between APOC1 ob/ob and ob/ob mice, whereas in APOC1 ob/ob mice the hepatic glucose production was significantly increased (22.4 ± 4.0 vs 16.4 ± 3.4 mg/kg/min).

Conclusion: APOC1 overexpression leads to hyperglycemia, insulin sensitivity and decreased adipose tissue development indicative for a fundamental role of APOC1 in the metabolic relationship between obesity, insulin resistance and hyperlipidemia.

**TuP20:W7**

**Influence of fenofibrate on apo B metabolism in familial combined hyperlipidaemia**

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Objectives: To examine the effect of 12 weeks treatment with micronised fenofibrate (FF) on apo B metabolism in 6 subjects with FCHL with chel 6.77 (0.95) and trig (TG) 2.59 (0.57) mmol/L.

Methods: The subjects underwent turnovers using tri-deuterated leucine. Isotopic enrichments in V1 (SF 60–400), V2 (SF 20–60), LDL (SF 12–20) and LDL (SF 0–12) apo B were determined by GCMS and kinetic parameters were derived by multicompartemental modelling.

Results: FF lowered TG by 35% (p = 0.04), VLDLc 50% (p = 0.04) and LDLc 15%. A 43% decrease in V1 was due to a doubling of the V1-V2 transfer rate (FTR), p = 0.05, and a fivefold increase in its clearance (FDC), p = 0.02. V2 direct production in these FCHL subjects was above normal at 384 mg/dL. The apo B apo B pool fell by 43% (p = 0.03) due to a 74% (p = 0.02) increase in V2 FTR. The LDL pool fell by 25% (p = 0.03), mainly due to an increase in FTR. The fall in LDL apo B of 20% (p = 0.09) was due to a 52% increase in LDL FDC (p = 0.02).

Conclusions: These FCHL subjects were characterised by a high production of V2 and a diminished catalolism of LDL. FF lowered V1 and V2 principally by promoting lipolysis but also by increasing the direct catalolism of V1, presumably by receptors. These effects are in concordance with a drug induced reduction in the apo CIIT content of VLDL – an apoprotein which inhibits catalolism and lipolysis of VLDL.

**TuP21:W7**

**Insulin resistance in the St. Thomas’ Mixed Hyperlipidaemic (SMHL) rabbit, a model for familial combined hyperlipidaemia**

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Objective: The SMHL rabbit exhibits an inherited combined hyperlipidaemia similar to that seen in FCHL. In this study we determined whether the SMHL rabbit is insulin resistant, a condition which is often found in FCHL patients.

Methods: Six young and six mature combined hyperlipidaemic SMHL rabbits, age/sex matched NZW controls and six young hypercholesterolaemic WHHL controls were fed a 0.08% (w/w) cholesterol-enriched diet for at least 3 months prior to the start of the experiment. We performed an oral glucose tolerance test after an overnight fast by providing the rabbits with a solution of 1 g of glucose per kg body weight. Blood was withdrawn just before and 15, 30, 45, 60 and 120 minutes after administration of the oral glucose dose.

Results: Both SMHL and WHHL rabbits did not show hyperglycemia. However, the area under the curve (AUC) for the insulin response was significantly increased for both young (p < 0.05) and mature (p < 0.01) SMHL rabbits compared to NZW (mean ± SD). The AUC for the ratio glucose/insulin response was increased in young and mature SMHL rabbits and in young WHHL rabbits (p < 0.05). Neither strain of rabbit showed a difference in the AUC for the NEFA response. In both young and mature SMHL rabbits, the log-value of plasma triglycerides was positively correlated with the AUC for the insulin response (p = 0.81 and p = 0.84, respectively; both p < 0.05).

Conclusion: SMHL rabbits are insulin resistant, and its severity appears to increase with age. Therefore, the SMHL rabbit offers a valuable animal model in which to study the relation between hypertriglyceridaemia and insulin resistance in FCHL patients.

**TuP22:W7**

**Plasma polyunsaturated fatty acid and threonine allele in codon 54 of the fatty acid binding protein 2 gene in obese Japanese children**

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We have studied the association between polymorphism of the intestinal fatty acid binding protein (I-FABP) and composition of plasma fatty acids.

The investigations were carried out in 32 obese children (10 girls and 22 boys) aged 11.2 ± 4.1 yrs (mean ± SD). Body weight relative to normal weight for height was 160 ± 24% in our outpatient clinics. Fatty acid composition in plasma phospholipids was determined by gas chromatography. The polymorphism of exon 2 in the I-FABP gene was analyzed by PCR-RFLP using HhaI restriction endonuclease.

Obese children with the Thr-54 allele revealed significantly lower proportions of arachidonic acid and Σ-n-6 long chain polyunsaturated fatty acids than those with Ala54 allele (p < 0.03 and p < 0.01).

These results suggested that Δ 6 desaturase activity might be decreased in obese Japanese children with Thr54 allele. The polymorphism in the I-FABP affects plasma fatty acid compositions in obesity.

**TuP23:W7**

**Mechanisms of hepatic VLDL-apoB overproduction in an insulin resistant hamster model**

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Objective: A novel animal model of insulin resistance, the fructose-fed Syrian golden hamster, was employed to investigate the mechanisms mediating the overproduction of VLDL in the insulin resistant states such as type 2 diabetes.

Methods & Results: Fructose feeding for a two week period induced significant hypertriglyceridaemia and hyperinsulinaemia, and the development of whole body insulin resistance was documented using the euglycemic-hyperinsulinemic clamp technique. In vivo triton WR-1339 studies showed evidence of VLDL-apoB overproduction in the fructose-fed hamster. Fructose feeding induced a significant increase in cellular synthesis and secretion of total as well as VLDL-TG by primary hamster hepatocytes. Increased TG secretion was accompanied by a 4.6 fold increase in VLDL-apoB secretion. Enhanced stability of nascent apoB in fructose-fed hepatocytes was evident in intact cells as well as in a permeabilized cell system. Analysis of newly-formed
lipoprotein particles in hepatic microsomes revealed significant differences in the pattern and density of lipoproteins. Immunoblot analysis of the intracellular matrix of human monocytes and polymorphonuclear neutrophils (PMNs), a key enzyme involved in VLDL assembly, showed a striking 2.1 fold elevation in hepatic lipids derived from fructose-fed vs. control hamsters. MTP mRNA levels were also significantly increased in fructose-fed hamster livers. Direct incubation of hamster hepatocytes with various concentrations of fructose did not directly stimulate apoB intracellular stability of extracellular secretion.

Conclusions: Hepatic VLDL-apoB overproduction in fructose-fed hamsters appears to result from increased intracellular stability of nascent apoB and an enhanced expression of MTP, which act to facilitate the assembly and secretion of apoB-containing lipoprotein particles.

**TuP24:W7**
The S1I polymorphism at the apolipoprotein C-III gene locus predict insulin sensitivity in healthy subjects

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The expression of insulin resistance, a condition related with type 2 diabetes mellitus, results from the interaction of environmental and genetic factors. We have examined the influence of apo C-III S1I polymorphism on insulin sensitivity in 59 healthy men and women (30 men and 29 females).

**Methods:** They were subjected to three dietary periods, each lasting four weeks. During the first period all subjects consumed a high SAT diet (38% of energy as fat, 20% SAT). The second and third dietary periods were administered following the randomized crossover design, and consisted of an NCEP step I diet (28% fat, <10% SAT) and high MUFA diet, (Mediterranean diet, with 38% MUFA). All food and drinks were prepared and provided in the research kitchen. We determined in vivo insulin resistance using the insulin suppression test with somatostatin.

**Results:** Steady-state plasma glucose (SSPG) concentrations (a measure of insulin sensitivity) were significantly lower in S1 male, compared with S2 male, after SAT diet (100 ± 10 vs 161 ± 23). NCEP-I diet (90 ± 11 vs 110 ± 11) and Mediterranean diet (86 ± 10 vs 130 ± 13). The apo C-III gene effect was independent of the Xba-I polymorphism of the glycogen synthase gene.

**Conclusions:** In summary, our results show an improvement in insulin sensitivity in males with the S1 allele of the apo CII polymorphism, independent of the improving effect observed with the substitution of MUFA and carbohydrates for SAT fat.

**TuP25:W7**
Impact of body mass index on the expression of hypertriglyceridemia in familial lipoprotein lipase (LPL) deficiency is related to the type of LPL gene mutation

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**Objective:** To assess the consequences of heterozygosity for LPL gene variants on the expression of hypertriglyceridemia (hyperTg) in individuals with normal to elevated body mass index (BMI).

**Methods:** Subjects, not taking drugs, were divided into quartiles based on BMI. Within each group, heterozygote carriers of LPL alleles leading to total (P200 or G188E) or partial (D99N) LPL deficiency were compared. All groups were paired for sex, age and BMI.

**Results:** Analyses revealed similar concentrations of plasma Tg in individuals with partial or total LPL deficiency and low BMI (<24). However, significantly higher and increasing levels of plasma Tg have been found in carriers of P200 or G188E mutations with BMI 24–27, 27–31 or 31. Thus, at BMI > 31, carriers of P200 or G188E mutations had plasma Tg 2.7-fold higher than carriers of D99N. Based on VLDL Tg, LDL and HDL cholesterol, it is of interest to note that deterioration of the lipoprotein profiles was more pronounced in subjects carrying the P200 or G188E gene mutation, as well as in males compared to females.

**VLDL Tg levels in heterozygotes carrying an allele with partial or total LPL deficiency by BMI quartile BMI**

<table>
<thead>
<tr>
<th>BMI (Part/Def)</th>
<th>20/13</th>
<th>25/26</th>
<th>30/24</th>
<th>22/29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial deficiency</td>
<td>2.1 ± 0.3</td>
<td>3.1 ± 0.3</td>
<td>3.4 ± 0.5</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>Total deficiency</td>
<td>2.1 ± 0.5</td>
<td>3.1 ± 0.3</td>
<td>4.3 ± 0.6</td>
<td>5.2 ± 1.0</td>
</tr>
<tr>
<td>Δ (total vs partial)</td>
<td>0.4</td>
<td>1.0</td>
<td>1.2</td>
<td>2.0</td>
</tr>
</tbody>
</table>

(Mean ± SEM, *p < 0.05*)

**TuP26:W7**
The effect of combined therapy of atenolol with simvastatin and with fenofibrate on the insulin resistance syndrome

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It is known that nonselective beta blockers aggravate the abnormalities of carbohydrate and lipid metabolism. On the other hand, the sympatho-adrenergic system plays a larger role in the pathogenesis of metabolic syndrome.

**Aim of Study:** To investigate the effect of a combined therapy of selective beta-blockers with statins and fibrates on the metabolic alterations of insulin resistance syndrome.

**Materials and Methods:** 37 men and women aged 40–59 (55 ± 2) with metabolic syndrome were randomized into 2 groups: I group of patients (n = 18) received simvastatin 10 mg (MID, USA) with atenolol 50 mg daily (Norton, UK), II group (n = 19) fenofibrot 200 ME (Fournier, France) in combination with atenolol 50 mg daily for a period of 8 weeks. The criteria for metabolic syndrome components were the following: AH-SBP 140–169 mm Hg and/or DBP 90–99 mm Hg; Abdominal obesity (AO)-waist to hip ratio > 1.0 for men and > 0.85 for women if BMI > 25 kg/m²; Hyperlipidemia (HLP)- Tg level 200 mg/dl and/or total Ch 250 mg/dl; Glucose intolerance – basal plasma glucose level up to 120 mg/dl and 2 hours after 75 g glucose load, 140–200 mg/dl.

**Results:** On treatment during 8 weeks SBP and DBP levels in patients of I group decreased by 18% and 16% (p < 0.001 and p < 0.01), in patients of II group by 14% and 13.8% (p < 0.01). Tg (20%), p < 0.05) and total Ch levels (29%, p < 0.001) in 1 group, 43% (p < 0.001) in II group statistically decreased. HDL Ch (16%, p < 0.05) increased only in I group. Like the basal levels, post prandial levels of glucose and immunoreactive insulin in both groups had tendency to decrease. At the same time in the I group post prandial insulin level decreased significantly by 7% (p < 0.01).

**Conclusion:** Both the combinations improves blood pressure and lipid profile changes involved in insulin resistance syndrome and have a neutral effect on carbohydrate metabolism. However the combination of fenofibrot with atenolol significantly decreases post prandial tissue insulin resistance. Selective beta blockers in mean doses combined with hypolipidemic drugs (statins and fibrates) can be administered to correct metabolic syndrome.

**TuP27:W7**
Effect of troglitazone on plasma lipid metabolism and lipoprotein lipase

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**Objective:** To clarify how troglitazone, an insulin-sensitizing agent, affects lipid metabolism and post-heparin plasma lipoprotein lipase (LPL).

**Methods:** Fifteen patients (3 male, 12 female) [the average age 62 ± 7; the mean body mass index, 25 ± 3 kg/m²] were recruited and the serum lipids and post-heparin plasma lipoprotein lipase (LPL) mass before and 4 weeks after oral administration of troglitazone (200 mg per day) were measured. Mouse preadipocyte cell line, 3T3-L1 cells were treated with this compound and LPL enzyme protein mass in the culture media was measured by an enzyme linked immunosorbent assay. A reverse transcription polymerase chain reaction (RT-PCR) and Northern blot analysis was conducted to investigate the effect of this compound on the expression of LPL.

**Results:** The average levels before treatment of fasting serum total cholesterol, triglycerides and high density lipoprotein-cholesterol, plasma glucose and glycylcohemoglobin A1c were 216 ± 34, 160 ± 84, 57 ± 19, 145 ± 30 mg/dl and 7.5 ± 1.6%. Four weeks after treatment, those levels were 209 ± 36, 105 ± 27 (p = 0.004), 63 ± 19 (p = 0.02), 139 ± 41 mg/dl and 7.3 ± 0.6% (p = 0.01), respectively. The postheparin plasma LPL mass increased from 226 ± 39 to 257 ± 68 ng/ml (p = 0.03) during that period. RT-PCR and Northern blot analysis revealed that in the cultured 3T3-L1 cells, the expression of LPL was enhanced in the presence of troglitazone.

**Conclusions:** These results suggest that troglitazone improves plasma triglyceride-rich lipoproteins metabolism by enhancing the expression of LPL in adipocytes.

TuP28:W7
Effects of ecosapentaenoic acid (EPA) and bezafibrate (BEZA) on serum lipids and blood pressure in insulin resistance
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Objective: Fructose feeding of rats causes insulin resistance involving hyperinsulinemia, hypertension, and hypertiglyceridermia. We have confirmed that hyperinsulinemia is associated with Na+/H+ exchanger (NHE) activation, resulting in elevation of blood pressure in salt-sensitive, borderline hypertensive rats (BHR). Some reported that EPA and BEZA, PPAR α stimulating lipid-lowering drugs, could reduce blood pressure. To elucidate the mechanism of this, we tested if lowering triglyceride (TG) level could decrease blood pressure and NHE activity in this model.

Methods: Male BHR at 8 weeks of age were fed with 60% fructose diet group (F) or standard diet group (C) for 4 weeks. Next, EPA (1000 mg/kg/day), BEZA (100 mg/kg/day), or none were administered with in each group for 4 weeks. Blood pressure was measured at every period of feeding. Finally, plasma levels of glucose, insulin (IRI), cholesterol (Cho), and TG were measured. Cysolic pH (pHc), [Na+]i, [Ca2+]i, and NHE activity of platelets were determined. NHE activity was evaluated by the recovery of pHi following addition of sodium propionate (Vmax).

Results: EPA significantly reduced systolic blood pressure in both F and C, but BEZA did not. Although EPA and BEZA decreased TG levels in both groups, they did not affect on levels of glucose, Cho, and IRL pH, [Na+]i, [Ca2+]i, and Vmax were not changed in each group except decreasing [Ca2+]i in fructose fed rats without EPA.

Conclusion: Lowering TG itself is not associated with regulation of blood pressure by NHE activation. EPA reduces blood pressure via NHE-independent mechanism.

TuP29:W7
Determination of reference values for fasting insulin levels in a representative sample of the adult Québec population
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Objectives: To determine the population distribution of fasting insulin levels and to identify the value corresponding to the 75th percentile.

Methods: A stratified random sample of men and women aged 18 to 74 years was selected from the province of Québec. Fasting plasma lipoprotein, lipid, insulin and glucose concentrations as well as anthropometric measurements were obtained in 1844 individuals.

Results: We first identified individuals with reported metabolic diseases (28%). We then classified untreated individuals with altered risk factors (33%). On that basis, 39% of the Québec population was considered in good metabolic health. Since we observed a positive correlation between fasting insulin levels and BMI (r = 0.41, p < 0.0001), we excluded healthy individuals with BMI > 25 kg/m² in order to identify the 75th percentile value for insulin levels in nonobese individuals, which corresponded to a value of about 60 pmol/L in both genders. Analyses among subgroups revealed that 50% of subjects with reported metabolic diseases and with altered risk factors had fasting insulin levels above 60 pmol/L.

Conclusion: Results of our study indicate that 60 pmol/L may be a useful reference value to define normal insulin levels (below the 75th percentile) in the Québec population.

TuP30:W7
Influence of etofibrate therapy on the insulinemia in the IGT/T2 in subjects with the polyolimetic syndrome depending on ace gene polymorphism
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Objectives: Changes in insulin secretion depending on ACE-gene polymorphism in subjects with polyolimetic syndrome during Etofibrate therapy.

Methods: the study was performed in 17 subjects aged 18-65 years, with polyolimetic syndrome. PCR was used to determine the ACE gene polymorphism. The ID genotype was found in 9 subjects, the genotype II and DD were represented in 4 subjects of each type. Insulin secretion was measured after a rapid, intravenous infusion (IVGTT).

Results: A decrease of insulin secretion during the early phase of insulin response (ER) up to 8th min. was observed in the treated subjects (443.2 ± 321.6 → 395.1 μU/mL × min., p < n.s.) as well as on the late phase (3625.6 ± 2667.6 to 2874.7 ± 1492.4 μU/mL × min., p < n.s.). Significant differences in insulin level were observed in the 10th min. (p < 0.05). The secretion profile was similar in group ID and II. An ER was followed by a linear decrease of secretion. In the DD group the concentration was stable, elevated up to the 60th min. and next an increase up to 120th min. was observed. The systolic blood pressure (BP) before treatment in the DD group was significantly higher in comparison to the ID group (p < 0.05). After therapy, a significant decrease of BP and glucose concentration (p < 0.05) in group ID was observed. Such a correlation in DD group was not found.

Conclusion: (1) Preliminary results of this study suggest that Etofibrate therapy influences on the insulin secretion in subjects with polyolimetic syndrome and the ACE gene polymorphism modifies this effect. (2) Hyperinsulinemia and insulin resistance in the polyolimetic syndrome is related with the DD genotype.

TuP31:W7
Association of visceral fat with coronary risk factors in population-based postmenopausal women
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Objective: To determine the amount of visceral fat (VF) by computer axial tomography (CAT) and its association with coronary risk factors in a population-based sample of postmenopausal women (PW).

Methods: Sampling design: A sample of 98 PW 50 to 65 years old was randomly selected from an eligible population of 17,587 in the northeastern region of Mexico City. Risk factors: Lipids, lipoproteins, insulin and glucose were quantified in a venous blood sample after a 12-hour fast. VF was determined at the L4-L5 intervertebral space with a single CAT scan. Physical activity was measured with Baecke’s questionnaire. Diet was assessed with the 24 hr prospective recall method.

Results: PW with increased IVF (VF) (VF > 118 cm³) had a significantly higher risk for HDL-C < 35, LDL-C/HDL-C > 3.5, hyperinsulinemia, diabetes mellitus (DM) and body mass index (BMI) > 27. In the PW without diabetes or hypertension (C) (n = 39), the group with IVF had significantly higher mean values of BMI, waist circumference, heart rate (HR), glucose and insulin. Multiple regression analysis showed VF explained tryglicerides (21%), apolipoprotein B (4.3%), and LDL-C (10.8%) in (C) and (HR) (15.6%) in hypertensive PW (n = 36).

Conclusions: Our results confirm the association of VF with coronary risk factors in a population-based sample of PW.

P/W8 GENE THERAPY AND OTHER NEW TREATMENTS
TuP1:W8
The preproendothelin-1 promoter as a tool for specific gene therapy to angiogenic vascular endothelial cells
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Gene therapy directed to the vascular wall, particularly to angiogenic endothelial cells should have an important role in vascular disease treatment. Angiogenesis is a major feature of many diseases including wound healing, solid tumors, developing metastases and ischemic heart diseases.

Objective: To develop a tool for directing gene therapy specifically to the vascular wall using an adeno-based vector.

Methods: Adeno-viral vectors, containing the murine PPE-1 promoter, and the reporter genes luciferase or Green Fluorescence Protein (GFP) were constructed. Their activities were compared to CMV based vectors in several angiogenic models.

Results: Genes activated by the PPE-1 promoter were highly expressed in Bovine Aortic Endothelial cells in vitro. Systemic injection of the adenoviral vectors AdPPE-1-Luciferase and AdCMV-Luciferase to normal C57BL/6 mice resulted in higher activity of PPE-1 promoter compared to CMV promoter in the aorta and vascularized tissues such as heart, kidney, lung and pancreas. Similarly, higher activity of the PPE-1 promoter compared to CMV promoter has been found in angiogenic rich tissues such as wound healing and angiogenetic.
Lewis Lung Carcinoma lung metastasis. Cellular distribution of the delivered gene revealed highest expression of GFP in angiogenic endothelial cells of the metastasis.

**Conclusions:** We expect that this approach of “vascular-directed” gene therapy will be applicable to both vascular diseases and cancer.

**TuP2:W8**

**Periodontal lacZ gene transfer to pig carotid arteries using a biodegradable collagen collar or a wrap of collagen sheet with adenoviruses and plasmid-liposome complexes**

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**Objective:** To develop periodontal gene therapy for the treatment of vessel wall thickening, stenosis and other complications in vascular surgery.

**Methods:** We compared lacZ gene transfer efficiency of DOTMA:DOPE (1:1 w/w) plasmid/liposome complexes and adenoviruses in pig carotid arteries using perivascular delivery with either a collagen collar or a wrap of collagen sheet. Clinical chemistry, tissue pathology and PCR analysis of lung, liver, kidney, spleen, skeletal muscle and gonads were used for assessing safety of the gene transfer.

**Results:** Gene transfer efficiency using the periodontal collar was 4-fold higher than using the collagen wrap with adenovirus at 7 days (10.22 ± 2.96 vs. 2.78 ± 1.28 positive cells/mm²; p = 0.18) and 4.3-fold at 14 days (13.46 ± 3.49 vs. 3.11 ± 0.88 positive cells/mm²; p = 0.03). Gene transfer efficiency at 7 days with adenovirus was 5-fold higher than with the plasmid/liposome complexes both using the collagen (10.22 ± 2.96 vs. 2.07 ± 0.95 positive cells/mm²; p = 0.01) and the collagen wrap (2.78 ± 1.28 vs. 0.45 ± 0.35 positive cells/mm²; p = 0.03). No lacZ activity was detected in plasmid/liposome transfected arteries at 14 days. A moderate systemic distribution of the transgene was detected in the major organs by PCR analysis despite the local gene delivery methods.

**Conclusions:** This study shows that (i) gene transfer vector delivered in the periodontal collar reaches the target tissue more efficiently than the vector in the collagen wrap; and (ii) adenoviruses delivered with the periodontal collar or the collagen wrap is well-tolerated and may become an efficient new tool in vascular gene therapy.

**TuP3:W8**

**PDGF-receptor-β antisense gene transfer decreases proliferation and PDGF binding of human primary smooth muscle cells**

A.-M. Turnunen, A. Krettek, F. Lustig, G. Bondjers, S. Yli-Herttuala1,2, A.I. Virtanen Institute; 2Department of Medicine, University of Kuopio, Kuopio, Finland; 3Faculty of Medicine, University of Göteborg, Göteborg, Sweden

**Objective:** We studied effect of retrovirus mediated PDGF-receptor-β antisense gene transfer to primary hSMC.

**Methods:** Cells (30% confluency) were transduced with antisense, sense or lacZ viruses. Next day the medium was changed into growth medium containing 20% FBS and the cells were analyzed by ligand binding analysis and BrdU ELISA proliferation assay.

**Results:** Introduction of high titer pseudotyped retroviruses with M.O.I. of 1 into primary hASMCs results in transduction efficiency of 20-30%. Ligand binding analysis shows that the PDGF-BB binding capacity of antisense-transduced hASMCs is 28% compared to control non-transduced cells. Also, the proliferation rate of the cells after selection was shown to be diminished in the antisense-cells.

**TuP4:W8**

**Adventitial ex vivo gene transfer to rabbit carotid artery using autologous vascular smooth muscle cells**

M.P. Turnunen, M.O. Hiltunen, P. Lehtolainen, J. Koponen, V. Pimenoff, A.-M. Turnunen, S. Yli-Herttuala1,2,3, A.I. Virtanen Institute; 4Department of Medicine, University of Kuopio, Kuopio, Finland; 5Gene Therapy Unit, Kuopio University Central Hospital, Kuopio, Finland

**Objective:** Our aim was to develop a new technique for performing ex vivo gene transfer to arterial wall using autologous smooth muscle cells (SMC).

**Methods:** The SMCs were harvested from rabbit ear artery, transfected in vitro with VSV-G pseudotyped lacZ retrovirus (1 × 10^6 CFU/ml) and returned back to adventitial surface of the carotid artery using a silicone collar placed around the artery. Animals were sacrificed 7, 14, 28, 42, and 84 days after the operation.

**Results:** The transduced SMCs implanted with high efficiency and expressed β-galactosidase marker gene at a very high level 7 and 14 days after the operation. The level of LacZ expression decreased thereafter, but was still easily detectable at all later time points and was exclusively localized to the site of cell implantation inside the collar.

**Conclusions:** The results indicate that the model can be used for a high-efficiency local gene transfer in arteries e.g. during vascular surgery.

**TuP5:W8**

**Adenovirus-mediated extracellular superoxide dismutase gene transfer in vitro and in vivo**

P. Turnunen, M.O. Lakkunen, P. Leppilainen, S. Yli-Herttuala, A.I. Virtanen Institute for Molecular Sciences, University of Kuopio, Kuopio, Finland

**Objective:** The aim of the study was to use a high titer of replication-deficient adenoviruses containing rabbit ec-sod cDNA or lacZ cDNA to investigate the production of active enzyme in atherosclerotic mice. Rabbit aortic SMCs and CHO-K1 cells were transduced in vitro to demonstrate post-transcriptional regulation of EC-SOD protein synthesis.

**Methods:** AdEC-SOD and AdlacZ (1 × 10^9 PFU) were injected intravenously into LDLR-/-Sc double knock-out mice. The total SOD activity was measured from plasma at time points 0, 3, 5, 7, 14, 17 and 20 days after gene transfer. Tissue distribution of adenovirus was determined by x-gal staining. In vitro synthesized enzyme was detected by Northern blot and enzyme activity analysis.

**Results:** Transgene expression was detected mainly in liver and spleen and in some extend also in kidney and heart. The production of active enzyme in plasma was detectable for two weeks and the highest enzyme activity was measured three days after gene transfer. Mean values for activities in plasma were 5924 U/ml in EC-SOD (n = 13) and 432 U/ml in lacZ (n = 7) group. The Northern blot analysis showed 62-fold higher mRNA amount in SMCs compared to CHO-K1 cells at 12 h after transduction where as the EC-SOD activity was only 2.5 times higher in SMC than in CHO-K1 cell culture medium suggesting a regulatory step.

**Conclusions:** These results demonstrate that rabbit EC-SOD is expressed in vivo after adenoviral gene transfer and is synthesized mainly in liver. In spite of high adenovirus doses, the EC-SOD activity in the plasma remains low, which may be at least partially due to the post-transcriptional regulation observed in vitro.

**TuP6:W8**

**Sterol regulation of human LDL receptor expression in gene therapy**

J. Heeren1, F. Schneiders2, U. Beisiegel1, 1Medical Clinic; 2Heinrich-Pette Institute, Hamburg, Germany

**Objective:** Gene therapy has potential in the treatment of familial hypercholesterolemia (FH). However, massive overexpression of LDL receptor (LDLR) induced by strong viral promoters results in extensive lipid accumulation and hence probable reduced cell viability. To avoid this problem, we produced adenoviral vectors containing the human LDLR cDNA plus approximately 600bp of the endogenous promoter (EP) containing the sterol regulated sequence (Ad. EP LDLR).

**Methods:** Adenoviral vectors for human LDLR gene transfer under control of the RSV promoter (Ad. RSV LDL R) and the Ad. EP LDLR were employed. Hepatoma cells were used for uptake experiments with radiolabeled and fluorescent LDL. At present we are investigating therapeutic effects in LDLR-/- mice.

**Results:** Western and northern blot analysis, as well as functional uptake experiments, reveal that expression of LDLR using Ad. EP LDLR is regulated by sterols. In contrast to the RSV driven LDLR expression, physiological
controlled LDLR expression prevents lipid accumulation and the formation of cholesteryl ester-based crystal-shaped structures.

**Conclusions:** In addition to vector-specific features, e.g., overcoming immuno-reactivity, the controlled tran-sgene expression might be required for safe and efficient therapeutic gene transfer. Here we show adenoviral gene transfer vectors that mediate stably regulated LDLR expression.

**TuP9:W8**

**Extracorpuscular fibrinogen-adsorption — A new procedure to improve plasma and whole blood viscosity**

W.O. Richter1, J.M. Schneidewind2, W. Ramlo2, R. Koll1, H. Klinkmann2.

1Institute of Lipid Metabolism and Hemorheology, Munich/Würndach; 2Dialysis Community North Rostock; 3Plasmapheresit, Teterow, Germany

**Objective:** To develop an effective, selective and safe procedure for extra-corpuscular removal of fibrinogen from plasma to improve hemorheology.

**Methods:** The pentapeptide glypro-arg-pro-lys was coupled to sepharose CL-4B. Columns containing 100 ml of Sepharose CL-4B were used to eliminate fibrinogen from the plasma of 8 healthy male subjects (mean age 27.4 ± 4.3 years, height 180.9 ± 8.3 cm, weight 85.1 ± 13.6 kg. Four treatments were performed in each proband (days 1, 2, 4 and 7).

**Results:** The pentapeptide bound only fibrinogen, fibrin and certain fibrinogen degradation products. Plasma fibrinogen was lowered from 221 ± 39 to 123 ± 22 mg/dl (2275 ± 477 ml plasma treated) by first treatment, from 173 ± 42 to 106 ± 16 mg/dl (1609 ± 761 ml) by the second, from 140 ± 19 to 99 ± 20 mg/dl (1224 ± 118 ml) by the third and from 160 ± 24 to 106 ± 10 mg/dl (1513 ± 521 ml) by the fourth. Plasma viscosity was improved from 1.40 ± 0.18 mPas before first treatment to 1.23 ± 0.06 mPas after fourth treatment, whole blood viscosity from 4.49 ± 0.36 mPas to 3.83 ± 0.27 mPas (P < 0.01). Microrcirculation was improved as measured by laser plethysmography. No clinical side effects and no clinically relevant change of laboratory parameters including in vitro tests on thrombine function were observed.

**Conclusion:** Affinity chromatography using the pentapeptide glypro-arg-pro-lys is an effective, selective and safe procedure to lower fibrinogen concentration in plasma thereby improving microcirculation. Therefore it could be a therapeutic option in severe atherosclerotic disease where drug therapy is not sufficient and invasive procedures cannot be applied.

**TuP8:W8**

**Hepatic levels of TNF-α, but not of IL-2 and IFN-γ correlate with DNA turnover and hepatotoxicity after adenoviral apo A-I transfer**

S. Van Linthout, D. Colen, B. De Geest. Center for Molecular and Vascular Biology, University of Leuven, Belgium

**Objective and Methods:** The mechanisms of the turnover of transgene DNA after adenoviral transfer are incompletely understood. Human apo A-I DNA copy number after transfer in C57BL/6 mice with 5 × 109 p.f.u. of a CMV-promoter driven construct (AdCMV) declined 2.5-fold (p = 0.01) between days 6 and 35, but did not decline between days 6 and 35 after transfer of an apo A-I promoter driven construct (AAd-I). Serum levels of transaminases were 10-fold higher 6 days after transfer with AdCMV than with AAd-I, suggesting that the decline of transgene DNA levels was not due to a cytokine immune response against virus transduced cells. Liver mRNA levels of IL-2 and IFN-γ, which stimulate a cytokine immune response, and of TNF-α, which can be produced by Kupffer cells, were measured by real-time PCR.

**Results:** TNF-α mRNA levels after gene transfer are indicated in the table (n = 3 to 4).

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 21</th>
<th>Day 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAd-I</td>
<td>1.0 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>0.6 ± 0.2</td>
<td>0.4 ± 0.01</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>AdCMV</td>
<td>1.0 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td>2.8 ± 1.4</td>
<td>16 ± 3</td>
<td>2.3 ± 0.7</td>
<td>0.7 ± 0.1</td>
</tr>
</tbody>
</table>

(*p < 0.05 versus day 0 and versus AAd-I)

Compared to control mice, neither IFN-γ mRNA levels nor IL-2 mRNA levels in the liver were significantly elevated at any time point after transfer with either AdCMV or AAd-I.

**Conclusion:** Transfer with AdCMV but not with AAd-I is associated with markedly elevated liver TNF-α mRNA levels. Because TNF-α may induce hepatocyte death, high liver TNF-α may be a direct cause of elevated liver enzymes and accelerated decline of transgene DNA.

**TuP9:W8**

**Sustained expression of human apo A-I in 3 different mouse strains**

B. De Geest, S. Van Linthout, D. Colen. Center for Molecular and Vascular Biology, University of Leuven, Belgium

**Objective and Methods:** Human apo A-I (HA-I) DNA copy number, mRNA and protein levels and immune response (I.R.) against HA-I were investigated after adenoviral (Ad) transfer of HA-I driven by an apo A-I promoter (AAd-I) or a CMV promoter (AdCMV) in 3 mouse strains. HA-I DNA copy number and mRNA levels in the liver were quantified by real-time PCR and HA-I levels and antibodies against HA-I by ELISA.

**Results:** HA-I DNA copy numbers, HA-I concentration at 35 and 180 days (mg/dl) and occurrence of I.R. against HA-I are indicated in the table.

<table>
<thead>
<tr>
<th></th>
<th>C57BL/6</th>
<th>BalBc</th>
<th>FVB</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAd-I</td>
<td>AdCMV</td>
<td>AdCMV</td>
<td>AdCMV</td>
</tr>
<tr>
<td>DNA 10 min</td>
<td>35 ± 3.5</td>
<td>30 ± 0.9</td>
<td>31 ± 3.9</td>
</tr>
<tr>
<td>DNA day 1</td>
<td>4.1 ± 0.9</td>
<td>4.6 ± 0.8</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>DNA day 35</td>
<td>0.1 ± 0.01</td>
<td>0.02 ± 0.02</td>
<td>0.0 ± 0.07</td>
</tr>
<tr>
<td>AAd-I day 35</td>
<td>4.1 ± 1.3</td>
<td>100 ± 8.4</td>
<td>&lt;1</td>
</tr>
<tr>
<td>AAd-I day 180</td>
<td>&lt;1</td>
<td>21 ± 3.3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>I.R.</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

DNA copy number and HA-I levels were significantly lower in Balb/c mice due to more rapid loss of DNA within day 1. After transfer with Ad-I, HA-I mRNA and protein levels correlated strongly with DNA copy number (not shown), no I.R. against HA-I occurred and expression was sustained for 6 months in all 3 strains. In contrast, after transfer with AdCMV, accelerated DNA loss and an I.R. against HA-I in FVB and Balb/c mice appeared within 35 days.

**Conclusions:** Adenoviral gene transfer of HA-I, when driven by its promoter, results in sustained expression for 6 months in 3 mouse strains.

**TuP10:W8**

**Simvastatin, HDL cholesterol and the regression to the mean. A reappraisal**

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**Objective:** Simvastatin has been reported to increase HDL-C in patients with low baseline HDL-C, but not in patients with high baseline HDL-C. This might suggest that the changes in HDL-C after simvastatin represent a statistical artifact.

**Methods:** The study was carried out on 428 patients treated with simvastatin 10 mg for 2 months and on 220 patients on stable diet in whom serum lipid pattern was determined 2 times at 2 months interval. Total cholesterol and serum triglycerides were measured with current enzymatic methods and HDL-C was determined after phosphotungstic acid precipitation. The calculation of the error due to the regression to the mean was made according to Trochim.

**Results:** On average, HDL-C significantly increased by 4% after simvastatin, the increase was however seen in only 227 patients (+20%; in 201 patients HDL-C did not increase (-12%). The patients in whom HDL-C increased had baseline HDL-C lower (48 ± 0.9 mg/dl) than patients in whom HDL-C did not increase (55 ± 1.0 mg/dl). The patients with baseline HDL-C < median had an increase in HDL-C of 5.3 mg/dl, while the patients with baseline HDL-C > median had a small decrease of 0.8 mg/dl. After correction for the regression artifact the changes in HDL-C of the 2 groups were 1.6 and 2.5 mg/dl, respectively. In the control group, the patients with HDL-C < median at the 1st measurement had an increase of 3.7 mg/dl at the 2nd measurement, while the patients with HDL-C > median at the 1st measurement had a decrease of 4.9 mg/dl at the 2nd measurement. The correction for the regression artifact resulted in a change of 0.0 mg/dl in both groups.

**Conclusions:** The regression artifact is present in simvastatin treated patients but it does not entirely explain the increase in HDL-C which must be considered a true pharmacological effect.
P:W9 GEOGRAPHIC EPIDEMIOLOGY OF
ATHEROSCLEROSIS

TuP1:W9
Risk factors for coronary atherosclerosis in men and women: Epidemiological study based on pathological assessment
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Objectives: The aims of this study are to abstract risk factors for coronary atherosclerosis in men and women respectively from autopsy cases of Japanese general population, and to examine the validity of the pathological assessment.

Methods: Two hundred and thirty three cases in Hisayama cohort study, who underwent a mass medical examination in 1988 and an autopsy at death during following period, were subjected to the study. According to AHA reporting system, epicardial coronary arteries were divided into 14 segments and the most narrowed site in each segment was sectioned for histological examinations. The coronary atherosclerosis was globally assessed and graded into 6 grades by qualifying the lesion and quantifying the stenotic ratio of the 14 sections. The risk factors were statistically analyzed by multiple regression analysis.

Results: There were significant correlations between the grade of coronary atherosclerosis and age, systolic blood pressure, serum cholesterol level and hemoglobin A1c in men, and age, systolic blood pressure and waist to hip ratio in women.

Conclusions: 1) The pathological examinations are useful for global assessment of coronary atherosclerosis. 2) The risk factors for coronary atherosclerosis are somewhat different between men and women in metabolic aspects.

TuP2:W9
Hyperhomocysteinemia in patients with ischaemic heart disease and mediterranean dietary pattern
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Objective: Hyperhomocysteinemia has been identified as an important risk factor for atherosclerotic vascular disease and may reflect deficiency states of folate, or vitamin B12. Dietary and environmental factors may modify homocysteine (Hcy) plasma concentrations. We investigated their relation to ischaemic heart disease (IHD) in Crete, where people consume the typical Mediterranean diet.

Methods: We examined 96 patients aged 62.2 ± 9.5 years old with established IHD and 60 healthy adults, matched for age and sex, as controls. All subjects underwent full clinical examination and laboratory testing (Homocysteine, Vitamin B12, Folate, Lipid Profile and serum Creatinine). They also gave a full medical history and completed questionnaire about their diet.

Results: Patients with IHD had significantly higher levels of Hcy and lower levels of HDL cholesterol when compared with controls (Median values: 16.74 ± 7.16 vs 11.60 ± 3.72 nmol/l (p < 0.001) and 38.77 ± 10.19 vs 51.87 ± 12.65 mg/dl (p < 0.001), respectively). Levels of B12, Folate, Creatinine, Total and LDL Cholesterol did not differ significantly between the two groups studied.

In conclusion, patient with IHD in Crete, consuming a typical Mediterranean Diet, tend to have increased plasma total Hcy levels when compared with healthy adults, offering the potential for therapeutic intervention.

TuP3:W9
Frequency distribution of apolipoprotein B and CETP polymorphism, plasma lipids and coronary heart disease in Taiwanese population
J. Wu1, Y.T. Lee2, H.C. Hsu2, 1Chang Gung University, 2National Taiwan University Hospital, Taiwan, ROC

Objective: To study the frequency distribution of apolipoprotein (apoB) and cholesterol ester transfer protein (CETP) gene polymorphisms and mutations and their association with plasma lipid level and coronary heart disease (CHD) in Taiwanese.

Methods: Polymere chain reaction (PCR) and restriction enzyme digestion were used to detect the genetic polymorphisms and mutations. When there is no restriction site involved, a primer designed to create a digestion site for a restriction enzyme that distinguishes wild type from mutant is used for the analysis. The mutated or polymorphic nucleotide was determined by direct sequencing of the gel-purified PCR fragments from different genotype. Plasma lipid levels were determined using commercial kits and lipoprotein levels were determined by ELISA or immunoturbidimetry.

Results: Very rare mutations were found for apoB R3500Q, R4019W, 265 promoter region C/T, intron 2 enhancer A/G and CETP intron 14 splice site. The population showed predominant apoB 431S (68.9%), CETP 442D (97.7%), intron 1 G allele (52%), intron 8 MspI (89%), intron 9 EcoNI (59.2%) and 4051 allele (61%) in the control group. CHD patients have more CETP EcomNI (25.3%) than the controls (13.6%) (p = 0.02). More CETP 442G and EcomNI alleles were found in hyper-high density lipoprotein (HDL) cholesterol (> 65 mg/dl) than their respective counterpart. The CETP 442G and apoB 431N were associated with high total cholesterol (TC) level.

Conclusion: We have determined the frequency distribution of some apoB and CETP mutations and genetic polymorphisms in Taiwanese, our CHD group showed increased CETP EcomNI frequency and increased EcomNI was found in HDL cholesterol greater than 50 percentile group. The CETP 442G and apoB 431N were associated with high TC, paradoxically, the CETP 442G was also associated with high HDL cholesterol level.

TuP4:W9
Insulin resistance syndrome and obesity in the Circassian population in Israel
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Objective: To estimate the prevalence of the traits for the insulin resistance (IR) syndrome and the association of this traits with BMI in the Circassian community in Israel. This study is the first to describe risk factors in a defined previously unstudied population.

Methods: This study sample comprised 450 women and 289 men aged 35 and over from both villages (70.5% of the total Israeli Circassian population). Data collection was carried out by means of personal questionnaires, laboratory tests, BMI and blood pressure measurements and information from medical files.

Results: The prevalence rate of obesity (BMI > 30) was 48% in the women and 35% in the men, higher compared to Israeli women and men (27% 17% p < 0.001 respectively). Age-adjusted prevalence rate of diabetes among Circassian men was 12.5% and among women 8% compared to Israeli population: 5.9% and 7.1% respectively (OR = 1.45 95 CI 1.29-5.91). In Circassian men (age < 50) HDL mean levels were lower than that of Israeli male subjects even after adjustment for obesity, smoking and other covariables (38.2 mg/dl vs. 40.3 p < 0.05). Plasma triglyceride levels in Circassian men and women (age < 50) were higher than those of Israelis (for man 214 mg/dl vs. 161 p < 0.001 and for women 186 vs 113 mg/dl p < 0.001). Prevalence rate of hypertension in Circassians (men 19.7%, women 29%) were particularly higher than Israeli women in all ages. In multivariate analysis levels of TG, systolic BP and fasting insulin were all positively associated with BMI. Among women without DM, BMI > 30 was the strongest risk factor for IR syndrome occurrence (OR = 2.6 CI 95% 1.23-4.60).

Conclusion: The high prevalence of diabetes, obesity, hypertension, hypertriglyceridemia and low HDL cholesterol concentrations in this homogenous population contribute to Syndrome X theory and may reflect genetic factors related to insulin resistance.

TuP5:W9
Is pulse pressure a predictor for the incidence of stroke in men? The Korea Medical Insurance Corporation Study
I. Suh1, S.H. Lee2, C.M. Nam3, I.S. Kim1, L.J. Appel1, 1Yonsei University College of Medicine, 2Graduate School of Business Science and Management, Yonsei University, Seoul, Korea; 3Johns Hopkins Medical Institution, Baltimore, USA

Objective: To examine whether pulse pressure (PP) is an independent predictor for stroke in male Koreans, a population which has high stroke mortality, and to determine whether blood pressure (BP) levels modify the relationship between PP and incidence of stroke.

Methods: We followed 106,745 Korean men, aged 35–59, who attended both the 1990 and 1992 Korean Medical Insurance Corporation health examination surveys over a period of 6 years (1990–1998). 1,267 developed stroke (469 hemorhhagic and 620 thrombotic). Cox proportional hazards models were used to assess the independent effect of PP on the incidence of stroke after
controlling for age, smoking, alcohol consumption, total serum cholesterol, fasting blood sugar and body mass index.

Results: The Cox proportional hazards models revealed significant hazards ratios (HR) for each 10 mm Hg increment of PP (1.69; 95% CI, 1.60–1.78). Stroke incidence was nearly four-fold greater in the highest level of PP (>55 mm Hg) than in the lowest (<40 mm Hg). This significant association between PP and stroke was shown both in hypertensives and normotensives. The HRs for each 10 mm Hg increment of PP for hypertensives and normotensives were 1.46 (95% CI, 1.37–1.56) and 1.37 (95% CI, 1.19–1.58), respectively.

Conclusions: In a large population of Korean men, a wide pulse pressure was a significant independent predictor for the incidence of stroke and the association was not modified according to BP level.

Conclusions: In Poland high 14 day case fatality is due to higher death rates for patients who have suffered a mild stroke. It may be caused by high prevalence of prestroke cardiac diseases and insufficient care in the early stages of stroke.

Epidemiology of high density lipoprotein levels in pre- and post-menopause: The Brisighella Heart Study

Atherosclerosis Centre "G.C. Descovich", University of Bologna, Italy.

Objective: To evaluate the trend of HDL-C in a large free-living female population in perimenopausal age.

Methods: The Brisighella Heart Study (BHS) is a longitudinal study (1972–still in progress) on the major cardiovascular risk factors in a North-Italian rural population. For this retrospective analysis of the BHS data, we considered only the population surveys carried out before the intervention phase (1976–1986). The metabolic and anthropometric parameters of 141 females in perimenopausal age (mean age = 47.75 ± 1.75 years) were analysed in premenopausal phase and, after 4 years, in postmenopausal phase. The Student's t-test for paired samples was carried out to determine when the variation of the metabolic and anthropometric parameters was significant (p < 0.01).

Results: Mean (±SD) lipoprotein plasma levels, glycemia and BMI in pre- and post-menopausal age are reported in the table.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Premenopause</th>
<th>Postmenopause</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>234.92 ± 14.98</td>
<td>263.63 ± 18.01</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>LDL-C</td>
<td>155.32 ± 11.21</td>
<td>175.69 ± 14.32</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>HDL-C</td>
<td>51.80 ± 7.12</td>
<td>50.83 ± 8.23</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>TG</td>
<td>132.82 ± 13.78</td>
<td>154.06 ± 11.92</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Glucose</td>
<td>75.01 ± 8.65</td>
<td>84.06 ± 7.98</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>BMI</td>
<td>25.04 ± 4.15</td>
<td>26.33 ± 4.34</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

Conclusion: In contrast with the results of epidemiological transversal studies (which suggest that the mean plasma levels of HDL-C level decrease in postmenopause) and according to the longitudinal Framingham and Taipe Studies our data confirm that mean HDL-C level significantly raises after menopause in a free-living female population.

Association between lipidic phenotype variability and CHD/CVD in a large rural population: The Brisighella Study

Atherosclerosis Centre "G.C. Descovich", Bologna, Italy.

Objective: To evaluate the association between lipidic phenotype variability (primitive and not) and cardiovascular diseases (CD): 410–414 and 430–438 codes by ICD (CVD) in a large rural Northern-Italian population.

Methods: The Brisighella Study is a longitudinal study (1972–in progress) on the major cardiovascular risk factors in a North-Italian rural population. At 1996's population control, 1303 subjects were tested in 4 or more four-yearly surveys (53.74 ± 14.97 years). The individual lipidic phenotype by Fredrickson classification was attributed for each survey on the basis of HDL-C and TG plasma levels suggested from De Bruin et al. for Gamilial Combined Hyperlipoproteinemia (FCH) detection. A primitive dyslipoproteinemia presence was suspected on the basis of: CD personal or familial history; No disease nor drugs assumption potentially affecting lipid metabolism; Cholesterol/lipids lowering drugs assumption; BMI < 27 for men and <25 for women; LDL-C plasma levels > 200 mg/dL; Correct dietary habit, χ² test with Yates correction was carried out to test the CD prevalence difference between the whole hyperlipoproteinemic population and the primitive hyperlipoproteinemic subgroup.

Results: Obviously, both Ila and variable phenotypes have a much higher CD prevalence in respect to normolipidemic subjects, while in the whole population CD is more frequent among Ila (2.94%) than among variable phenotypes (1.83%) [χ² = 26.39, 1 DF, p < 0.001]. Comparing the same prevalences among the whole dyslipidemic group and the primitive dyslipidemic subgroup it appears that in Ila subjects there is a similar CD prevalence, while in primitive variable phenotypes it is much superior (5.26%) [χ² = 10.05, 1 DF, p < 0.001]. In primitive hyperlipidemias CD prevalence is significantly higher in variable phenotypes than in Ila subjects, too (5.26% Vs. 2.90%).

Conclusion: In our population the CD prevalence is more strongly associated to a primitive variability of the lipidic phenotype (partly related to FCH) than to a constant primitive lipid phenotype.

**TuP10:** Low incidence of xanthomas in heterozygous familial hypercholesterolemia (FH) in Spain


The diagnosis of FH is based on identification of high LDL levels, with evidence of an autosomal dominant mode of inheritance, high incidence of ischaemic heart disease (IHD), xanthomas, xanthelasmas and corneal arcus. Xanthomas are present in 34-75% of patients with FH in North-European countries, this incidence seems to be lower in South-European countries. In our study we wanted to characterize clinical manifestations of FH in Spain.

**Patients and Methods:** We studied 301 cases of FH from central and north regions in our country. Inclusion criteria were: total cholesterol over 300 mg/dl and triglyceride below 250 mg/dl in the proband and in at least one of the first-degree relatives of the proband.

**Results:** Plasma lipids levels (means ± SD) and clinical manifestation were:

<table>
<thead>
<tr>
<th>Total (n = 301)</th>
<th>Men (n = 140)</th>
<th>Women (n = 161)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>154 ± 58</td>
<td>154 ± 53</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>125 ± 69</td>
<td>124 ± 67</td>
</tr>
<tr>
<td>LDLC (mg/dl)</td>
<td>149 ± 64</td>
<td>147 ± 64</td>
</tr>
<tr>
<td>HDLC (mg/dl)</td>
<td>53 ± 13</td>
<td>50 ± 12</td>
</tr>
<tr>
<td>Xanthomas</td>
<td>23 (8%)</td>
<td>19 (13%)</td>
</tr>
<tr>
<td>Xanthelasmas</td>
<td>18 (6%)</td>
<td>5 (4%)</td>
</tr>
<tr>
<td>Aortic Coarct</td>
<td>60 (22%)</td>
<td>55 (20%)</td>
</tr>
<tr>
<td>IHD</td>
<td>60 (20%)</td>
<td>42 (29%)</td>
</tr>
<tr>
<td>Familial history of IHD</td>
<td>155 (51%)</td>
<td>70 (50%)</td>
</tr>
</tbody>
</table>

With a total cholesterol level of 346 ± 58, only 8% of the patients have xanthomas and 20% IHD. 51% have a familial antecedent of IHD.

**Conclusions:** Contra to that shown in international literature, xanthomas are very infrequent in FH in Spain and the diagnosis should be suspected for other aspects. The high prevalence of familial antecedent of IHD supports the usefulness of this aspect as a marker for diagnosis and prevention.

**TuP11:** Peripheral vascular surgery in women – Trends and outcome changes in the Stockholm area (Sweden)

R. Hultgren, Per Olsson, Eric Wahlberg, *Department of Vascular Surgery, Karolinska Hospital, Stockholm, Sweden*

**Objectives:** To investigate possible gender differences over time in: a) the number and type of procedures performed, b) outcome, in patients with PAOD undergoing vascular interventions.

**Methods:** Data from the inpatient-register of the National Board of Health and Welfare and data from the Cause of Death-register for Stockholm County (population 1.7 million 1994) have been analyzed for all patients subjected to vascular intervention 1970-1994.

**Results:** The total number of interventions rose from 18 in 1970 to 786 interventions/million inhabitants 1994. While the number of embolectomies has diminished over time, the number of infrainguinal procedures increased. The fraction of female patients also grew significantly (p < 0.001) from 34% to 48% 1984. Mean age, increased from 63 to 71 years with a steady five year age difference between women and men. (71 vs. 66 years, p = 0.01).

The strongest negative predictor for survival was increasing age (p < 0.001), followed by male sex (p < 0.001). Comparing 30-day survival among the infrainguinal and supringuinal procedures, shows no significant difference between women and men, although women are older (6 and 3 years older).

**Conclusions:** This study presents a dramatic increase in the number of vascular operations in Stockholm with a parallel expansion of the proportion performed on female patients. It is unknown whether this development is a result of an increased incidence of PAOD in women. It is probable, however, that in 10-15 years more peripheral vascular procedures will be infrainguinal interventions in women over 70 years old. Previous assumptions, that the outcome of vascular surgery is poor in women, with both elevated mortality rates and more complications, are challenged in this study. Male sex was a predictor of poor survival, suggesting that gender aspects still need to be considered in the preoperative evaluation of the patients, but with different premises.

**TuP12:** Dramatic increase of premature myocardial infarction in Isfahan, Iran

F.A. Sayed-Tabatabaei, N. Sarraf-Zadegan, M. Boshtam, Sh. Hoseini, *Isfahan Cardiovascular Research Center, Isfahan, Iran*

**Objective:** Cardiovascular diseases (CVD) are the leading causes of death in US and most of the western countries, as well as developing countries. Increasing incidence of coronary artery disease (CHD) is observed in most of developing countries in recent years. This study was performed to determine the trend of incidence of premature myocardial infarction (MI) (ie. in population less than 60 years old) from 1986 to 1995 according to official statistics on hospital discharges.

**Methods:** As there was no official MI registry unit in our country, a special register has been established in Isfahan Cardiovascular Research Center and data was collected from all hospitals which may admit the CHD patients in CCUs or cardiology wards. MI was considered to have occurred when at least two of the three WHO standard criteria for MI diagnosis were fulfilled.

**Results:** An increase in incidence of MI was seen in both sexes and it was more clear between 1990 to 1995. The absolute increase in men was from 95.9 to 175 per 100,000. It seems to be greater than women which was from 13.2 to 64.7 per 100,000, but the values themselves are greater in men. Indeed, the relative increase (incidence ratio) was more dramatic in women (4.9 fold vs. 1.8 fold). The relative increase in total population was 2.2 fold.

**Conclusions:** Such a huge increase in values suggests the need for large comprehensive preventive campaigns for primary prevention and risk factor control especially in women. To avoid the possible sources of bias, establishing the standard registry units based on MONICA protocol in all developing countries is strongly suggested.

**TuP13:** Coronary atherosclerosis in subjects 60-69 years old in three European cities and its changes over a 25 year period

V.S. Zhdanov, N.H. Sternby, A.M. Vikhert, *Russian Cardioiology Complex, Moscow, Russia; 2 Malmo University Hospital, Malmo, Sweden*

**Objectives:** To study the evolution of coronary atherosclerosis (Athr) in subjects 60-69 years old over a 25 year period.

**Methods:** We have previously reported the evolution of atherosclerosis in subjects 20-59 years old in different populations. The main coronary arteries were collected at autopsy in subjects 60-69 years old in Malmo, Riga and Yalta and during two periods, the early sixties (the 1st study) and the late eighties (the 2nd study). During the 1st study 1536 males and 1114 females were included, during the 2nd 410 and 319 subjects, resp. Ath was estimated as extent of raised lesions (RL).

**Results:** In Malmo males extent of RL was decreased in the 2nd study whilst it was significantly increased in males of Riga and Yalta. In Malmo females the extent of RL decreased in the 2nd study, in Riga and Yalta the extent of RL was the same in 40-59 year old females, but was increased in females 60-69 years old.

**Conclusions:** Extent of Ath in Malmo males and females decreased in the 2nd study whilst in Riga and Yalta Ath increased in the 2nd study. This changing pattern was also found for younger age groups.

**TuP14:** Appropriate treatment of dyslipidaemia in angiographically defined British Columbians

M. Francis, J. Frohlich, *Healthy Heart Program, St. Paul’s Hospital, University of British Columbia, Vancouver, Canada*

**Objective:** To assess the appropriateness of dyslipidaemia treatment in British Columbians with known angiographic, lipid, and risk factor profiles.

**Methods:** Three hundred and fourteen patients were followed up 4 years after having angiography, lipid profile, and risk factor assessment. Positive angiograms were seen in 253 individuals. Through a mailed questionnaire, information was collected on changes in smoking, diet, and other lifestyle risk factors. Availability of diet, exercise, and smoking cessation counselling was assessed. Most recent cholesterol measurement was noted, along with use of lipid-lowering and other medications.

**Results:** The National Cholesterol Education Program (NCEP) guidelines were used to determine appropriateness of treatment for dyslipidaemia. Based on these guidelines, 274 patients should have had annual cholesterol mea-
The impact of landmark clinical trials on secondary prevention of acute myocardial infarction (AMI) in Spain. Preveze 98 study

M. de Oya, J.L. Lopez-Sendín, E. de Teresa, J. Velasco-Rami, E. Villa, J. Cosín. On behalf of PREVESE 98 Study Group; Madrid, Spain

In 1994 an epidemiological survey (PREVESE 94), was conducted in 39 Cardiology Departments in Spain, to analyse retrospective risk factors and pharmacological therapy, at hospital discharge, of 1242 patients with diagnosis of AMI during the period 1994–1998. In order to find out any new risk factors we compared our results with other studies.

Methods and Design: In the present survey 74 hospitals have been involved, 36 out of the 39 previously participating. In this edition (1998) 2054 patients with AMI diagnosis were identified retrospectively.

Results: Significant changes, between 1994 and 1998, in the treatment at hospital discharge are shown in the table. These modifications have mostly involved a 6-fold increase in the utilisation of statins, besides a more frequent use of ACE inhibitors and beta-blockers.

<table>
<thead>
<tr>
<th></th>
<th>1994</th>
<th>1998</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACEI</td>
<td>32.5%</td>
<td>46.7%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium Antagonists</td>
<td>26.4%</td>
<td>17.8%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>33.2%</td>
<td>45.1%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Statins</td>
<td>4.5%</td>
<td>29.2%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Conclusions: Availability of decisive results from landmark trials on secondary prevention of AMI, together with new guidelines which have emphasised cholesterol as a key modifiable risk factor, may account for significant modifications in the pattern of use of statin therapy. Despite this improvement, under-utilisation of these drugs in AMI patients is still evident, as the level of statin prescription at discharge was even lower than the percentage of patients considered hypercholesterolaemic (35%) before the AMI.
**P:W11 RESTENOSIS**

**TuP1:W11** Inhibitory effect of TS-962, an acetyl inhibitor, on intimal thickening of carotid artery in a rabbit balloon injury model


**Objective:** To determine the inhibitory effect of TS-962 on intimal thickening caused by balloononing in hyperlipidemic and normolipidemic rabbits.

**Methods:** Ballooning of the right common carotid artery of 2-month-old male WHHL and NZW rabbits was performed. TS-962 was added to the diet of WHHL rabbits (n = 4 in each group) for 4 weeks and to the diet of NZW rabbits (n = 9–10 in each group) for 2 weeks after balloononing. For NZW rabbits, pre-medication was given for one week before balloononing. The effects of protocol on WHHL rabbits and transplant on NZW rabbits were also evaluated. Intimal thickening was evaluated as the intimal/medial area (iM) ratio.

**Results:** Smooth muscle cell rich-intima featured lipid accumulation in the WHHL rabbits, and TS-962 (0.0003 and 0.003% in the diet) reduced the iM ratio below that in the control group (0.45 ± 0.19 and 0.58 ± 0.05 vs 1.00 ± 0.23 in the control). The iM ratio with protocol administration (0.35%) was 0.43 ± 0.06. In the normolipidemic NZW rabbits as well, TS-962 (0.0001, 0.0003 and 0.003%) reduced the iM ratio below that in the control group (0.230 ± 0.056, 0.126 ± 0.028 and 0.145 ± 0.032 vs 0.279 ± 0.039 in the control). The iM ratio with transplant administration (0.167%) was 0.184 ± 0.050. TS-962 induced no change in serum cholesterol level at the end of the study in both WHHL and NZW rabbits.

**Conclusions:** TS-962 inhibited intimal thickening induced by balloononing, in both hyperlipidemic and normolipidemic rabbits. TS-962 may influence cell migration or proliferation associated with intimal formation independent of its effect on lipid metabolism, and may be effective in preventing arterial restenosis.

**TuP2:W11** Genetic risk factors in coronary heart disease (CHD) and restenosis

L. Previtali, F. Sandrelli, S. Stefanato, A. Codemo, R. Razzolini1, I. Cortella, S. Martini, C. Gabelli, G. Cepaldu. Clinica Medica 1; 1 Dept. of Cardiology, University of Padua, Italy

**Introduction:** Many different genetic polymorphisms of proteins involved in lipoprotein metabolism, platelet aggregation and coagulation have been pro-posed as risk factors for atherosclerosis. The relevance of these polymorphisms in restenosis after percutaneous coronary interventions remains controversial.

**Methods:** In order to study their role in CHD and restenosis we have analyzed the genetic polymorphisms of platelet glycoprotein IIb/IIIa (GpIIb/IIa), Angiotensin Converting Enzyme (ACE), Methylenetetrahydrofolate Reductase (MTHFR), coagulation Factor VII (intrin 7) and apolipoprotein E (apoE) in an Italian population of 115 CHD subjects (P) with single vessel disease and successful coronary angioplasty; clinical follow-up of this group, including stress test and/or myocardial nuclear scintigraphy and/or coronary angiography, detected restenosis in 51 patients. A group of 117 subjects, randomly selected among the workers of a local factory was also studied as control (C).

**Results:** Frequency distribution of the GpIIb/IIa genotype showed a significant difference between C and P (p = 0.0178) and between patients with (R) and without restenosis (NR) (p = 0.0118), with a higher frequency of the PIA2 allele in P and R subjects. ApoE4 had an higher prevalence in P compared with C (p = 0.038), while no difference was observed between R and NR. Difference in the distribution of the Factor VII polymorphism approached statistical significan-ce (p = 0.067) in comparison between R and NR only. We observed no difference in ACE and MTHFR polymorphism distribution in any of the groups analyzed.

**Conclusions:** These results confirm the suggested role of GpIIb/IIa and apoE genetic variations as risk factors in CHD while only GpIIb/IIa polymorphism shows a relationship with the restenosis process.

**TuP3:W11** Nitrate tyrosine in porcine stented and unstented vein grafts

P. Gadsdon, A. Yim, S. Wan, A.C. Newby, G.D. Angelini, J.Y. Jeremen. Liverpool John Moores University, Liverpool; Division of Cardiothoracic Surgery, The Chinese University of Hong Kong, Department of Cardiovascular Surgery, University of Bristol, UK

**Objectives:** Peroxynitrite (ONO0) formed by the reaction of nitric oxide (NO) with superoxide (O$_2^-$/O$_2$) may play a role in the aetiology of vein graft failure due to neointima formation. We therefore investigated the distribution and content of nitrate tyrosine (NT: an index of ONOO formation) in porcine vein grafts, with and without external stents (reduces neointima formation). 

**Methods:** Saphenous vein-coronary artery interposition grafting was performed in Large White pigs (22–36 kg, n = 8), one graft being externally supported with an oversize Dacron stent. After four weeks the grafts were removed and the distribution of NT and nitric oxide synthase (NOS) established using immunocytochemistry and tissue levels of NT measured with an ELISA.

**Results:** NT was found to be present in large amounts in the adventitia of vein grafts but was mainly concentrated around microvessels which also expressed high levels of both eNOS and iNOS. Absolute NT levels were greater in all regions of stented grafts compared to unstented grafts.

**Conclusion:** Since external stents prevent neointima formation and stented grafts contain greater levels of NT that unstented grafts, these data mitigate against NT as playing a role in vein graft failure. On the contrary, the dense localisation of NT around microvessels points to a role for NO in angiogenesis, a mechanism proposed to be axiomatic in mediating the beneficial effect of the external stent.

**TuP4:W11** Elevated C-reactive protein (CRP) after percutaneous transluminal coronary angioplasty (PTCA) is a predictor of restenosis

S. Morimoto, Y. Fujisaka, S. Tsuibo, T. Okamura, M. Masai, J. Tatsuki, M. Manatani, T. Iwanski. Hyogo College of Medicine, Nishinomiya, Japan

**Objective:** Recent evidence suggests that C-reactive protein (CRP) might be a candidate of risk factors of coronary artery disease. CRP and fibrinogen are acute-phase reactants induced by inflammatory processes, which is associated with cell proliferation. We studied if elevation of CRP in intervention of acute myocardial infarction or unstable angina can be related to restenosis after PTCA.

**Methods:** We selected 25 patients with restenosis (R) and 25 patients without restenosis (NR) found on control months after the urgent PTCA. In all patients, Serum CRP and fibrinogen levels were measured everyday in a week after the urgent PTCA.

**Results:** Summation of CRP values of initial 7 days (7CRP) in R was significantly higher that in NR (p < 0.05). Taking 20 mg/dl as a cut-off point, the risk ratio for restenosis of 7CRP ≥ 20 mg/dl was 8.07 (95% CI 1.04–62.3, p < 0.05). On the other hand, there was no difference in 7FIB between NR and R. Peak values of CRP and fibrinogen were observed at 3rd or 4th day after intervention. Taking 10 mg/dl for peakCRP or 450 mg/dl for peakFib as a cut-off point, the risk ratio of peakCRP ≥ 10 mg/dl for restenosis was 3.50 (95% CI 1.02–11.96, p < 0.05) or that of peakFib ≥ 450 mg/dl was 4.71 (95% CI 1.05–21.27, p < 0.05). The ratio of peakCRP ≥ 10 mg/dl combined with peakFib ≥ 450 mg/dl was 3.94 (95% CI 1.08–14.32, p < 0.05).

**Conclusions:** Persistent elevation of CRP and fibrinogen levels in acute phase could be powerful predictors of late restenosis after the urgent PTCA.

**TuP5:W11** Homocysteine, copper and caeruloplasmin in patients undergoing coronary artery bypass graft surgery

J.Y. Jeremen, A. Lotto, A. Doy, N. Shukla, R. Aschione, I. Wan, D. Stansbie, G.D. Angelini. Departments of Cardiovascular Surgery and Chemical Pathology, Bristol Royal Infirmary, Bristol, UK

**Objective:** Copper (Cu) and the Cu-binding protein, caeruloplasmin (CP) augment the generation of superoxide (O$_2^-$/O$_2$) from homocysteine (HC). O$_2^{-}$ is proatherogenic through several mechanisms, including the negation of NO bioactivity via formation of peroxynitrite. In turn, impaired NO formation has been implicated in graft failure following coronary artery bypass graft surgery (CABG). The plasma concentrations of HC, Cu and CP were therefore measured in patients undergoing CABG.

**Methods:** Blood was taken from 12 patients undergoing CABG before and after surgery and plasma levels of Cu, CP and HC measured.
**Results:** There were significant increases in plasma Cu and CP which were sustained for two months after CAGB, whereas there were no significant changes in HC (table). It is likely that the increase in Cu is due to increased CP, an acute phase response.

<table>
<thead>
<tr>
<th></th>
<th>1 day pre-op</th>
<th>6 days post-op</th>
<th>60 days post-op</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HC (µmol/L)</strong></td>
<td>14.6 ± 1</td>
<td>16 ± 2</td>
<td>13.9 ± 2</td>
</tr>
<tr>
<td><strong>Cu (µmol/L)</strong></td>
<td>16 ± 2</td>
<td>22.6 ± 6.3</td>
<td>21.5 ± 3.3</td>
</tr>
<tr>
<td><strong>CP (g/L)</strong></td>
<td>0.3 ± 0.03</td>
<td>0.63 ± 0.04*</td>
<td>0.41 ± 0.04*</td>
</tr>
</tbody>
</table>

* p < 0.05; compared to 1 day pre-operative (mean ± SEM, n = 122)

**Conclusions:** Although there are no changes in the levels of HC after CAGB, the elevation of Cu and CP may increase superoxide formation from HC. These alterations are potentially important in the pathophysiology of both early and late vein graft failure due to thrombosis and neointimal/medial thickening, respectively.

**TuP6:W11**

**The proliferation profile and histology of symptomatic coronary in-stent restenosis lesions**

E. O’Brien, C. Glover, X. Ma, M. Labinaz, J. Veinot. University of Ottawa Heart Institute, Canada

**Objective:** In-stent restenosis is currently a major limitation of interventional cardiology. Typically, neointimal proliferation is suspected as the cause of this problem, yet little is known about the nature of this tissue. This study examines the proliferation profile and histology of in-stent restenosis lesion.

**Methods:** In contrast to existing reports that summarize the post-mortem histology of stented arteries that are free of in-stent stenosis, atherectomy devices were used to obtain tissue from 8 patients with symptomatic coronary in-stent restenosis lesions. Using standard histological stains, and in situ hybridization for histone H3 mRNA (a sensitive and specific marker of cell proliferation) these tissues were characterized.

**Results:** Directional or pullback atherectomy catheters removed 1–4 large tissue specimens from each lesion. The in-stent restenosis tissue consisted largely of proteoglycans, with a uniform population of stellate shaped smooth muscle cells. Expression of TGF-b related factors was noted. There was a noticeable absence of collagen, microvessels and inflammatory cells in this tissue. Organizing thrombus was noted in a minority of specimens. Low levels of cell proliferation were documented.

**Conclusions:** In-stent restenosis largely consists of an abundant extracellular matrix that is rich in proteoglycans. Understanding the histopathology of these lesions may be useful for predicting the efficacy of novel treatment modalities (e.g., radiation therapy) and developing specific gene-directed therapies for modifying this disease process.

**TuP7:W11**

**BO-653, a novel antioxidant suppressed VSMC proliferation and neointimal thickening in rabbit and porcine models**


**Fujiigotemba Research Labs. Chugai Pharma Co., Ltd., Gotemba; 2Juntendo Univ., Tokyo; 3Univ of Tokyo, Tokyo, Japan; 4Mayo Clinic and Foundation, Rochester, USA**

Antioxidants have been associated with oxidative stress and modify neointimal formation. A new powerful antioxidant, BO-653 has anti-atherogenic effects. This study demonstrates that BO-653 can suppress the proliferation of vascular smooth muscle cells (VSMC) induced by FBS in vitro, and that it can prevent neointimal thickening after balloon injury in cholesterol fed rabbits and stented injury in porcine.

In vitro, BO-653 suppressed both ³H-thymidine incorporation and cell number increase in serum-starved VSMC (A7r5) stimulated with 2% FBS. In a rabbit balloon injury model, BO-653 daily administration suppressed neointimal thickening in iliac arteries of rabbits fed high cholesterol diet in a dose-dependent manner, the effective dose being >30 mg/kg/day.

In a porcine coronary artery stented model, BO-653 (500 mg/day) was administered from 7 days before stent implantation until the 28th day after stent implantation and then the intimal thickening was evaluated. Analysis of the correlation between of neointimal thickness on stented injury severity exhibited that the regression line of BO-653 group shifted below that of placebo group, which revealed the reduced neointimal thickening by BO-653 treatment (P < 0.01). Regarding neointimal area, BO-653 reduced neointimal thickening induced by stented injuries (placebo group: 2.04 ± 0.19 mm², (mean ± SE) BO-653 group: 1.50 ± 0.16 mm², P = 0.04).

**TuP1:W12**

**Green tea catechins beneficially modify cholesterol metabolism in the hypercholesterolaemic rabbit**

C.A. Burlall1, M. Abbey1,2, P.D. Rous2,1 University Adelaide; 2CSIRO Health Sciences and Nutrition, Adelaide, Australia

**Objective:** To determine the mechanism by which green tea catechins exhibit their hypocholesterolaemic effects.

**Methods:** Twenty-four New Zealand White rabbits were initially made hypercholesterolaemic by feeding them cholesterol (0.25% w/w) for a period of two weeks. This cholesterol enriched diet was then supplemented with a crude catechin extract from green tea at concentrations of either 0, 0.5, 1 or 2% (w/w) for four weeks. At the end of the treatment period, plasma and liver lipids were determined using enzymatic methods. The hepatic LDL receptor binding activity was measured by the calcium-dependent binding of colloidal gold-LDL to solubilised liver membranes on nitrocellulose. Relative amounts of LDL receptor protein were measured by Western Blotting with a specific polyclonal antibody. The plasma ratio of lathosterol to cholesterol was determined and used as an index of cholesterol synthesis.

**Results:** Administration of the crude catechin extract from green tea resulted in significantly lower plasma total (−60%) and LDL (−80%) cholesterol concentrations (p < 0.05) in the 2% (w/w) treatment group. Total (−25%) and unesterified (−15%) cholesterol in the liver were also significantly lowered. There was a significant reduction in cholesterol synthesis (−60%) and a significant increase in LDL receptor binding activity (−80%). The relative amount of LDL receptor protein was increased by 70% but this did not reach significance (p = 0.057). Lastly, the cholesterol concentration in the thoracic aorta was significantly reduced.

**Conclusions:** This study suggests that green tea catechins exhibit their hypocholesterolaemic effects by increasing the LDL receptor and lowering cholesterol synthesis. Green tea catechins may therefore be protective against heart disease.

**TuP2:W12**

**Dietary trans fatty acids lower HDL cholesterol and impair endothelial function**

N.M. de Roos, M.L. Bots, M.B. Katan2,1 Division of Human Nutrition & Epidemiology, Wageningen University, Wageningen; 2Julius Center for Patient Oriented Research, University Medical Center, Utrecht, The Netherlands

**Objective:** Some cholesterol-lowering diets decrease both low density (LDL) and high density lipoprotein (HDL) cholesterol. It is unknown whether diet-induced decreases in HDL cholesterol increase the risk of cardiovascular disease.

**Methods:** We investigated whether decreasing HDL cholesterol impaired endothelial function in a controlled dietary trial of trans fatty acids and saturated fats in 29 healthy men and women.

**Results:** Replacing 9.2 energy percent saturated fats by trans fats decreased HDL cholesterol by 0.39 mmol/L (14.2 mg/dL) while HDL and triglycerides remained stable. Flow-mediated vasodilatation (FMD) of the brachial artery, a marker of endothelial function, decreased from 6.2 to 4.4 percent (mean decrease 1.8; 95% CI, 0.4 to 3.2).

**Conclusion:** Diet-induced decreases in HDL cholesterol coincided with impaired endothelial function and might therefore increase the risk of cardiovascular disease.
TuP3.W12 Dietary trans fatty acids adversely affect the composition of postprandial lipoproteins
S. Samman1, L.M. Gatto1, D.R. Sullivan2. 1Human Nutrition Unit, Dept of Biochemistry, University of Sydney, Sydney; 2Department of Clinical Biochemistry, Royal Prince Alfred Hospital, Camperdown, Australia
Objective: To investigate the effect of consuming fatty meals rich in either trans fatty acids or oleic acid on the composition and metabolism of triacylglycerol-rich lipoproteins (TRL).
Methods: Nineteen normolipidaemic male volunteers consumed two test meals identical in composition except for a 6% energy exchange between oleic acid (CIS meal) and TFA (TRANS meal) in random order. The oral fat load was 1 g fat per kg body weight. Blood samples were collected prior to consuming the meal then at 4, 6 and 8 h. TRLs were isolated by ultracentrifugation (d < 1.006 g/mL) and apo[a] and apo[a] concentrations were measured by RIA (Mercodia, Sweden). Plasma lipids were determined enzymatically.
Results: Consumption of the CIS and TRANS meals resulted in similar concentrations of cholesterol and triglyceride in both plasma and TRL. Despite this, the rate of transfer of cholesteryl ester from HDL to TRL was higher (28%, P < 0.005) after the TRANS meal compared with the CIS meal. Similarly, the transfer of apo[a] from the d > 1.006 g/mL to the d < 1.006 g/mL fraction was greater (30%, P < 0.02) after consumption of the TRANS meal compared with the CIS meal.
Conclusions: Our data indicate that consumption of meals containing TFA at 10% of energy results in postprandial lipoproteins which contain more cholesteryl ester and apo[a] compared with those produced after consumption of meals containing similar amounts of oleic acid.

TuP4.W12 Adipose tissue trans fatty acid levels and first myocardial infarction
Peter M. Clifton, Manny Noakes. CSIRO, Adelaide, SA, Australia
Objective: To assess whether trans fatty acid intake and adipose tissue level is related to cardiovascular disease.
Methods: In cohort and case control studies in the USA trans fatty acid intake has been strongly associated with coronary artery disease but data from Europe has been less convincing. We performed a case control study of first myocardial infarction with assessment of dietary intake and adipose tissue levels of trans fatty acids. Cases had no past history of coronary artery disease, diabetes or elevated lipids. Healthy controls were selected from the electoral roll to match the cases by age, sex and postcode. 174 cases of first myocardial infarction and 174 controls were recruited.
Results: Cases had a higher total trans intake (3.5 vs 3.0 g/day, p = 0.002) and in the highest quintile of trans fatty acid intake (median 5.5 g/day) the risk ratio was 2.25 (p < 0.05). Consumption of trans fatty acids from margarines was not different between cases and controls. As trans fatty acids were removed from some margarines from March 1996 onwards we will present only those subjects with a fat biopsy performed before this date. In this group of 44 cases and 34 controls the cases had a greater amount of elaidic acid [18:1 beta delta 9] (0.83% vs 0.64%, p = 0.03) and trans vaccenic [18:1 delta 11] (0.74% vs 0.60%, p = 0.0003) in their adipose tissue. The cases and controls did not differ in average age, BMI or social class. In the highest tertile of adipose tissue elaidic acid the risk ratio was 5.9 (p < 0.01) which remained significant after adjustment for other coronary risk factors and fat intake.
Conclusions: Despite Australian diets containing only 1.2% of calories from trans fatty acids a high intake or a high adipose tissue level of trans fatty acids is associated with an increased incidence of myocardial infarction.

TuP5.W12 Genistein, a bioactive component found in soybeans, enhances relaxation to porcine coronary artery, in vitro
M.Y.K. Lee, R.Y.K. Man. Department of Pharmacology, Faculty of Medicine, The University of Hongkong, Hong Kong SAR, China
Objectives: Genistein, a phytoestrogen found in soybeans is well absorbed through the gastrointestinal tract and enter into the systemic circulation. Since it is weakly estrogenic and carries antioxidant properties, it can exert cardio-protective effects to human. Hence, our research objectives are to investigate the direct and indirect effects of genistein on vascular relaxation in porcine coronary artery and the role of endothelium in its action.
Methods: Vascular functions were assessed using the organ bath techniques. Firstly, direct relaxing ability of genistein was assessed after pre-contracted with U46619. After that, the indirect effects of genistein (3 μM) to different relaxing agents were studied. Endothelium-dependent (bradykinin (BK) and A23187) and-independent vasodilators (sodium nitroprusside (SNP) and cromakalim) were used to perform the dose-response relaxation. Lastly, LDL-C and Triton-X100 were employed to study the involvement of nitric oxide synthase (NOS) and endothelium to the enhancement effects of genistein.
Results: High concentration of genistein (10^-5 M-10^-4 M) had direct relaxing properties; At 3 μM, it enhanced the relaxation to SNP and cromakalim in porcine coronary artery but not to BK or A23187. Enhancement of relaxation to SNP was preserved in rings treated with L-NAME and Triton-X100.
Conclusions: Genistein can exert indirect relaxation to porcine coronary artery at vascular smooth muscle level and is independent of the activity of NOS.

TuP6.W12 Green tea and epigallocatechin gallate modulate cholesterol homeostasis in HepG2 cells
C.A. Bursill1, P.D. Roach1. 1University of Adelaide; 2CSIRO Health Sciences and Nutrition, Australia
Objective: To investigate the effects of green tea and its major antioxidant constituent epigallocatechin gallate (EGCG) on cholesterol metabolism in HepG2 cells.
Methods: HepG2 cells were incubated for 48 h with media containing increasing concentrations (0, 50, 100 or 200 μM) of either freshly brewed green tea or EGCG. At the end of the treatment period the cells were harvested and the LDL receptor binding activity was determined by measuring the calcium-dependant binding of colloidal-gold LDL to the intact cells. The relative amounts of LDL receptor protein were measured using Western Blotting with a specific polyclonal antibody against the LDL receptor. Cholesterol and lathosterol (an index of cholesterol synthesis) were also determined in the cells and media using gas chromatography.
Results: Green tea and EGCG significantly increased LDL receptor binding activity and the relative amounts of LDL receptor protein. They also decreased intracellular total cholesterol at all treatment concentrations, however only green tea, at 200 μM, significantly reduced unesterified cholesterol. A biphasic "down then up" change in intracellular cholesterol synthesis was observed; lathosterol concentrations were reduced in the lower dose treatment groups and cholesterol in the highest dose group. In the highest dose treatment group, green tea and EGCG also increased total cholesterol concentrations in the cell media.
Conclusion: Green tea and EGCG upregulated the LDL receptor in HepG2 cells. This effect may have been due to a reduction in intracellular total cholesterol. This in turn may have been due to a reduction in intracellular cholesterol synthesis at the lower doses but it was more likely due to an increase in cholesterol efflux at the highest dose.

TuP7.W12 Eicosapentaenoic acid and docosahexaenoic acid have differential effects on serum lipids and lipoproteins, LDL-particle size, glucose & insulin in dyslipidaemic men
T.A. Morri, V. Burke, I.B. Pudley, G.F. Watts, D.N. O’Neill1, J.D. Best1, J.T. Bellin. Department of Medicine, The University of Western Australia and West Australian Heart Research Institute, Perth, WA; 1Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia
Objective: To determine whether pure eicosapentaenoic acid (EPA, 20:5 n 3) and docosahexaenoic acid (DHA, 22:6 n 3) have differential effects on fatty acids, serum lipids and lipoproteins, glucose and insulin in humans.
Methods: Using a double-blind, placebo-controlled trial of parallel design, 59 overweight, non-smoking, mildly hyperlipidaemic men were randomised to 4 g/day of purified EPA, DHA, or olive oil (placebo) capsules while continuing their usual diets for 6 weeks.
Results: Fifty-six men aged 48 ± 1.1 years completed the study. Relative to the olive oil group, triacylglycerols fell 0.45 ± 0.15 mmol/L (~20%, P = 0.003) in the DHA group and 0.37 ± 0.14 mmol/L (~18%, P = 0.012) in the EPA group. Neither EPA nor DHA affected serum total cholesterol. In the EPA group, there were no significant effects on LDL-C, HDL-C and LDL-C/HDL-C, but HDL-C was significantly reduced (6.7%, 0.05 ± 0.02 mmol/L, P = 0.032). Although HDL-C was only marginally increased by DHA (3.1%, 0.03 ± 0.03 mmol/L, NS), HDL-C was increased by 0.07 ± 0.02 mmol/L (~29%, P = 0.004). Although DHA increased LDL-C significantly (8%, 0.34 ± 0.14 mmol/L, P = 0.019), LDL-particle size increased by 0.25 ± 0.08 mm (P = 0.002). EPA did not alter LDL-particle size. Both EPA and DHA significantly increased fasting insulin (1.58 ± 0.73 pmol/L, P = 0.035 and
TuP8:W12
Response of small, dense low density lipoprotein (LDL) to fish oil is influenced by apo E genotype
A.M. Minihane1, P. Talmud2, J. Wright3, M. Murphy3, C. Williams1, B. Griffin1, 1University of Reading, Reading; 2University College London, London; 3University of Surrey, Guildford, UK
Objective: To examine the impact of apo E polymorphism on responsiveness of small, dense LDL to fish oil supplementation.
Methods: 50 male subjects, aged 30-70 years, with moderately raised plasma triglyceride (TG) (1.5-5.4 mmol/l), a low high density lipoprotein cholesterol (HDL-C) (<1.1 mmol/l), and predominance of small, dense LDL (LDL-C density 1.044-1.060 g/ml > 50%) entered a double blind, placebo controlled, cross-over study. Subjects consumed 6 g of fish oil daily (6 x 1 g capsules containing 3 g eicosapentaenoic acid/docosahexaenoic acid (‘Pika-soft’), or 6 g of an olive oil ‘placebo’ for 6 weeks. After a 12 week washout, subjects switched to the opposite supplement. Total cholesterol (TC), TG and HDL-C were measured by enzymatic, colorimetric assays, HDL by anion precipitation and LDL-C by the Friedewald formula. LDL-C was determined by density gradient centrifugation and apo E genotypes by microplate-array diagonal gel electrophoresis.
Results: The group expressed a higher than expected frequency of the ε4 allele (40%). Fish oil decreased plasma TG (~35%, p < 0.05) and small, dense LDL-C (~26%, p < 0.05). The effect on TG was independent of apo E genotype. Conversely, carriers of the ε4 allele (ε4/ε3 n=18, ε4/ε4 n=2) showed a disproportionate decrease in LDL-C (~36%) and increase in LDL-C (+16%). Plasma TC and HDL-C showed no overall response to fish oil.
Conclusions: Carriage of the ε4 allele may account for the increase in LDL-C often associated with fish oil supplements. This effect can be explained, in part, by a more pronounced redistribution of LDL subclasses in ε4 carriers towards LDL particles which are potentially less atherogenic.

TuP9:W12
Pomegranate juice consumption reduces oxidative stress and low density lipoprotein atherogenic modifications: studies in the atherosclerotic apolipoprotein E deficient mice and in humans
B. Fuhrman, N. Volkova, L. Dornfeld, M. Rosenblat, M. Kaplan, T. Hayek, D. Presser, M. Aviram. The Lipid Research Laboratory, Technion Faculty of Medicine, The Rappaport Faculty of Medicine, Institute for Research in the Medical Sciences and Rambam Medical Center, Haifa, Israel
Objective: To analyze the effect of pomegranate juice (PJ) consumption by humans or by the atherosclerotic apolipoprotein E deficient (E-) mice on: 1) lipoprotein atherogenic modifications (oxidation, aggregation and retention); 2) macrophage atherogenic responses (lipoprotein uptake and LDL oxidation); 3) atherosclerotic lesion development.
Results: Consumption of PJ for 2 or 14 weeks by humans or by the atherosclerotic mice, respectively, exhibited antioxidative effects against lipid peroxidation in whole plasma and in isolated lipoproteins, and increased the activity of serum paraoxonase. PJ consumption also reduced two other related modifications of the lipoprotein: i.e. its retention to proteoglycan, and its susceptibility to aggregation. The effect of PJ on macrophage atherogenic responses was studied in mouse peritoneal macrophages (MPMs) from E- mice. Following PJ consumption, the capacity of the macrophages to oxidize LDL was reduced by 88%, and this effect was associated with reduced cellular lipid peroxidation, reduced superoxide anion release and elevated content of macrophage glutatione. Furthermore, the uptake of oxidized LDL and that of native LDL by MPMs that were obtained after PJ administration was significantly reduced by about 20%. Finally, PJ supplementation to E- mice reduced the size of their atherosclerotic lesion, and also the number of foam cells.
Conclusions: This study clearly demonstrated a potent anti-atherogenicity of pomegranate juice consumption in healthy humans and in atherosclerotic mice.

TuP10:W12
Effect of a standardized grape seed extract on LDL-susceptibility to oxidation in smoking men
Objective: We evaluated the effect of a capsule formulation of a polyphenolic extract of grapes (Leucoselect-Phytosyme, LP) on LDL susceptibility to oxidation in a group of smoking human volunteers.
Methods: A randomized, double blind, crossover study was undertaken in 24 healthy smoking men, aged 30 or more. Enrolled subjects were given 2 capsules bid, each containing 75 mg of a grape procyanidin extract, or placebo, daily for 4 weeks (phase 1). A washout period of 3 weeks was then followed by 4 weeks of the opposite treatment (phase 2). Blood samples were taken at baseline and at the end of each phase and assayed for plasma lipids and LDL susceptibility to oxidation.
Results: Pharmacological compliance was good and no adverse effects were recorded. Subjects did not disclose significant modification of total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol, during LP treatment. Among oxidative indices, malondialdehyde (MDA) concentration was significantly reduced in subjects taking LP (~14.7% ± 2.1% vs. +5.0% ± 18.1%, p < 0.01), and the Log phase prolonged (+15.4% ± 24.4% vs. -0.1% ± 16.9%, p < 0.05) compared to placebo and basal values (table).

<table>
<thead>
<tr>
<th>Leucoselect Phytosyme</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>52.9 ± 7.6</td>
</tr>
<tr>
<td>4 week</td>
<td>59.0 ± 13.0</td>
</tr>
<tr>
<td>MDA (nmoles/mg protein)</td>
<td>0.64 ± 0.11</td>
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<td></td>
<td>0.56 ± 0.10</td>
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</table>
*p < 0.005 ‘4 week’ vs. ‘basal’

Conclusions: The antioxidant potential of grape-extract polyphenols may be converted in a pharmacological preparation provided of good tolerability and significant efficacy in a common model of oxidative stress (smoking).

TuP11:W12
Effect of mercury, iron and antioxidants on atherosclerosis in LDL-R-deficient mice
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Objective: Lipid peroxidation, which is effected by free radicals, pro-oxidative catalytic metals and antioxidant defense mechanisms has a major role in atherogenesis. We tested the effect of the low vitamin E and C, iron or mercury supplementation combined with moderate atherogenic diet in LDL-R-deficient mice with a special interest to investigate the role of dietary mercury.
Methods: Five groups of 20 LDL-R-deficient mice were kept 4 months on a western type diet without or with supplementation with either combined antioxidants (0.02% vitamin E + 0.05% vitamin C) or iron (0.02% ferrousphate). Half of the mice in the iron and control groups had also mercury supplemented water (methylmercury 1 mg/l).
Results: All groups had equivalent plasma cholesterol levels. Low levels of vitamins, mercury or iron supplementation had no statistical effect on lesions size. Unlike others the group with combined dietary mercury and iron supplementation led to significant progression of atherosclerosis in LDL-R-deficient mice. The mean lesion size of the aortic tissue increased by 27% (p = 0.05) compared to controls and the cross-sectional lesion area by 20-27% compared to all groups (p = 0.001).
Conclusions: The results show no atheroecrosis effect of the low vitamins supplementation, but the effect of combined mercury and iron seems to be over 3-fold more atherogenic than iron alone.

TuP12:W12
Cholesterol increases DNA methylation in rabbit tissues
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Objectives: Changes in DNA methylation level and their effects have been widely studied. Our group has found a connection between hypermethylation and cell proliferation at local as well as genome level. The aim
of this study was to find out whether cholesterol is responsible for the hypomethylation.

Methods: In the study we used NZW rabbits having 30 days cholesterol diet (n = 8), WHHL rabbits having heritably high cholesterol: level (n = 6) and as controls NZW rabbits having normal chow (n = 3). In order to analyse methylation level, DNA was isolated from brain, lungs, heart, spleen, liver and kidney. After that DNA samples were prepared for HPLC analysis.

Results: The results indicate that cholesterol increases DNA methylation especially in lungs (cholesterol diet NZW 3.92% and WHHL 3.87% vs control 2.75%) and heart (3.68% and 3.52% vs 2.79%). Methylation level was slightly increased also in other tissues except in liver and kidney.

Conclusions: According to the study, cholesterol does not decrease DNA methylation. Instead, elevated cholesterol level seems to increase genomic methylation, which can affect regulation of gene expression. This can be caused by enhanced activity of methyltransferases or increased S-adenosymethionine.

TuP13:W12 Effects of combination of tall oil sterols and n-3 fatty acids on atherosclerotic lesion development in cholesterol-fed (0.2%) w/w apo E-KO mice
T. Lukic1, E. Novak2, M.H. Moghadassian3, J. Frohlich1, H. Pritchard1
1University of British Columbia, 2Forbes Medi-Tech Inc., Canada

Objective: To determine the effect of polysaturated fatty acids and phytoestrogens on atherosclerotic lesion development in apoE-deficient (apoE-KO) mice.

Methods: 28 apoE-KO mice were fed an atherogenic diet supplemented with 1% non-hydrogenated phytosterols (Phytrol®); 1% hydrogenated phytosterols; 1% omega-3 fatty acids, or combination of 1% of each phytosterols with 1% omega-3 fatty acids for 14 weeks. Aortic roots were sectioned and stained with oil red O and Movat's pentachrome. Atherosclerotic lesion size was determined using a digitizing morphometry image-analysis. Control mice consumed identical diet that did not contain the phytosterols or omega-3 fatty acids.

Results: Compared to controls, combination of 1% non-hydrogenated phytosterols with 1% omega-3 fatty acids significantly (57%; p < 0.05) reduced aortic atherosclerotic lesion size.

Conclusions: Combination of non-hydrogenated phytosterols with fish oil significantly reduces atherosclerosis in apoE-KO mice while a combination of hydrogenated tall oil phytosterols with fish oil was not as effective.

TuP14:W12 A novel linoleic acid derivative shows a marked inhibition of platelet aggregation
I. Minullina, O. Ibragimov. Kazan State Medical Academy, Kazan, Russia

Objective: The lipoxigenase products of polysaturated fatty acids of plant origin are known for their marked antiaggregatory properties. In the present study we investigated the effect of the recently described linoleic acid derivative (LAD) isolated from garlic bulbs on aggregation of human platelets.

Methods: Platelet aggregation to various agonists was monitored by a standard turbidimetric technique.

Results: When stimulated with thrombin (0.5 U/ml) platelet aggregation was inhibited by LAD in concentrations ranging from 50 mmol (p < 0.05). LAD also decreased platelet aggregatory response to ionophore A 23187 (1 mmol) at concentrations starting with 100 mmol (p < 0.01). Addition of LAD in concentrations of 30-40 mmol prior to platelet stimulation with arachidonic acid (1 mmol) reduced their aggregation by 50%. Platelet aggrega-gation to arachidonate was completely abolished by LAD in concentrations of 100 mmol and more (p < 0.01). An application of LAD in concentrations of 25-200 mmol also caused dose-dependent inhibition of the aggregation induced by ADP (2.5 mmol) or adrenaline (1 mmol). Addition of LAD in concentrations of 100 mmol produced a 2-fold decrease in platelet aggregatory responses to this agonists. This inhibition rate was comparable to the effect of 500 mmol acetylsalicylic acid. However, when LAD and acetylsalicylic acid were used together, they had a very weak synergistic effect on platelet activation.

Conclusions: These observations suggest that antiaggregating properties of LAD may be mediated via its action on cyclooxygenase-dependent pathway of arachidonic acid metabolism.

TuP15:W12 Decrease in plasma LDL-C and apolipoprotein-B by stiostanol-ester in Japanese
Y. Honna1, T. Ishikawa2, M. Tateno3, A. Matianyama4, 1Dept. of Internal Medicine, Tokai Univ., Isehara, 259-1193; 2Dept. of Gerontology, Keio Univ., Tokyo, 3Clinical Pharmacology Center; 4Nisshii Shiki Chuo Sogo Hosp, Japan

Objective: The effect of a stiostanol-ester (SE) containing spread on plasma level of lipids and apolipoproteins was investigated in a randomized, placebo controlled study in Japanese.

Methods: 105 volunteers were divided into 3 groups (control; N = 35, 2 g/day SE; N = 34, 3 g/day SE; N = 36) by randomization. 4 weeks of test period were followed by a 4 week observation period without spread. Plasma levels of lipids and apolipoproteins were taken at weeks 0, 2, 4 and the 4 week follow up visit.

Results: Plasma levels of LDL-C and apolipoprotein B were significantly reduced by 9.5% and 7.1% after 4 weeks of treatment with 2 g/day SE group, respectively, and LDL-C and apolipoprotein B levels returned to baseline during the 4 week follow up period without spread. No further reduction was observed in the 3 g/day SE group. Plasma levels of TG, HDL-C, apolipoprotein AI and apolipoprotein E were not changed with use of SE.

Conclusions: SE significantly decreased plasma levels of LDL-C and apolipoprotein B. However, the decreases were similar for the 2 g/day and the 3 g/day SE groups.

TuP16:W12 Light to moderate alcohol intake and the metabolic syndrome in 60-year old men and women
M. Rosell1, M.-L. Hellénius2, U. de Faire1,3, 1Institute of Environmental Medicine, 2Division of Medicine, Karolinska Institute, Stockholm, Sweden

Objective: To investigate the relationship between the alcohol intake and the prevalence of the metabolic syndrome in 60-year old men and women.

Methods: Every third 60-year old man and woman in Stockholm County was invited to the study, 2039 men and 2193 women participated (78%). A physical examination was performed and fasting blood samples were taken. A questionnaire including five questions concerning alcohol was filled in. The metabolic syndrome was defined when three of the criteria were fulfilled: WHR ≥ 0.95 and ≥0.85, HDL cholesterol < 1.0 and 1.1 mmol/l, f-insulin ≥ 15.5 and ≥11.7 mU/l (fourth quartile) for men and women respectively, f-glucose ≥ 6.1 mmol/l, s-triglycerides ≥ 1.7 mmol/l, blood pressures diastolic ≥ 90 and systolic ≥ 140 mmHg. Alcohol intake (light to moderate vs non or occasional drinking) in men and women with the metabolic syndrome was compared with a reference group with no criteria fulfilled. Prevalence odds ratios (POR) were calculated with logistic regression.

Results: Thirty percent of the men and 15% of the women fulfilled the criteria for the metabolic syndrome. The POR’s are adjusted for physical activity, education, smoking and the intake of fruit and vegetables.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Men (POR, 95% CI)</th>
<th>Women (POR, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light to moderate alcohol intake</td>
<td>0.79 (0.51-1.22)</td>
<td>0.54 (0.40-0.74)</td>
</tr>
<tr>
<td>Light to moderate wine intake</td>
<td>0.84 (0.64-1.09)</td>
<td>0.49 (0.37-0.65)</td>
</tr>
<tr>
<td>Light to moderate spirits intake</td>
<td>1.74 (1.22-2.49)</td>
<td>1.19 (0.60-2.38)</td>
</tr>
<tr>
<td>Light to moderate beer intake</td>
<td>1.38 (1.06-1.74)</td>
<td>12.78 (3.47-47.03)</td>
</tr>
</tbody>
</table>

Conclusions: Moderate intake of total alcohol and wine reduces the risk of having the metabolic syndrome with approx. 50% among 60-year old women whereas moderate intake of spirits and beer increases the risk among men.
TuP17:W12
Dietary monounsaturated fatty acids and carbohydrates increase VLDL-cholesterol and atherosclerosis in mice via an apo E-dependent pathway
M. Merkel1,2, W.V. Carasco3, L.C. Hudgins2, J.L. Breslow2, 1Univ. Hosp. Eppendorf, Hamburg, Germany; 2Rockefeller University, New York, USA

Objective: In most dietary recommendations saturated fatty acids are exchanged for carbohydrates. However, the relative benefit of replacing saturated fats with monounsaturated and polyunsaturated is not clear.

Methods: Four isocaloric diets have been designed with 78% of calories identical and 22% derived from either saturated (SAT), monounsaturated (MONO), polyunsaturated (POLY) fat or carbohydrate (CARB). Wild type (WT), LDL receptor deficient (LRKO) and apo E deficient (EKO) mice were fed these diets for 12 weeks and examined for lipoprotein profile and atherosclerosis.

Results: VLDL-cholesterol was about doubled on MONO and CARB (36 ± 12 and 18 ± 4 mg/dl) compared to SAT and POLY (12 ± 5 and 6 ± 2 mg/dl) in WT mice (ANOVA: p < 0.0005). In LRKO mice, VLDL cholesterol was more than doubled on MONO (831 ± 261 mg/dl) vs. SAT (373 ± 154 mg/dl) and closed to tripld on CARB (730 ± 288 mg/dl) vs. POLY (257 ± 53 mg/dl); p < 0.0001). In EKO mice, SAT resulted in the highest VLDL-cholesterol (1220 ± 285 mg/dl), with lower values on MONO (944 ± 203 mg/dl) and lowest on both POLY (739 ± 147 mg/dl) and CARB (728 ± 131 mg/dl), p < 0.0001. Aortic root atherosclerotic lesion size was in LRKO mice significantly higher in MONO and CARB (200678 ± 32982 and 201861 ± 67536 μm²) than in SAT and POLY (111028 ± 38817 and 137933 ± 35199 μm², p < 0.0001). Atherosclerotic lesion size was positively correlated with VLDL-cholesterol (R² = 0.27, p < 0.001). No significant differences in atherosclerosis were seen in EKO mice (SAT 195829 ± 57995, MONO 152166 ± 51400, POLY 149184 ± 64911 and CARB 210051 ± 87772 μm²).

Conclusion: Dietary monounsaturated fatty acids and carbohydrates are able to dramatically increase VLDL-cholesterol in wild type and LRKO mice. This effect was completely abolished in the absence of apo E. After further characterization of this phenomenon, dietary recommendations may be adapted.

TuP18:W12
Inhibition of low-density lipoprotein oxidation by fish protein and antioxidants
K. Kondo1, T. Iwamoto2, K. Hosoda3, M. Kaniyama1, R. Hirano4, T. Kidoo5, A. Matsumoto6, S. Watambe6, H. Iakura7, 1Ochanomizu University; 2Nat. Inst Health and Nutrition; 3Suntory Ltd.; 4Kagawa Nutrition University, Tokyo, Japan

Objective: Fish contains not only fish oil but also fish protein and other components. However, studies in terms of dietary fish intake have been focused on the fatty acid content of fish oil but some adverse effects are oxidation EPA or DHA. Oxidation of LDL is implicated in the development of atherosclerosis. We therefore determined the ex vivo effects of fish protein and other component on LDL oxidation. As other component, we have paid attention to astaxanthin which is a carotenoid and antioxidant and produced by fish.

Method: The oxidation of LDL was measured in a 1 ml reaction system consisting of V-70 and LDL.

Results: At first we requested 16 volunteer females (mean age 19.4 [SD 0.9] years) to initially consume casein (control period) and then fish protein (experimental period), corresponding to a dose of 16.9 g protein per day for 10 days. Fasting venous blood samples were taken at days = 10, 0, +10. Oxidation of LDL was longer at day 10 (lag time 14.3%) than at day 0.

Second, 19 volunteers (11 males, 8 females, mean age 25.5 [SD 4.9] years) consumed astaxanthin, at dose 3 ranging from 1.8, 3.6 and 14.4 mg for 14 days. Fasting venous samples were taken at days 0, +14. LDL lag time was longer (5.0, 26.2 and 42.3% respectively) compared with day 0 after consuming astaxanthin at doses of 1.8, 3.6 and 14.4 mg for 14 days.

Conclusion: Our results provide evidence that dietary fish intake inhibit LDL oxidation and possibly contribute to the prevention of atherosclerosis.

TuP19:W12
Red wine polyphenolic compounds preserve normal arterial relaxation by preventing α-tocopherol consumption, cholesterol oxidation, and endothelium dysfunction
V. Deckert, A. Athias, C. Desrumaux, V. Palleau, P. Gambert, D. Masson, L. Lagrost. INSERM U 498, Faculty of Medicine, Dijon, France

Objective: To determine whether red wine polyphenolic compounds (RWPC) can play a significant protective effect on both oxidation of low density lipoprotein (LDL) and vascular endothelium dysfunction.

Methods: RWPC were obtained from the Cabernet-Sauvignon grape variety. Isolated LDL from human plasma were oxidized in the presence of CuSO4. Lipid peroxidation and a-tocopherol consumption of LDL were assayed by HPLC. Oxidized LDL forms were analyzed by capillary gas chromatography. Vascular reactivity studies were conducted on rabbit aortic rings mounted in organ baths.

Results: RWPC markedly reduced the formation of lipid peroxidation, 7β-hydroxycholesterol and 7-ketocholesterol in oxidized LDL. The ability of RWPC to prevent the emergence of oxidized cholesterol was directly dependent on the LDL α-tocopherol content. Once the LDL α-tocopherol has been consumed, RWPC became no more effective indicating that RWPC act by sparing endogenous α-tocopherol. RWPC prevented the inhibitory effect of oxidized LDL on the endothelium-dependent (EDRF) and rabbit aorta. RWPC could also exert a direct and remnant inhibitory effect on the contractile response of rabbit aorta to norepinephrine, as well as a direct relaxing effect on precontracted rings.

Conclusion: RWPC can preserve a normal vascular reactivity by acting at different stages of the cascade that leads to lipid oxidation, endothelium dysfunction and vasoconstriction.

TuP20:W12
Exploring new functions of polyunsaturated fatty acids on the gene expression using DNA chip
A. Matsumoto1, Y. Fujii2, S. Hanaka1, H. Itakura1, M. Ishii3, S. Tsutsumi4, H. Aburantani5, 1Natl Inst of Health and Nutr; 2Ochanomizu Univ; 3RCAST, Tokyo Univ, Tokyo, Japan

Objective: A reduction in plasma lipid levels, often atherogenic, is achieved by intake of polyunsaturated fatty acids (PUFAs) which reportedly affects the expression of enzymes and proteins involved in lipid metabolism. In this study, we have investigated the PUFA induced regulation on the expression of thousands of genes simultaneously, using DNA chip technology.

Methods: HepG2 cells were treated with either 0.25 mM of arachidonic (20:4, n-6), eicosapentaenoic (20:5, n-3) or docosahexaenoic acids (22:6, n-3) for 24-hr at 37°C. Following isolation of polyadenylated RNA, mRNA levels of genes were detected by GeneChip FL. Affymetrix (R) of rabbit aorta. RWPC could also exert a direct and remnant inhibitory effect on the contractile response of rabbit aorta to norepinephrine, as well as a direct relaxing effect on precontracted rings.

Results: PUFAs decreased mRNA levels of enzymes related to lipid metabolism, including HMG-CoA reductase, mevalonate pyrophosphate decarboxylase, squalene synthase, fatty acid synthase and steroyl CoA desaturase. PUFAs also decreased mRNA levels of PAI-1, INF-gamma receptor and connective tissue growth factor (as well as other unknown genes). These results indicate that PUFAs affects the expression of a large number of genes known to influence lipid metabolism, as well as other genes not yet identified with function. Consequently, this approach enables us to explore novel functions of fatty acids on gene regulation.

TuP21:W12
Effect of an oleic acid-rich diet on postprandial apolipoproteins in type 2 diabetes
D. Ongas, C. Madigan, M. Ryan, P. Collins, A. Johnson, G.H. Tomkin, Dept of Clinical Medicine, Trinity College Dublin, Dept of Biochemistry, Royal College of Surgeons in Ireland, Adelaide/Meath Hospital, Dublin, Ireland

Objective: Postprandial lipoproteins are thought to be particularly atherogenic and this study examines the effect of monounsaturated as compared to polyunsaturated fatty acid diet on the apoproteins which regulate postprandial lipoprotein clearance.

Methods: Eleven type 2 diabetic patients were treated with a polynsaturated and monounsaturated isoicotic diet in a 2 week crossover study. Blood was taken at the end of each dietary period fasting and for up to 8 hours following a high fat meal. Chylomeric and very low density lipoprotein (VLDL) were isolated by ultracentrifugation. Apo B100, B48 and apo E were measured by polyacrylamide gradient gel electrophoresis and apo C1 and C11 were determined by isoelectric focussing. Fatty acids were measured by gas/lc chromatography.

Results: The diet resulted in a significant alteration in the ratio of monounsaturated fats (p < 0.05) confirming adherence to diet. There was no difference in weight but fasting blood sugar was significantly lower on the monounsaturated fat diet (7.8 ± 0.4 vs 9.0 ± 0.4 p < 0.02) as was fasting insulin (13.0 ± 0.8 vs 14.7 ± 1.3 p < 0.05). Postprandial apo B48 and B100 area under curve (AUC) were significantly lower on the monounsaturated fat diet (4.9 ± 0.8 vs 14.2 ± 3.6 p < 0.05 and 36.3 ± 7.1 vs 77.9 ± 21.9 p < 0.05). Apo E AUC was significantly higher (945 ± 203 vs 495 ± 102 p <
0.01) and apo C-III (16.2 ± 3.6 vs 22.4 ± 4.4) and apo C-III (93 ± 13 vs 127 ± 20) were significantly lower on monounsaturated diet (p < 0.05). Apo C-III is apo A-E, the ligand for receptor-mediated clearance of VLDL.

**Conclusions:** The monounsaturated fat diet improved clearance of post-prandial particles due to reduction in apo C-III and increase in apo A-E. The study suggests a mechanism that might explain the beneficial effect of a Mediterranean-type diet in the prevention of atherosclerosis.

**TuP22.W12**

**Macrophage-mediated oxidation of LDL may be counteracted by phenolic compounds contained in extra-virgin olive oil**

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Phenolic antioxidants may play a key role in the prevention of cardiovascular disease. The aim of this study was to investigate the capability of phenolic compounds contained in extra-virgin olive oil in inhibiting the oxidation of LDL mediated by J774 A-1 murine macrophage-like cell line. Cells (1.5 × 10⁶ /25 mm dish) were incubated with LDL (0.2 mg of protein/ml) in DMEM, phenol red-free, supplemented with 2 mM CuSO₄, for 24 h at 37°C. To evaluate the effect of phenol compounds on cell-mediated oxidation of LDL, tyrosol, protocatechuic acid or oleuropin (range 0.25–1 mM) were added to the cells 2 h before. The extent of LDL oxidation was measured in the medium by TBARS assay. In addition, changes in cholesterol contents of LDL on agarose gel were evaluated. J774 A-1 cells significantly increased the amount of TBARS when compared with the cell-free control (44.5 ± 7 vs 3.67 ± 1 nmol/mg of protein LDL, p < 0.001). Protocatechuic acid and oleuropin, at the lower concentration tested, completely inhibited TBARS formation, while tyrosol was slightly effective even at 1 mM concentration (34.5 ± 2.8 nmol/mg of protein LDL). In the presence of cells, the electrophoretic mobility of LDL was two times higher than native LDL. This increase was completely prevented by protocatechuic acid or oleuropin, but not by tyrosol. In conclusion the presence of protocatechuic acid and oleuropin may prevent the oxidation of LDL mediated by J774 A-1 cells. This finding might be related to the two hydroxyl groups on the phenolic ring of those compounds. Our investigation suggest that dietary intake of extra-virgin olive oil might contribute to lower the risk of coronary heart diseases.

**TuP23.W12**

**Influence of Apo A4 genotypes (ApoA4-347 mutation) on the lipid response to diet in familial hypercholesterolemia**

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**Objective:** To analyze the influence of Apo A4 genotypes (Gln300 → His) on the lipid response to an hypercholesterolemic diet (NCEP-I) in familial hypercholesterolemia.

**Subjects and Methods:** A total of 67 FH heterozygotes (43 women) were studied at baseline and after 3 months of consuming NCEP-I diet. Lipids and lipoproteins were measured with standard methods and apo A4 genotype by PCR and restriction analysis.

**Results:** Genotype 1/1 was found in 51 subjects while 16 subjects had genotype 1/2. No sex-related differences in response to diet were detected. Apo A4-A2 allele was associated with lower LDL-C (p = 0.049) and apo B levels (p = 0.027), independently of the response to diet. After diet, apo A4-A2 carriers showed lower reductions in apo B levels (6.2% p = 0.036) but not in LDL-C.

**Conclusion:** In FH subjects, presence of apo A4-A2 allele is associated with lower lipid levels at baseline and after the NCEP-I diet period, indicating a beneficial interaction with LDL receptor alterations.

**TuP24.W12**

**Tocotrienols from rice bran oil have no effects on levels of LDL cholesterol and markers for cholesterol synthesis and absorption**

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**Objective:** Tocotrienols from rice bran oil (T-RBO) have been reported to lower LDL cholesterol by reducing HMG-CoA reductase activity, but results are controversial. We have therefore compared the effects of T-RBO with those of other tocopherols (α-Toc, γ-Toc, δ-Toc, EPA, an HMG-CoA reductase inhibitor) on LDL cholesterol and markers (in terms of μmol/mmol of cholesterol) for cholesterol absorption and synthesis in hypercholesterolemic subjects.

**Methods:** 33 men and 38 women followed a cholesterol-lowering diet for three weeks. For the next six weeks, subjects continued their diets and were randomly divided into three groups, consuming a placebo (PL; n = 24), T-RBO (42 mg/day, n = 24) or PRA (40 mg/day, n = 23). In a subset of 50 persons, serum lathosterol as an indicator of cholesterol synthesis, and serum campesterol, a plant sterol, as a marker for intestinal cholesterol absorption, were determined by gas chromatography.

**Results:** As compared to the PL-group, no favourable effects of T-RBO were observed on serum LDL cholesterol concentrations. Also, serum lathosterol and campesterol levels did not change. In the PRA-group, LDL cholesterol decreased by 32% (P < 0.001) and 28% (P < 0.001) as compared to the PL-group and T-RBO-group, respectively. Lathosterol was lowered with PRA by 50% (P < 0.001) compared to the PL-group, and by 57% (P < 0.001) compared to the T-RBO-group. Campesterol increased with PRA by 15% (P < 0.001) versus the PL-group.

**Conclusions:** In contrast to PRA, T-RBO do not affect lipid metabolism as evidenced from unchanged concentrations of serum LDL cholesterol, lathosterol and campesterol.

**TuP25.W12**

**Effect of dietary fatty acid modification on blood lipids, lipoproteins and lipoprotein subclasses in men**


Small, dense low density lipoproteins (LDL) (d > 1.044 mg/dl) are associated with increased atherogenic risk. The aim of the study was to investigate the effect of two modified test fats (A and B) on lipid profile in healthy men. Sixteen men (age 35–75 y) substituted 80 g of their normal dietary fat intake were fat during two periods of 21 days in a double blind, randomized cross over study. Both test fats were low in cholesterol raising saturated fatty acids. Test fat A contained 5% w/w long chain n-3 fatty acids matched by oleic acid in test fat B. Before, during and between the study periods the participants’ habitual diet was assessed by four days weighed food records. Fasting blood samples were drawn before and in the end of the study periods.

Preliminary results show that plasma triacylglyceride (TG) was lower after fat A than B (P < 0.05), no other significant differences were observed. However, compared to baseline values test fat A decreased plasma triacylglyceride (TG) from 1.59 to 1.16 mmol/l (P < 0.001), apolipoprotein-B in small dense LDL from 22.77 to 17.69 mg/dl (P < 0.01) and plasma cholesterol from 5.69 to 5.24 mmol/l (P < 0.01). LDL cholesterol and high density lipoprotein (HDL) cholesterol was unchanged. Compared to baseline values test fat B decreased plasma cholesterol from 5.80 to 5.32 mmol/l (P < 0.001) and LDL cholesterol from 3.61 to 3.32 mmol/l (P < 0.01). There was no effect on TG, HDL cholesterol or small dense LDL. We conclude that compared to the habitual diet, both dietary regimens had a beneficial effect on lipid profile, with fish oil fat resulting in a considerable decrease in total TG and concentration of small dense LDL, whereas modified fat without fish oil decreased LDL cholesterol. Supp. by FØT/KE/Danish Research Agency.

**TuP26.W12**

**Meal-induced factor VII activation and thrombin formation in atherosclerotic patients**

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**Objective:** To study whether meal-induced factor VII (FVII) activation leads to thrombin formation in atherosclerotic patients.

**Methods:** Thirty patients with angiographically verified coronary artery disease consumed low-fat (5% of total dietary energy) breakfast (8.30 h) and lunch (11.00 h) and isoeenergetic high-fat (40%) breakfast and lunch on two separate days in a randomized cross-over design. Blood samples were drawn at 8.15, 12.30, 14.00, 15.30, and 16.45 h. Plasma triglycerides, activated FVII (FVIIa), FVII protein (FVIIlag), prothrombin fragment 1 + 2 (F1 + 2), and soluble fibrin were determined.
TuP27/W12 Interaction between ApoE polymorphism and dietary factors in modifying serum lipids

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Objective: To evaluate the interaction between ApoE polymorphism and dietary factors in modifying serum lipid concentrations.

Methods: Altogether 415 patients with coronary heart disease (CHD) aged 61 (33–74) years (mean [range]) participated in EUROASPIRE study in Finland. The serum lipid concentrations were measured and ApoE genotype was determined. Dietary intake was examined once with a four day food record.

Results: Patients were grouped by ApoE genotype: e2 (E2/E2, E2/3, n = 21, 5.1%), e3 (E3/E3, n = 245, 59.2%) or e4 (E4/E3, E4/2, E4/E4, n = 148, 35.7%). The mean ± (SD) serum total cholesterol (TC) and triglyceride (TG) concentrations (mmol/l) were 5.57 ± 1.00 and 2.38 ± 1.19 in patients with e2, 5.15 ± 1.17 and 1.94 ± 1.68 in patients with e3 and 6.16 ± 2.12 and 1.90 ± 1.23 in patients with e4 (p = 0.123 and p = 0.073), respectively. Body mass index, age and lipid lowering drugs were included as confounding factors in the analyses. The TC rising effect of high saturated fat intake was not seen in patients with e2 (TC [mmol/l]) in tertiles of saturated fat intake were 5.03 ± 0.89, 5.85 ± 0.70 and 5.33 ± 1.60 (p = NS) in patients with e2, 5.97 ± 1.02, 5.96 ± 1.17 and 6.47 ± 1.19 (p = 0.095) in patients with e3 and 6.07 ± 1.25, 6.06 ± 1.11 and 6.30 ± 1.23 (p = NS) in patients with e4. Interestingly, a high saccharose intake was associated with high TG only in patients with e2 (TG [mmol/l]) in tertiles of saccharose intake were 1.60 ± 0.69, 2.46 ± 1.10 and 3.09 ± 1.31 (p = 0.035) in patients with e2, 2.04 ± 1.29, 1.95 ± 2.54 and 1.79 ± 0.82 (p = NS) in patients with e3 and 2.17 ± 1.35, 1.75 ± 0.76 and 1.71 ± 1.28 (p = 0.007) in patients with e4.

Conclusion: Hypertriglyceridemic effect of high saccharose feeding is confined to CHD patients with allele e2. On the other hand, they show no hypercholesterolemic response to high saturated fat intake.

TuP28/W12 Phytosterols from tall oil (Phytrol®) prove very effective for cholesterol reduction in gerbils fed various diets

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Objective: To establish dietary factors relevant to tall oil phytosterols’ (Phytrol®) efficacy in fat/cholesterol responsive animal models.

Methods: Study 1. Gerbils and hamsters were fed purified diets containing 0.05% cholesterol and 30% energy from fat for 4 weeks to assess plasma cholesterol (TC). Hamsters were divided between experimental (0.5% Phytrol® in a fat mixture) and control (fat mixture alone) groups. Gerbils were separated into 3 groups, 0.5% Phytrol®, 0.5% phytostanol esters from margarine, or control (fat mixture alone). Study 2. Gerbils were fed purified diets containing 0.05% cholesterol for 4 weeks. The TC response to graded doses (0.25%, 0.5%, and 1.00% of diet) of Phytrol® was examined. Study 3. Gerbils were fed 0.5% Phytrol® at three levels of dietary fat (19%, 35%, 42% energy) and two levels of dietary cholesterol (0.02% and 0.08%) to assess possible interactions.

Results: Study 1. In gerbils Phytrol® reduced TC on average 30%, in contrast to a 15% decrease following consumption of phytostanol esters. In hamsters the Phytrol®-induced TC decrease was only 16%. Study 2. The TC response in gerbils to graded doses of Phytrol® (0.25%, 0.5%, 1.0%) was decreased by 35%, 46%, and 66%, respectively, relative to the TC value at 0% Phytrol®. Study 3. Dietary cholesterol (0.02% and 0.08%) had no impact on TC in the presence of 0.5% Phytrol®. As fat intake increased from 19% to 42% of the diet, Phytrol® efficacy increased with respect to its TC-lowering capacity.

Conclusions: The data demonstrate the enhanced sensitivity of the gerbil TC response to dietary phytosterol manipulation. Secondly, Phytrol® (free sterols and stanols) was twice as effective as an equal weight of phytostanol esters from margarine. The capacity of Phytrol® to minimize TC was increased at higher fat intakes.

TuP29/W12 The effect of short chain fatty acids on some metabolic risk factors in westernised black men

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Objective: To determine the effect of a combination of short chain fatty acids (SCFA) resembling the products of oat bran fermentation (acetate: propionate: butyrate) on some lipid and haemostatic coronary risk factors in Westernised black men.

Methods: 21 healthy, non-smoking, not alcoholic dependent subjects (20-45 y), with higher normal fibrinogen levels (>2.5 g/L) were included in a randomised, placebo-controlled, double-blind, clinical trial (experimental group: 11; control group: 10). Supplementation of SCFA (6 g/d) was continued for five weeks. Dietary intake, anthropometry, blood pressure, lipid and haemostatic risk factors were also studied.

Results: Habitual dietary intake showed a tendency towards a high-fat low fibre diet. Mean total serum cholesterol levels (4.5 mmol/L) in both groups support the trend towards Westernisation. After supplementation a statistical significant increase in the HDL cholesterol was observed. Although mean fibrinogen levels (E: 2.8 g/L; C: 2.9 g/L) did not change, a significant decrease in fibrin monomer levels, network fibrin content, factor VII and factor VIII activity were found. Compaction of fibrin networks increased significantly and a tendency to an increased mass to length ratio of fibrin fibres within the networks were shown.

Conclusions: SCFA supplementation may have a direct effect on haemostasis, especially the fibrin network characteristics, factor VII and factor VIII activity, as well as fibrin monomer concentration. This suggests that SCFA supplementation may have a strong protective effect against atherosclerosis and thrombosis.

TuP30/W12 The effect of Oolong tea on LDL oxidation in hyperlipidemia

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Objectives: Oolong tea is a traditional Chinese tea that is also popular in Japan. In China, Oolong tea has long been believed to be beneficial to health. We already reported that Oolong tea has a lowering effect on serum lipids and a reducing effect on body weight in humans. In this study, we evaluate the effect of Oolong tea on LDL oxidation.

Methods: The subjects were 34 Chinese between the age of 28 and 60 years with hyperlipidemia. The subjects were instructed to keep their usual diet during the whole period of the experiment. After two weeks of pre-observation, they were asked to take Oolong tea twice a day for the subsequent 8 weeks. The subjects were allowed to take water freely. The Blood samples were collected twice. The first sample was taken at the beginning of the experiment (T1) and the second (T2) at the end of the experiment. The LDL fraction of the blood extracted from plasma and oxidation of LDL eiluted by 40 mL of 2-2'-Azobis-(4-methoxy-2, 4-dimethylvaleronitrile) (V70) was assessed by spectrophotometer at 234 nm. The time until the onset of oxidation (lag time) was measured. Plasma lipids were also measured.

Results: The lag time of T2 was prolonged significantly compared with T1 (T1: 6.20 ± 12.9 min., T2: 70.1 ± 14.3 min.; p < 0.05). Both total cholesterol and triglyceride of T2 were decreased significantly compared with T1 (total cholesterol: −19.5%; p < 0.001, triglyceride: −25.4%; p < 0.01). However, there was no significant difference in HDL cholesterol between T1 and T2.

Conclusions: These results suggest that Oolong tea significantly reduces LDL oxidation, total cholesterol and triglyceride and may be beneficial in the prevention of coronary heart disease.

TuP31/W12 Intake of antioxidant vitamins and carotoid plaques in women

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Objective: To evaluate the association between the intake of antioxidant vitamins and an ultrasound end-point of atherosclerosis (i.e. intima-media thickening as evaluated by high-resolution B-mode echography).

Patients and Methods: A cohort of free living women (n = 310) recruited in an LABCAN (longitudinal study done in Naples, Southern Italy, age 30-69, was evaluated for early signs of carotid atherosclerosis by noninvasive ultrasound in relation to the level of intake of antioxidant vitamins. Participants were asked to answer a food-frequency questionnaire. The questionnaire has been tested as for validity and reproducibility. B-mode imaging (Biosound 2000 s.a.) was performed on common carotid arteries and carotid bifurcation in all women and intima-media thickness (IMT) was evaluated (plaque or thickening: IMT > 1.2 mm).

Results: At the level of carotid bifurcation there was a significant inverse relationship between vitamin E intake and the prevalence of plaque or thickening after adjustment for age and energy intake. Odds ratios for tertiles of vitamin E intake were: tertile III = 1; tertile II = 1.27 (95% CI. 0.70-2.32); tertile I = 2.13 (95% CI. 1.10-4.31). Test for linear trend across tertiles of vitamin E intake was: χ² = 4.84 (p = 0.028). No linear trend was found for the intake of retinol, beta-carotene and vitamin C.

Conclusions: The intake of vitamin E in free-living women was inversely related to the prevalence of carotid plaques or thickenings as detected by high-resolution B-mode echography.

TuP32:W12 Effect of soy isoflavones on nitrate concentration and antibodies to heat-shock proteins (HSP60, HSP70, HSC70) in hypercholesterolemic induced in rabbits by J.R.O. Pereira, E. Apolinário, F.P. Gonçalves, J.M. Inácio, D.S.P. Almeida. Departments of Food Science and Clinical and Toxicological Analyses, Faculty of Pharmaceutical Sciences, University of Sao Paulo, Sao Paulo, SP, Brazil.

Objective: To verify the effect of soy isoflavones on concentrations of cholesterol and nitrate in blood plasma and the formation of antibodies against heat shock proteins (HSP60, HSP70, HSC70) in casein-induced hypercholesterolemia.

Methods: One group of young male New Zealand White rabbits was fed with a cholesterol-free casein-based diet supplemented with isoflavones extracted from soy molasses (ISF) and compared to another group with the same diet (CAS) without supplementation, during 6 months. The nitrate concentration was analysed by chemiluminescence in gaseous phase. Antibodies titers were determined in rabbit sera by ELISA. All the analysis were done monthly.

Results: Both groups had a peak of total plasma cholesterol (TPC) at 60 days of feeding. The ISF group had a significant decrease of TPC (37.7%) and LDL-cholesterol (36.2%) and a increase of HDL-cholesterol (2.1 times) at 60 days in relation to the CAS group. The nitrate concentration increased in both groups after 30 days of feeding. However, this increase was less intense in ISF group as compared to CAS group (CAS: 54 ± 18.9% and ISF 26.4 ± 22.2%). After 60 days of diet, the nitrate of CAS group returned to the basal levels and that of the ISF group was reduced (17.5 ± 10%) in relation to baseline. The antibodies titers anti-HSP60, 70 and HSC70 were increased in both groups, however the increases were less intense in ISF group as compared to CAS.

Conclusions: Soy isoflavones may induce a reduction of cholesterol and nitrate concentrations, as well as, of anti-HSP60, HSP70 and HSC70 titer in casein-fed hypercholesterolemic rabbits. Supported by FAPESP.

TuP33:W12 Dietary sodium chloride restriction in Wistar rats impairs the plasma triacylglycerol removal rate S. Catanoso1, J.C. Rocha1, E.R. Nakandakare1, M. Passarelli1, C.H. Mesquita2, A.A. Silva3, M.S. Dolnikoff3, L.M. Harada3, E.C.R. Quintans1, J.C. Heimann1. 1Lipids Lab (LIM 10); 2Exp. Hypertension Lab (LIM 16); 3Inst. Nuclear Research, Univ. Sao Paulo Medical School, Sao Paulo, SP, Brazil.

Objective: Studies in humans have indicated that dietary salt restriction raises plasma levels of total cholesterol (TC) and triacylglycerols (TAG). In order to explain the mechanisms involved, a rat experimental model was developed consisting of chronic feeding ad libitum isocaloric diets with variable sodium chloride contents.

Methods: Rates of synthesis of plasma TAG were measured either as the increase of plasma TAG after blocking its removal rate by the intra-arterial pulse infusion of Triton-WR 1339, or as the rate of incorporation of [14C]-oleic acid into plasma [14C]-TAG. Plasma TAG removal rate was determined by the intra-arterial pulse infusion of a TAG-rich lipid emulsion.

TuP34:W12 Dietary folate and the risk of cardiovascular disease mortality: Results from the multiple risk factor intervention trial (MRFIT) D. Chee, J. Stamler. For the MRFIT Research Group: Northwestern University Medical School, Chicago, IL, USA.

Background: Available data indicate associations between elevated plasma homocysteine levels and atherosclerotic disease. Folate is a cofactor in homocysteine metabolism; folate supplementation reduces levels of plasma homocysteine.

Aim: To assess prospectively relationship of dietary folate to risk of mortality from coronary heart disease (CHD), cardiovascular disease (CVD), and all causes.

Methods: Cohort of 6,426 men from the MRFIT Usual Care Group. Data on dietary folate from five 24-hour dietary recalls at years 0, 1, 2, 3 and 6. Analyses: baseline data and 22-year mortality; data on means from all five recalls and 16-year mortality. Cox proportional hazards models adjusted for age only, and for age, BMI, BMI squared, cigarette smoking, serum cholesterol, ethnicity, education, special diet, calories (with folate per day), alcohol intake, SBP, non-fatal CVD, and anti-hypertensive medication.

Results: Mean dietary folate was inversely and significantly related to 16 year mortality from CHD, CVD, and all causes. Relative risk of CVD death was 0.72 for men with folate one standard deviation (SD) higher compared with men with folate one SD lower (mcg per 1,000 kcal). Baseline folate was inversely and significantly related to 22 year mortality from CHD, CVD, and all causes.

Conclusions: Dietary folate is inversely associated with mortality from the coronary heart disease and cardiovascular diseases. These results suggest that higher folic acid intake can contribute to reducing mortality from cardiovascular diseases.


Objective: To assess contemporary intake – in Chinese, Japanese, U.K., U.S.A. middle-aged men and women from diverse population samples – of multiple dietary variables known to be related to blood pressure, blood cholesterol, and/or risks of the major adult cardiovascular diseases (CVD).

Design and Methods: Cross-sectional epidemiologic study of 17 population samples in China, Japan, U.K., U.S.A., men and women ages 40-59, 260+ people/sample (total: 4,674); dietary data from 4 24-hour recalls and 24-hour timed urine collections/person.

Results: Average height and body mass index were greater from Western (BMI 26.5±29.0 kg/m²) than Asian (22.9±24.0) samples. Kcal/day were highest for Americans (2286.0), lowest for Chinese (2051.1). Total at was 18% of kcal for Chinese, 24% for Japanese, 32-33% for Western samples. Other macronutrients and micronutrients (e.g., antioxidants) also varied sizably across these samples, including protein (total, vegetable, animal), as did alcohol and dietary cholesterol – 68 mg/1,000 kcal for the Chinese, 119-129 for the Western, 188 for the Japanese samples. Na and Na/K intakes were higher for Asian than Western samples, K lower.

Conclusions: In the late 1990s, dietary patterns continue to prevail in China, Japan, U.K., U.S.A. that have been implicated in contrasting East Asian and Western patterns of CHD and stroke incidence.

TuP36:W12  Role of dietary variables in the inverse relation of education to blood pressure: INTERMAP Epidemiologic Study

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Objective: To test the hypothesis that several dietary variables play an important role in accounting for the inverse association – found in many populations – between educational attainment of individuals and their blood pressure (systolic, diastolic – SBP, DBP).

Design and Methods: Cross-sectional epidemiologic study of 17 population samples in China, Japan, U.K., U.S.A., men and women ages 40-59, 260+ people/sample (total: 4,674); dietary data from 4 24-hour recalls and 2 24-hour timed urine collections/person; 8 BP measurements/person at 4 visits; data on multiple possible confounders; multiple linear regression analyses by county, adjusted for sample within county, and pooling of country coefficients weighted by inverse of variance.

Results: With control for age-sex, there was a significant inverse relation of education (years) to BP, especially SBP, multiple linear regression coefficient -0.2560 (Z score -4.59); this coeff. was -0.2287 (~3.056) with pulse, history of high BP treatment, and of diet change also in the model (i.e., 6 factors). Addition of 24-hr urine Na and K, or Na/K, sizably reduced the coeff. (Z < 2.576, p < 0.01); inclusion of BMI or dietary vegetable protein in the 6-factor model had a similar effect. With several diet variables in the model, the education-SBP coeff. further decreased (Z < 1.960, p < 0.05).

Conclusions: These data are consistent with those of INTERSALT in indicating that several dietary factors play a role in the education-BP inverse association present in many populations. They support the inference that improved nutrition can contribute to prevention and control of the more unfavorable BP levels of less educated strata.

TuP37:W12  Cholesterol reducing effects of a food containing oat bran and soy germ in mildly hypercholesterolemic subjects

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Objective: To evaluate the hypocholesterolemic effects of oat beta-glucan in the form of oat bran (OGB), combined with soy germ (SG), when administered in a food.

Methods: 52 men and 18 women aged 21 to 70 with total cholesterol levels between 5.2 and 8.0 mmol/L were recruited to the study. The study was double-blind, parallel arm, placebo controlled. After a 2 week run in period with counselling for a reduced fat and cholesterol diet (AHA 1) subjects were randomly assigned to the test or placebo group. OBG and SG were incorporated into 1 cereal bar (1 portion) and 3 biscuits (1 portion) at a level of 1.5 g OBG and 1.5 g SG per portion for the treatment group. Subjects in the placebo group consumed identical foods that did not contain OBG and SG. Subjects consumed 1 bar and 3 biscuits per day for 28 days. The final dose of both OBG and SG was 3 g/d each. Fastinng blood lipids were measured at weeks ~1 and 0 and again at week 3.5 and 4. Comparisons between groups were made using ANOVA. The averages of the 2 baseline measures were used as covariates, and averages of the 2 postrandomization values were used as dependent variables.

Results: 34 individuals in the treatment and 36 in the control group completed the study. The daily ingestion of 3 g OBG and 3 g SG resulted in a 0.40 mmol/L decrease (8%, p < 0.05) in LDL, treatment relative to placebo group. Total cholesterol dropped by 0.34 mmol/L, whereas in HDL no significant difference was seen.

Conclusion: Functional foods containing 3 g OBG and 3 g SG lower LDL and total cholesterol and so may reduce the risk of a coronary heart disease.

TuP38:W12  Sustained plasma TG-lowering may be obtained with fish oil, in HIV+ patients who develop hyperlipidemia under highly active antiretroviral therapy


Objective: To analyse the long term plasma lipid-lowering effect of Omega-3 polyunsaturated fatty acids in HIV+ patients developing hypertriglyceridaemia under stable highly active antiretroviral therapy.

Methods: During the time period between Jan. 1997 and Jan. 1999, 51 HIV+ patients (M/F = 45/6, age 43±4.5 years) under highly active antiretroviral therapy (2NRTI: 25%; 2NRTI + 1PI: 63%) who had received fish oil, were analysed retrospectively. They were selected for plasma TG levels > 1.5 g/L and a CD4 count > 200 cells/mm3 at entry. Lifestyle and dietary advice had been given together with a continuous supplementation of 6 g/d MaxEPA®. Plasma TC, TG, liver function, CD4 and CD8 cell count, were monitored at 1, 3, 6, 9 and 12 mo, and compared with baseline by paired t-test.

Results: At entry, patients had been under stable antiretroviral therapy for a median time period of 13 mo, during which CD4 and CD8 cell counts had risen of +36% and +19% (p = 0.0004). Their plasma TC and TG levels were 2.5 ± 1.5 g/L (med = 2.2, max = 12) and 4.5 ± 4.2 g/L (med = 3.2, max = 21), 37% had a TC > 2 g/L. Most pts (80%) had at least one disturbed parameter of liver function. At all time points under MaxEPA, TC decreased (11 to -20%, p = 0.02-0.0008) in pts with increased TC. In parallel, a significant TG lowering of -20 to -32% (p = 0.04-0.0001) was observed in pts with moderate TG (2.45 ± 0.7 g/L), even more pronounced -42 to -68% (p = 0.02-0.0002) in pts with high TG (8.34 ± 5 g/L) whatever the type of antiretroviral drug combination. Immune and liver functions were unchanged.

Conclusions: These preliminary data suggest that fish oil supplementation with MaxEPA may significantly and steadily lower plasma lipid levels in HIV+ pts under HAART, fostering further controlled studies.

TuP39:W12  Effect of diets enriched in saturated and hydrogenated fat on HDL metabolism

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Objective: To determine the mechanism(s) responsible for the differential effect of saturated and hydrogenated fat/trans fatty acids on high density lipoprotein (HDL) cholesterol (C) levels.

Methods: Study subjects over the age of 50 years (n = 36) with fasting LDL-C levels > 130 mg/dl were studied during each of six 5-day diet phases. All diets contained 30% of energy as fat, 15% protein, 55% carbohydrate, 62 mg/100 kcal and 11 g fiber/100 kcal. Two-thirds of the fat in each diet was either soybean oil, semi-liquid margarine, tub margarine, shortening, stick margarine or butter.

Results: As previously reported, LDL-C levels were 153b, 157b, 160a, 178a and 185a and HDL-C levels were 47b, 48b, 47b, 46b and 50 mg/dl, oil, semi-liquid, tub, shortening, stick and butter, respectively (means sharing same superscript not significantly different at p < 0.05). Differences in HDL-C levels were attributable primarily to changes in HDL-2. Apo A-I levels mirrored those of HDL-C (apo A-I particles both with and without apo A-II). Cholesterol ester transfer protein (CETP) activity was 13.12b, 13.28b, 14.83b, 13.76b, 15.74b and 14.35b nmol/h mL1; phospholipid transfer protein (PLTP) activity was 5.33, 5.20, 5.50, 5.33, 5.65 and 5.35 nmol/h mL1; and the fractional esterification rate of HDL was 21.0, 21.0, 21.7, 20.8, 21.5 and 21.1%/hour; after consumption of the oil, semi-liquid, tub, shortening, stick and butter enriched diets, respectively.

Conclusion: The present data suggest that relative to saturated fat, hydrogenated fat intake including trans fatty acids contributes to lower HDL-C levels via changes in CETP activity.

TuP40:W12  Taurine prevents atherosclerotic lesion development in WHHL rabbits


Objective: The hypocholesterolemic action of sulfur amino acid, taurine has been extensively investigated in animal models. As there are few studies on the anti-atherosclerotic effects of taurine, we examined effects of taurine on development of atherosclerotic lesions in Watanabe heritable hyperlipidemic (WHHL) rabbits.

Methods: Taurine (0.3%) was dissolved in tap water, and provided for drinking by male WHHL rabbits (2 months-old) fed a regular chow for 24 weeks. Serum and aortic lipid contents were determined enzymatically, using commercial kits. Contents of thiobarbituric acid reactive substances (TBARS) were also measured in serum and aorta, as a marker of lipid peroxidation. For histochalastic analysis, the carotid artery was fixed with formalin and stained.
for macrophages and smooth muscle cells, using monoclonal antibodies, RAM-11 and IA4, respectively.

**Results:** Treatment of WHHL rabbits with taurine significantly reduced cholesteryl ester accumulation in the aortic arch, thoracic, and abdominal aorta by 35, 43, and 54%, respectively, concomitant with decrease in acyl-CoA: cholesterol acyltransferase activity. Taurine had no apparent effects on serum cholesterol and blood pressure during the experimental period. TBARS levels were reduced in serum (~29%) and aorta (~50%). Histochemical studies showed that taurine decreased the number of macrophages in the intima without affecting the number of smooth muscle cells.

**Conclusions:** These results demonstrate the anti-atherosclerotic effects of taurine that are independent of serum cholesterol levels and suggest that antioxidant effects may be involved in the prevention of atherosclerosis when taurine is ingested.

**TuP41-W12**

**Comparison of cholesterol-lowering effects of hydrogenated vs unhydrogenated tall oil phytosterols in apo E-KO mice**

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**Objective:** To compare the effect of hydrogenation of tall oil phytosterols (PS) on their cholesterol lowering effect.

**Methods:** 20 male apo E-KO mice were fed with an atherogenic diet supplemented with either 2% (w/w) hydrogenated tall oil sterols (97.5% total stanols) (n = 6) or 2% (w/w) unhydrogenated sterols (Phytrol® 17%, stanols) (n = 6) or without PS supplementation (controls, n = 8). Plasma total cholesterol levels determined at baseline, week 4 and week 8.

**Results:** Both treatments significantly reduced plasma total cholesterol compared to controls. However, unsaturated (non hydrogenated) phytosterols were more efficient in reducing plasma cholesterol levels as compared to saturated. Compared to controls, unsaturated phytosterols decreased cholesterol levels by 48% and 53% over 4 and 8 weeks, respectively, whereas the reduction in plasma cholesterol by phytosterols were only 25% and 32%.

**Conclusions:** Hydrogenation of the PS to phytostanols reduces the cholesterol lowering effect of tall oil phytosterols in this animal model.

**TuP42-W12**

**Daily consumption of non-esterified phytosterols from tall oil (Phytrol®) in a chocolate matrix significantly lowers LDL cholesterol in moderately hypercholesterolemic individuals**

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**Objective:** To investigate the effect of dietary phytosterols or plasma lipid levels when consumed in chocolate.

**Methods:** 70 men and women aged 21-70 were recruited to the study who had an LDL cholesterol greater than 3.5 mmol/L. Non esterified phytosterols from Tall oil (Phytrol®) (0.6 g) were incorporated into 10 g chocolate minibars during. Subjects consumed 1 chocolate with each of three meals daily for 28 days. The final dose of total phytosterols was 1.8 g/day. Fasting blood lipids were determined at weeks −2, −1 and at randomization and again at week 3 and 4. Control subjects consumed identical chocolates that did not contain the phytosterols.

**Results:** 33 individuals in the Phytrol regime and 32 controls completed the study. The reduction of 0.43 mmol/L of LDL was highly significant (p < 0.00003) as determined by paired t-test. This represented a 8.9% reduction. LDL did not change significantly in the control group.

**Conclusions:** 1.8 g/day of non esterified phytosterols from Tall oil significantly reduce LDL cholesterol when consumed in chocolate.

**TuP43-W12**

**Influence of phytosterols versus phytostanols on plasma lipid levels in hypercholesterolemic subjects**

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**Objective:** To examine the effect of unesterified sitosterol and stigiansterol supplementation on plasma cholesterol levels in hypercholesterolemic subjects consuming a fixed foods diet.

**Methods:** Fifteen healthy hypercholesterolemic subjects consumed each of four dietary treatments for 21 days in a crossover design. Phytosterols were blended into the butter component of the diet. Diets, high in saturated fat, contained either (i) butterfat (B), (ii) butterfat with non-saturated plant sterols (BNS) (0.8 g/day), (iii) butterfat with saturated plant sterols (BSS) (1.8 g/day), or (iv) butterfat with a 50:50 mix of non-saturated plant sterols and saturated plant sterols (BNSS) (1.8 g/day). All meals were prepared and consumed under supervision within a metabolic research unit.

**Results:** There were no significant changes in weight across the four treatment phases. Plasma total cholesterol level at day 21 was lower (p < 0.01) for BNS (−6.3%), BSS (−10.3%), and BNNS (−11.6%), versus B (1.5%). Similarly, LDL-C mean reductions were larger (p < 0.03) for BNS (−9.1%), BSS (−11.2%), and BNSS (−13.8%), compared to B (2.2%). Plasma TG and HDL-C levels did not differ across diets.

**Conclusion:** These data indicate that the unesterified sterols and stanols lowered plasma total and LDL-C equivalently in the context of a saturated fat diet. Supported by Dairy Farmers of Canada and Forbes Medi-Tech Inc.

**TuP44-W12**

**Effects of dietary unsaturated fatty acids and natural antioxidants on markers of lipoprotein metabolism and lipid oxidation**

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**Objective:** To compare the effects of oleic (OA) and linoleic (LA) acids on markers of lipoprotein metabolism and lipid oxidation in diets either high or low in natural antioxidants from vegetables, berries and apple.

**Methods:** Healthy female and male volunteers (n = 77, 19-52 yr) had one of the four strictly controlled 6-week diets rich either in OA (18% of energy en%) or LA (11 en%) and containing either large or small amounts (815 vs. 170 g/10 MJ) of vegetables, berries and apples. The proportions total fat, saturated fat, carboxylates and protein were similar in all diets. Nineteen healthy volunteers served as controls.

Blood and 72-hour urine samples were collected in the beginning and at the end of the 6-week period. Several markers of dietary compliance (plasma fatty acids, vitamin C, carotenoids, quercetin), lipoprotein metabolism (serum total, LDL and HDL cholesterol, triglycerides, lipid transfer proteins), and lipid oxidation (LDL oxidation in vitro plasma and LDL malondialdehyde, paraoxonase) were measured.

**Results:** The compliance to the diets was good. However, in the markers of lipoprotein metabolism or lipid oxidation no significant differences were found between the diets.

**TuP42-W12**

**Effects of dietary unsaturated fatty acids and natural antioxidants on markers of lipoprotein metabolism and lipid oxidation**
Conclusions: The results suggest that in healthy volunteers with adequate vitamin intakes, 6-week diets differing markedly in the amounts of OA and LA and natural antioxidants from plant sources do not differ in their effects on lipoprotein metabolism or lipid oxidation.

P:W13 EXTRACELLULAR MATRIX

TuP1:W13 The A3 adenosine receptor has a crucial role in determining blood pressure and matrix deposition in the vasculature: Studies in knock out mice
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Objective: Adenosine administration has been reported to lower blood pressure by activating specific membrane receptors. The rat and human heart and aorta have been previously found to express both A2 type adenosine receptors, which activate adenyl cyclase, and A3 adenosine receptors (A3AR), which inhibit adenyl cyclase. In the current study, we used A3AR knock out mice to examine the hypothesis that the relative levels of the A2 type adenosine receptors and A3AR determines the steady state levels of cAMP in the cells and may affect vascular function.

Methods and Results: In situ hybridization demonstrated that the level of A3AR is high in the vascular smooth muscle (VSM) layer of aortas derived from wild type mice, but is not detectable in the knock out mice in all tissues, while the A1 and A2 receptors are not altered. The steady state level of cAMP is elevated in the aorta and heart of knock out mice, as compared to wild type mice. When challenged with adenosine, the A3AR knock out mice display a further increase in cAMP levels in the heart and VSM and a significant decrease in blood pressure, as compared to wild type mice. Moreover, the knock out mice exhibit higher levels of the elastin cross-linking enzyme, lysyl oxidase (LO), as well as an altered deposition of elastin in the vasculature. This is in accordance with the known ability of high levels of cAMP to induce LO gene expression.

Conclusions: Our studies point to the A3AR as a crucial regulator of the steady state level of cAMP in the vasculature and of blood pressure in response to adenosine, as well as of matrix production.

TuP2:W13 Effect of TS-962, an ACAT inhibitor, on histological composition of aortic lesions in WHHL rabbit long-term atherosclerotic model

Objective: To determine the direct effect of TS-962 on the composition of advanced aortic lesions in old WHHL rabbits.

Methods: TS-962 was added to the diet of male WHHL rabbits at a concentration of 0.0005% to 0.05% (n = 9−10 in each group) from 2 months of age for 18 months. At the end of the study, the aortas were removed and the changes in composition of lesions were evaluated by histopathological assay and scored from 0 to 4. The macrophage-induced contraction response of

TuP3:W13 Decorin is produced by capillary endothelial cells during angiogenesis associated with inflammation
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Objective: Decorin, a member of the small leucine-rich proteoglycan family, has previously been shown to be involved in the angiogenesis-like behaviour displayed by macrovascular endothelial cells (ECs) in vitro. In this study we sought to examine whether decorin is also associated with angiogenesis in vivo.

Methods: Temporal artery biopsy specimens from patients with giant cell (temporal) arthritis (TA) and uninvolved control subjects were analyzed by immunohistochemistry using the following antibodies/antiserum: a monoclonal mouse antibody to CD31 to identify ECs, a monoclonal mouse antibody to CD68 to identify macrophages, and a polyvalent rabbit antisemur LF-30 specific for the decorin core protein. Similar analyses were performed for other human tissue samples in which the pivotal role of angiogenesis is more generally accepted. These samples included sections of the granulation tissue (GRT) during wound healing, the pyogenic granuloma (PGR) and the ovary (OVR) during follicle formation.

Results: Immunohistochemical analyses demonstrated that in TA sections there are CD31 positive microvessels not only in the adventitia as in uninvolved temporal artery wall, but also in the media and in the external one-third of the thickened intima. When the adjacent sections were stained with the LF-30 decorin antisemur, a positive reaction was detected in all the three main layers of the vessel wall. In addition, ECs of the microvessels within the thickened intima stained for LF-30 indicating endothelial decorin production. As regards the GRT, PGR and OVR sections, the CD31 immunostaining revealed that new capillary blood vessels are widely distributed in all of them. However, capillary ECs only in the GRT and the PGR specimens, but not in those of the OVR, stained positive for decorin. To investigate whether the difference in the staining pattern of decorin in capillary ECs is associated with the degree of inflammation, adjacent sections of TA, GRT, PGR and OVR specimens were stained with CD68 to assess the presence of macrophages. The analyses demonstrated that in TA, GRT and PGR specimens, in which the production of decorin by capillary ECs was evident, a large number of macrophages was found in capillary-rich areas. In contrast, in the OVR specimens, which all possessed decorin negative capillaries, there were significantly less macrophages as compared to TA, GRT and PGR specimens. Furthermore, in the OVR specimens the macrophages were not present in areas rich in capillary blood vessels.

Conclusions: Our results provide evidence that decorin is involved not only in angiogenesis in vitro as has previously been established, but also in vivo. Furthermore, the results suggest that decorin production by ECs in man is restricted to angiogenesis in which the inflammatory component is likely to dominate.

TuP4:W13 Effects of Ox-LDL on metalloproteinase release and enzymatic activity in human endothelial cells
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cells were pre-treated with Ox-LDL for 24 hours and then TPA was added for another 24 hours the gelatinolytic activity was reduced by 49% ± 23% at 50 µg/mL to 74% ± 7% at 80 µg/mL while if cells incubated with TPA for 24 hours and then Ox-LDL were added the gelatinolytic activity was reduced by 22% ± 9% at 50 µg/mL to 57% ± 12% at 80 µg/mL. As TPA is not a physiologic stimuli we also evaluated the effect of Ox-LDL on TNP2 (20 ng/mL) induced secretion of MMP-9. Ox-LDL reduced the MMP-9 secretion in a concentration dependent manner (by 29% ± 6% at 10 µg/mL to 73% ± 8% at 80 µg/mL vs cells incubated with TNFα alone for 18 hours). Moreover TNP2-1 release was also reduced by Ox-LDL in a concentration dependent manner. These observation suggest the possibility that Ox-LDL acts on activated endothelium as a negative regulator of neo-angiogenesis, we are currently addressing the mechanisms underlying these effects.

**TuP5.W13**

**Association of native and naturally occurring multiple-modified LDL with proteoglycans from intimal sublayers and media of uninvolved and atherosclerotic human aorta**

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**Objective:** The aim of this study is to investigate the association of native LDL and naturally occurred multiple-modified LDL (nomiLDL) to proteoglycans isolated from proteoglycan-rich and muscle elastic sublayers of intima as well as media of grossly normal and atherosclerotic human aorta.

**Methods:** Native LDL and nomiLDL were divided by lectin chromatography on RCA120-agarose. Proteoglycan preparations were isolated by guanidine-HCl extraction with consequent ultracentrifugation in CsCl gradient and cetylpyridinium chloride precipitation from intimal sublayers and media of uninvolved and atherosclerotic areas of human aorta. The glycan composition of obtained proteoglycans was analysed by HPLC method. The LDL binding to proteoglycans was measured by ELISA technique.

**Results:** The binding of nomiLDL to proteoglycans from proteoglycan-rich and muscle elastic sublayers of normal intima was 2- and 3-fold, correspondingly, higher than native LDL. The binding of native LDL and nomiLDL to proteoglycans of both intimal sublayers decreased in order: normal > intima > lesions > fatty streaks > fibroatheroma. The similar changes in lipoprotein binding were observed for the proteoglycans from media layer of human aorta. The binding of native LDL and nomiLDL correlated positively with proteoglycan level of chondroitin sulphate A and C, and negatively with dermatan sulphate content.

**Conclusions:** NomiLDL association with proteoglycans may play important role in early stages of vascular extravascular lipid binding and retention.

**TuP6.W13**

**Binding of native and naturally occurring multiple-modified LDL with elastin of uninvolved and atherosclerotic human aorta**

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**Objective:** Several years ago we have found and isolated a subfraction of naturally occurred multiple-modified LDL (nomiLDL) which produced lipid accumulation in human aortic intimal smooth muscle cells and macrophages. In present study we studied the comparative binding of native LDL and nomiLDL with elastin from early and developed atherosclerotic lesions to evaluate the possible role of nomiLDL in extracellular lipid deposition.

**Methods:** Native LDL and nomiLDL were isolated by lectin chromatography and iodinated using ICI-procedure. Elastin samples were isolated by NaOH extraction from proteoglycan-rich and muscle elastic sublayers of intima and media of uninvolved and atherosclerotic human aorta. The LDL binding was determined by radioactivity measurement or by ELISA technique.

**Results:** The binding of nomiLDL with elastin from proteoglycan-rich sublayer of normal intima was 2-fold higher as compared to native LDL. NomiLDL was bound to elastin from proteoglycan-rich sublayer of intimal lesions and fatty streaks 2- and 5-fold effectively than with normal elastin. The association of nomiLDL with elastin from proteoglycan-rich sublayer of fibroatheroma was 2-fold lower than to elastin from fatty streaks. The close changes in lipoprotein binding were observed for the elastin from muscle elastic sublayer of human aorta. The binding of native and nomiLDL with elastin from media of normal and atherosclerotic areas was similar. Retention time for nomiLDL in preformed lipoprotein-elastin complexes was longer than for native LDL. Binding of nomiLDL was correspondent to positive charged amino acid level in aortic elastin.

**Conclusions:** NomiLDL binding to elastin may be involved in intracellular lipid deposition in human blood vessels.

**TuP7.W13**

**PDGF-BB negatively regulates the expression of perlecan in rat vascular smooth muscle cells**

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**Objective:** Compare quantitative and qualitative differences of perlecan and other proteoglycans (PGs) between quiescent and growing rat arterial smooth muscle cells (SMC) in vitro.

**Methods:** SMC were serum starved for 48 h and then stimulated with PDGF-BB 10 ng/mL. The PGs were labeled with 35S-Methionine, 35S-sulfate, analyzed on molecular sieve columns and SDS-PGE. Total mRNA was collected for analysis with Northern Blotting.

**Results:** PDGF-BB induced a 2 to 2.5-fold increase in cell number over a period of 48 hours. Time-course experiment showed a peak in total 35S-sulfate incorporation between 6–12 hours after stimulation. Samples were collected from this time window for further analysis. Perlecan was the main PG and the majority of it was found in the cell-layer, eluting at Kav = 0.29 on CL-4B column. Perlecan mRNA and core protein levels decreased by approximately 30% and 5%, respectively. The chain length of the heparan sulfate attached to perlecan remained unchanged. PDGF-BB stimulation did, on the other hand, increase the core protein expression and chondroitin sulfate chain length of versican, biglycan and decorin. Synadecan core showed a slight increase in expression.

**Conclusion:** PDGF-BB selectively reduce perlecan expression. This may be a mechanism to facilitate growth since perlecan and heparan sulfate containing molecules are growth inhibitory to SMC.

**TuP8.W13**

**Metabolic evidence for sequestration of low density lipoprotein in abdominal aorta of normal rabbits**

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**Objective:** To determine whether metabolic evidence would support the hypothesis that LDL is "sequestered" (present in a form that exchanges slowly with plasma LDL) in abdominal aorta of normal rabbits and that more LDL is sequestered at branch sites.

**Methods:** Thirty three normal rabbits were injected with LDL labeled with 125I-tyramine cellobiose (125I-TC) to trace both undegraded LDL and aortic LDL degradation products. For 25 rabbits, LDL was also labeled with trace undegraded LDL alone. The time dependent aortic 125I-TC and 131I accumulation was determined from 0.6 to 120 hours after injection. Sequestration of LDL was investigated by comparing fits to aortic data for mathematical models with and without sequestered LDL.

**Results:** This study provided metabolic evidence for sequestration of LDL at branch (p < 0.01) and uniform (p < 0.005) abdominal aorta. Concentrations of sequestered LDL were 109 ± 28% higher (p < 0.0005) for branch sites. LDL mean residence time was 23.5 ± 3.1 hours for branch sites, 7.6 ± 3.5 hours longer (p < 0.05) than for uniform abdominal aorta.

**Conclusions:** The enhanced retention of higher concentrations of sequestered LDL at branch sites could predispose these aortic sites to cholesterol accumulation and atherogenesis after onset of hypercholesterolemia and macrophage entry into the aorta.

**TuP9.W13**

**Laminar shear stress induced the basement membrane collagen synthesis and inhibited the collagenase activity by cultured endothelial cells**

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**Objective:** Endothelial cells (EC) may behave as hemodynamic sensors, translating mechanical information from the blood flow into biochemical signals. The purpose of this study was to examine the influence of EC under shear stress on collagen synthesis and collagenase activity those balance was important to estimate the basement membranes, which provides adherence and integrity for the endothelium, may serve an important signaling function in atherogenesis.

**Methods:** Confluent EC were exposed to laminar shear stress up to 30
The first morphologic sign of atherogenesis is accumulation of small lipid droplets within the extracellular matrix of the arterial intima. Fusion of modified LDL particles in one mechanism leading to formation of such lipid droplets. Secretory non-pancreatic phospholipase A2 (snPLA2), an enzyme capable of hydrolyzing LDL phospholipids, is found in arterial extracellular matrix and in contact with extracellular lipid droplets. Here we studied the effects of snPLA2 and bee venom PLA2 (bVPLA2) on the integrity of LDL particles and on their interaction with human aortic proteoglycans. In addition, the capacity of proteoglycans to bind native and PLA2-treated LDL particles were compared in a microtiter well assay. We found that treatment of LDL with both kinds of PLA2 induced aggregation and fusion of proteoglycan- and glycosaminoglycan-bound LDL particles. The aggregated and fused particles were found to bind to proteoglycans more avidly than native LDL. Most importantly, lipolysis of LDL with snPLA2 and bVPLA2 increased the capacity of a proteoglycan matrix to bind LDL by 3-fold, respectively. Taken together, the results suggest that PLA2 present in the arterial intima has a role in retention and accumulation of LDL-derived lipid droplets in this tissue site.

**TuP13/W13**  
**The effects of oxidative modification on the interaction of low density lipoprotein with collagen**

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Objective: To study the effects of both low density lipoprotein (LDL) and collagen oxidation on the binding of LDL to collagen.

Methods: Immunohistochemical and in vivo methods have shown LDL to deposit not only in macrophages, smooth muscle cells and endothelial cells but also in the extracellular matrix (ECM) of the arterial tunica intima. This binding process is thought to play a key role in atherogenesis. LDL has been shown to bind several collagen types and so we have developed an in vitro method, based on existing protocols, for the study of these interactions.

LDL isolated from plasma by density gradient ultracentrifugation was labelled using a europium (Eu³⁺) chelate of N²-(p-iodosobenzoyl)-diethylenetriamine-N¹,N⁰,N²,N⁴-tetracetic acid (DTTA). Microtitre plates were coated with various collagen solutions, blocked with bovine serum albumin and then incubated with Eu³⁺-LDL solutions. Following addition of enhancement solution, plates were read on a DELFIA research fluorimeter (Wollic Ox). LDL (50 μg/mL protein) was oxidised in a 2 mM CuCl₂ solution. Collagen was modified by incubation with 0.5 M malondialdehyde (MDA).

Results: The binding of LDL to collagen types I, III, IV and V all found in the intimal matrix) was shown to increase with increasing degrees of LDL oxidation, whilst MDA modification of collagen (type I, the main component of the atherosclerotic artery), was shown to decrease Eu³⁺-LDL binding. Binding activities of both native and oxidised LDL were greater with collagen types I and III than with types IV and V.

Conclusions: LDL oxidation increases binding of this lipoprotein to the intimal ECM, thus leading to an increased rate of atherogenesis. In addition, LDL shows elevated binding to collagen types I, III, IV and V, indicating that these ECM components may play a key role in ‘trapping’ LDL. It appears that MDA modification of collagen I may inhibit this atherogenic binding process.
TuP14:W13 Iron laden macrophages in human atheroma are associated with erythropoietin expression. Effects of oxLDL and HDL on the secretion of iron and ferritin

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Iron has been implicated in atherogenesis mainly due to its capacity to initiate severe oxidative stress, which is supported by some of epidemiological and experimental studies. We have previously proposed that released lysosomal iron from iron-laden macrophages may contribute to lipoprotein oxidation, ceroid formation, and lipid-induced cellular injury. In this study we aim to (i) characterize expression and regulation of ferritin gene in the iron-laden macrophages, (ii) study the interaction between such cells and lipoproteins, and (iii) search for the evidence of erythropoietin expression in atheroma. We found that iron-uptake into macrophages, via transferrin-receptors (iron-loading) or scavenger receptors (erythropoietic), leads to accelerated synthesis of ferritin mRNA and protein. The desferrioxamine (DFO) enhanced the iron regulatory proteins (IRP-1 and IRP-2) binding to the iron responsive elements (IREs) binding activity, while iron salt and hemin dramatically decreased the binding activity. Although both iron-loading and erythropoietin-cytosis result in an increase in cellular iron content, more lysosomal iron was seen in phagocytic vacuoles of macrophages exposed to oxidized red blood cells. Such iron-laden macrophages secrete ferritin and iron into the culture medium. The release of iron and ferritin was enhanced by oxidized low-density lipoprotein (oxLDL) while inhibited by high-density lipoprotein (HDL). In human atherosclerotic lesions there is a lesion-dependent iron-macrophage activity with hemoglobin antibodies in the areas rich in ferritin-accumulated macrophages, suggesting that the degradation of uptake-erythrocyes within these cells result in iron accumulation in atheroma. Based on these data we conclude that iron-loading and erythropoietic activity are helpful models for understanding the role of cellular iron and ferritin in lipid-oxidation or oxidative cellular damage in atherogenesis, and that erythropoietic activity by macrophages within atherosclerotic vessels may be one of major origin for iron-accumulation in atherosclerotic lesions.

TuP15:W13 The effect of a dihdydropyridine and a non dihdydropyridine calcium antagonist on plasma levels of active extracellular matrix metalloproteinases 2 and 9 in patients with essential mild to moderate hypertension


Objective: The ability of some antihypertensive drugs to protect from hyper- vascular tension damage might be partially due to their capacity to control particular matrix metalloproteinase (MMPs)-mediated extracellular matrix metabolism. This study was designed to investigate if six months antihypertensive treatment with the dihdydropyridine calcium antagonist felodipine or the non dihdydropyridine calcium antagonist ditlazem has any effect on plasma levels of active MMP-2 and active MMP-9, two enzymes that share similar substrate specificity (collagen IV and gelatin A and B) in patients (pts) with mild to moderate essential hypertension.

Methods: Forty six pts with never-treated mild to moderate essential hypertension (HP) were evaluated for plasma concentrations of MMP-2 and MMP-9 (both measured by ELISA) before and after six months treatment with felodipine (23/46) or ditlazem (23/46) and compared to 25 normotensive control subjects (CS). No significant differences were noticed in blood pressure (BP), total peripheral resistances (TPRs), left ventricular mass index as well as plasma levels of both MMP-2 and MMP-9. HP pts treated with felodipine and those treated with ditlazem. Blood pressure and TPRs were both normalized after treatment with felodipine (160/102 to 121/80 mmHg, p < 0.001) and 1742 to 1167 dynes.cm.sec^-3, p < 0.001, respectively) as well as after treatment with ditlazem (158/104 to 124/83 mmHg, p < 0.001 and 1718 to 1244 dynes.cm.sec^-3, p < 0.001, respectively). Felodipine-treated pts attained a significant increase in MMP-2 plasma concentrations (138 ± 16 vs 108 ± 12 ng/mL, p < 0.001 compared to respective values before treatment) but a non significant increase in MMP-9 plasma concentrations (3.5 ± 1.3 ng/mL vs 3.2 ± 0.8, p = NS compared to respective values before treatment). Ditlazem-treated pts attained non significant increases in both MMP-2 and MMP-9 plasma concentrations (MMP-2: 114 ± 22 vs 109 ± 21 ng/mL, p = NS and MMP-9: 1.9 ± 2.2 vs 3.6 ± 1.8 ng/mL, p = NS, both compared to respective values before treatment).

Conclusions: These findings suggest that in mild to moderate essential hypertension, although treatment with felodipine or ditlazem both normalize BP and TPRs, however treatment with felodipine may be more effective than that with ditlazem in regulating at least plasma levels of active MMP-2. Thus, it could be speculated that depressed active MMP-2 and MMP-9 plasma levels in HP pts may not be related to changes in volume density of extracellular matrix components which usually increases TPRs, but they could be related to changes in extracellular matrix architecture or cell-matrix attachment which increases or reduces vascular stiffness, depending on vascular bed, and remains to be established.

TuP16:W13 Depressed plasma levels of active extracellular matrix metalloproteinases 2 and 9 in patients with essential mild to moderate hypertension


Objective: Previous studies on extracellular matrix metabolism in hypertension have focused on particular matrix metalloproteinases (MMPs) (e.g. MMP-1) mainly related to cardiac remodeling (lefl venricular hypertrophy). This study was designed to quantify in a non invasive way the plasma concentrations of active MMP-2 and MMP-9, two enzymes that share similar substrate specificity (collagen IV and gelatin A and B), mainly related to vascular remodeling usually influencing peripheral resistance, in patients (pts) with mild to moderate essential hypertension before and after chronic treatment with the calcium antagonist amloprimide.

Methods: Forty two pts with never-treated mild to moderate essential hypertension (HP) were evaluated for plasma concentrations of MMP-2 and MMP-9 (both measured by ELISA) before and six months treatment with amloprimide and compared to 25 normotensive control subjects (CS). Before treatment depressed values in HP pts over CS were observed for MMP-2 (102 ± 18 vs 124 ± 15 ng/mL, p < 0.01) and MMP-9 (6.5 ± 1.8 vs 15.9 ± 3.2 ng/mL, p < 0.002). HP pts with total peripheral resistances (TPRs) < 1550 dynes.cm.sec^-3 exhibited higher values of MMP-2 and MMP-9 than HP pts with TPRs > 1550 dynes.cm.sec^-3 (MMP-2: 111 ± 12 vs 97 ± 10 ng/mL, p < 0.006 and MMP-9: 9.5 ± 1.8 vs 5.4 ± 1.2, p < 0.001). Blood pressure and TPRs were both normalized after treatment (158/102 to 124/80 mmHg, p < 0.001) and 1742 to 1167 dynes.cm.sec^-3, p < 0.001, respectively). Treated pts attained a non significant increase in MMP-2 plasma concentrations (105 ± 12 ng/mL) but a significant increase in MMP-9 plasma concentrations (16.4 ± 2.3 ng/mL, p < 0.01 compared to respective values before treatment).

Conclusions: These findings suggest that plasma concentrations of active MMP-2 and MMP-9, mainly related to vascular extracellular matrix metabolism, are depressed in HP pts. This may reflect decreased extracellular degradation of IV collagen and gelatines A and B which could contribute to increased TPRs-related vascular remodeling, since plasma concentrations of these particular MMPs are higher in HP pts with baseline than those without baseline TPRs and are normalized when TPRs are normalized after treatment. Six months treatment with amloprimide can normalize MMP-9 but not MMP-2-mediated extracellular matrix metabolism.

TuP17:W13 Extracellular matrix proliferation in iliac artery remodeling after balloon injury in miniature pig

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Objective: To study quantitatively extracellular matrix (ECM) proliferation and cellular expression of transforming growth factor-β (TGF-β) during arterial remodeling after balloon injury. Methods: Hema-toxilyn and eosin (HE) stain, histochemistry, immunohistochemistry, and image analysis were performed on iliac artery of 19 miniature pig induced by high cholesterol diet followed by balloon injury. Antibodies to the 5 major components of ECM were used. TGF-β1 mRNA was illustrated by in situ hybridization. Results: The total amount of ECM was increased during arterial remodel-

Table: Quantity of 5 ECM components during arterial remodeling (x ± SD)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Collagen I</th>
<th>Collagen III</th>
<th>Collagen V</th>
<th>Fibronectin</th>
<th>Laminin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7.2 ± 2.8</td>
<td>6.0 ± 1.8</td>
<td>6.1 ± 1.6</td>
<td>6.9 ± 1.8</td>
<td>9.9 ± 2.3</td>
</tr>
<tr>
<td>Early</td>
<td>9.2 ± 2.2</td>
<td>8.2 ± 2.0</td>
<td>7.2 ± 1.8</td>
<td>10.8 ± 1.6</td>
<td>11.6 ± 1.7</td>
</tr>
<tr>
<td>Late</td>
<td>14.1 ± 1.9</td>
<td>12.2 ± 2.1</td>
<td>11.3 ± 2.4</td>
<td>8.4 ± 1.3</td>
<td>6.8 ± 1.7</td>
</tr>
</tbody>
</table>
ing. The quantity of the 5 ECM macromolecules was illustrated in the table. TGF-β1 mRNA was detected with nuclear stain pattern in endothelial cells, macrophages/foam cells, and smooth muscle cells and extensively expressed in early lesions.

**Conclusion:** Collagen fibers were the main extracellular matrices and TGF-β1 was suggested as the major cytokine in arterial remodeling.

**TuP16:** Morphometric studies on extracellular matrices during atherosclerotic lesion progression

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**Objective:** Increased deposition of extracellular matrix (ECM) within arterial intima is the major morphologic finding during atherosclerotic lesion progression from fatty streak (FS) to fibrous plaque (FIP). This study quantitatively showed the distributions of major ECM molecules in normal intima (NI), fatty streak (type II lesion), fatty plaque (FAP) (type III lesion), and fibrous plaque (type V lesion) of human coronary artery.

**Methods:** Hematoxylin and eosin (H&E) stain, histochemistry, immunohistochemistry, and image analysis were performed on coronary artery from 160 human autopsy cases. Antibodies to types I, III, V collagen, fibronectin (FN), and laminin (LN) were employed to demonstrate the major components of ECM.

**Results:** The total amount of ECM was increased as the atherosclerosis developed and advanced. The quantity of the 5 ECM macromolecules was significantly different among NI, FS, FAP, and FIP. FN and LN were predominantly in FS, while collagen fibers were remarkably increased in FIP. Furthermore, type V collagen was the most predominant one while the type I collagen is the least. Morphologically, the 5 ECM proteins deposited in FS as the fine fibril-like structure. In FAP and FIP, types I, III collagen were unevenly distributed in patch or cord pattern among different plaques and different areas of the same lesion.

**Conclusions:** The amount of types I, II, V collagen was increased during atherosclerosis development and progression. Collagen fibers were the main extracellular matrices in fibrous plaque. The role of fibronectin and laminin in the formation of fatty streak needs to be defined.

**TuP19:** An in vitro coculture model of transmigrant monocyte and foam cell formation

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**Objective:** To observe precise process from monocyte transmigration to foam cell formation, simple in vitro co-culture system using arterial wall cell and monocyte were developed.

**Method:** Rabbit arterial endothelial cell (RAEC) and smooth muscle cell (RASMCC) were primarily and separately cultivated. In method I system, RAEC monolayer was formed on collagen matrix layer on microporous filter of chemotaxis chamber (Falcon). In method II system, RAEC smooth muscle cell layer was previously cultivated on the filter and it produced de novo extracellular matrix (ECM). Monocyte prepared from human peripheral blood by magnet beads associated antibody cell sorting (MACS, Miltenyi Biotec.) was added onto the both system. Transmigration of monocyte was observed scanning microscopy and confocal microscope. Differentiation of monocyte was observed by immunohistochemistry using anti CD68, AM-3K, and anti-SK-A III antibody. Morphology of subendothelial macrophage was observed by transmission electron microscopy. Lipid accumulation was confirmed by oil red O staining of frozen section of coculture.

**Results:** In the presence of MCP-1 in cultivation medium, precise morphological change of monocyte during transmigration was observed within 2 hours after the addition of monocyte. By day 3 differentiation into macrophage was observed. In the presence of oxidatively modified LDL within collagen matrix, mononuclear cell with foamy appearance, large cytoplasm and lipid accumulation in it appeared on day 7.

**Conclusion:** We have constructed in vitro coculture system of arterial wall, and where early phenomena of atherogenesis could be observed.

**TuP16:** Plaque instability and acute coronary syndromes

**TuP21:** High density lipoproteins protect the heart against myocardial stunning

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**Objective:** The incidence of acute coronary events is strongly inversely related to the plasma concentration of high density lipoproteins (HDL). In the present study we investigated whether HDL may exert a direct cardioprotection against ischemic injury, independent of their function in lipid metabolism.

**Methods:** Plasma-derived HDL and synthetic HDL (SHDL) made with phosphatidylcholine and apoprotein A-I (apoA-I), lipid-free apolipoproteins and phosphatidylcholine liposomes have been injected into isolated rat hearts in which a myocardial stunning was induced by a brief period of low-flow ischemia followed by reperfusion.

**Results:** The administration of plasma-derived HDL during the last 10 minutes pre-ischemia caused a dose-dependent reduction of myocardial ventricular dysfunction at reperfusion, and reduced by 75% the release of CK into the perfusate. sHDL also protected the heart against reperfusion stunning, but less effectively than plasma-derived HDL. Equivalent doses of phosphatidylcholine liposomes or lipid-free apolipoproteins were instead ineffective.

**Conclusions:** The present findings demonstrate that plasma-derived or synthetic HDL protect the heart against ischemia-reperfusion damage. The protection is direct and dose-dependent, and occurs at physiological HDL concentrations. A low plasma HDL level increases cardiovascular risk not only by preventing lipid removal from the arterial wall, but also by exposing the heart to excessive ischemia-reperfusion damage.

**TuP22:** Elevated levels of plasma total homocysteine associated with coronary events, but not with coronary atherosclerosis

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**Objective:** Elevated levels of total homocysteine (tHcy) have been recognized as an important risk factor for cardiovascular diseases, but the association between tHcy levels and coronary events remains unclear.

**Methods:** We determined plasma tHcy and folate, vitamin B6, vitamin B12 levels and C677T polymorphisms in methylenetetrahydrofolate reductase (MTHFR) in 383 male patients with angiographically verified coronary artery disease (CAD) and 83 male control subjects. CAD (+) was defined as ≥50% stenosis in any major coronary artery. Severity of CAD was classified by number of diseased vessel; single, double, triple vessel disease. Patients who had a history of myocardial infarction were defined as coronary events (+) (CE+)(n = 165) and other patients were defined as coronary events (−) (CE−)(n = 135). tHcy were expressed as median levels.

**Results:** Median tHcy level in all subjects was 12.4 nmol/ml (range: 3.8–36.3 nmol/ml). tHcy level were not different between CAD (+) and CAD (−) and not significantly associated with the severity of CAD. However, tHcy levels in CE (+) were significantly higher than CE (−) (13.1 vs 11.1 nmol/ml, p = 0.01). There was no significant difference in MTHFR genotypes in any groups.

**Conclusions:** These findings suggest that elevated levels of tHcy associate with coronary events.

**TuP3:14** Aggressive lipid-lowering therapy in patients with unstable angina

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**Objective:** It is supposed that rapid correction of plasma lipids levels can improve prognosis of unstable angina patients (UA pts). The speed of plasma lipids lowering may be important for the success of therapy. We attempted to accelerate the lipids lowering (total serum cholesterol (TC) ≤ 4.2 mmol/l and low-density lipoprotein cholesterol (LDL-C) ≤ 2.6 mmol/l) by combined treatment with pravastatin (P) and plasmapheresis (PA).

**Methods:** Fourteen UA pts (Gr I) with baseline TC of 6.11 ± 1.08 mmol/l and LDL-C of 4.02 ± 0.96 mmol/l were treated with PA and P 80 mg PO daily since the first day. Pts had 1.9 ± 0.6 sessions of PA an average (from 1
to 3 sessions). The sessions were repeated until TC level decreased to at least 4.2 mmol/l, as measured 18–20 hours after the last session. Patients of control group (Gr C) (12 UA pts with baseline TC of 6.26 ± 0.92 mmol/l and LDL-C of 4.24 ± 0.96 mmol/l) were treated without PA.

**Results:** During one session of PA plasma TC decreased by 23.4 ± 9.6% (p < 0.005), LDL-C by 32.4 ± 13.2% (p < 0.002). On 10th day of the study the mean TC in Gr 1 decreased to 3.62 ± 0.69 mmol/l (−40.8%, p < 0.001), LDL-C to 1.84 ± 0.66 mmol/l (−54.2%, p < 0.001). In Gr C plasma TC and LDL-C decreased to 5.82 ± 1.32 (−6.9%, p > 0.5) and to 3.82 ± 0.92 mmol/l (−9.9%, p < 0.1) respectively. In 2 months in Gr 1 plasma TC and LDL-C remained significantly lower, than in Gr C: 4.64 ± 0.65 vs 5.25 ± 0.68 and 2.71 ± 0.71 vs 3.11 ± 0.54 mmol/l respectively. Nevertheless in Gr C significant reduction of plasma TC by 16.1% (p < 0.002) and LDL-C by 26.7% (p < 0.001) were noted. Since 4 month plasma TC and LDL-C were identical in both groups.

**Concluded:** PA+P diet is well-tolerable by UA pts and allows to achieve much faster reduction of plasma TC and LDL-C than P+ diet.

**TuP4W14**

**Relation of C-reactive proteins (CRP) and subacute restenosis after coronary angioplasty in acute myocardial infarction**

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**Objective:** Increasing evidences suggest that inflammation has an important role in both the initiation and the progression of atherosclerosis. Elevated levels of acute phase proteins and their determinants occur in acute coronary syndrome, and may also predict future cardiovascular events. Elevated plasma concentrations of C-reactive protein (CRP), a sensitive marker of underlying systemic inflammation, have been reported in patients with acute ischemia or myocardial infarction. We evaluated whether CRP levels of post-percutaneous transluminal coronary angioplasty (PTCA) patients might relate with subacute restenosis.

**Methods:** We studied 55 male patients (age, 59.1 ± 10.1 years) who admitted because of acute myocardial infarction (AMI). They all underwent urgent PTCA, and have been reviewed angiographically for 1 month follow up. Blood samples for CRP determination were collected 1 month later from onset of AMI.

**Results:** Statistically significant differences were observed between restenosis group and no restenosis group in CRP levels (0.69 ± 0.82 mg/dl vs 0.23 ± 0.26 mg/dl, p < 0.005). There were no difference in age and risk factors for coronary artery disease.

**Conclusions:** Increased CRP levels are associated with subacute restenosis and may have clinical benefits in predicting cardiovascular disease.

**TuP5W14**

**C-RP, fibrinogen and WBC in diagnosis and prognosis of patients with unstable angina evolved in non Q-AMI**

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**Objective:** Acute phase proteins play an important role in pathogenesis and prognosis of acute coronary syndromes; in particular their clinical application could be of great utility in unstable angina.

**Methods:** In this study we enrolled 96 patients (43% women, 34% non insulin diabetes, 3% insulin diabetes), admitted in our Coronary Unit for unstable angina. The study provided analysis of family anamnesis, clinical evolution, serum inflammatory indexes (CRP, WBC and Fibrinogen on admission, then after 6, 12, 24, 36 hours and then on dimission), myocardial lesion enzymes (CK and CK.m) and intercurrent complications within 15 days from hospital discharging.

**Results and Conclusions:** On admission, we observed a direct relationship between leukocytes (1000/mm3), Fibrinogen (mg/dl) and CRP (mg/dl) and risk of evolution in non Q AMI (WBC over 5,000,000/mm3, risk increased of 14% per 1,000,000/mm3) (see table).

In patients with a first non Q-AMI and with Fibrinogen peak value > 500 mg/dl we also evaluated a significant reduction of Left Ventricular Ejection Fraction on dimission (m.v. 50.88% vs 58.25% p = 0.041) and a greater incidence of complications post-event (43% vs 6%).

**TuP6W14**

**Plasma CRP levels and QT dispersion in acute coronary syndromes**


**Objective:** To evaluate the predictive value of C reactive protein (CRP) levels and QT dispersion (QTD) at presentation in determining the presence of myocardial damage and the risk of post-infarction angina in acute coronary syndromes (ACS).

**Methods:** Plasma CRP levels at 0, 12 and 24 hours and QTD on admission were evaluated in 32 patients with chest pain suggesting ACS. The patients were divided into acute myocardial infarction (AMI) or unstable angina pectoris (USAP) depending on ECG changes and cardiac enzyme profile. AMI (23 Q-wave and 11 non-Q wave) group was further subdivided into two according to the occurrence of post-infarction angina during hospitalization period.

**Results:** Thirty-four patients with AMI (age 58 ± 11 years, range 43–75) and 18 patients with USAP (age 55 ± 11 years, range 36–72) were similar with respect to peak CRP levels in the first 24 hours (4.0 ± 2.2 mg/dl vs 3.4 ± 1.7 mg/dl, p > 0.05) and QTD at presentation (72 ± 21 ms vs 71 ± 32 ms, p > 0.05). Nineteen out of 34 AMI patients had recurrence of chest pain during hospitalization. Peak CRP levels in the first 24 hours and baseline QTD were significantly higher in patients who developed recurrent chest pain during follow-up.

<table>
<thead>
<tr>
<th>Post-MI angina (n = 15)</th>
<th>Post-MI anagia (n = 19)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/dl)</td>
<td>2.6 ± 1.3</td>
<td>5.1 ± 2.6</td>
</tr>
<tr>
<td>QTD (ms)</td>
<td>63.3 ± 17.0</td>
<td>78.6 ± 22.1</td>
</tr>
</tbody>
</table>

**Conclusion:** In ACS, CRP levels and QTD do not differentiate AMI from USAP at presentation, however in AMI they can have predictive value in post-infarction risk stratification during short term follow-up.

**TuP1W15**

**Nitric oxide synthase polymorphisms and coronary heart disease**

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**Background:** The Nitric Oxide (NO), synthesized from L-arginine by the actions of the enzyme Nitric Oxide Synthase (NOS), is recognized as a principal factor in the regulation of vascular tone. Due to the fact that the constitutive eNOS isoform may be related with early coronary heart disease, the relationship between eNOS polymorphisms and coronary heart disease was investigated.

**Methods:** Prospectively we studied the eNOS polymorphisms in a Spanish group of 200 male patients (mean age 43 ± 5 years) diagnosed as having unstable angina or myocardial infarction and also in a sex and age-matched control group of 250 healthy men. DNA polymorphism at the eNOS gene was Polymerase Chain Reaction analysed. Statistical analysis was carried out using the Chi square.

**Results:** Data are summarized in the following table.

<table>
<thead>
<tr>
<th>eNOS</th>
<th>Patients</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>146 (73%)</td>
<td>179 (72%)</td>
<td>ns</td>
</tr>
<tr>
<td>45</td>
<td>48 (24%)</td>
<td>56 (23%)</td>
<td>ns</td>
</tr>
<tr>
<td>44</td>
<td>4 (2%)</td>
<td>7 (3%)</td>
<td>ns</td>
</tr>
<tr>
<td>56</td>
<td>2 (1%)</td>
<td>0</td>
<td>ns</td>
</tr>
<tr>
<td>35</td>
<td>0</td>
<td>5 (2%)</td>
<td>ns</td>
</tr>
</tbody>
</table>
TuP2:W15

Paraoxonase-1 and miR-192 polymorphism modulates the non-fatal myocardial infarction risk associated with decreased high density lipoproteins

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Objective: Serum paraoxonase (PON1), a high density lipoprotein (HDL)-linked enzyme, appears to have a role in the protection of low density lipoproteins from oxidative stress. PON1-192 polymorphism comprises low and a high activity alleles (Q and R, respectively). The present study examined the possible influence of the PON1-192 polymorphism on the risk of MI associated with a classic risk factor such as low HDL cholesterol concentration.

Methods: Two hundred and twenty consecutive micrordial infarction (MI) patients and 311 age- and sex-matched control subjects were studied. PON1-192 polymorphism was determined by PCR.

Results: Our results did not support the existence of a significant association between the paraoxonase-1 and genetic polymorphism and MI risk. However, subjects carrying the QQ genotype showed markedly increased MI risk associated with low HDL cholesterol levels compared with R carriers. With paraoxonase-1 and HDL confirmed a MI risk of 3.04 (P < 0.0001) compared with normal HDL levels in the entire population, the risk increased to 5.52 (P < 0.0001) in QQ homozygous subjects with low HDL concentrations relative to QQ homozygotes with normal HDL levels and decreased to 2.04 (P = 0.027) in R carriers.

Conclusions: The non-fatal MI risk associated with low HDL cholesterol concentrations as a classical risk factor resulted remarkably more pronounced in subjects with the low activity QQ genotype than those carrying the R allele.

TuP3:W15

ApoE genotype does not predict cholesterol response to pravastatin in a large sample of ambulatory patients


Objective: Most studies that have evaluated the influence of apoE genotype in the cholesterol response to hypolipidemic drugs have been performed in carefully controlled conditions, mainly in subjects with familiar hypercholesterylomea, with contradictory results. The aim of the study was to evaluate if apoE genotype had a practical implication in the prediction of cholesterol reduction in a representative sample of hypercholesterolemic subjects attended in an ambulatory setting.

Methods: Subjects with hypercholesterolemia and triglycerides below 350 mg/dl despite a lipid lowering diet, were treated with 20 mg of pravastatin during 16 weeks according to NCEP guidelines. Plasma levels of total and HDL-cholesterol, triglycerides and apoE genotype were measured in a centralized laboratory.

Results: 654 subjects were recruited for the study. 376 (57.5%) patients who fulfilled all the inclusion and exclusion criteria and completed the study with a compliance > 75% were selected for the analysis. Apo E allele distribution on the entire population was e2 1.8%, e3 86% and e4 12.2%. Patients were grouped according to their genotype in E2(E2/E3), E3(E3/E3) and E4(E4/E4).

TuP4:W15

The importance of the G/A→A polymorphism of the β-fibrinogen gene in relation to myocardial infarction risk

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Objective: To establish the association between the G/A→A polymorphism of the β-fibrinogen gene and plasma fibrinogen levels and myocardial infarction (MI) respectively.

Methods: Data for this case-control study of subjects aged 45–70 living in Stockholm was collected between 1992 and 1994. Cases were first-time acute MI patients, 837 men and 340 women, and referents were 1012 men and 485 women randomly chosen from the Stockholm County population registers. Odds ratios (OR) with 95% confidence intervals (CI) were calculated using a software program for logistic regression analysis.

Results: The OR, adjusted for age and residential area, of MI for men with plasma fibrinogen level > 90th percentile value of referents was 2.1 (95% CI 1.6–2.8) and for women 2.5 (95% CI 1.6–3.9). Plasma fibrinogen levels have been suggested to be determined partly by the G/A→A polymorphism of the β-fibrinogen gene. In our data, high fibrinogen levels are associated with presence of the A allele at this polymorphic site; the mean plasma fibrinogen value of the AA genotype group compared to the GG genotype group is 0.2 g higher for men (p < 0.1) and 0.3 g higher for women (p < 0.05). However, the presence of this allele does not imply an increased risk of MI for either men or women, OR 0.9 (95% CI 0.7–1.0) and 1.1 (95% CI 0.8–1.5) respectively.

Conclusion: High plasma fibrinogen level is clearly associated with MI in both sexes, and also associated with presence of the A allele of the G/A→A polymorphism of the β-fibrinogen gene. The presence of this allele is not associated with an increased risk of MI.

TuP5:W15

Polymorphisms in upstream region of angiotensin I-converting enzyme (ACE) gene regulating ace promoter activity


Objective: Plasma ACE levels are associated with insertion/deletion (I/D) polymorphism of the ACE gene. We investigated relationships between 2 polymorphisms (A240T and T93C) in the 5′ upstream region of the gene and the I/D polymorphism, and the effect of A240T and T93C on promoter activities of ACE.

Methods: In genome DNA from 133 patients with coronary artery disease and 125 control subjects, I/D, A240T, and T93C polymorphisms were identified using PCR-RFLP methods. Luciferase reporter vectors with the upstream region, including A240T and T93C, were transfected to HUVECs. After incubation at 37°C for 48 h, luciferase activities were measured.

Results: The -240T (A) was completely linked to the -93C (T). There was no significant effect on the luciferase activity.

Conclusion: The -240T→93C allele was linked to the D allele that is associated with high plasma ACE level. However, the promoter activity of the -240T→93C allele was lower than that of the -240A→93T allele. It seems that these polymorphisms in the upstream region of the ACE gene regulate plasma ACE level independently of I/D polymorphism.

TuP6:W15

Repeatedly elevated non-HDL cholesterol concentration in childhood emerges in infancy and is often a sign of familial dyslipidaemia

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Objective: Non-sporadic dyslipidaemia in childhood may be a sign of genetic dyslipidaemia. It could be found already during infancy.

Methods: Population of 463 families (the STRIP study) was used. In children non-fasting samples (fasting from the age of 5 years) were gathered from the age of 7 and 13 months and then annually. Serum total cholesterol and high density lipoprotein cholesterol (HDL-C) concentrations were measured providing non-HDL cholesterol. Lipid phenotypes of both parents

were defined on the base of gender-age specific 90th percentiles of fasting low density lipoprotein cholesterol (LDL-C) and triglycerides. The parents were classified according to the affected parent to high LDL-C only (type IIA), hypertriglyceridemia only (type IV) or both high LDL-C and high triglycerides (type IIB) and normal parents. Children were classified by the non-HDL cholesterol values in the highest age-gender specific quintile to frequent dyslipidemia (seen in at least half of the samples), to sporadic dyslipidemia or non-dyslipidemia. At least 5 samples were required, which excluded 8% of children.

Results: Frequent dyslipidemia of children was seen in 15% of families with normal, 26% with IIA, 46% with IIB and 11% with IV parents (P = 0.002). If all measurements of a child during the follow-up were independent on each other, parents would have had 5.9% frequently dyslipidemic children by chance. If one elevated non-HDL cholesterol was found between the age of 13 months and 7 years, the child had 60.2–62.0% probability of having frequent dyslipidemia.

Conclusion: Elevated non-HDL cholesterol of a child is a marker of repeated dyslipidemia, which in turn may be a marker of familial dyslipidemia.

TuP7:W15

Can Cholesterol Ester Transfer Protein (CETP) TaqIB genotype affect the impact of standardized cardiac rehabilitation program (CRP) on lipid risk markers?

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Objective: To study the effect of genotype on response to lifestyle intervention and to investigate the influence of TaqIB polymorphism (PM) on changes in lipid profile during CRP.

Methods: Patients (n = 226) with ischemic heart disease who participated in a 4-month medically supervised CRP were compared for lipid profile changes in the program.

Results: The frequencies of the B1 and B2 alleles for TaqIB genotype were 0.553 and 0.447, respectively. Serum TC, LDL-C and TG of non-B2 carriers were significantly reduced at the end of CRP, while the increase in HDL-C was not significant. By contrast, the only significant change in B2 carriers was an increase in HDL-C. Comparison of mean relative change (MRC) in serum lipids between B2 and non-B2 carriers showed a significant difference in TC and TG.

Δ TC (%) | Δ LDL-C (%) | Δ HDL-C (%) | Δ TG (%)
---|---|---|---
Non-B2 | -5.18 ± 1.44* | -3.91 ± 2.67* | 4.30 ± 2.13 | -7.94 ± 3.01*
B2 | -0.22 ± 1.24 | -0.86 ± 1.81 | 4.65 ± 1.44* | 8.29 ± 6.17

*p < 0.05, intra-allele analysis B2 vs non-B2; p = 0.05, inter-allele MRC analysis

Conclusions: Our data demonstrate that: 1) The benefits of CRP, in this cohort, are associated with the absence of the B2 allele. 2) This "heterogeneic" property of CETP TaqIB polymorphism confirms its importance in lipid outcomes in the B1 carriers. This is not limited to pharmacological intervention alone but rather due to global lifestyle changes incorporated in a CRP.

TuP8:W15

Low hepatic lipase activity is a risk factor for coronary artery disease

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Objective: HDL cholesterol is inversely correlated with the presence of coronary artery disease (CAD). HDL levels are modulated by lipoprotein lipase (LPL) and hepatic lipase (HL). While LPL and HL play important roles in lipid metabolism there has been conflicting data on their role in the development of CAD.

Methods: We measured LPL and HL activities in post hepatic plasma of 190 patients undergoing elective coronary angiography. The extent of CAD was quantitated by the "extent score" which has been shown to correlate better with known risk factors than other measures of CAD extent.

Results: No association was observed between LPL concentration (r = -0.06, p = 0.46) or activity (r = -0.01, p = 0.90) and CAD extent. In contrast, HL activity showed a significant inverse association with the extent of CAD (r = -0.19, p = 0.011). If separated into quartiles, lower HL activity was consistently associated with increasing CAD extent (HL > 348 nmoi/ml/min: extent score: 31.7, HL 272–348: 37.7, HL 200–271: 39.4, HL < 200: 44.5). To test the association of HL with CAD without potential confounding factors, we also analyzed the −514C/T polymorphism in the HL promoter.

Compared with wild type, heterozygotes for the T allele had a 15% lower HL activity (253 ± 85 vs. 296 ± 99 nmoi/ml/min, p < 0.05), homozygotes 47% lower HL activity (158 ± 73, p < 0.001). The presence of −514T was associated with a higher extent of CAD (r = 0.16, p = 0.05).

Conclusions: 1) We found no association of LPL concentration or activity with the extent of CAD. 2) Low HL activity was associated with a greater extent of CAD. 3) A polymorphism in the HL promoter leading to lower HL activity was also associated with CAD. 4) These data suggest that low hepatic lipase activity is a risk factor for coronary artery disease.

TuP9:W15

Lp(a) levels and apo(a) genotype are both important for CHD risk: Results from two case control studies in India

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Lipoprotein(a) levels and apo(a) K IV-2 genotypes were determined in Coronary Heart Disease (CHD) patients (N = 254) and controls (N = 480) from two Asian-Indian populations (North and South). In both populations and also in the pooled data set mean Lp(a) levels were significantly lower in the patients (27.4 mg/dl) compared to the controls (17.6 mg/dl). Odds ratios for being in the CHD group rose contiously with increasing Lp(a) but were independent of the apo(a) K IV-2 repeats.

The inverse relation between apo(a) K IV-2 number and Lp(a) levels was highly significant in patients and controls from both Asian-Indian populations. However apo(a) K IV-2 allele frequencies were not different between CHD patients and controls and hence do not explain the elevated Lp(a) levels in CHD patients. Rather allele associated Lp(a) levels were higher in the patients. The best predictor of CHD status was an Lp(a) concentration above the expected mean level for the respective K IV-2 repeats. These results allow for two alternative explanations: 1) Lp(a) elevation is secondary to CAD in Asian-Indians or 2) elevations of Lp(a) above an apo(a) type specific level determine CHD risk. These alternatives can only be distinguished by a prospective study.

TuP10:W15

ecNOS (−786 T/C) and the ACE (I/D) polymorphisms in the risk for early coronary artery disease

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Background: DNA polymorphisms at the endothelium constitutive nitric oxide synthase (ecNOS) gene have been linked to the risk for coronary artery disease (CAD) and coronary spasm. In vitro, a polymorphism in the 5' region of the ecNOS gene (−786 T/C) influences promoter activity. The genetic variation at the angiotensin converting enzyme (ACE) is associated with plasma-ACE activities, and has also been linked with susceptibility to cardiovascular disease. We studied if the −786 T/C polymorphism in the 5' flanking region and the 27-base pair repeat polymorphism in the intron 4 of the ecNOS gene were associated with the risk for CAD.

Methods: A total of 170 male smokers CAD-patients younger than 50 years, and 300 controls were genotyped for the ecNOS and the ACE (I/D) polymorphisms, and frequencies were compared by the Chi square test. Odds ratios (OR) were also calculated.

Results: Only the −786 T/C polymorphisms in the 5' flanking region of the ecNOS gene was significantly associated with early CAD in our population. The frequency of the CC genotype was significantly increased (p = 0.039) in patients compared to controls (OR = 1.67; 95% CI = 0.99, 2.72). We found a synergistic effect between the ecNOS-CC and the ACE-DE genotypes in the risk of early CAD. The frequency of CC + DD was significantly increased (p = 0.002) among patients. Thus, among those with a CC genotype, to be ACE-DE increased significantly the risk of suffering an early CAD episode (OR = 2.82; 95% CI = 1.40, 5.70).

We also analysed ecNOS transcription (RT-PCR) in lymphocytes from 9 healthy volunteers (3 CC, 3 TC, 3 TT). Individuals who were CC showed lower ecNOS-mRNA levels compared to CT and TT.
TuP11:W15 The 447 truncation of lipoprotein lipase, lipids and lipoproteins, and risk of ischemic heart disease. The Copenhagen City Heart Study and updated meta-analysis

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Objective: The Asp9Asl, Gly188Glu, and Asn291Ser mutations in lipoprotein lipase appear to increase triglycerides, reduce HDL cholesterol and possibly increase the risk of ischemic heart disease. The 447 truncation of two amino acids of this enzyme may have opposite effects. We tested this hypothesis.

Methods: We genotyped 8,499 women and men from a general population sample, The Copenhagen City Heart Study, and 885 women and men with ischemic heart disease. Participants carrying the Asp9Asl, Gly188Glu, and Asn291Ser mutations in lipoprotein lipase were excluded from this study.

Results: Frequencies of heterozygotes and homozygotes in the general population sample were 11.7% (n = 1,629) and 1.1% (n = 103), respectively. Compared with female and male non-carriers, plasma triglycerides were 0.12 mmol/L and 0.20 mmol/L (p = 0.001 and p < 0.001) lower in heterozygotes, and 0.18 and 0.41 mmol/L lower in homozygotes (p = 0.37 and p < 0.06), respectively. Similarly, HDL cholesterol was increased 0.07 and 0.05 mmol/L (p = 0.001 and p = 0.02) in heterozygous women and men, and 0.03 and 0.17 mmol/L in homozygous women and men (p = 0.59 and p = 0.04), respectively. In spite of these effects on triglycerides and HDL cholesterol in both genders, the 447 truncation had no statistical significant effect on risk of ischemic heart disease in our study alone. However, when incorporated into meta-analysis, this study contributed toward an overall 15% decrease in risk of ischemic heart disease among carriers of the 447 truncation.

Conclusions: Like other common mutations in lipoprotein lipase, the 447 truncation has moderate, but in contrast to these beneficial effects on triglyceride and HDL cholesterol levels, and possibly protected against ischemic heart disease.

TuP12:W15 Angiotensinogen mutations and hypertension in the general population. The Copenhagen City Heart Study

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Objective: Hypertension is linked to the angiotensinogen gene. We tested the hypothesis that the Thr174Met and Met235Thr mutations in this gene increase the risk of hypertension.

Methods: We genotyped 9,100 women and men from the Danish general population, of which 54 percent had hypertension.

Results: 12 and 41 percent carried the 174M and 235T mutations, respectively; the 174M mutation always occurred on the same allele as the 235T mutation. On multifactorial logistic regression analysis, women homozygous for 235T had an odds ratio for hypertension of 1.29 (95% CI: 1.05-1.58), which increased to 1.50 (1.15-1.96) if they in addition were homozygous for 174T. Women homozygous for 235T also had increased risk of isolated systolic hypertension (OR: 1.37; 1.02-1.84), and of mild hypertension (OR: 1.40; 1.10-1.77). Finally, women homozygous for both 235T and 174T had increased risk of being on antihypertensive medication (OR: 1.53; 1.12-2.09).

We found no effects on risk of hypertension as a function of genotype in men. Homozygosity for both 235T and 174T was associated with a 10 percent increase in plasma angiotensinogen levels in both genders; however, systolic and diastolic blood pressure was positively correlated to plasma angiotensinogen concentration only in women.

Conclusions: In the population at large, double homozygosity for 235T and 174T in the angiotensinogen gene increase angiotensinogen levels by 10 percent, and may be a weak risk factor for hypertension in women, but not in men.

TuP13:W15 Small low density lipoprotein particles in familial combined hyperlipidemia

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Background: The influence of genetic and environmental factors on LDL particle size in patients with familial combined hyperlipidemia (FCHL) is under debate.

Methods and Results: We determined LDL particle size with non-denaturing gradient gel electrophoresis from 553 Finnish subjects from 48 pedigrees ascertained for FCHL. Individuals with high triglyceride concentrations or mixed hyperlipidemia had smaller LDL particles than those with hypercholesterolemia or nonaffected subjects (LDL size 25.1 ± 1.6, 25.3 ± 1.5, 26.2 ± 1.3, and 26.7 ± 1.2 mm, respectively, P < 0.001). In multivariate regression analysis serum triglycerides (r² = 0.43%, P < 0.001) and HDL cholesterol (r² = 4.5%, P < 0.001) were the only significant predictors of LDL particle size. Complex segregation analyses of the quantitative trait for LDL size revealed 1) ample evidence that LDL size is inherited as a polygenic trait with environmental factors involved, 2) no ample evidence that LDL size is influenced by a major gene in FCHL.

Conclusions: In Finnish FCHL families small, dense LDL is a polygenic trait that appears to be influenced by environmental factors.

TuP14:W15 Polymorphisms in the insulin response element of APOC-III gene promoter influence the correlation between insulin and triglycerides or triglyceride-rich lipoproteins in humans

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The goal of the present study was to assess whether the –455 and –482 mutations in APOC-III gene Insulin Response Element (IRE) affect the relationships between plasma insulin and triglyceride-rich lipoprotein levels. The population sample was composed of 983 subjects (485 men and 498 women), aged between 35 and 65 years, randomly sampled from the electoral rolls in Northern France and stratified on gender and ten-year age groups. Plasma triglyceride, apoC-III, apoB, Lp-CIII-B, Lp-Eβ lipoprotein particles and insulin levels were measured. Two polymorphisms in APOC-III gene IRE (T > C at –455 and/or C > T at –482) were determined. Plasma insulin was positively correlated to triglyceride (p < 0.0001), apo C-III (p < 0.0003), Lp-C-III-B (p < 0.0001), apo B (p < 0.0001) and Lp-Eβ (p < 0.0001) levels. This association differed significantly according to APOC-III IRE polymorphisms. The relationship between insulin and Lp-C-III-B (p < 0.02) or apoB (p < 0.02) was greater in women bearing the C allele of –455 than the T allele. Similarly, the relationship between insulin and Lp-C-III-B (p < 0.02) or Lp-Eβ (p < 0.02) was greater in women bearing the C allele of –482 than the T allele. There was no evidence for any effect in men. These results suggest that the relationship between plasma insulin and triglyceride-rich lipoprotein levels is partly influenced by polymorphisms in APOC-III IRE.

TuP15:W15 Relation of risk factor levels in adult offspring to parental history of myocardial infarction (MI): The Reykjavik Offspring Study

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Background: A family history of heart disease has been reported to increase risk of coronary heart disease. There is also familial aggregation of known cardiovascular risk factors. Most family studies, however, rely on self-reported information on parental disease.

Methods: We have compared the cardiovascular risk profile in a large cohort of adult offspring with well-documented parental history (case) of MI to offspring without such a history (control). Their parents have all attended a prospective population survey (Reykjavik study, started in 1968). Risk factors studied included systolic and diastolic blood pressure (SBP, DBP), total cholesterol (TC), HDL cholesterol, triglycerides (TG), and body mass index (BMI).

Results: To date, 3384 offspring have been studied (1563 males (1093 cases/470 controls) and 1821 females (1236 cases/585 controls)). Mean age is 47 ± 9 years for men and 48 ± 9 for women.

Table. Risk factor levels of cases and controls. Values are age adjusted mean ± SD. TG is log transformed for analysis.

<table>
<thead>
<tr>
<th></th>
<th>SBP</th>
<th>DBP</th>
<th>BMI</th>
<th>TC</th>
<th>HDL</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male case</td>
<td>111 ± 15*</td>
<td>81 ± 10*</td>
<td>27.0 ± 3.8*</td>
<td>5.7 ± 1.0</td>
<td>3.8 ± 0.30*</td>
<td>1.50 ± 1.0</td>
</tr>
<tr>
<td>Control</td>
<td>128 ± 15</td>
<td>79.4 ± 7.8</td>
<td>25.5 ± 4.2</td>
<td>5.8 ± 1.1</td>
<td>3.3 ± 0.32</td>
<td>1.38 ± 1.0</td>
</tr>
<tr>
<td>Female case</td>
<td>125 ± 16</td>
<td>77 ± 8</td>
<td>26.5 ± 4.7</td>
<td>5.6 ± 1.1</td>
<td>3.7 ± 0.27*</td>
<td>1.16 ± 0.7</td>
</tr>
<tr>
<td>Control</td>
<td>132 ± 15</td>
<td>76.8 ± 8</td>
<td>25.4 ± 4.5</td>
<td>5.4 ± 1.0</td>
<td>1.53 ± 0.38</td>
<td>1.10 ± 0.6</td>
</tr>
</tbody>
</table>

*Statistically significant differences between case and control (P < 0.05)

Conclusion: A positive parental history of MI is associated with a worse cardiovascular risk profile in both men and women. These observations may have important implications for prevention.

TuP16:W15 Lipoprotein subclasses measured by NMR spectroscopy help explain the sex differential in coronary artery disease: Results from the Framingham Offspring Study J. Orvos 1, E. Jeyarajah 4, J. Ordovas 2, J. McNamara 1, E. Schafer 2, F. Wilson 2
1. NC State University, Raleigh, NC; 2. Tufts University, Boston, MA; 3. Framingham Heart Study, Framingham, MA, USA

Objective: To determine whether there are gender differences in VLDL, LDL and HDL subclass distributions independent of those attributable to male/female differences in lipid levels that would help explain the lower incidence of coronary artery disease (CAD) in women.

Methods: Frozen plasma samples were analyzed from 1684 men and 1753 women from cycle 4 (1988/89) of the Framingham Offspring Study. Lipoprotein subclass profiles were measured by NMR spectroscopy and included levels of VIDL, LDL subclasses (V1*-V6; larger numbers denoting larger particles), LDL, 3 LDL (L1-L3) and 5 HDL (H1-H5) subclasses, plus average VIDL, LDL, and HDL particle sizes.

Results: As reported previously, women had lower triglycerides (TG) and higher HDL-C than men. From established relations between lipid levels and subclass distributions, these differences would be expected to produce a lower-risk subclass profile of smaller-size VIDL, and larger-size LDL and HDL. Our results confirmed this prediction, and showed that gender differences in the sizes of all 3 lipoproteins persist (P < 0.001) even after controlling for levels of TG, LDL-C, and HDL-C. HDL showed the largest male/female difference, with women having a mean particle size ~1 SD unit (0.4 nm) greater than men, resulting from more large (H4, H5) and less small (H1) LDL. LDL particle size was smaller for men (by 0.4 nm), due to lower levels of large (L3) and higher levels of small (L1) LDL. VIDL size was lower in women, due mainly to fewer large particles (V4, V5, V6).

Conclusions: Women have a less atherogenic subclass profile than men, even after accounting for differences that can be attributed to lipid levels.

TuP17:W15 Investigation of polymorphisms in the immune system in patients with coronary heart disease C. Szalaj 1, G. Füts 1, J. Duba 1, J. Kramer 1, L. Romics 2, Z. Prohászka 2, A. Csádár 2
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Objectives: There are several lines of evidence that inflammatory components could be involved in the pathogenesis of coronary heart diseases (CHD). We hypothesized that some polymorphisms in the immune system might be associated with a susceptibility to CHD. We determined the distribution of polymorphisms in the TNF-α promoter, in CCR5, CCR2 chemokine receptors, in a chemokine SDF1 and in the C3 and C4 complement components in patients with CHD and control subjects.

Methods: Genotypes were determined by polymerase chain reaction with or without restriction fragment length polymorphism. 320 patients with severe CHD and 230 healthy controls were investigated.

Results: Carriers with the silent complement factor C4B*Q0 and the functionally deficient C4A*6 alleles were found to be subject to increased risk of CHD development. No other differences were found in the allelic frequencies between cases and controls. Strong allelic associations were found in patients with CHD between TNFα -308A and the C4A*Q alleles, and between TNFα -238A and C4A*6. There were no allelic associations in control patients. The functionally deficient C3F* allele, the TNFα -308A alone and together with the C4A*Q0 occurred significantly more frequently in patients with myocardial infarction (MI) than without MI.

Conclusions: The C4A*Q0, the C4A*6 alleles alone and the TNFα -308A + C4A*Q0 and the TNFα -238A + C4A*6 combination of alleles might be associated with a susceptibility to CHD. The C3F* allele, the TNFα -308A alone and together with the C4A*Q0 were found to be associated with a significantly higher relative risk for development of MI.

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Background: The role of monocytes in the atherosclerotic process is well established. These cells are attracted to the intima by means of some proinflammatory chemokines, such as MCP-1 and RANTES, which interact with the CCR2 and CCR5 receptors. In this work the relationship between ischemic coronary artery disease and proinflammatory receptor polymorphisms was investigated.

Methods: We genotyped the CCR5-D32 and CCR2-I64V polymorphism receptors in a Spanish population of 250 men younger than 50 years old and diagnosed with coronary artery disease. An age and sex-matched group of 500 healthy subjects served as control. Statistical analysis was carried out using the Chi square.

Results: Data are summarized in the following table.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Controls</th>
<th>Patients</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) CCR2 64VV</td>
<td>370 (74%)</td>
<td>203 (81%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>64IV</td>
<td>641</td>
<td>122 (24%)</td>
<td>45 (18%)</td>
</tr>
<tr>
<td>64II</td>
<td>8 (2%)</td>
<td>2 (1%)</td>
<td>ns</td>
</tr>
<tr>
<td>B) CCR5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>We/We</td>
<td>410 (82%)</td>
<td>208 (83%)</td>
<td>ns</td>
</tr>
<tr>
<td>We/D2</td>
<td>84 (17%)</td>
<td>42 (17%)</td>
<td>ns</td>
</tr>
<tr>
<td>D2/D2</td>
<td>6 (1%)</td>
<td>0</td>
<td>ns</td>
</tr>
</tbody>
</table>

Conclusions: The gene and genotype frequencies of the CCR5 64VV polymorphism were not different between cases and controls. However, the 64VV genotype of CCR2 64VV polymorphism resulted to be significant in higher cases as compared to controls. Also, none of the patients was homozygous for the CCR5-D32.

TuP19:W15 Genetic markers in women with angiographically documented coronary artery disease from Brazil L.A. Salazar 1, A.L. Cunha 1, N. Forti 2, J. Diamant 3, S.D. Giannini 4, M.H. Hirata 5, R.D.C. Hirata 5, 1. Faculty of Pharmaceutical Sciences; 2. Heart Institute (InCor), University of Sao Paulo, Medical School, Sao Paulo, SP, Brazil

Objective: To evaluate the possible association between Psll (intron 15) promoter polymorphism at the low-density lipoprotein receptor (LDLR) locus and a-1-to-4 truncated (C247-T44) in the methyleneinodiolate reductase (MTHR) gene with susceptibility to coronary artery disease (CAD).

Methods: Twenty five Brazilian women (mean age, 49 ± 8 years) proven to have CAD by angiography (coronary artery stenosis greater than 70%) and 50 healthy women (mean age, 52 ± 7 years) were recruited for this study. Blood samples were collected after 12 h fast for the determination of lipid profile and DNA extraction. LDLR and MTHR genotypes were detected by PCR-RFLP. Chi-square analysis was used to test Hardy-Weinberg equilibrium, and for comparison of allele frequencies and genotype distribution between the studied groups.

Results: The frequency of the P111 homozgyous genotype for Psll polymorphism at the LDLR gene was greater in CAD group compared to controls (56% vs. 18%, P = 0.0054). On the other hand, CAD group presented similar frequency of MTHR genotypes when compared to controls (x² = 0.509, P = 0.7755). The relative risk for CAD in women carrying the P111 genotype was 5.8 (95% confidence interval, 3.24–10.3).

Conclusions: These data suggest that Psll polymorphism at the LDLR gene remain an useful genetic marker to predicting CAD risk in Brazilian women.

Financial Support: FAPESP-Brazil

**TuP20:W15**

Dissection of genetic factors in cardiovascular disease in a mediterranean population


**Objective:** Detect possible combinations of common predisposing alleles ("haplotypes") which modifies the individual level of susceptibility to coronary artery disease (CAD).

**Methods:** Study population: 352 subjects with CAD (109 women/243 men) (150 angor pectoris/202 myocardial infarction) and 148 healthy subjets (56 women/92 men). Both groups are matched in race, age, sex and Body mass. Pathways, genes and polymorphisms studied: Lipid metabolism (lipoproteins-lipase (LPL)(6-7 and 8-9), oxidation (paraoxonasa (paraox)(GlIn-Arg 192), inflammation - insulin resistance - apoptosis (Tumor Necrosis Factor-alpha (TNF)), platelet aggregation (Glycoprotein IIIa receptor (GpIIa/Ib)) and fibrinogen (B-fibrinogen (B-fibr)) and fibrinolysis (Inhibitor of the tissue-type plasmonogen activator (PAI)). Genotypes have been studied in peripheral blood samples by restriction fragment length polymorphism using PCR amplification and agarose gel electrophoresis with bromide staining. Statistical Analysis: Software SPSS 6.0 and Logistic regression analysis.

**Results:** A) genotype GG (B-fibr) + genotype A1A1 (GpIIa/Ib) = 0.012 OR: 1.81 (1.13-2.89). B) allele B (paraox) + allele T2 (TNF) p = 0.017 OR: 2.35 (1.15-4.92). C) genotype P-P (LPL 6-7) + genotype (++) (LPL 8-9) + genotype A1A2 (GpIIa/Ib) p = 0.007 OR: 0.20 (0.05-0.73) D) allele - LPL (6-7) + allele A2 (GpIIa/Ib) + allele T1 (TNF) p = 0.014 OR 0.49 (0.27-0.88). E) allele A2 - LPL (6-7) + allele (--) (LPL 7-8) + allele A2 (GpIIa/Ib) + allele T1 (TNF) p = 0.008 OR: 0.44 (0.23-0.82). F) allele - (LPL 8-9) + allele A2 (B-fibr) + allele A2 (GpIIa/Ib) + allele T1 (TNF) p = 0.011 OR: 0.44 (0.23-0.75). G) allele A2 - LPL (7-6) + allele (++) (LPL 8-9) + allele B (B-fibr) + allele A2 (GpIIa/Ib) + allele T1 (TNF) p = 0.007 OR: 0.40 (0.20-0.80).

**Conclusions:** There are several "haplotypes" that modulate the susceptibility threshold of CAD. It’s necessary to make big "polymorphism libraries" to study such haplotypes.

**TuP21:W15**

Association of MTHFR and ESR1 polymorphism with atherosclerotic changes in coronary arteries

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**Objective:** To study whether the Ala222Val mutation of methylenetetrahydrofolate reductase (MTHFR) and estrogen receptor 1 (ESR1) PvuI polymorphisms are associated with the atherosclerotic changes in the main coronary arteries.

**Methods:** The atherosclerosis of 286 men (mean age 52 ± 9.6 years) was studied in post-mortem examination. The percentage of fatty streaks, fibrous plaques, calcification and complicated lesions from the walls of descending coronary, left circumflex artery and right coronary artery were measured. The MTHFR Ala222Val polymorphism was as well as ESR1 PvuI polymorphism were assayed by PCR and restriction enzyme digestion.

**Results:** In the most obstructed coronary artery the cases with mutated V/V genotype of MTHFR and P/P genotype of ESR1 had significantly (P < 0.0020) largest area of complicated lesions than the other genotype combinations.

**Conclusions:** There may be an interaction between the MTHFR and ESR1 polymorphism in development of atherosclerosis in coronary arteries.

**TuP22:W15**

Different amounts of the angiotensin-converting enzyme mRNA originating from the alleles with deletion and insertion


**Objective:** The insertion/deletion (I/D) polymorphism in intron 16 of the angiotensin-converting enzyme (ACE) gene is involved in the development of cardiovascular diseases. We compared the ACE mRNA expression originating from the D allele and the I allele in the human white blood cells from ID heterozygotes.

**Methods:** We identified the mRNA from the I allele using the G2215A polymorphism in exon 15 that was linked to the I/D polymorphism. RNA samples were obtained from 12 healthy heterozygotes of both I/D and G2215A, and every insertion was shown to be linked to 2215G. ACE mRNA was amplified by the RT-PCR method with an end-labeled antisense primer. The PCR products were digested with HaelII and separated by electrophoresis, and the relative radioactivities of the 2215A and 2215G bands were measured using an auto-image analyzer.

**Results:** In every cases, the intensity of the 2215A product (D allele origin) was higher than that of the 2215G product (I allele origin). The mean ratio of 2215A to 2215G was 1.79 (1.11-2.82).

**Conclusion:** The D allele led to higher expression of the ACE mRNA and may affect the renin-angiotensin system in local regions.

**TuP23:W15**

Preliminary report: Endothelin-1 gene polymorphism in malignant ventricular arrhythmias

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**Objective:** Endothelin-1 (ET-1), one of the most potent vasoconstrictors, is known to induce ventricular arrhythmias. The aim of this study was to investigate the frequency distribution of the common polymorphism of the ET-1 gene and its possible relation to states leading to hemodynamical collapses during malignant ventricular arrhythmias (MVA) in patients with structural heart disease (SHD) and left ventricular dysfunction.

**Methods:** The TaqI polymorphism (in intron 4, position 8002) of the ET-1 gene was determined using PCR with wthout restriction digestion in 26 consecutive Caucasian patients with a history of MVA treated with an implantable cardioverter defibrillator (ICD). In this group, 19 patients were receiving amiodarone because of high incidence of MVA after ICD implantation and 7 were not. All persons had low left ventricular ejection fraction of <40%.

**Results:** The frequency of (+) allele was 0.788 and (-) allele was 0.212, genotype distributions were (+) (+) = 57.7%, (+) (-) = 42.3% and (-) (-) = 0%, respectively. Of 26 patients, 9 (34.6%) had recurrent palpitations, and 8 (30.8%) had syncope. The risk for syncope was associated with the (+) genotype of the ET-1 gene (p = 0.010). Patients with chronic amiodarone therapy had also significantly higher frequency of the (+) genotype than patients without this antiarrhythmic treatment (p = 0.011).

**Conclusions:** Patients with SHD and MVA carrying (+) genotype of the ET-1 gene are at increased risk for syncope as worse hemodynamic manifestation of their malignant arrhythmias. They also more frequently require chronic amiodarone therapy than persons without this genotype. This results could have clinical implications for the most efficient therapy of patients with MVA.

**TuP24:W15**

Influence of apolipoprotein E polymorphism on vitamin A and vitamin E levels in a Spanish adult population

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**Objective:** To study the effect of the apolipoprotein (apo) E phenotype on plasma vitamin (vit.) A and vit. E levels in men and women from a population of Madrid.

**Methods:** Men (n = 117) and women (n = 244) were randomly selected from a working population. Venous blood was drawn after an overnight fast to determine plasma concentrations of vit. A and vit. E, lipids and other metabolites by standardised methods. Apo E phenotyping was performed by isoelectric focusing of delipidated VLDL. Body weight and height were measured. Smoking habits, menstrual status and oral contraceptive use were recorded. Dieticians assessed alcohol consumption and dietary intake trough a week-based food frequency questionnaire. Subjects taking any hypolipidemic medication or vitamin supplements were not included in the study.

**Results:** Vit. A and vit. E levels were higher in men than in women, although differences in vit. E disappeared after adjusting for lipid levels. The variation in vit. E levels was not associated with the apo E phenotype in either men or women, even though in the latter this polymorphism was associated with plasma cholesterol levels, which was the variable that best correlated with vit. E (r = 0.6514, p < 0.0001). In women, but not in men, the variation in vit. A levels was significantly associated with the apo E phenotype, the e32 subjects having a higher concentration than the e33 and e4 carriers. This was true also after adjusting for lipid levels. Stepwise variable selection analysis was performed in women, with vit. A levels as the dependent variable. The
apo E phenotype (coded with two dummy variables) entered the model after plasma triglycerides, plasma urea nitrogen, oral contraceptive use and alcohol intake, in that order, and it explained 4% (p < 0.01) of the variance of vit. A levels in women.

Conclusion: Allele s2 increases vit. A levels in women independently of its influence on lipid levels.

TuP25:W15 Renin-angiotensin system polymorphisms and risk of cardiovascular disease in familial hypercholesterolemia
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Objective: The role of renin-angiotensin system polymorphisms as risk factors for coronary heart disease (CHD) is controversial. This study investigated their role in patients with heterozygous familial hypercholesterolemia (FH).

Methods: Polymorphisms frequencies for angiotensin-I converting enzyme insertion/deletion (ACE ID), angiotensinogen M235T and angiotensin-II type I receptor (AGTR2) A1166C were determined in 112 patients with FH and 72 patients with polygenic hypercholesterolaemia of whom 26.7% and 41.6% respectively had established CHD.

Results: None of the polymorphisms were associated with risk of CHD in patients with polygenic hypercholesterolaemia. Logistic regression analysis of risk factors for CHD in patients with FH identified male sex (OR = 3.03), smoking (OR = 2.91), diastolic blood pressure (OR = 3.70), plasma glucose (OR = 3.51) and the AGTR2 A1166C polymorphism (OR = 3.10) as risk factors for CHD in patients with FH.

Conclusion: The angiotensin II receptor A1166C polymorphism may interact with severe hypercholesterolaemia, and other risk factors to increase risk of CHD in FH patients.

TuP26:W15 Additional effect of Chlamydia pneumoniae infection to genetic factors in the development of coronary artery disease

Objective: To test the hypothesis that Chlamydia pneumoniae (CP) infection may have some additional effects to genetic factors (the angiotensin converting enzyme (ACE) gene D/I polymorphism and the platelet glycoprotein (GP) IIb/IIIa gene 157Thr/Met polymorphism) in the development of coronary artery disease (CAD).

Methods: We analyzed serum IgG antibody to CP in 125 consecutive patients (pts) who had cardiac catheterization. The cut off index of IgG titer was measured by ELISA, the index > 1.10 being considered positive. The ACE and GP IIb/IIIa polymorphisms were analyzed using polymerase chain reaction after DNA extraction from peripheral blood.

Results: Of the 125 pts, 92 had significant CAD (>50% stenosis). The prevalence of CP IgG seropositivity was 46% in pts with CAD and 24% in those without CAD (p = NS). The frequencies of ACE D-allele and GP IIb/IIIa Met-allele carriers were 64% and 23% in pts with CAD versus 55% and 18% in those without CAD, respectively (p = NS). Notably, the percentage of CP IgG-seropositive pts who had ACE D-allele was significantly higher in pts with CAD (26%) than in those without CAD (6%) (p < 0.05). However, the percentage of CP IgG-seropositive pts who had GP IIb/IIIa Met-allele was 9% in pts with CAD versus 3% in those without CAD (p = NS).

Conclusion: CP infection may have some additional effects to genetic factors, especially ACE D/I polymorphism, in the development of CAD.

TuP1:W16 NEW ASPECTS OF PHARMACOLOGICAL TREATMENT

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Objective: This study was to test the effect of lipid reduction on the cardio-intima-media thickness (IMT) in hypercholesterolemic patients in Fukuoka, Japan.

Methods: A total of 157 asymptomatic hypercholesterolemic patients (mean age 69.4 years) were assigned to probucol (500 mg/day, n = 75), pravastatin (10 mg/day, n = 61) or matched control (diet alone, n = 21). The 2-year change in lipids and in IMT in the common carotid artery and incidence of major cardiovascular events were surveyed. Ultrasound-detected carotid atherosclerosis (2A) as defined by a mean maximum IMT (MMaxIMT) ≥ 1.3 mm.

Results: The serum LDL cholesterol level was significantly reduced by 31% in probucol group and by 43% in the pravastatin group and by 26% in the diet alone group (P < 0.0001, P < 0.0001, P < 0.01, respectively). Probucol and pravastatin groups with CA resulted in a significant reduction of the MMaxIMT (p < 0.01, p < 0.05, respectively) and a significant MMaxIMT in the diet alone group after 2 years (p < 0.001). There was a significant relationship between the reduction of MMaxIMT and serum LDL cholesterol in the probucol group (p < 0.05). Moreover, major cardiovascular events were significant fewer in the probucol group than in the other groups (p < 0.05).

Conclusion: These observations suggest that lipid reduction prevents from the progression of MMaxIMT in the common carotid artery, and that probucol is more effective in reducing the risk of major cardiovascular events in hypercholesterolemic patients than pravastatin.

TuP2:W16 Colesevelam HCl (Welchol™), a new, potent, well tolerated, non-systemic, lipid lowering agent
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Objective: To develop a safe, non-absorbed, LDL cholesterol reducing drug that provides convenient monotherapy and can be an effective component of combination therapy.

Methods: Seven placebo-controlled trials involving 1,350 diet stable patients explored the safety and efficacy of colesevelam alone and in combination with HMG-CoA reductase inhibitors (HMGRI). Patients with Fredrickson type IIa hyperlipoproteinaemia constituted the primary population. Combination therapy studies were conducted with concurrent administration of lovastatin, simvastatin and atorvastatin.

Results: Treatment with colesevelam demonstrated dose dependent statistically significant reductions in LDL cholesterol and was effective whether the dose was administered once or twice per day (Figure). Side effects, including gastrointestinal, were comparable between treatment and placebo groups. Colesevelam demonstrated at least additive efficacy in reducing LDL-C when combined with an HMGRI, compared with individual agents in the same trial.

Conclusion: Colesevelam hydrochloride is a safe, convenient and effective therapy for reducing LDL-C alone or in combination with an HMGRI. Compared to historical data with other bile acid sequestrants, colesevelam hydrochloride was 4-6 times more potent.

TuP3:W16 F 12511, a novel ACAT inhibitor, and atorvastatin regulate endogenous hypercholesterolemia in a synergistic manner in the casein-fed rabbits

F 12511, a novel ACAT inhibitor, lowers plasma cholesterol levels in New-Zealand white rabbits fed a cholesterol-free casein-rich diet. Endogenous hypercholesterolemia (EH) pre-established for 8 weeks was used to compare treatments with F 12511 and atorvastatin for a further 8-week period, and
to verify whether both agents act synergistically. F 12511 appears to be 3-4 fold more potent than atorvastatin for reducing total plasma cholesterol (active doses ranging from 0.16 to 2.5 and 1.25 to 10 mg/kg/day, respectively) while the hypocholesterolemic efficacy of both compounds at 2.5 mg/kg/day reaches 70 and 45%, respectively. A striking reduction up to 75% of esterified cholesterol in liver mediated by F 12511 accounts for the decrease of plasma VLDL, LDL, and apo B-100 whereas a reduction of the LDL production rate has been described as the main mechanism underlying atorvastatin effect. In a specific experiment, the co-administration of threshold doses of F 12511 and atorvastatin (0.63 and 1.25 mg/kg/day, respectively) lowers plasma total cholesterol and apo B-100 containing lipoproteins to a greater extent and more rapidly than either agent alone. In the liver, a decrease in free cholesterol substrate for ACAT may emphasize the effect of F 12511 on cholesteryl ester content leading to a diminution at least in an additive manner of the assembly and secretion of atherogenic lipoproteins in the EH rabbit model. Therefore, the combination of the ACAT inhibitor F 12511 with atorvastatin can represent a better approach than either agent alone to regulate lipoprotein secretion in certain pathophysiological situations.

TuP4:W16

**Antithrombotic effect of the ACAT inhibitor F 12511 in casein-fed New-Zealand rabbit**


The putative anti-atherosclerotic properties of F 12511, a novel ACAT inhibitor, were addressed in New-Zealand rabbits fed a cholesterol-free casein-rich diet which develop fibro-fatty pre-atheroma lesions. During the endogenous hypercholesterolemia (EH) established for 32 weeks, an endothelial abrasion was carried out in abdominal aorta by balloon denudation at 6 weeks of casein feeding, and F 12511 was administered in diet (8-10 mg/kg) for the last 24 weeks. Total plasma cholesterol level rose up to 250-300 mg/dl in both groups of rabbits before starting the pharmacological treatment; F 12511 time-dependently reduced total plasma cholesterol by about 50%. Incidence of lesions in uninjured aorta (thoracic arch, coeliac bifurcation) assessed by immunostaining were 50% decreased after F 12511 treatment; lesions in this treated-group are characterized by few macrophages localized principally in the endothelium and by a larger content of collagen and smooth muscle cells suggesting features of plaque stability. Quantitative image analysis of standardized sections of mechanically injured abdominal aorta (renal to iliac artery) revealed a 20% surface covered by pre-atheroma lesions in the placebo group while F 12511 almost suppressed them. These data suggest that the combination of EH with endothelial injury in the rabbit may be an useful model to test antithrombotic compounds, lipoprotein lowering, by the ACAT inhibitor F 12511 not only reduces the incidence of vascular lesions and macrophage infiltration but also may reinforce the fibrous skeleton of the atheroma.

TuP5:W16

**Dose-dependent cholesterol-lowering effects of D-003 on normocholesterolemic rabbits**

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D-003 is a mixture of higher primary aliphatic acids, wherein octacosanone acid represents the major component. The present study was undertaken to evaluate the effects of D-003 (5, 25, 100 and 200 mg/kg) administered orally for 30 days on lipid profile of normocholesterolemic New Zealand rabbits as well as the effects of the withdrawal of the treatment for the next 30 days. Animals were adapted to experimental conditions for 15 days and randomized in 5 experimental groups: a control group, only receiving orally equivalent volumes of the vehicle and 4 groups treated with the different doses of D-003 (5-200 mg/kg). After 30 days, D-003 significantly (p < 0.01) and dose-dependently reduced both total cholesterol (27%–37%) and low density-licpoprotein cholesterol (LDL-C) (64.5%–84.4%). In addition, doses from 25 to 100 mg/kg significantly increased (p < 0.01) HDL-C values, although such increase was not dose-dependent. No drug-related effects on triglycerides were observed. The control group did not show significant changes on lipid profile during the experiment. The effects of D-003 on TC, LDL-C and HDL-C were reversible after washout, with a complete recovery to baseline values after 30 days on washout period, but no rebound effects were observed. In addition, no evidences on any drug-related toxicity regarding to mortality, food consumption, weight gain and blood biochemistry safety indicators were observed. It is concluded that D-003 shows reversible cholesterol-lowering effects characterized by a dose-dependent reduction of TC and LDL-C accompanied by a not dose-dependent increase in HDL-C levels.

TuP6:W16

**Inhibition of cholesterol biosynthesis in cultured fibroblasts by D003, a mixture of very long chain saturated fatty acids**

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The present study was undertaken to investigate the effects of D003, a mixture of very long chain saturated fatty acids isolated and purified from sugar cane wax, on cholesterol biosynthesis in cultured fibroblasts. Treatment of cultured fibroblasts with D003 (0.05–50 μg/ml) inhibits in a dose-dependent manner cholesterol biosynthesis from 14C-labeled acetate (33–68%). Also, the addition of D003 at concentrations that inhibited cholesterol biosynthesis (5 μg/ml) significantly decreased incorporation of radioactivity from 14C into sterols, but not 14C-cholesterol. These data suggest an effect on 3-hydroxy-3-methylglutaryl coenzyme A (HMGC-CoA) reductase, the main regulatory enzyme in the cholesterol biosynthesis pathway. However, when added at the incubation mixture (5–50 μg/ml), D003 did not suppress preformed reductase. Thus, there was no evidence for a competitive or non-competitive inhibition of enzyme activity by D003. Treatment of intact fibroblasts with D003 at concentrations inhibiting cholesterol biosynthesis, also inhibits HMGC-CoA reductase activity (65%). This suggests that D003 is effective in regulating reductase when added to the medium of cultured cells. Since the suppressive action of D003 was observed in metabolic conditions under which reductase up-regulation was enhanced, an interference with enzyme synthesis and/or degradation can not be ruled out. However, the precise mechanism for the inhibition of cholesterol biosynthesis is not yet clear and further studies are needed to clarify the precise mechanism of the inhibitory action of D003 on cholesterol biosynthesis.

TuP7:W16

**Desirable lipid-lowering profile of novel MTP inhibitor**

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Objective: Microsomal triglyceride transfer protein (MTP) plays an important role in the assembly and secretion of apoB (apoB)-containing lipoproteins. MTP inhibitors could be used to treat hyperlipidemic patients. In general, excessive apoB appears to be degraded when apoB-containing lipoprotein secretion is reduced by the MTP inhibition. However, the inhibition of MTP might be attended by the risk of hepatic triglyceride (TG) accumulation. It is undesirable to induce liver TG accumulation in hyperlipidemic treatment and such accumulation might cause fatty liver. In the present study, we found that novel MTP inhibitor successfully lowered serum TG levels without excessive accumulation of liver TG in rats.

Methods: To investigate whether MTP inhibitors should induce accumulation of liver TG, previously-reported MTP inhibitor or our novel MTP inhibitor (0.3–90 mg/kg) was orally administered to high fructose-fed rats. After 8 days treatment serum and liver TG levels were enzymatically measured. We also studied the effects on incorporation of [(14)C]-acetate or [(14)C]-glycerol into cellular lipid fractions. HepG2 cells were incubated with labeled-substrate and compounds for 4 or 24 hr. The cellular lipid fractions were extracted and separated by thin layer chromatography.

Results: Both of MTP inhibitors significantly reduced serum lipid levels. Only in the case of our compound the liver TG accumulation was not observed at effective doses. Our compound also reduced newly synthesized TG accumulation in HepG2 cells.

Conclusions: Our novel MTP inhibitor showed desirable lipid lowering profile, which meant potent serum TG lowering activity without liver TG accumulation in high fructose and rats at pharmacological dose. These effects might depend on the abilities of our MTP inhibitor that possesses inhibitory actions of TG biosynthesis.
TuP8:W16  Safety and efficacy of micronised fenofibrate 267 mg once daily for one year in type II dyslipidaemic patients at risk for coronary heart disease

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The risk of coronary heart disease was set up by the European Atherosclerosis Society (EAS) according to plasma cholesterol concentration and/or to the association of other risk factors. The safety and efficacy of micronised fenofibrate 267 mg once daily for 1 year were evaluated in an open study in 199 patients (92 women/107 men, mean age 51.9) with total cholesterol (TC) above 7.8 mmol/l (300 mg/dl) and/or LDL-cholesterol above 5.5 mmol/l (215 mg/dl) after 2 month diet, both considered as major risk factors according to EAS guidelines.

Among 28 patients prematurely withdrawn, 11 were withdrawn for mild adverse events. Creatinine increased by 9.8% and 9.2% after 6 and 12 months of treatment, respectively. ALAT and/or ASAT significantly increased in 9 patients. Conversely, 95% of the patients did not present with any ALAT values above twice the normal limit over the treatment period.

Baseline values and per cent changes of lipids are given below:

<table>
<thead>
<tr>
<th>TC</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>Triglycerides</th>
<th>LDL-C/HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline mean (SD)</td>
<td>8.28 (0.98)</td>
<td>6.04 (0.99)</td>
<td>1.40 (0.44)</td>
<td>1.92 (1.18)</td>
</tr>
<tr>
<td>% change at 6 months</td>
<td>-25.8 (11.5)</td>
<td>-31.2 (14.8)</td>
<td>+6.2 (17.4)</td>
<td>-34.5 (26.5)</td>
</tr>
<tr>
<td>% change at 12 months</td>
<td>-25.9 (12.4)</td>
<td>-31.2 (15.6)</td>
<td>+7.1 (19.2)</td>
<td>-35.5 (26.9)</td>
</tr>
</tbody>
</table>

a+12.1% and +15.1% at 6 and 12 months, respectively, in patients with baseline TG > 2.3 mmol/l

The mean decrease in apo B was 27% both after 6 and 12 months of treatment. The mean values for all lipid parameters after 6 and 12 months of treatment were statistically different from baseline (p < 0.001). At the end of treatment, 41.5% and 43.5% out of 193 patients had a TC below 5.9 mmol/l (230 mg/dl) and a LDL-C below 4 mmol/l (155 mg/dl), respectively.

Micronised fenofibrate 267 mg once daily was well tolerated and with a sustained efficacy over one year in the treatment of patients at a high risk for coronary heart disease related to major increase of LDL-C.

TuP9:W16  BAY 13-9952 (Impitaplate): Pharmacodynamic effects of a new and highly active inhibitor of the microsomal-triglyceride-transfer-protein (MTP)

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Objective: BAY 13-9952 has shown to potentily inhibit the MTP-activity and secretion of apoB-containing particles in vitro. Studies in rats and dogs should demonstrate the pharmacological profile of this new therapeutic principle and the pharmacodynamic effects of BAY 13-9952.

Methods: The postprandial (pp) TG rise was investigated after oral olive oil loading in rats up to 3 hours pp. The hepatic VLDL secretion was studied after intravenous injection of lipoprotein lipase inhibitor Triton WR 1339 and determination of plasma TG. Acute and subchronic lipid lowering (TG and CHOL) effects of BAY 13-9952 were investigated in genetically hypertriglyceridemic fa, fa-Zucker rats and normolipidemic dogs.

Results: The pp TG absorption was reduced by 50% with 0.3 mg/kg body weight p.o. (ED50-value). The hepatic VLDL secretion in rats was decreased by 50% after 1 mg/kg body weight p.o. After acute administration to fa, fa-Zucker rats BAY 13-9952 effectively reduced plasma TG and CHOL concentrations by 50% after 1.5 mg/kg and TG up to 80% after 4.5 mg/kg b.w. In subchronic 4-week studies only 0.5 mg/kg/day reduced the TG levels by 84%. In subchronic dog studies, 4 mg BAY 13-9952 lowered plasma TG levels by 60%.

Conclusion: In acute and subchronic rat and dog studies BAY 13-9952 effectively reduced pp serum TG rises as well as plasma TG and CHOL concentrations. This pharmacological approach of inhibiting MTP-activity and secretion of apoB-containing particles may offer a new therapeutic principle for the treatment of combined hyperlipidemia and arteriosclerosis.

TuP10:W16  Effect of pentoxifylline on ROS generation in patients with transient myocardial ischaemia

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The aim of our study was to estimate the effect of pentoxifylline (PTX) on reactive oxygen species (ROS) generation by unprimed neutrophils and those primed with tumour necrosis factor (TNF-α) in patients with transient myocardial ischaemia (exercise test and PTCA procedure). Chemiluminescence of unprimed as well as primed with TNF-α neutrophils was measured. Forty patients with stable angina were examined in whom exercise test was applied on cycloergometer and, after three days, PTCA was performed. The patients were randomly allotted into two groups. The study group consisted of 26 patients who were administered PTX orally (3 × 400 mg/day) and the control group of 14 patients with no drug administration. Our study has been approved by the local Ethics Committee. Blood samples were collected for examination from cubital vein just before and 10 min after the exercise test or PTCA procedure. Chemiluminescence of fornyl-Met-Leu-Phe (fMLP) stimulated neutrophils was measured. Transient myocardial ischaemia caused the increase in ROS generation by neutrophils in the control group. In the study group PTX diminished ROS generation by unprimed neutrophils stimulated by fMLP before and after the exercise test and PTCA procedure, in comparison to the control group. Moreover, PTX diminished stimulated by fMLP chemiluminescence of TNF-α primed neutrophils before the exercise test and PTCA procedure. No TNF-α effect on neutrophils observed after transient myocardial ischaemia caused by exercise test or PTCA procedure can point to endogenous release of this cytokine during transient myocardial ischaemia.

TuP11:W16  The potent cholesterol absorption inhibitor, ezetimibe, ablates hypercholesterolemia and hypertriglyceridemia in a model of combined hyperlipidemia

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Objective: Ezetimibe (SCH458235) is a member of a unique class of compounds that potently and selectively inhibits cholesterol absorption in the intestine, thereby reducing plasma cholesterol levels in preclinical models of hypercholesterolemia, and in humans. We assessed the effect of ezetimibe on diet-induced combined hyperlipidemia.

Methods: Hamsters were fed chow, chow with 0.12% cholesterol, or the same 0.12% cholesterol diet containing 15% (w/w) dietary triglycerides comprising C14:0, C18:1, or C18:2 fatty acids in the absence or presence of 1 mg/kg ezetimibe for 84 days. Cholesterol and triglycerides in serum and lipoprotein fractions, and liver cholesterol ester and free cholesterol, were analyzed as endpoints for efficacy of the drug.

Results: Moderate cholesterol and moderate cholesterol/high-fat diets with or without ezetimibe induced combined hyperlipidemia to varying degrees compared with a diet of chow alone. The presence of ezetimibe in the diet normalized serum cholesterol to nearly chow-fed levels under all dietary conditions. Ezetimibe normalized VLDL cholesterol to chow-fed levels and significantly decreased LDL cholesterol to below chow-fed levels. The ratio of HDL to LDL cholesterol increased significantly with the addition of ezetimibe. Hypertriglyceridemia induced by the presence of dietary fat was also vastly reduced to nearly chow-fed levels in ezetimibe-treated hamsters. The presence of ezetimibe completely eliminated the accumulation of cholesterol ester and free cholesterol in the liver that was induced under the various dietary conditions.

Conclusion: Ezetimibe is very effective in blocking the combined hyperlipidemia induced by the combination of moderate cholesterol and high-fat in hamsters, and may be an effective therapy for ameliorating combined hyperlipidemia in humans.

TuP12:W16  The effect of metoprolol CR/XL on atherosclerosis

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Objective: To investigate whether metoprolol CR/XL treatment might affect subclinical atherosclerosis in subjects with hypercholesterolemia.

Methods: Subjects with primary hypercholesterolemia (total cholesterol ≥ 6.5 and LDL cholesterol ≥ 5.5 mmol/L) and also fulfilling the following ultrasound criteria: a maximum intima-media thickness (IMT) > 1.0 mm and/or a measurable plaque in the far wall of the carotid artery were recruited.
to the present three-year prospective, randomised, placebo controlled, double-blind study. A total of 103 subjects started double blind treatment: 47 in the metoprolol CR/XL (100 mg) group and 56 in the placebo group. Twelve patients in each group withdrew from treatment. Results are reported for the remaining 79 subjects. Subclinical atherosclerosis was measured by B-mode ultrasound in the carotid artery each year during follow-up.

Results: Thirty-nine subjects in the placebo group and 33 subjects in the metoprolol CR/XL group were treated with statins with similar dose levels and total cholesterol was reduced from 9.4 to 6.0 mmol/L in the metoprolol CR/XL group and from 8.7 to 6.2 mmol/L in the placebo group. Furthermore, IMT of the common carotid artery in the metoprolol CR/XL group decreased from 0.905 ± 0.232 mm to 0.869 ± 0.169 mm and increased from 0.892 ± 0.177 mm to 0.922 ± 0.188 mm in the placebo group (p < 0.05 for difference in change between groups). The decrease in IMT in the metoprolol CR/XL group was obvious already after the first year of follow-up.

Conclusion: In subjects with hypercholesterolemia treated with lipid lowering drugs, the addition of 100 mg of metoprolol CR/XL seems to beneficially affect atherosclerosis development, as measured by IMT. This may reflect direct effects of metoprolol on arterial tissue.

TuP13W16

The cholesterol absorption inhibitor ezetimibe inhibits the development of atherosclerosis in apo E knockout (−/−) mice fed low fat and western diets

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Objective: To determine if ezetimibe (SCH58235) reduces cholesterol absorption and inhibits atherogenesis in apo E knockout mice fed western and low fat diets containing cholesterol.

Methods: Ezetimibe (1, 3, and 10 mg/kg) was evaluated for inhibition of 14C-cholesterol absorption (acute, 2 h) in apo E−/− and −/− mice. Atherosclerosis and lipoprotein changes were determined in apo E−/− mice fed diets containing 0.15% cholesterol and 40% lard fat (western) or 10% corn oil (LDL cholesterol) for 6 months.

Results: Apo E−/− and −/− mouse cholesterol absorption was inhibited by >90% by ezetimibe at doses > 3 mg/kg. The plasma cholesterol levels in the apo E−/− mice were reduced from 964 mg/dl to 374 mg/dl in the western diet groups and from 726 mg/dl to 231 mg/dl in the low fat groups by ezetimibe (5 mg/kg/day) after 6 months of treatment. These reductions occurred in the VLDL and LDL lipoprotein fractions, while HDL levels were increased by ezetimibe. Aortic atherosclerotic lesion surface area was reduced from 20.24% to 4.06% in the western diet group and from 24.11% to 7.04% in the low fat group by ezetimibe. The cross sectional area of the carotid artery lesions were reduced 97% by ezetimibe from 0.098 mm2 to 0.003 mm2 and 0.142 mm2 to 0.004 mm2 in the western and low fat groups, respectively.

Conclusion: Ezetimibe inhibits cholesterol absorption, reduces plasma cholesterol, and increases HDL levels in apo E−/− mice under both western and low fat dietary conditions. Ezetimibe inhibited the progression of atherosclerosis in both western and low fat cholesterol-fed apo E−/− mice. Ezetimibe may inhibit atherogenesis clinically in individuals consuming restricted fat or western diets.

TuP14W16

Increased serum concentrations of sitostanol in volunteers after feeding of sitostanol olate enriched margarine

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Objective: Previous studies have indicated that sitostanol is not absorbed in man in contrast to other plant sterols ingested with a normal diet. The present study was undertaken to measure serum concentrations of sitostanol before and after consumption of margarine enriched with sitostanol olate.

Methods: Blood samples from 12 male normolipemic volunteers (aging 23–32 yrs.) were taken before and one week after the ingestion of 1.5 g sitostanol/day. Total and LDL cholesterol in serum were measured enzymatically and plant sterols by gas-liquid chromatography. Serum concentrations of sitostanol were measured by a new sensitive isotope dilution method using gas-liquid chromatography/mass spectrometry with 5, 6, 22, 23 [3H]-sitostanol as internal standard.

Results: Serum total and LDL cholesterol decreased significantly by 7.3% (p < 0.005) and 12.4% (p < 0.02), respectively. The ratio of campesterol to cholesterol, a marker for cholesterol absorption, declined significantly from 2.62 μg/mg to 2.26 μg/mg (p < 0.001). Serum concentrations of sitostanol increased from 2.2 μg/dl to 9.4 μg/dl (p < 0.001), and decreased to pre-treatment levels two weeks after stopping margarine ingestion.

Conclusion: The results confirm that sitostanol olate reduces serum cholesterol by inhibiting the efficiency of intestinal cholesterol absorption. However, for the first time, it could be demonstrated by a sensitive isotope dilution method that sitostanol is present in serum of healthy volunteers and increases significantly during feeding of sitostanol olate. After stopping sitostanol olate intake normal serum concentrations of sitostanol appear already two weeks later.

Supported by a grant of BMBF (01EC0402).

TuP15W16

Low-density lipoprotein cholesterol reduction by SCH 58235 (ezetimibe), a novel inhibitor of intestinal cholesterol absorption in 243 hypercholesteremic subjects: Results of a double-blind study


Objective: A multicenter, double-blind, randomized, placebo-controlled, parallel group study of SCH58235 (ezetimibe), a novel inhibitor of intestinal cholesterol absorption, was designed to confirm a prior dose-ranging study of the effect of ezetimibe on low-density lipoprotein cholesterol (LDL-C).

Methods: Following dietary stabilization on National Cholesterol Education Program (NCEP) Step 1 or stricter diet, lipid-lowering-drug washout, and a 6-week placebo lead-in period, 243 subjects with baseline LDL-C > 130 mg/dl, ≤250 mg/dl, and triglycerides ≤ 300 mg/dl, were randomized to placebo, or to ezetimibe 0.25, 1, 5, or 10 mg dosed daily for 12 weeks.

Results: Ezetimibe at all doses tested significantly reduced directly measured LDL-C from baseline compared with placebo (P < 0.01). This effect was significant following 1 week of therapy, the first timepoint examined, and achieved maximal or near maximal response by 2 weeks. The mean percent reduction in directly measured LDL-C at study endpoint is presented below:

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Mean % Change in LDL-C (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n=51)</td>
<td>4.3 ± 1.4</td>
</tr>
<tr>
<td>Ezetimibe 0.25 mg (n=46)</td>
<td>9.9 ± 1.5</td>
</tr>
<tr>
<td>Ezetimibe 1 mg (n=40)</td>
<td>12.6 ± 1.5</td>
</tr>
<tr>
<td>Ezetimibe 5 mg (n=49)</td>
<td>16.4 ± 1.4</td>
</tr>
<tr>
<td>Ezetimibe 10 mg (n=46)</td>
<td>18.7 ± 1.5</td>
</tr>
</tbody>
</table>

The safety profile of the ezetimibe-treated groups was no different from that of the placebo group.

Conclusion: Ezetimibe is safe and effective, and its effect to lower LDL-C is rapid in onset.

TuP16W16

Efficacy of combination of atorvastatin and micronized fenofibrate for the treatment of severe mixed lipid disorders

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Objective: To evaluate the efficacy of fenofibrate in combination with a high dose of atorvastatin in patients with severe mixed lipid abnormalities.

Methods: This was a 18-week, open-label study conducted in our lipid clinic. After a 6-week dietary baseline phase we implemented a treatment phase, during which patients received 200 mg/day micronized fenofibrate for 6 weeks. After the end of this period the subjects stopped this treatment and took 40 mg/day atorvastatin for 6 weeks. Finally 200 mg/day of micronized fenofibrate were added to the statin therapy. Serum lipid profiles, including levels of lipoprotein(a) and fibrinogen, as well as muscle and liver enzymes, were measured during screening, and at weeks 4, 8, 12, and 18 of the treatment period.

Results: Administration of fenofibrate reduced significantly serum triglycerides from 380 ± 25 mg/dl to 230 ± 40 mg/dl, total cholesterol (TC) from 340 ± 35 mg/dl to 290 ± 30 mg/dl and LDL-cholesterol (LDL-C) from 230 ± 35 mg/dl to 210 ± 40 mg/dl (p < 0.05 for all parameters). Moreover, a significant increase in serum HDL-cholesterol levels (p < 0.05) was observed. When the patients received atorvastatin alone a more pronounced decrease of TC and LDL-C (220 ± 25 and 145 ± 24 mg/dl, respectively) was observed. However, the plasma levels of TG although significantly lower compared
to baseline values (275 ± 32 mg/dl vs 380 ± 25 mg/dl) were higher than the values observed during the treatment with fenofibrate. Moreover, serum HDL cholesterol concentrations were higher and the plasma fibrinogen levels lower during the fibrate therapy phase compared to the statin therapy phase. During the combination treatment the lipid profile was significantly better than that obtained on single-agent therapy as TC, TG and LDL-C were 196 ± 32 mg/dl, 205 ± 24 mg/dl, 133 ± 22 mg/dl, respectively.

Conclusions: Combination of high dose of atorvastatin and micronized fenofibrate is useful for the treatment of severe mixed lipid disorders.

**TuP17/W16** Is micronised fenofibrate as effective on HDL-C in women and in men?

D. Delaval, H. Salomon. **Laboratoires Fournier, Garches, France**

**Objective:** Low HDL-C levels (HDL) predict an increased risk of coronary heart disease (CHD). The recognized level for increased CHD risk in EAS guidelines (1998) is <40 mg/dl. The HDL increase with Micronised Fenofibrate (Feno 200M) observed in a large open label study (n = 7098), was shown to be baseline dependent, reaching a 90.2% increase in patients with baseline HDL < 25 mg/dl. The present analysis aims at describing in the same study the efficacy of Feno 200M on HDL according to gender in the at-risk sub-groups of HDL < 35 and <40 mg/dl.

**Methods:** From 4014 men (mean age 55, BMI 27) and 3035 women (mean age 60, BMI 27) respectively, subgroups of patients were described on mean HDL level at baseline and after 12 weeks of Feno 200M treatment.

**Results:**

<table>
<thead>
<tr>
<th>HDL</th>
<th>Baseline</th>
<th>HDL &lt; 35 mg/dl</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>29.8</td>
<td>42.2 (±8.35)</td>
<td>33.1</td>
</tr>
<tr>
<td>Total</td>
<td>29.4</td>
<td>41.5 (±4.43)</td>
<td>32.5</td>
</tr>
</tbody>
</table>

**Conclusions:** Feno 200M is similarly effective in low HDL dyslipidemic women and men in increasing HDL, with endpoint mean levels greater than 40 mg/dl. Low HDL dyslipidemic patients should benefit from therapy which raises their HDL levels.

**TuP18/W16** Efficacy and safety of usual doses of atorvastatin in primary coronary artery disease. **Short study**

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**Introduction:** The clinical benefits of reducing LDL cholesterol (LDL-C) have been well established in several large-scale controlled interventions with statins. Current guidelines, designed in accordance with these results, stress the importance of achieving the targeted LDL-C goals in order to obtain the best results. The higher the global risk the lower the targeted LDL-C. However, few data are available about the results of lipid-lowering therapy under usual clinical conditions.

**Objectives:** To assess the efficacy and safety profile of Atorvastatin at usual doses (10–20 mg) in the context of the routine by Spanish primary care physicians and its effectiveness to achieve LDL-C goals according to the guidelines of the Spanish Arteriosclerosis Society (SEA).

**Methods:** This 6 months observational, multicentre, prospective, pharmacovigilance study included patients with primary hypercholesterolemia or combined hyperlipidemia. The physicians prescribed them Atorvastatin 10 mg because of their inadequate LDL-C levels, according to Spanish guidelines. Two months later, if LDL-C target was not achieved, the dose was doubled. A final control was done at the end of the 6th month.

**Results:** A group of 1351 physicians initially recruited 6769 patients, 5317 were included and 4033 finished the study. Twenty nine percent of included patients (1564 cases) were receiving previously lipid-lowering drugs. Only 1 patient was withdrawn due to lack of efficacy. Mean total cholesterol was 288 ± 28 mg/dl (range 201–414). Treatment with Atorvastatin reduced total cholesterol, LDL-C and total triglycerides by 27.6%, 36% and 19% respectively, with increases of 17% in HDL cholesterol. Eighty-nine patients (1.7%) had adverse events related to the drug and only 2 of them (0.04%) had serious adverse events. At the end of the 6th month, according to SEA guidelines, 66% of the treated patients achieved targeted LDL-C goals, ranging from 26% for very high risk patients (previous coronary heart disease) to 100% for low risk participants.

**Conclusions:** Atorvastatin is a safe and effective lipid-lowering drug to be used in usual clinical practice. Atorvastatin also has a great effectiveness in achieving LDL-C goals recommended by the Spanish Arteriosclerosis Society.

**TuP19/W16** The ACAT inhibitor avasimibe increases the fractional clearance rate of postprandial triglyceride-rich lipoproteins

J.R. Burnett, P.H.R. Barret, D.E. Telford, M.W. Hutf, J. Royal Perth Hospital; University of Western Australia, Perth, Australia; The John P. Roberts Research Institute, University of Western Ontario, London, Canada

Previously, we have shown, in vivo, that the acyl coenzyme A: cholesterol acyltransferase (ACAT) inhibitor avasimibe decreases hepatic apolipoprotein B (apoB) secretion into plasma. To test the hypothesis that avasimibe modulates exogenous triglyceride-rich lipoprotein (TRL) metabolism in vivo, an oral fat load (2 g fat/kg) containing retinol was given to 9 control miniature pigs and to 9 animals after 28 d treatment with avasimibe (10 mg/kg/d, n = 5; 25 mg/kg/d n = 4). The kinetic parameters for plasma retinyl palmitate (RP) metabolism were determined by multi-compartmental modeling using SAAM II. Avasimibe decreased the 2-h TRL (d < 1.06 g/ml; S<sub>2</sub> > 20) triglyceride concentrations by 34% (p < 0.01). The TRL triglyceride 0–12 h area under the curve (AUC) was decreased by 21% (p < 0.06). In contrast, avasimibe had no effect on peak TRL RP concentrations, time to peak, or its rate of appearance into plasma, however, the TRL RP 0–12 h AUC was decreased by 17% (p < 0.05). Analysis of the RP kinetic parameters revealed that the TRL fractional clearance rate (FCCR) was increased 1.4-fold (p < 0.002) with avasimibe. Avasimibe decreased the percent conversion of TRL RP from the rapid to the slow turning-over compartment by 35% (p < 0.09). The TRL RP FCCR was negatively correlated with very low density lipoprotein (VLDL) apoB production rate measured in the fasting state (r = -0.504; p = 0.0001). No change in total intestinal lipid concentrations were observed. Thus, although avasimibe had no effect on intestinal TRL secretion, plasma TRL clearance was significantly increased, an effect that may relate to a decreased competition with hepatic VLDL for removal processes.

**TuP20/W16** Extended-release niacin versus gemfibrozil for treatment of low HDL cholesterol


**Objective:** To compare agents that raise plasma levels of high density lipoprotein cholesterol (HDL-C).

**Methods:** In a randomized, double-blind trial, we compared an extended-release niacin, Niaspan<sup>®</sup>, at doses from 1000 mg to 2000 mg h.s., versus gemfibrozil 600 mg b.i.d. in 173 patients selected for HDL-C ≤ 40 mg/dl. Patients also had low density lipoprotein cholesterol (LDL-C) > 160 mg/dl or <130 mg/dl with atherosclerotic disease, and triglycerides ≤ 400 mg/dl.

**Results:** Niaspan at 1500 and 2000 mg raised HDL-C (21% and 26%, respectively) more than gemfibrozil (13%), raised apoA-I more (9% and 11%, versus 4%), reduced lipoprotein(a) (7% and 20%, versus no change for gemfibrozil), and had no adverse effect on LDL-C (-2% and 0% change) versus a 9% increase in LDL-C with gemfibrozil. Gemfibrozil reduced triglycerides more than Niaspan (-40% for gemfibrozil, -16% to -29% for 1000 to 2000 mg Niaspan). All comparisons were statistically significant. Gemfibrozil also increased fibrinogen (5% to 9%) and Niaspan tended to decrease fibrinogen (-1% to -6%, p < 0.02 for difference between two treatments).

**Conclusions:** In patients with low baseline HDL-C, Niaspan provided a 2-fold greater HDL-C increases, decreases in lipoprotein(a), and lower fibrinogen compared to gemfibrozil. Gemfibrozil reduced triglycerides more, but also increased LDL-C, which did not occur with Niaspan.

**TuP21/W16** Colestilan: A new bile acids sequestrant resin, A review of its clinical study in hypercholesterolemia in Japan

N. Nakao<sup>®</sup>, Y. Goto<sup>®</sup>. MCI-196 study group; Fussa Hospital; Tokai University, Tokyo, Japan

**Objective:** To report the efficacy and safety of Colestilan; INN (colestamide; JAN; CHOLEBINE<sup>®</sup>; MCI-196), a new bile acids sequestrant resin, in the clinical study performed in Japan.
**TuP22:W16** Differences between cholestrylamine and colestidin (Colestidin), a novel hypolipidemic resin concerning regulation of hepatic cholesterol metabolism

K. Suzuki, N. Kaneko, A. Nemoto, H. Shimada, Mitsubishi-Tokyo Pharmaceutical Inc., Yokohama, Japan

**Aim:** Hypolipidemic activity of Colestidin (CLT) is four times more potent than that of Cholestrylamine (CSA) whereas in vitro binding affinities to bile acids of CLT is about two times less than CSA. To elucidate the differences, effect on the activity of cholesterol 7α-hydroxylase (C7H) and HMG-CoA reductase (HMG-R) activity by prolonged treatment (14, 28 days) of both resins.

**Method:** Rat's hepatic microsomal C7H and HMG-R activity was determined by using [14C]-cholersterol and [14C]-acetate as a substrate.

**Result:** At dose of 250 mg/kg in rats, both resins inhibited re-uptake of bile acids in the intestine and equally lowered their portal vein bile acids concentration by about 40%. Administration of CLT and CSA for 14 days significantly increased C7H activity by 282%, 230%, respectively and HMG-R activity by 250%, 180% (Table). In 28 days treatment of CLT, C7H and HMG-R activity was kept high levels (189%, 235%). Treatment of CSA increased HMG-R by 183%, whereas C7H activity reverted to normal (102%).

**Table:** Effect on hepatic C7H and HMG-R activities in rats

<table>
<thead>
<tr>
<th>Duration days</th>
<th>Chol 7αH Act, %</th>
<th>HMG-R Act, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CLT CSA</td>
<td>CLT CSA</td>
</tr>
<tr>
<td>0</td>
<td>100 100</td>
<td>100 100</td>
</tr>
<tr>
<td>14</td>
<td>282* 230*</td>
<td>230* 180*</td>
</tr>
<tr>
<td>28</td>
<td>180* 102</td>
<td>102 183*</td>
</tr>
</tbody>
</table>

Dose of CLT, CSA: 250 mg/kg/day; Values are expressed % of non-treatment control

**Conclusion:** These results suggested that one of the possible reasons for potent hypolipidemic efficacy of CLT is due to maintain increasing C7H activity, namely bile acids production. There could be the different regulation on cholesterol metabolism between CLT and CSA.

**TuP23:W16** RAY 13-9952 (Impiltapide), an inhibitor of the microsomal triglyceride transfer protein (MTP), inhibits atherosclerosis and prolongs lifetime in apoE knockout mice

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**Objective:** To demonstrate anti-atherosclerotic effects of the MTP-inhibitor Bay-13-9952 and to investigate whether MTP inhibition has a positive effect on life expectancy in apoE knockout mice.

**Methods:** All animals were fed with a Western-type diet containing 0.5, 15 or 45 ppm of Bay 13-9952. In an atherosclerosis study animals (n = 15/group) were sacrificed after 14 weeks of treatment and cross-sectional plaque areas of the aortic root were determined by a computer-aided morphometric system. In a subsequent study, the animals (n = 25/group) were checked daily for survival.

**Results:** Bay 13-9952 led in all dosages to a significant reduction of lipid concentrations and atherosclerotic lesions. The average cross-sectional plaque area in the 0 ppm control group was 0.135 mm². The corresponding values in the 5, 15 and 45 ppm Bay 13-9952-treated groups were 0.046 mm², 0.034 mm² and 0.009 mm², corresponding to reductions of 66%, 75% and 93%, respectively. In the ongoing survival study only one animal was still alive in the 0 ppm group after 18 months (4% survival). The number of surviving animals dose-dependently increased in the treated groups: 5 animals were still alive in the 5 ppm group, 15 in the 15 ppm group and 24 in the 45 ppm group. This corresponds to survival rates of 20%, 60% and 96%, respectively.

**Conclusion:** The MTP-inhibitor Bay 13-9952 dose-dependently suppresses atherosclerotic plaque formation in apoE knockout mice and improves survival.

**TuP24:W16** Pharmacodynamic interaction between the new selective cholesterol absorption inhibitor ezetimibe and simvastatin

T. Kosoglu1, I. Meyer2, B. Musiol3, L. Mellars1, P. Statkeych1, M.P. Miller1, P.P. Som1, M.B. Affrime1, 1Schering-Plough Corp., Kenilworth, NJ, USA; 2QuintilesInnovex, GmbH, Freiburg, Germany

**Objective:** We evaluated a potential pharmacokinetic and/or pharmacodynamic interaction between ezetimibe (EZE) and simvastatin (SIM).

**Methods:** In a randomized, evaluator-blind, placebo-controlled, parallel-groups study S8 healthy male hypercholesterolemic (screening LDL-C ≥ 130 mg/dl) stabilized and maintained on a NCEP Step 1 Diet were randomized to one of the following five treatments: SIM 10 mg, SIM 10 mg plus EZE 0.25, 1 or 10 mg or placebo orally, every morning for 14 days.

**Results:** No serious or unexpected adverse events (AEs) were reported. Reported AEs were generally mild, non-specific and similar between treatments. There were no significant increases in clinical laboratory tests associated with any of the treatments, particularly those tests assessing liver function. EZE had no effect on the pharmacokinetics of SIM. The mean (S.E.) Day 14 %change from Baseline in serum lipids was:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LDL-C</th>
<th>Total-C</th>
<th>HDL-C</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo, n = 11</td>
<td>-3.2 (5.1)</td>
<td>-4.1 (5.3)</td>
<td>-20.0 (4.6)</td>
<td>34.5 (7.5)</td>
</tr>
<tr>
<td>SIM 10 mg, n = 12</td>
<td>-34.9 (3.2)</td>
<td>-28.4 (2.4)</td>
<td>-8.1 (3.3)</td>
<td>-19.2 (5.8)</td>
</tr>
<tr>
<td>SIM 10 mg + EZE 0.25 mg, n = 11</td>
<td>-37.5 (4.6)</td>
<td>-30.8 (3.6)</td>
<td>-9.5 (3.6)</td>
<td>1.1 (5.1)</td>
</tr>
<tr>
<td>SIM 10 mg + EZE 1 mg, n = 12</td>
<td>-40.1 (3.1)</td>
<td>-32.2 (2.3)</td>
<td>-9.4 (2.5)</td>
<td>-14.1 (5.9)</td>
</tr>
<tr>
<td>SIM 10 mg + EZE 10 mg, n = 11</td>
<td>-51.9 (2.6)</td>
<td>-36.6 (3.3)</td>
<td>-12.6 (4.1)</td>
<td>-6.2 (10.4)</td>
</tr>
</tbody>
</table>

**Conclusions:** The coadministration of EZE and SIM 10 mg was safe and well tolerated. EZE combined with SIM 10 mg caused a dose-dependent reduction in LDL-C, with no apparent effect on HDL-C or triglycerides. The coadministration of EZE 10 mg and low dose SIM has the potential to produce clinically significant reductions in LDL-C, comparable to maximum doses of sim alone, with a favorable safety profile.

**TuP25:W16** A comparison of fenofibrate and statins in raising low HDL-C

D. Delaval, Laboratoires Fournier, Garches, France

**Objective:** To compare the ability of fenofibrate and 3 commonly used statins to raise low HDL-C (HDL) and to assess the relative ability to raise HDL above 40 mg/dl, the suggested CHD risk threshold in 1998 EAS guidelines.

**Methods:** 3 randomised controlled published studies comparing fenofibrate to 3 commonly used statins (simvastatin, pravastatin, atorvastatin) at usual doses in low HDL baseline populations (<40 mg/dl), treated during 12–16 weeks, were selected for analysis.

**Results:** At usual doses, fenofibrate was statistically significantly superior to all 3 commonly used statins in raising low HDL. All the fenofibrate treatment groups were able to achieve an endpoint level greater than 40 mg/dl. All three statins had a relatively minor effect.

**Studies & populations**

- **HDL-C (mg/dl)**
  - Steinsmetz ($20.00 lb$)
  - Ducub (P20.00 lb)
  - Osi (A10)
Conclusions: Low HDL is a prevalent independent risk factor for CHD. Fenofibrate ability to raise low HDL to above 40 mg/dl in a manner superior to commonly used statins makes it as a good treatment option for patients with low HDL.

P:H3 IMPLEMENTATION OF PREVENTION PROGRAMMES

TuP1:H3 Extensive lifestyle management intervention following cardiac rehabilitation: Study design

Long-term risk factor and lifestyle management is crucial for the prevention of cardiovascular events. Current cardiac rehabilitation programs (CRP) are not adequately designed for long-term management. Adherence to diet, exercise, smoking cessation and medications declines significantly in the years following CRP. Implementation of a resource sparing follow-up risk factor and lifestyle management program may successfully reduce cardiovascular risk and health care utilization.

Hypothesis: A 4 year risk factor and lifestyle management intervention (LMI) following a standard CRP will result in greater adherence to beneficial lifestyle behaviours, reduction of global cardiovascular disease risk, reduction of health care utilization and reduction of cardiovascular events compared to usual care.

Methods: Over a 2 year period, 300 men and women, with ischemic heart disease will be recruited following a CRP and randomized to a LMI or usual care group. Intervention: Participants will attend additional cardiac rehabilitation sessions, receive telephone follow-up calls and risk factor and lifestyle counselling sessions. Health professionals and participants will work together to set appropriate risk factor and lifestyle behaviour goals using the stages of change model. Telephone follow-up calls will provide direction and assess progress between counselling sessions. Family physicians will be updated on participant’s progress and involved in participants’ management. Usual Care: Participants will return to the care of their family physician and return annually for outcome assessment.

Outcomes: Lifestyle adherence, global risk, health care utilization and cardiovascular events at year 4.

TuP2:H3 The design and background characteristics of the study on the primary prevention of coronary events with pravastatin among Japanese with mildly elevated cholesterol levels. (Japanese Mega Study)
Haruo Nakamura. Japanese Mega Study Group; Tokyo, Japan

Objectives: Although cholesterol-lowering treatment has been shown to reduce total and nonfatal coronary heart disease (CHD) in subjects with or without coronary disease, it is still uncertain whether benefit from the reduction of cholesterol in subjects extends to Japanese with mildly elevated serum cholesterol. Characteristic features of Japanese are lower death rate of CHD, relatively low serum cholesterol levels with relatively high HDL cholesterol, diets with less saturated fat and cholesterol, and relatively small physical constitutions.

Design: Japanese Mega Study is a randomized, diet-controlled trial designed to test the hypothesis that treatment with pravastatin will reduce risk of major vascular events in men (40 to 70 years old) and women (post-menopause to 70 years old) without preexisting vascular disease. A total of 2560 men and 5449 women with total cholesterol ranging from 220 mg/dl to 270 mg/dl, have been randomized equally to treatment with 10–20 mg Pravastatin/day or to dietary treatment alone. Mean total cholesterol was 242.6 mg/dl, mean LDL cholesterol was 155.9 mg/dl, mean HDL cholesterol was 57.3 mg/dl and median triglyceride levels were 128 mg/dl. Following an average 5 year intervention period, primary assessment will be made of the influence of this therapy on major vascular events (a combination of CHD, death, nonfatal myocardial infarction, and fatal and nonfatal stroke.)

TuP3:H3 Reassessing European attitudes about cardiovascular treatment (REACT) – Physicians’ survey
E.D.R. Hobbs, L. Erhardt. ’The Medical School, University of Birmingham, UK; ’Malmö University Hospital, Sweden

Objective: The REACT survey was designed to assess acceptance of and/or implementation of CHD and cholesterol treatment guidelines by primary care physicians. A separate arm of the survey assessed attitudes and behaviors of the general public towards cholesterol and CHD.

Methods: Telephone interviews were conducted with 754 physicians in five countries (France, Germany, Italy, Sweden and Great Britain).

Results: Some 90% of physicians agree with the content of current guidelines for management of high cholesterol. The majority of physicians believe cholesterol is managed well in existing CHD patients (80%) and in those at risk of CHD (68%), although lower figures were reported in Germany (73%) and 61%, respectively) and Great Britain (63% and 57%). A total of 45% of physicians felt that cholesterol treatment is not sufficiently aggressive; higher figures were reported in Sweden (52%) and the UK (56%). Most physicians (92%) believe their patients know that cholesterol is associated with cardiovascular disease. However, only 51% of the general public were aware that high cholesterol increases CHD risk. In addition, most physicians (94%) report discussing target cholesterol numbers with their patients, whereas only 47% of the public recall ever having discussed cholesterol levels with their doctor. When physicians were asked how cholesterol management could be improved the most common reply was the need for better patient education (49%).

Conclusions: Most physicians believe that cholesterol levels in CHD patients are managed well. However, this is not consistent with data from studies such as EURASPIRE which show that under-treatment of hypercholesterolemia in CHD patients is widespread. Physicians also appear to be underestimating their patients’ knowledge of cholesterol and associated risk of CHD. A significant opportunity to improve the management of CHD therefore remains.

TuP4:H3 A comparison of cardiovascular risk calculation algorithms
A. Wierzbicki, T. Reynolds, K. Gill, S. Alg, M. Crook. St. Thomas’ Hospital, London; Queen’s Hospital, Burton-on-Trent, UK

Objective: This study compared 9 different algorithms for determining the need for treatment in patients without cardiovascular disease.

Methods: Risk factor data was obtained on 400 patients and risks were assessed by the Framingham model, National Cholesterol Education Program (NCEP), European, British, New Zealand, British Regional Heart Study, St epithelial, Munster Heart Study (PROCAM) and Dundee guidelines, and a local general rule for atheroma treatment (GREAT).

Results: Patients were 56% male, aged 53.8 ± 2.13 years, 38% smoked, 55% had a family history, 37% familial hypercholesterolaemia (FH), 33% hypertension, 5.2% left ventricular hypertrophy; 31% moderate obesity and 15% were diabetic. The average cholesterol was 7.9 ± 2.6 mmol/L. Median Framingham risk was 1.66%/year and >2%/year in 37% and >3%/year in 17.5%. Treatment was required in 86% by NCEP, 70% by GREAT or European, 61% by New Zealand, 58% by British, 42% by Dundee, 40% by PROCAM, 25% by BRHS and 16% by Sheffield guidelines.

Conclusions: Different algorithms vary widely in their predicted need for drug treatment in this high-risk population with hyperlipidaemia.
Tuesday June 27, 2000: Read by Title Abstracts

**TU1W7**  
**FATTY ACIDS: THE LINK BETWEEN INSULIN RESISTANCE AND DYSLIPIDAEMIA**

**Tu1T:W7**  
**High protein, low carbohydrate weight loss diets in overweight subjects with the insulin resistance syndrome**

Peter M. Clifton, Manny Noakes. CSIRO, Adelaide, SA, Australia

**Objective:** To assess the role of high protein weight loss diets in subjects with insulin resistance.

**Methods:** High carbohydrate, low fat diets are commonly recommended for weight loss. In very low calorie weight loss diets a high protein level is used to minimise muscle loss. We have explored the question of whether a high protein level confers any advantages in a diet that has a modest 30% restriction of caloric intake in overweight subjects with one or more components of the insulin resistance syndrome. Forty nine subjects (10 males, 39 females) with a BMI of >30 (mean BMI 34.4) and either a triglyceride of >2 mmol/L (n = 29) or a fasting glucose of >6 mmol/L (n = 6) or a blood pressure of >140/90 (n = 11) were selected for the 12 week study and randomised into two groups matched for age, BMI, plasma glucose, cholesterol and triglyceride. One weight loss diet contained 15% of calories as protein while the other contained 30%. Both diets contained 35% fat with the same fatty acid composition and 6000 KJ/day.

**Results:** Weight loss was 9.0 kg (9.7%) with no difference between diets. Weight loss induced a 9% fall in total cholesterol, a 31% fall in plasma triglyceride and a 13% fall in HDL cholesterol. Fasting insulin fell by 46% and fasting glucose by 7%. Insulin levels during a 75 g oral glucose tolerance test were reduced by 25% on the normal protein diet and by 47% on the high protein diet (p <0.02 for diet effect) and a sex/diet interaction (p <0.02) with males showing almost all of the enhanced insulin fall on the high protein diet.

**Conclusions:** A high protein weight loss diet may enhance insulin sensitivity and prevent progression to impaired glucose tolerance.

**Tu2W7**  
**Association of body mass index and waist circumference with coronary risk factors in adolescents**

C. Posadas-Romero1, J. Zamora-González1, L. Yamamoto-Kimura, R. Posadas-Sánchez2, G. Carbos-Saldaña1, A. Hernández-Ortiz1,1. Depto de Endocrinología del Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico

**Objective:** To investigate the association of general obesity and central adiposity with the different coronary risk factors in adolescents.

**Methods:** Sampling design: A cross sectional study of junior high school students. **Risk factors:** Anthropometry and blood pressure (BP) were measured. Lipids, lipoproteins and glucose were quantified in a venous blood sample after a 12-hour fast.

**Results:** We studied 1281 boys and 1703 girls with a mean age of 13.5 ± 1.2. Systolic BP (SBP), diastolic BP (DBP) and triglycerides had the highest correlation with both body mass index (BMI) and waist circumference (WC). However, the highest correlations were with WC in boys. Multiple regression analysis showed that BMI was associated independently with SBP and DBP in boys and with SBP and LDL-C in girls. WC was independently associated with SBP, DBP, TC, LDL-C and TG in both sexes.

**Conclusions:** Our results confirm that in the adolescent population, the association with coronary risk factors is different for general and central obesity. Furthermore, waist circumference is a better predictor for coronary risk factors than BMI.

**Tu3W7**  
**Insulin resistance, obesity, and dyslipidemia as a predictor of cardiovascular diseases**

O. Kovaliowa, T. Asheuolova. Department of Internal Medicine, Kharkiv State Medical University, Ukraine

**Objective:** To investigate the relationships between insulin (INS) sensitivity, obesity (body mass index: BMI), blood pressure (BP) levels and lipid alterations as a predictor of cardiovascular diseases.

**Design and Methods:** Fasting blood glucose and INS after overnight fast and 2 hrs after 75 g oral glucose tolerance test (OGTT), BML, BP, total cholesterol (TC), HDL-cholesterol (HDL-C), triglycerides (TG) in 31 subjects were measured. They were classified in 2 groups: 1) gr (n = 15) with normal fasting (24.14 ± 4.3 μU/mL) and 2 hrs (29.61 ± 1.92 μU/mL) INS, 2) gr (n = 16) with hyperinsulinemia (55.4 ± 6.78, 58.93 ± 4.37 μU/mL, respectively). None was diabetic.

**Results:** In 1 normoinsulinemic group BP (SBP 118.5 ± 2.35, DBP 77.5 ± 0.78 mmHg), BMI (19.2 ± 0.72 kg/m²), fasting and 2 hrs glucose (5.06 ± 0.16, 5.76 ± 0.49 mmol/L, respectively), TC (3.92 ± 0.31 mmol/L), TG (2.26 ± 0.33 mmol/L) HDL-C (1.04 ± 0.04 mmol/L) were detected. In 2 hyperinsulinemic group statistically higher were mean BP (SBP 164.3 ± 5.66, DBP 91.9 ± 2.5 mmHg, p < 0.001), BMI (26.8 ± 1.87 kg/m², p < 0.001), fasting and 2 hrs glucose (5.88 ± 0.13, 7.54 ± 0.36 mmol/L, respectively, p < 0.001), HDL-C was lower (0.97 ± 0.02 mmol/L, p < 0.001)/No statistical differences were observed in TC 4.47 ± 0.3 mmol/L, p > 0.1/).

**Conclusions:** Obtained data suggest that an increase of fasting and 2 hrs post-load INS levels associated with an adverse CV risk profile. Our results indicate that already fasting insulinemia level is a predictor of hypertension and atherogenic dyslipidemia.

**Tu4W7**  
**Effect of atorvastatin on chylomicron remnants metabolism in obese subjects studied with a stable isotope breath test**

D. Chan1, G.F. Watts1, P.H.R. Barrett1, T. James1, J. Mamo1, T.A. Mori1, T.G. Redgrave2. University Departments of Medicine: 1Physiology, University of Western Australia; 2Department of Nutrition, Dietetics and Food Science, Curtin University of Technology, Perth, Western Australia, Australia

**Objective:** To examine the effect of an inhibitor of cholesterol synthesis on chylomicron remnants (CR) metabolism in viscero obese men with dyslipidaemia.

**Methods:** 8 viscero obese men (plasma triglycerides ≥ 1.5 mmol/L, cholesterol > 5.2 mmol/L, waist circumference > 100 cm and BMI > 29 kg/m²) were studied. Atorvastatin (40 mg/day) was given for 6 weeks. Chylomicron remnant (CR) metabolism was measured by the breath test using intravenous bolus injection of chylomicron-remnant like particles labelled with cholesteryl 13C-oleate, with subsequent measurement of 13C02 in the breath over 10 hrs by isotope ratio mass spectrometry. The fractional clearance rate (FCR) of the CR-like particles was derived from the appearance of 13C02 in breath using a multi-compartmental model (SAAM-II). Fasting lipids and lipoproteins including remnant-like particles cholesterol (RLP-C) and apolipoprotein B-48 were also measured before and after intervention.

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Atorvastatin</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride, mmol/L</td>
<td>2.25 ± 0.92</td>
<td>1.71 ± 0.82</td>
<td>0.016</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.91 ± 0.48</td>
<td>3.59 ± 0.32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.95 ± 0.14</td>
<td>0.90 ± 0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.91 ± 0.25</td>
<td>1.81 ± 0.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RLP-C, mmol/L</td>
<td>0.45 ± 0.33</td>
<td>0.30 ± 0.38</td>
<td>0.041</td>
</tr>
<tr>
<td>Apo B, μg/mL</td>
<td>23.0 ± 8.3</td>
<td>18.1 ± 8.8</td>
<td>0.011</td>
</tr>
<tr>
<td>FCR of CR, 1/pool/hr</td>
<td>0.053 ± 0.040</td>
<td>0.103 ± 0.050</td>
<td>0.043</td>
</tr>
</tbody>
</table>

**Conclusions:** The findings suggest that atorvastatin decreases the plasma concentration of chylomicron remnants in obese subjects as a consequence of increased particle clearance as measured by the breath test. This may be due to upregulating hepatic LDL/CR receptors or decreased competition with VLDL for clearance by these receptors.

**Tu5W7**  
**Fat distribution and dyslipidemia**

N. Mohammadifard, N. Sarrafzadegan, F. Sajadi. Isfahan Cardiovascular Research Center, Isfahan, Iran

**Objective:** To investigate the relation between hypertriglyceridemia/low HDL-cholesterol (HDL-C) and central obesity as two prevalent risk factors in Mediterranean regions.

**XIIth International Symposium on Atherosclerosis, Stockholm, Sweden, June 25-29 2000**
Increased diurnal triglyceridemia in obese insulin resistant subjects is associated to abdominal fat


Background: Metabolic ward studies have shown that abdominal obesity is linked to postprandial triglyceride (TG) metabolism in insulin resistance (IR). We evaluated whether this is also the case for diurnal triglyceridemia.

Methods: We analyzed diurnal capillary TG (TGC) profiles in 30 obese subjects (15 males, BMI 31.0 ± 8.8 kg/m², 47 ± 2 with IR (fasting insulin 15.5 ± 2.5 mU/L, glucose 6.7 ± 0.5 mmol/L) to diurnal triglyceridemia in 30 age and sex matched lean controls (BMI 22.8 ± 0.3 kg/m²) without IR (fasting insulin 7.2 ± 0.4 mU/L, glucose 5.3 ± 0.1 mmol/L). Subjects measured capillary TG on 3 different days, 6 times each day in an out-patient clinic setting. The diurnal TG profile was calculated as area under the TGC curve (TGC-AUC) and as incremental area (dTGC-AUC). Food intake was recorded and fasting blood was drawn once at the start of the study.

Results: Fasting plasma lipids in obese subjects were within normal limits albeit higher than in controls (TG: 1.8 ± 0.5 vs 1.0 ± 0.1 mmol/L, p < 0.001, cholesterol: 5.5 ± 0.2 vs 4.9 ± 0.2 mmol/L, p < 0.05). TGC-AUC was elevated in obese subjects (34.6 ± 2.9 vs 20.1 ± 1.2 mU/min, p < 0.001), and dTGC-AUC tended to be higher (7.9 ± 1.2 vs 5.1 ± 0.7 mU/min, p = 0.05). In obese subjects, TGC-AUC and dTGC-AUC were significantly correlated to fasting TG (r = 0.85 and r = 0.68, respectively), HLD-C (r = -0.66 and r = -0.53) and waist-hip ratio (r = 0.57 and r = 0.72), but not to BMI or self-reported dietary energy intake. In controls, no associations were found with anthropometric parameters, but the best correlations for TGC-AUC and dTGC-AUC were found with fasting TGc (r = 0.88 and r = 0.40) and apo B (r = 0.46 and r = 0.41).

Conclusion: Increased diurnal triglyceridemia in obese insulin resistant subjects is linked to abdominal obesity, which is in agreement with metabolic ward studies under strictly standardized conditions. We suggest that diurnal triglyceridemia may serve to study the effects of interventions on atherosclerotic profiles in obese subjects.

Lipoprotein (a) levels in hypertensive patients with abdominal obesity

N. Shchelintseva, I. Ozerya, O. Offeriev, O. Alexandrovich, N. Perova. National Research Center for Preventive Medicine, Moscow, Russia

Objective: To reveal whether serum lipoprotein (a) (Lp(a)) level associated with metabolic disturbances in hypertensive patients with abdominal obesity (AO).

Methods: Seventy hypertensive men aged 35–66 with AO: waist-to-hip ratio > 0.9 (0.98 ± 0.006) and body mass index > 26 kg/m² (30.8 ± 0.4) were included in this study. All patients were grouped according to serum Lp(a) levels: I group (n = 46) with Lp(a) ≤ 20 mg/dL (6.4 ± 0.8), II group (n = 24) with Lp(a) > 20 mg/dL (65.2 ± 8.6). Lp(a) levels were measured by "rocket" immunoelectrophoresis.

Results: In both groups we obtained glucose/insulin ratio < 6.0 in 2 h post-glucose loading. Total C and LDL C mean values were not different between the two groups. Patients with high Lp(a) levels had lower concentration of Tg (139 vs 195 mg/dL, p < 0.01) and higher HLD-C (46 vs 42 mg/dL, p = 0.05). Apo AI levels were similar in both groups, but at high Lp(a) HDL phospholipids (PL) were lower (76 vs 92, p < 0.01), that was associated with higher HLD-C/apo AI ratio (0.36 vs 0.33, p < 0.05) and HLD-C/HDL PL ratio (0.64 vs 0.48, p < 0.05).

Conclusion: High Lp(a) level is associated with disturbances in HDL-mediated cholesterol transport system in hypertensive patients with abdominal obesity.

Prevalence of overweight and obesity in old people

E. Resta, S. Cavaliere, S. Cappetti, A. Innominato. Dept. of Internal Medicine, Infectious Diseases, Section of Geriatrics, University of Bari, Bari, Italy

Objective: The aim of this study was to evaluate the prevalence of overweight and obesity in a free-living old population.

Methods: We studied 484 subjects (253 M, 231 F), aged 65–84 yr., randomly selected in Casamassima, a rural village of Southern Italy. The nutritional status was assessed with anthropometric variables (BMI, waist circumference, and TSF), serum parameters (albumin, prealbumin, and ApoB), and Mini Nutritional Assessment (MNA).

Effect of bezafibrate on relationship between insulin resistance and fatty acids

T. Onuma, S. Kato, N. Takayanagi, K. Abe, Y. Tanaka, R. Kawamori. Dept. of Internal Medicine, Juntendo University, Tokyo, Japan

Objective: Insulin resistance (IR) is noticed to be an important risk for type 2 diabetes mellitus and atherosclerosis. Bezafibrate (BF) which improves serum lipid-activities seems to reduce IR. This study is performed to elucidate the effect of BF on the relationship between IR and serum fatty acids in patients with hypertriglyceridemia.

Methods: Thirty patients with hypertriglyceridemia were given 200 mg of BF twice a day for two months. Fasting plasma glucose and insulin levels were measured and calculated HOMA index was significantly reduced after BF-administration. Percentage of tristearin, linoleic, linolenic and icosadienic acids were significantly decreased and palmitoleic, γ-linolenic, arachidic, 5-8-11 icosatetraenic, bishomo-γ-linolenic, arachidonic, behenic, docosatetraenoic, lignoceric and docosahexaenoic acids were significantly increased after BF-administration. There was significantly positive correlation between the decrease in HOMA index and the decrease in percentage of icosadienic acid.

Conclusion: These results indicate that the improvement of IR after BF-administration might not be closely associated with the change of serum lipids and fatty acids composition in hypertriglyceridemic patients.

Wait to hip ratio as a marker of insulin resistance in healthy subjects

J.T. Real, I. Martinez-Usó, P. Ascaso, A. Rodrigo, J.F. Ascaso, R. Carmen. Dept of Endocrinology and Nutrition, Hospital Clinico Universitario, University of Valencia, Spain

Objectives: Our objective was to analyze, in healthy subjects, the relationship of...
IR and other coronary risk factors with the waist to hip ratio (WHR) in order to identify a possible surrogate marker of IR.

Subjects: 30 healthy males, age 30-60 y, non-smokers and non-diabetics were studied.

Methods: WHR, Blood pressure, TC, TG, HDL-C, glucose and peripheral insulin sensitivity (SI) determined with the minimal model method.

Results: Subjects were divided into two groups: SI > 2 x 10^{-4} mU/min (No IR) and SI ≤ 2 x 10^{-4} mU/min (IR). SI = 3.25 ± 0.96 vs 1.24 ± 0.48; T/TW9 Geographic Epidemiology of Atherosclerosis

TuT1/W8 GENE THERAPY AND OTHER NEW TREATMENTS

Reperfusion treatment for acute myocardial infarction

Chi Zhao, Shumin Chen, Hao Wang. Department of Cardiology, Dalian Central Hospital, Dalian, China

Objective: To compare outcomes of primary angioplasty with the intravenous urokinase and the intracoronary urokinase intervention for acute myocardial infarction (AMI).

Methods: 110 patients were followed: 1) 45 patients received direct angioplasty, 2) 45 patients were treated by intravenous thrombolysis, 3) 20 patients were treated by intracoronary thrombolysis. Reperfusion rate, mortality, re-infarction rate, and left ventricular ejection fraction were monitored.

Results: The reperfusion rate (92.3%) in the direct angioplasty group was significantly higher than that in intravenous thrombolysis (63.6%) and intracoronary thrombolysis (63.2%) groups. The mortality and re-infarction rate in the former markedly lower than the other two groups. Compared with the intravenous and the intracoronary thrombolytic treatments, the patients received direct angioplasty showed higher left ventricular ejection fraction.

Conclusion: The present data suggest that direct angioplasty results in a reduction of short-term mortality and nonfatal reinfection and therefore advocate the routine use of coronary angioplasty as a primary reperfusion strategy for acute myocardial infarction.

TuT2/W9 Lifestyle behaviours and coronary risk factors in adolescence

C.A. Paterno. For the investigators of FRICELA Study (Factores de Riesgo Coronario En La Adolescencia y Coronary risk factors in adolescence); Society of Cardiology & Society of Pediatrics. Buenos Aires, Argentina

Objective: To know the prevalence of smoking, hypertension, hypercholesterolemia, the associations among them, and with other variables in adolescence. Lifestyle, behaviours and coronary risk factors detected in the adolescence may result in cardiovascular risk factors in adulthood.

Methods: 2.599 teen-ager with no previous related disease, between 12 to 19 years, male and female, mean age: 15.07 ± 1.87 years old, were studied from August 1994 to July 1997, with a specialized questionnaire pointed to knowing: physiological functions, daily and weekly tasks, recreation, toxic habits, familial history of coronary risk factors and coronary heart disease, and body parameters. An epidemiological, national and multicentric study was developed in 30 medical centres of 12 provinces and the Federal District.

Results: A multivariate analysis adjusted to other risk factors was made, taking into account results statistical significance.

Smoking was inversely associated with hours of study at home and positively associated with nap, with parents smokers, and strongly association with alcohol intake. Hypertension was directly associated with overweight, obesity; and family history of hypertension, and inversely associated with gym. Hypercholesterolemia showed a strong association with hypertension, furthermore, was associated positively with overweight, obesity; and sedentarism.

Conclusion: Lifestyle and behaviours changes (control of overweight, obesity and hypertension; avoidance of sedentarism, tabaquism and alcohol intake) should be performed in the adolescence, in order to reduce the incidence of coronary heart disease in adulthood.

TuT3/W9 The risk profile of ablebodied Ukrainian male population as a factor of formation of national health 20-years monitoring

I. Smyrnova, O. Kvaska, I. Gorbas, N. Davydenko, V. Volikov. Research Institute of Cardiology, Kyiv, Ukraine

Objective: The investigation of the risk profile dynamics in ablebodied male population during 20 years as a factor of formation of health.

Methods: Data are presented of 4 cross-sectional epidemiological studies of representative male samples of age 20-59 years executed at 5 years interval. Average number of population was 2000.

Results: The received data testify about: 1) the increase in specific gravity of persons with arterial hypertension in population on the whole (by 10.5%) is more vivid in the decades 40-49 (by 6.6%) and 50-59 (by 17.6%) years (p < 0.01); 2) the increase in prevalence of persons with atherogenic range of lipoproteins is more vivid in young decades 20-39 years (by 7.3 and 8.2% accordingly); 3) the stable prevalence of smoking (about 50% in population on the whole); 4) the part of persons without risk factors decreased as related to the age by 4.5; 6.3; 10.1; 2.2% correspondingly as a result of the growth in the associativeness of internal risk factors.

Conclusions: The result obtained not only allow to explain the increase of premature death of ablebodied male age in Ukraine also to point to the priority measures in elaboration of the strategy and policy of action in elaboration of cardiovascular diseases prevention in the health care reforming strategy of health service.

TuT4/W9 Characteristics of patients with early-onset coronary disease and without smoking habits

A. Batalla1, G.I. Cubero2, J.J.R. Reguero2, S. Hevia2, E. Merino2, S. Braga2, E. Bustillo2, J.C. Sammartín1, A. Cortina1. Department of Cardiology, 1Hospital de Cabechas (Gijón); 2Hospital Central de Asturias (Oviedo), Spain

Purpose: To determine common characteristics of patients under 50 years of age with coronary disease and without smoking habits.

XIIth International Symposium on Atherosclerosis, Stockholm, Sweden, June 25-29, 2000
Methods: Consecutively, 230 male patients before 50 years of age (mean age 43 ± 5 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease, were prospectively studied. Smoking habits, arterial hypertension, diabetes and dyslipidemia were determined during the acute phase by means of a questionnaire, physical examen and fasting analysis. Levels of Total cholesterol (Tchol), HDL cholesterol (HDL), triglycerides (TG), apolipoprotein A1 (Apo AI), apolipoprotein B (Apo B), Lp(a), LDL cholesterol (LDL) and total corrected cholesterol (TCC) were analysed after patients had fasted for more than 12 hours. In order to determine new coronary events a mean follow-up of 32 ± 13 months was carried out.

Results:

<table>
<thead>
<tr>
<th></th>
<th>mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smokers (214)</td>
</tr>
<tr>
<td>ApeoB</td>
<td>100 ± 26</td>
</tr>
<tr>
<td>LDL</td>
<td>151 ± 48</td>
</tr>
</tbody>
</table>

No differences were found in the levels of Apo AI, Tchol, HDL, TCC, Lp(a) and TG nor in the prevalence of hypertension and diabetes.

No differences were seen during follow-up in relation to the number of coronary events, mortality and prevalence of angina, myocardial infarction, heart failure and the need for coronary revascularisation.

Conclusions: Patients with early-onset coronary disease under the age of 50 years and without smoking habits present higher levels of LDL and Apo B than those patients with the same characteristics and smokers, which suggest that the control of lipid profile in smokers must be higher.

TuT5W9 Familiar hypercholesterolemia phenotype in Spain
S. Castillejo, A. Cenarro, P. Mozas, G. Reyes, M. Pocovi, P. Mata, R. Alonso, P. Pazo, F. Civeira. On behalf of the Spanish Group of FH; Departamento de Bioquimica, Universidad de Zaragoza, Spain

Familial Hypercholesterolemia (FH) is a common autosomal disorder associated with increased LDL-cholesterol levels, presence of xanthomas and premature coronary artery disease (CAD). In the last two years, the Spanish FH Foundation has been developing a national register of this lipid metabolic disease.

Objective: To determine FH phenotype in a Mediterranean sample and to confirm if the paradox that CAD is less frequent in the Mediterranean countries is applicable for this hyperlipidemia.

Methods: 231 index cases, selected by application of MedPed clinical criteria to diagnose a definite FH, were studied. The sample was representative from the Spanish FH population.

Results: The main results are indicated in the next table.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>LDL-C</th>
<th>TG</th>
<th>HDL-C</th>
<th>CHD %</th>
<th>Xanth. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n = 231)</td>
<td>51.2</td>
<td>340</td>
<td>124</td>
<td>50.0</td>
<td>31.1</td>
<td>47.2</td>
</tr>
<tr>
<td>Males (n = 106)</td>
<td>48.8</td>
<td>316</td>
<td>135</td>
<td>44.6</td>
<td>42.4</td>
<td>40.9</td>
</tr>
<tr>
<td>Females (n = 125)</td>
<td>53.9</td>
<td>344</td>
<td>115</td>
<td>55.6</td>
<td>22.3</td>
<td>48.8</td>
</tr>
<tr>
<td>&lt;41 yrs M (n = 23)</td>
<td>30.8</td>
<td>363</td>
<td>117</td>
<td>42.5</td>
<td>30.4</td>
<td>47.8</td>
</tr>
<tr>
<td>M (n = 19)</td>
<td>30.4</td>
<td>334</td>
<td>94</td>
<td>46.4</td>
<td>16.7</td>
<td>38.9</td>
</tr>
<tr>
<td>41–50 yrs M (n = 36)</td>
<td>45.6</td>
<td>329</td>
<td>124</td>
<td>47.3</td>
<td>25.0</td>
<td>58.3</td>
</tr>
<tr>
<td>F (n = 24)</td>
<td>45.5</td>
<td>327</td>
<td>114</td>
<td>59.9</td>
<td>22.0</td>
<td>54.2</td>
</tr>
<tr>
<td>51–60 yrs M (n = 26)</td>
<td>55.5</td>
<td>317</td>
<td>152</td>
<td>43.5</td>
<td>61.0</td>
<td>46.2</td>
</tr>
<tr>
<td>F (n = 39)</td>
<td>50.8</td>
<td>357</td>
<td>124</td>
<td>54.6</td>
<td>30.8</td>
<td>63.5</td>
</tr>
<tr>
<td>&gt;60 yrs M (n = 21)</td>
<td>65.8</td>
<td>326</td>
<td>155</td>
<td>43.6</td>
<td>61.9</td>
<td>47.6</td>
</tr>
<tr>
<td>F (n = 39)</td>
<td>68.8</td>
<td>354</td>
<td>116</td>
<td>55.1</td>
<td>20.5</td>
<td>35.9</td>
</tr>
</tbody>
</table>

Conclusions: The incidence of CAD in Spanish FH males is similar to that reported for other occidental countries. No significant differences have been found between subjects with or without xanthomas and CAD with respect to lipid values.

TuT6W9 Cardiovascular risk factors of the Circassian population in Israel
Y. Haron1, O. Hussein2, S. Linn1. 1Unit of Epidemiology, The Bruce Rappaport Faculty of Medicine, Technion Institute of Technology, Haifa; 2Internal Medicine A, Rebecca Sieff Government, Safed, Israel

Objective: To estimate the prevalence of cardiovascular risk factors in the Circassian community in Israel. Circassians, an indigenous people originating in the northern Caucasus region of eastern Europe. In Israel, they live as an endogamous community in cultural and geographical isolation for over a century.

Methods: Study sample comprised 450 women and 289 men aged 35 and over from both villages (70.5% of the total Israeli Circassian popula-
tion). Data collection was carried out by means of personal questionnaires (cigarette smoking, Leisure time physical activity), laboratory tests (Total Cholesterol (TC), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Triglycerides (TG), Glucose, mutation in three genes: MTHFR 677C>T, APOC1 191G>A, Prothrombin 20210A). Blood pressure and BMI measurements and information from medical files.

Results: The rate of CHD in the Circassian population > 35 is 7.3% (men 11.4% and women 4.6%) according to medical files. Age-adjusted prevalence rate of diabetes was 12.5 (according to new instructions of the ADA) and prevalence rate of hypertension (according to WHO) was 25%. The prevalent risk factors among the Circassian population was obesity (BMI > 30) (48% of the women and 35% of the men), physical inactivity (81% of the women and 52% of the men), and smoking (33% among men and 10% of women). Genotype distribution for three mutations in the Circassian population was within the described rates in white populations and conformed to the Hardy-Weinberg equilibrium. In multidisciplinary analysis prevalence of CHD among Circassians was significantly and independently related to age, diabetes, BMI and smoking. Diabetes was the strongest risk factor.

Conclusions: Prevalent behavioral risk factors in the Circassian population combined with familial risk factors (diabetes and hypertension) in this endogamous community, present a population at risk for cardiovascular disease.

TuT7W9 Hypertension and diabetes prevalence, awareness, treatment and control among the eastern mediterranean countries
N. Sarrafzadeh, F. Sajadi. Isfahan Cardiovascular Research Center, Isfahan, Iran

Objectives: As policy makers need more data (preferably age and sex-based) on the prevalence, the number of diagnosed, treated and controlled hypertensive or diabetic patients to use for the future needs for health care and preventive actions, this study has been done.

Methods: We reviewed all the studies performed since 1971 in each country in the region using MEDLINE database, publications in medical international and national journals etc. Also, the results were given for each gender separately.

Results: Results showed that although the percentage of aware and treated patients were relatively good, however the control rates are very low and far from developed countries. Of particular interest was the fact that although hypertension and diabetes were more prevalent among women compared to men, but women tended to be more aware and achieved better treatment than men. The control rate was similar between men and women. The following tables showed some of the main results obtained from this study.

Frequency of hypertension awareness, treatment and control rates among hypertensive men and women in some countries in the region.

Country | Sex | Age | No Def | Prevalence % | Awareness % | Treatment % | Control % | Period
-------|-----|-----|-------|---------------|--------------|--------------|------------|--------
Saudi Arabia | Men | >10 1956 | 1400 | 10.6 | – | – | – | 1995
Women | >10 2033 | 1400 | 11.5 | – | – | – | – | 1995
Bosn | >10 4000 | 1400 | 11.1 | – | – | – | – | 1995
Pakistan | Men | >3 151 | 1400 | 26 | 38 | 24 | 8 | 1997
Women | >3 1400 | 25.7 | 34.2 | 21 | 72 | 1995
Egypt | Men | >3 | 1400 | 25.7 | 34.2 | 21 | 72 | 1995
Women | >3 | 1400 | 26.9 | 38.9 | 200 | 8 | 1995
Ind | Men | >19 3276 | 1600 | 16.8 | 43.8 | 39.6 | 8 | 1996
Women | >19 5300 | 1600 | 19.4 | 60.6 | 56.4 | 126 | 1996
Both | >19 5300 | 1600 | 18 | 55 | 50 | 127 | 1996

Prevalence rate of diabetes and IGT and the level of awareness among men and women in different population in the EME.

Country | Prevalence (% of the study) | Age | Sample Size | Period | Defini- | IGT* | Awareness
-------|-----------------------------|-----|-------------|--------|--------|------|----------
Men | Women | Total | Size
--- | --- | --- | --- | --- | --- | --- | ---
Bahrain | 36.4 | 3 | 35 | 153 | 1996 | 3pp | 18.9 | 16.7 | 14.7 | 16.7 | 18.4 | 17.8
Iran | 4.6 | 5 | 30 | 69 | 900 | 1997 | 3pp | 7.8 | 7.7 | 2.4 | 43 | 60 | 65
Kuwait | 14.7 | 14.8 | 40 | 100 | 1996 | **PP** | – | – | – | – | – | –
Saudi | 3.6 | 5 | 4.6 | 10 | 1450 | 1992 | 3pp | 14 | 74 | 106 | 36 | 36


Conclusion: We conclude that although higher awareness level was observed among hypertensive and diabetic women than men, still the figures are far from western population and public education programs are needed.
Cardiovascular risk in Romanian doctors from Sibiu
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Objective: To assess the prevalence of the cardiovascular risk factors and to evaluate the global cardiovascular risk in the medical staff of the Academic Hospital from Sibiu.

Methods: Each subject was investigated about lifestyle, personal and family history (multiple choice original questionnaire), somatometric data, blood pressure (BP), metabolic disorders. Stress level was self-reported on a 1 to 10 score scale. Global cardiovascular risk was assessed from the current-use European charts.

Results: In n = 62 subjects (mean age = 41.3), both sexes (32 female, 30 male), dislipidemia is the most frequent risk factor (54.8%). Hypertension was found in 12.9%, high BMI in 35.4%. 41.9% have positive familial history in cardiovascular disease. 16.1% subjects were under treatment for known conditions (high blood pressure, chest pain), 25.8% are smokers (more than 10 ciggarettes/day). 35.4% report frequent overeating, only 22.5% practice regular exercise and stress mean for the group is 7.67.

Conclusions: Global cardiovascular risk is increased in this professional group mean risk is in 12.9%, moderate in 19.3%, and 3.2% of subjects have high risk.

Correlation between the number of risk factors and the occurrence of acute coronary events – An epidemiological study in 1200 patients from North-Western Transilvania
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Objective: Previous epidemiological studies are pointing out the cumulative effect of several risk factors on the progression of ischemic heart disease. We carried out our study on a population from North Western Transylvania with a high incidence of atherosclerosis partially explained by traditional high cholesterol diet.

Methods: We studied two groups of patients over four years, group A: 1200 pts having multiple risk factors (overweight, dyslipidemia, smoking, daily stress), and group B: 1300 pts having only one of the above mentioned risk factors. None of these patients (group A or group B) had any sign of overt heart or vascular disease, and they did not require medical treatment. The groups were homogeneous regarding age and sex. At baseline, all patients underwent lab examinations (total cholesterol, HDL-cholesterol, LDL-cholesterol, tryglicerides, glyceremia) and EKG. All investigations were repeated every six months, adding appropriate treatment whenever needed.

Results: After four years of survey in group A (having several risk factors) we noticed 321 (26.7%) major coronary events (unstable angina, acute myocardial infarction) in group B the occurrence of coronary events was lower – 140 (11.6%).

Conclusions: The presence of several risk factors is correlated with a higher prevalence of major coronary events in a population with a traditional high fat diet.

Clarification of changes of serum lipids of aging in men and women who requested a medical health check up
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Objective: To clarify changes of serum lipids in men and women, according to age.

Methods: The study population consisted of 54,701 men and 40,529 women who visited us from 1986 to 1996. Serum total cholesterol (TC), triglyceride (TG), and high-density lipoprotein-cholesterol (HDL-C) were determined enzymatically. Atherosclerosis index (AI) were calculated by standardized calculating formulae. Statistical analysis was performed by Welch’s t-test, Fisher’s Z transformation and an analysis of variance (ANOVA). A p value of less than 0.05 was considered to indicate statistical significance.

Results: TC, AI and TG changes were completely different in men and women. Compared with in men, in women those levels increased extremely in the fifth decade making a plateau in the seventh decade. HDL-C showed a plateau for all ages in men and women, but its level in women remained higher than in men. The ratio of undesirable to desirable increased with age for TC in men and women, more than half of its population were on the undesirable level in the sixth decade. For HDL-C, in men it remained higher than in women and its fluctuation with age was small. For AI, in both men and women it was small and stable with age. For TG, in men increased with age until the fifth decade and decreased with age thereafter, and in women increased with age making a plateau from the fifth decade.

Conclusions: We should emphasize changes of serum lipids in men and women, according to age.

Triglycerides in children and youngsters with familial diabetes, hypertension and coronary heart disease
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The importance of hypertriglyceridemia in diabetes, hypertension (HT) and coronary heart disease (CHD) still rises some controversy and the information is particularly scant on its relation to the extent of asymptomatic atherosclerosis in young people.

So the authors decided to quantify triglycerides in children and youngsters in two groups, A and B, respectively with and without diabetes, HT and CHD in their parents.

A total of 216 children and adolescents, both sexes, 5-17 years old were considered. The laboratory method used for triglyceride measurement was a totally enzymatic one with Roche reagents.

Results: The results have shown an higher triglyceride mean concentration in group A than in group B, statistically significant (p < 0.05) when CHD was increased. The pathology considered in group A had no significant difference between triglyceride mean levels in groups A and B when diabetes and HT were in the parents’ history of group A children and youngsters.

Conclusion: The authors conclude that preventive measures should be implemented in children and adolescents with hypertriglyceridemia and CHD in their families.

Risk factors in Croatian population

Objective: To determine the risk factors in an example of adult healthy population in Croatia.

Methods: The sample was formed by 3003 adults, both sexes, aged between 18-65 years, coastal and continental regions. The anthropometric data were collected by interviewing the population in order to complete the questionnaires. The blood specimens were analyzed using colorenzymatic methods for total cholesterol and triglycerides. The plasma fibrinogen level was determined by the standard photometric optic method on the basis of chromogenic substrate.

Results: As much as 54.7% of population is overweight and 26% is hypertensive. The prevalence of both, obesity and hypertension rises with age and are higher in male than in female population. Hypertension shows higher prevalence in coastal (29%) in relation to continental region (25%). Overall 38% of adult are smokers, 35% male and 25% female. The intensity of smoking is higher in males where it generally increases with age, and is higher in the coastal region. The intensity of physical activity in both sexes are low 3% female and 12% male The mean total cholesterol is in males (5.8 mmol/L) and in females (5.7 mmol/L) while triglycerides is higher in males (2.21 mmol/L) than in females (1.52 mmol/L). The fibrinogen levels are higher in females (3.16 g/L) than in males (2.29 g/L). Coastal population has higher levels of total cholesterol, triglycerides and plasma fibrinogen.

Conclusions: High prevalence of risk factors in Croatian population need for preventive measures, in order to achieve lifestyle modifications. This includes cessation of smoking, regular physical activity, maintenance of healthy weight, blood pressure and plasma lipids control.
frequently inaccurate and need to be reinforced. The purpose of this study was to evaluate of relationship between plasma high density lipoprotein levels and patient’s compliance.

Methods: The study group consist of 1239 consecutive patients (pts) (570 male, 669 female, mean age: 54 ± 8 years range 38–74) with hyperlipidemia who met NCEP criteria for drug treatment have been enrolled in this study from January 1997 to December 1998. They all were given lipid lowering drug therapies in once a day formulation to reduce their lipid levels. Enrolled patients were divided into two main groups based on HDL-C levels; Group A HDL-C < 0.35 (n = 465) and group B HDL-C > 0.35 (n = 774). We put patients on a follow-up program and gave them appointment times on 3, 6, and 12 Months of therapy.

Results: Number of patients who did return was 261 (85%), 176 (93%), 103 (82%) in group I and 712 (92%), 671 (98%), 589 (97%) in group II at the 3., 6., 12. Months of follow-up, respectively. These findings suggest that patients with low HDL-C levels group A were compliant when compared with group B (p < 0.05)

Conclusion: These findings suggest that non-compliant patients has lower HDL-C levels when compared with compliant patients. Sedentary lifestyle is a very important problem in hypercholesterolemic patients particularly low HDL-C states may negatively affect patient’s compliance. A comprehensive education of these patients are urgently needed in order to improve his knowledge about hypolpidemic diet, drug, lifestyle modification and to obtain his motivation which is essential for long term compliance.

TuT4:W9 Mortality of myocardial infarction with the relation to cholesterol and economy in Japan
N. Nakazawa. Fujieda Municipal Hospital, Fujieda Medical Association Fujieda Health Promotion Center, Japan

Objective: To clarify the relation among mortality of myocardial infarction (MI), serum cholesterol levels and economy state in Japan.

Methods: Mortality of MI was examined from 1987 to 1997 in Fujieda city. Serum cholesterol levels were determined every year amounted 215, 744 (male 70,872 and female 144,872) during 1987 to 1997 in Fujieda residents. Economic state was examined from economic white paper in Japan.

Result: Mortality of MI was highest at 1994 and lowest 1987 as the number of 40 and 18 subjects per 100,000 population. Two high peaks were noted at 1991 and 1994. Serum cholesterol was noted average about 200 mg/dl. Highest cholesterol was observed at 1992 at the levels of 203.8 mg/dl. Lowest at 1987 at the levels of 196.8 mg/dl. Two high peaks were observed at 1992 and 1995. Index of business condition growth rate of medical expense to a year before and change of economic growth showed two peaks of like a word M type also.

Conclusion: Mortality of MI and serum cholesterol were closely related to social economic changes in Japan.

TuT1:W10 TRANSPANTATION AHEROSCLEROSIS

TuT1:W10 Plasma homocysteine levels in renal transplanted patients. Effect of treatment with folic acid
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Objective: To evaluate homocysteine levels in renal transplanted patients (RTP) with stable function treated with ciclesporine (CyA) or tacrolimus, and the changes observed in the two groups of patients after treatment with folic acid.

Methods: Forty two RTP (21 treated with CyA and 21 with tacrolimus) were studied. Controls were 40 healthy subjects matched by age and gender with patients.

Results: In RTP homocysteine levels were increased compared with controls (16.4 ± 5.2 vs 8.0 ± 1.8 μmol/L; p < 0.001). Hyperhomocysteinaemia was found in 33 patients and one control (78.5% vs 2.5%; p < 0.001). By univariate analysis patients treated with CyA had higher homocysteine than those treated with tacrolimus, but multivariate analysis did not confirm these results. Homocysteine levels decreased significantly after treatment with folic acid (5 mg/daily for 3 months) with a median reduction of 31%, and with no differences in patients treated either CyA or tacrolimus.

Conclusions: Hyperhomocysteinaemia is very frequent in RTP. Folic acid therapy produces a significant decrease in homocysteine concentrations, without differences in relation to immunosuppressant therapy.

TuT2:W10 Lipoprotein and glucose profile in liver transplant
E. Papadopoulou1, M. Tsotis1, G. Iovimos1, A. Papageorgiou1, A. Arrvamide1, M. Elia1,2, A. Antoniades1, Departments of 1Endocrinology; 2Transplantation, Hepotipication Hospital, Thessaloniki; 2Department of Internal Medicine, Ioannina, Greece

Objective: to study the lipoprotein and glucose profile throughout the year after liver transplantation.

Methods: Plasma lipoproteins (pre-beta (VLDL), beta (LDL)) and fasting blood glucose were determined in 10 patients (8 M, 2 F mean age 42.3 ± 10.4 years) at baseline, 3 hours, 7 days, 3, 6, and 12 months after liver transplantation.

Results:

<table>
<thead>
<tr>
<th>N</th>
<th>10</th>
<th>0</th>
<th>3 h</th>
<th>7 d</th>
<th>3 mo</th>
<th>6 mo</th>
<th>12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-b</td>
<td>122.2 ± 5.6</td>
<td>102.4 ± 4.1</td>
<td>105.0 ± 4.1</td>
<td>115.8 ± 6.2</td>
<td>96.3 ± 3.5</td>
<td>93.5 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>Pre-b</td>
<td>70.5 ± 3.0</td>
<td>63.8 ± 2.6</td>
<td>64.7 ± 2.7</td>
<td>56.8 ± 1.3</td>
<td>63.9 ± 1.0</td>
<td>66.7 ± 7.5</td>
<td></td>
</tr>
<tr>
<td>glucose</td>
<td>135.4 ± 4.4</td>
<td>140.4 ± 5.7</td>
<td>149.4 ± 5.4</td>
<td>101.3 ± 28</td>
<td>97.2 ± 2.0</td>
<td>108.1 ± 30</td>
<td></td>
</tr>
</tbody>
</table>

P < 0.0001

Pre-b lipoprotein levels were normal at baseline, were slightly decreased at 3 hours and 7 days whereas after transplant. The decrease was even more pronounced at 6 and 12 months. The b-lipoprotein levels were above normal at baseline and they decreased at 3 hours and 7 days after surgery. A slight increase was observed at 6 and 12 months. One year after surgery the b-levels remained above normal. Hyperglycemia was observed before surgery. There was statistical significant decrease to normal values at 3 to 12 months after transplantation.

Conclusions: In hepatic patients, pre-b levels remained above normal one year after surgery. In contrast, glucose metabolism returned to normal faster after surgery. The implications for atherosclerosis in these patients are open to discussion.

TuT3:W10 Apolipoprotein changes and liver transplantation
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Objective: To investigate the changes of apolipoproteins, total cholesterol (TCHOL) and HDL-c, before and up to one year after liver transplantation.

Methods: Orthotopic liver transplantation was successfully performed in 10 patients (8 M, 2 F, mean age 42.3 ± 10.4 years). Serum levels of apolipoproteins (Apo A1, ApoB, Lp(a)), total Cholesterol and HDL-c were determined in all patients, at baseline, 3 hours, 7 days, 3, 6, 12 months after liver transplantation.

Results:

<table>
<thead>
<tr>
<th>N</th>
<th>10</th>
<th>0</th>
<th>3 h</th>
<th>7 days</th>
<th>3 mo</th>
<th>6 mo</th>
<th>12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoA1</td>
<td>96.88 ± 47</td>
<td>92.4 ± 22.8</td>
<td>85.8 ± 30.5</td>
<td>109.7 ± 40</td>
<td>130.1 ± 35.1</td>
<td>131.1 ± 31</td>
<td>131.1 ± 34.4</td>
</tr>
<tr>
<td>ApoB</td>
<td>47.5 ± 33</td>
<td>74.8 ± 4.9</td>
<td>92.6 ± 43.7</td>
<td>85.5 ± 19</td>
<td>103.7 ± 4.32</td>
<td>121.2 ± 52</td>
<td>121.2 ± 52</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>10.5 ± 5.6</td>
<td>10.3 ± 3.4</td>
<td>10.4 ± 4.4</td>
<td>9.4 ± 2.2</td>
<td>16.6 ± 4.9</td>
<td>16.4 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>TCHOL</td>
<td>275.5 ± 72</td>
<td>155.7 ± 56</td>
<td>160.2 ± 2.5</td>
<td>197.2 ± 47</td>
<td>219.1 ± 55</td>
<td>216.2 ± 65</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.001

Apolipoprotein A1 (ApoA1) was decreased from the day before surgery to 7 days after transplantation. A significant progressive rise of ApoA1 was observed at 6 and 12 months after transplantation. ApoB levels, normal before and immediately after surgery, were subsequently decreased until third month and then increased at 6 and 12 months after surgery. Lp(a) levels increased posttransplant at 6 and 12 months.

Conclusions: Apolipoprotein changes (ApoA1, ApoB) had the same course throughout the study: they were decreased at 3 h and 7 d after surgery and then significantly increased at the 6th month presumably due to the recovery of liver function.
TuT4-W10  Anti-atherothrombotic support treatment after orthotopic heart transplantation

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Atherothrombogenic conditions provides the coronaryogenic perfusion's injury that decrease life-time of patients after orthotopic heart transplantation (OHT). The Aim was to find effective treatment and/or prevention of disturbances in myocardial perfusion in transplanted heart.

Patients and Methods: Nineteen patients were examined twice per year during 8 years after OHT: in therapy antiatherosclerotic drugs and antiaggregants and anticoagulants were used alone or together. Also plasmaphersis was applied in each patients. The lipoprotein profile, blood coagulation, haemorheology and myocardial perfusion by scintigraphy were studied.

Results: Antiaggregants alone didn't reduce atherothrombotic risk. The last was slightly less when antplatelet drugs were combined with fibrates but not with statins. Short courses with high doses of antiaggregants or unfractionated heparin were unsuccessful too. In contrary, 2-weekly low molecular weight heparin (LMWH) – Frixaparine and rheologic correctors had been more effective because reduced thrombotic risk was already in 3–4 days. Additional decrease of atherothrombotic risk was marked by Lipanur use.

Plasmaphersis allowed a normalization of the lipoproteins level and blood rheology and to increase the myocardial perfusion of the left ventricle by the elimination of temporary hyperpufered areas. Thus plasmaphersis could improve coronary perfusion and therefore reduce the chronic ischemia. Also plasmaphersis prolonged total lipolytic effects of Lipanur.

Conclusions: Any methods alone are low effective against atherothrombotic disturbances in transplanted heart. The therapy should be combined with LMWH + rheological correctors + plasmaphersis twice per year. Between these courses antiaggregants and fibrates should be used continuously.

T:W11 RESTENOSIS

TuT1-W11  C-reactive protein and its relation with restenosis after percutaneous transluminal coronary angioplasty

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Percutaneous transluminal coronary angioplasty (PTCA) activates neutrophils and platelets, triggers inflammatory response. Mechanisms of restenosis have not been completely understood yet. It is thought that inflammation contributes to restenosis. Aim of this study was to evaluate the elevation of C-reactive protein (CRP) levels in patients (pts) who underwent elective PTCA and the relation between CRP levels and the restenosis at 24 hours.

Methods: 52 (Group I) who underwent diagnostic coronary angiography and 47 pts (Group II) to elective PTCA were included in this study. CRP, CPK, CK-MB levels were measured at baseline, 8 and 24 hours after the procedure. 24 hours later control coronary angiographies were performed in 22 of 47 pts. Serum CRP levels increased in 32 percent of Group I pts and 49 percent of Group II pts. CRP elevations did not correlate with the number of the lesions and balloon inflations and total inflation durations. CRP increased 78.5% of LAD and 23 percent of CX lesions (p < 0.01). After 24 hours, control coronary angiographies were performed in 14 LAD, 10 CX, and 1 diagonal lesions. Restenosis were determined in 8 pts (32%) and CRP increased in 75 percent of these pts. But CRP increase was present only 35.2% of the nonrestenotic pts. CRP levels increased in all restenotic LAD lesions but interestingly none of the restenotic CX lesions. CPK and CK-MB levels were normal in all pts.

We found that the inflammation which was determined by CRP elevation was significantly higher in Group II pts with LAD lesions than those with the other vessel lesions. We can explain the higher rate of the early restenosis in LAD intervention with the increased inflammatory response. CRP elevation may be a noninvasive marker of the restenosis in LAD vessel.

TuT2-W11  Angiographic findings in non-Q wave myocardial infarction: Differences in relation with age


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Purpose: To determine the angiographic findings in non-Q wave myocardial infarction according to age.

Methods: From a cohort of 48 patients who were consecutively admitted to the hospital with a diagnosis of non-Q wave myocardial infarction and underwent a coronarographic study a group of 26 patients less than 65 years old (Group A) and a group of 22 patients older than 65 years old (Group B) were made. We examined differences between both groups.

Results: The significant data are presented

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 26)</th>
<th>Group B (n = 22)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco consumption</td>
<td>78%</td>
<td>26%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ECG changes in anterior wall</td>
<td>39%</td>
<td>57%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Three vessels disease</td>
<td>21%</td>
<td>42%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Coronary calcification</td>
<td>17%</td>
<td>42%</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Conclusions: Tobacco consumption is more frequent in patients older than 65 years old with non-Q wave myocardial infarction compared with younger. However, ECG changes in anterior wall, three vessels disease and coronary calcification were significantly more frequent in patients less than 65 years old.

TuT3-W11  Relation of coronary calcium score measured by electron-beam computed tomography to results of coronary angioplasty

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Objective: The large studies of last years have shown close link between coronary calcium and atherosclerotic coronary narrowings, however influence of this factor on results of percutaneous transluminal coronary angioplasty (PTCA) is insufficiently studied. Aim of the study was to evaluate association between degree of coronary calcification measured by electron-beam computed tomography (EBCT) with outcomes of PTCA.

Methods: 55 patients with 67 coronary artery stenoses were subjected to PTCA (11 – usual balloon angioplasty and 56 – angioplasty with stent implantation). The residual stenosis was estimated at the end of the intervention by computerised quantitative analysis of angiograms. All patients before intervention were examined with EBCT. All patients before PTCA, and after 1 and 6 months after it fulfilled stress-test and Holter ECG monitoring.

Results: The coronary calcium score in a segment subjected to PTCA was on the average 39.6. The average residual stenosis in calcified segments was 9.4%, and in noncalcified – 8.1% (n.s.). Significant differences in the percentage of residual stenosis were heavily dependent on type of the intervention: 4.2% – in stent group and 27.5% – in balloon angioplasty group (p < 0.001). Restenosis was detected in 18.1%. In segments with restenosis the average Calcium score was 107.5 comparing with 26.3 in segments without restenosis (p < 0.02).

Conclusions: The high calcium score in a segment of coronary arteries subjected to PTCA was associated with increased risk of restenosis. The presence of calcium in dilated segment didn’t influence on the degree of residual stenosis and success of PTCA.

TuT4-W11  Monocyte activation markers in coronary restenosis

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Objective: Regarding restenosis as a result of nonspecific inflammatory process in a vessel wall we analyzed the expression of monocyte surface antigens involved in inflammatory reactions, in patients with stable angina who had undergone coronary angioplasty.

Patients and Methods: Ten patients, five without angiographically verified restenosis angiography, were included, and all receiving standard therapy (aspirin and β-blocker), were included. By flow cytometry we measured the monocyte expression of integrins Mac-1 and VLA-4 (CD49d), urorosinase receptor (uPARI) and membrane-bound urorosinase (uPA), the number of monocyte-thrombocytic complexes (MTC) after cell activation “in whole blood”. Spontaneous and LPS-induced tissue factor activity (TFsp and TFst) of isolated monocytes was determined by chromogenic assay.

Results: No between-group difference was found in Mac-1 expression or in the number of MTC, while VLA-4 integrin expression was elevated in the restenosis group. Patients with restenosis tended to have higher expression of uPARI and, to a lesser extent, higher quantity of membrane-bound uPA. The ratio of TFsp/TFst activity was about 3-fold higher in patients with restenosis when compared with patients without restenosis. Some data (mean ± S.D.) are summarized in the table (* p < 0.05).

Conclusion: According to preliminary data the expression of TF, VLA-4 and uPAR by activated monocytes can be altered in patients, prone to the development of restenosis.

T:W12 DIET AND BIOACTIVE COMPONENTS OF FOOD

TuT1:W12 Effect of diet and wine on hepatic lipase (HL) and cholesteryl ester transfer protein activity (CETP)
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Objective: To evaluate the effect of diet composition and wine consumption on plasma lipoproteins, HL and CETP activity.

Methods: 42 men, were assigned during three months to two different diets: half of them consumed a Mediterranean diet (MD) and the rest a western diet (WD). At second month both groups drank 240 ml of red wine at dinner. Plasma lipoproteins, apoprotein (Apo) AI, Apo B, Subfractions of HDL, and HL and CETP activity were measured at baseline (TO) and after the 1st (T1), 2nd (T2) and 3rd month (T3) on diet.

Results: No significant changes in total cholesterol (chol), LDL-chol, HDL-chol, triglycerides, and Apo AI were observed associated to the diet or wine consumption. Apo B increased significantly in the subjects that received the WD [85.1 ± 20.4 mg% (TO) vs 97.7 ± 19.8 mg% (T1); p < 0.05]. HDL-3 increased significantly with the wine consumption in the subjects on MD [29.1 ± 3.8 mg% (T1) vs 33.7 ± 4.5 mg% (T2); p < 0.05]. A non-significant increase of HDL-3 was detected in the group on WD. HDL-2 was unchanged with the diets and neither with the wine. CETP decreased slightly after wine consumption in both groups and was not changed with the diets. CETP correlated significantly with HDL-chol (r = 0.05, p < 0.000) and HDL-3 (r = -0.2, p < 0.02). HL increased with the WD [36.6 ± 20.3 pmol/ml/min (TO) vs 49.1 ± 25.2 pmol/ml/min (T1); p = 0.04], but was not changed with the MD. After wine consumption, both groups had a decrease of HL, but more significant in the MD [48.9 ± 13.8 pmol/ml/min (T1) vs 33.8 ± 9.4 pmol/ml/min (T2); p = 0.001 for MD and 49.1 ± 25.2 pmol/ml/min (T1) vs 36.2 ± 16.1 (T2); p = 0.05 for WD].

Conclusions: WD induced an increase in Apo B plasma levels. Wine consumption was associated to an increase in HDL-3 plasma levels that can be partially attributed to a lower activity of CETP and HL. WD may attenuate the beneficial effects of the wine consumption on lipoprotein metabolism.

TuT2:W12 The in vitro inhibitory effect of tannin derivatives on 3-hydroxy-3-methylglutaryl coenzyme a reductase on vero cells
P. Chan1, Fung-Lin Hsu2. 1Department of Medicine; 2School of Pharmacy, Taipei Medical College, Taipei, Taiwan, China

Objective: Multiple primary or secondary intervention trials to lowering serum cholesterol in human resulted in significant reduction of coronary events and death, one of the major reason attributes to developing of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor such as pravastatin. Developing new inhibitors of cholesterol synthesis is still common in pharmaceutical industry. Tannin is a large group of natural polyphenolic compound possessing beneficial antioxidant effect by previous studies.

Methods: The methods for analysis of specific inhibitors of mevalonate biosynthesis have already well-established by using Vero cells, a cell line obtained from the kidney of African green monkey. Tannin derivatives isolated from different traditional Chinese herbs were dissolved in 1% DMSO and incubated with Vero cells with or without the addition of 1 mM mevalonate or 5 mM sodium acetate for 24 h in order to observe cell growth. Pravastatin a specific HMG-CoA reductase inhibitor, was used as positive control which could inhibit Vero cells growth effectively and cell growth inhibition was reversible after adding 1 mM mevalonate.

Results: More than 50 tannin derivatives were used for study, only two compounds: proanthocyanidin A-2 (belongs to flavan-3-5ol group) and 1, 2, 3, 6-tetra-O-galloyl-β-D-glucose (belongs to galloatin group) showed significant growth inhibition of Vero cells.

Conclusions: This study showed that some isolated tannin derivatives from traditional herbs were effective HMG-CoA reductase inhibitors which might be developed into new hypcholesterolemic agents.

TuT3:W12 The combined use of pravastatin omega-3-polyunsaturated fatty acids (PuFA) and antioxidants in the therapy of coronary heart disease (CHD)
J.G. Fomin1, O.L. Belaya1, V.I. Kalmykova1, I.P. Rudakova2, E.N. Gavriliova1, E.V. Zakharova1. 1Moscow Medical Academy; 2State Research Institute of Vitanomology, Moscow, Russia

Objective: Try to correct undesirable changes in liver and thrombocytes function, such as cytosis and increase of aggregation of thrombocytes, caused by therapeutic application of 40 mg pravastatin (lipostat).

Methods: To treat CHD with dyslipidemia in 50 patients in history myocardial infarction aged 41–72; 40 mg of pravastatin (lipostat) was applied daily in combination with 20 mg “Eicosavitol” (fish oil) containing 14% of omega-3-PuFA, 20 mg alpha-tocoopherol and 80 mg antioxidant benzoquin - a prolonged antioxidant form of riboflavin for 1–3 months.

Results: The use of standard methods caused a decrease in the level of cholesterol (Ch)-LDL by 40%, triacylglycerols by 35%, thrombocyte aggregation by 30% as well as an increase in Ch-LHD by 7%, erythrocyte omega-3-PuFA by 85%, and erythrocyte membrane resistance by 30%. There was also a normalisation of lipid peroxide level.

Conclusions: Such combination of pravastatin and domestically produced omega-3-PuFA and antioxidant preparation causes a decrease in liver cell cytosis, thrombocyte adhesion and aggregation and an improvement in the functional state of thrombocytes.

TuT4:W12 Clinical effect of omega-3-polyunsaturated fatty acids (PuFA) and antioxidants in the therapy of coronary heart disease (CHD)
O.L. Belaya1, V.I. Kalmykova1, I.G. Fomin1, L.A. Ivanova1, E.V. Zakharova1, E.N. Gavriliova2, I.I. Korf1. 1Moscow Medical Academy; 2Inst. of Nutrition; 3Russian Academy of Medical Science, Moscow, Russia

Objective: To study the clinical efficiency and safety of using of PuFA preparation "Eicosavitol" (fish oil, containing 14% omega-3-PuFA) produced domestically with antioxidants.

Methods: The 72 patients in history myocardial infarction with dyslipidemia were given the preparation (20 ml “Eicosavitol”, 20 mg alpha-tocoopherol and 80 mg antioxidant benzoquin - a prolonged antioxidant form of riboflavin) per day for person during 1–3 months. The effect of treatment on the lipid composition, lipid peroxidation (LP), ADP-stimulated thrombocyte aggregation and clinical symptoms of CHD were assessed.

Results: The use of standard methods demonstrated a decrease of the level of total cholesterol (Ch) by 12%; Ch-LDL by 15%; VLDL by 12%; triacylglycerols by 10%; LP products by 10%, as well as increase of Ch-LHD by 7%; erythrocytes omega-3-PuFA by 85%; thrombocytes aggregation by 40% and erythrocyte membrane resistance by 30%.

Conclusions: Such combination of preparation, both in vitro model systems and under clinical conditions, exerted a marked antioxidant and therapeutic effect. It is economically beneficial and must be applied life-long.

TuT5:W12 Long term fasting effect on coronary heart disease
Yu. Slyvka, L. Radecka. Ternopil Medical Academy, Ternopil, Ukraine

Objective: Previously was evaluated positive effect of long term fasting (LTF) on plasma cholesterol level during treatment of different internal diseases. It was the basis of investigation of the LTF effect in patients with coronary heart disease.

Methods: 53 patients aged 43–65 years with coronary heart disease and alimentary obesity of degree I–II were studied. The treatment consists of 14–21 days fasting and the same period of subsequent recreation diet. All the patients easily tolerated fasting. Drugs were administered only in cases of angina or increased blood pressure. Total cholesterol, LDL and HDL cholesterol and triglycerides levels in blood plasma were estimated before and after treatment.

Results: During treatment was significant decreasing of body weight (from 105.3 ± 2.1 to 94.1 ± 4.0 kg). Blood pressure decreased from 165 ± 5
mm Hg before to 138 ± 6 mm Hg after LTF. Cases of angina decreased. General condition of patients improved. Total cholesterol, LDL-cholesterol and triglycerides levels in blood plasma decreased.

<table>
<thead>
<tr>
<th></th>
<th>Before LTF</th>
<th>After LTF</th>
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<tbody>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>7.90 ± 0.06</td>
<td>6.24 ± 0.01*</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.24 ± 0.05</td>
<td>1.18 ± 0.03</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>4.32 ± 0.04</td>
<td>3.74 ± 0.03*</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.29 ± 0.05</td>
<td>1.82 ± 0.02*</td>
</tr>
</tbody>
</table>

* - p < 0.05 (2-tail) compared with results before treatment.

**Conclusion:** LTF have positive effect on blood plasma cholesterol concentration and general condition in patients with coronary heart disease. Remote results of such treatment must be studied.

**TuT6:W12** The effects of diacylglycerol loading orally on postprandial hyperlipidemia in man

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1 Ayo Hospital Jikei University; 2 Coll. Liberal Arts & Sciences Tokyo Med & Dental University; 3 Kao Corporation, Japan

**Objective:** Elevated plasma triacylglycerol (TG) concentrations in the postprandial state are associated with an increased risk of coronary heart diseases. Diacylglycerol (DG) is digested into glycerol and free fatty acid through 1-monacylglycerol. And 1-monacylglycerol is supposed to be resistant to the reconstitution of TG in the intestine. The present study is to evaluate the difference effect of orally loading of DG and TG on dynamics of postprandial serum lipids, especially remnant-lipoprotein particles.

**Methods:** DG used in the study is consisted of 1.3-DG and 1.2-DG isomers in the ratio of 7:3 and the fatty acid compositions of DG and TG were prepared to be similar. Either DG- or TG-cream (30 g fat/m² of body surface area) were loaded in 6 healthy male volunteers (31–40 yr) after 12 hrs fasting. Blood samples were collected before and 2, 3, 4, 6 and 8 hrs after the loading. And then, serum lipids and apolipoproteins, and remnant lipoproteins (remnant-like particles, RLP) were measured. Cross-over experiment was performed after one month to test both DG and TG-cream in the same subjects.

**Results:** Serum TG concentration remarkably increased after lipid loading in both groups, but was lower in the DG group compared to the TG group at each point of measurement. Differences between two groups at 2, 3 and 8 hr were statistically significant. But, serum cholesterol was not significantly difference between two groups. Both RLP-TG and RLP-C concentrations were increased after fat loading in both groups, but the values in DG group were lower compared to those in TG group at each point. The differences were significant between two groups at 2, 3 and 8 hr in RLP-C. Area under the curve (AUC) of serum TG, RLP-TG and RLP-C and TG loading showed decrecent of 12.9%, 27.6%, and 17.4%, respectively, from those after TG loading. And the decrement of AUC of RLP-TG in DG group was significant compared to TG group.

**Conclusions:** The dynamics of lipids in the remnant lipoprotein after the fat loading was reflected more sensitively the metabolic difference of DG and TG ingestion. And DG loading has less potent activity than TG to promote postprandial hyperlipidemia in man.

**TuT7:W12** Alcohol consumption effects on postprandial changes in triacylglyceride transporting lipoproteins

J. Kovar, D. Korberová, R. Poledne. Institute for Clinical and Experimental Research, Prague, Czech Republic

To determine the effects of alcohol ingestion on postprandial lipoprotein metabolism, changes in triglyceride-rich lipoproteins (TRL) (d < 1.006 g/mL) and IDL (1.006-1.019 g/mL) were determined for 7.5 hours after standardized breakfast in 8 normolipemic healthy men in three settings. Alcohol (0.6 g/kg) was administered either with breakfast (A) or in the evening before breakfast (B); no alcohol was administered in a control experiment (C).

When given with breakfast (A), alcohol induced more pronounced postprandial lipemia evaluated as the area under the increment curve (AUC) of TRL and TRL-C; it also induced TG accumulation in IDL during the postprandial phase (Fig) while postprandial changes in IDL-C concentration were unaffected by alcohol consumption. Alcohol consumption in the evening before the test (B) induced an increase in baseline TRL and had no effect on baseline IDL. Alcohol likewise had no effect on postprandial changes in TRL or IDL concentrations.

Such effects of alcohol consumption on postprandial lipemia cannot be explained by its action on lipoprotein lipase – its activity measured using intravenous fat tolerance test at the end of the postprandial test was not affected by alcohol consumption in both settings.

It can be concluded that alcohol given with a meal affects not only postprandial TRL but, also, postprandial IDL-TG; its effect is quite different from that of alcohol given in the evening before the test.

**TuT8:W12** Diet and omega-3 fatty acid intervention trial on atherosclerosis. (DOIT-study)

E. M. Hjerpe, H. Arnesen, I. Hjerpe. Ullevål University Hospital, Oslo, Norway

Data from epidemiological studies suggest that lipid lowering diet and high levels of N-3 PUFA in the diet retard atherosclerosis and/or its clinical consequences. In recent years new methodology has made it possible to quantitate atherosclerosis non-invasively. In addition, methods for evaluation of vascular reactivity and endothelial function have become available.

**Aim of the Study:** To evaluate the effects of diet intervention and/or PUFA supplementation on atherosclerosis progress in a high risk population, by studying pre-and post-intervention markers of atherosclerosis: a) Intima media thickness of the carotid artery (Anatomy), b) Fingerphotoplethysmography (Physiology), c) Markers of endothelial dysfunction (Biochemistry).

**Methods:** 800 survivors from the follow-up of the Oslo study diet and smoking intervention trial (men born 1922–1932) have been asked for participation. The design is a randomised 2 × 2 factorial study with diet intervention and placebo-controlled, double blinded supplementation with 2.4 g N-3 PUFA/d (Piscol, Lube, Denmark), planned for 3 yrs. The intima thickness evaluations (using B-mode ultrasound) are analysed at a core laboratory (MAS, Malma, Sweden). Atherosclerotic plaque scores are also included. Pletysmographically, pulse wave velocity and predefined pulse curve features are registered and computerised. Biochemically, soluble markers of endothelial function like von Willebrand factor, thrombomodulin, tissue plasminogen activator antigen, and the adhesion molecules VCAM-1, ICAM-1, E- and P-selectins are determined. Serum free fatty acids are measured as background and compliance variables.

**Conclusion:** The effect of diet and N-3 PUFA supplementation on progression of atherosclerosis is being assessed by anatomical, physiological and biochemical variables in the DOIT study. 563 men at high risk of atherosclerosis have been randomised and have passed the 18 mths (mid-study) visit.

**TuT9:W12** Soy protein diet significantly improves plasma lipids

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**Objective:** Although the effects of polyunsaturated-monounsaturated and saturated fats on the lipid profile have been well documented, the effects of soy protein diet are not well known.

**Methods:** We evaluated 14 male hypercholesterolemic patients (age 51 ± 11, range 30-69) with a normal body mass index who were nonsmokers. After calculating their daily requirements, a diet with 25-30% of energy from fats, 10-12% from proteins and the rest from carbohydrates was instituted. 60% of the proteins was obtained from soy. All anthropometric and lipid parameters were assessed at baseline and 6 weeks after diet in the same patients.

**Results:** There was a significant improvement in plasma cholesterol and low density lipoprotein (LDL) levels, whereas the decrease in triglyceride and Apoprotein B remained borderline. HDL, Apoprotein A and lipoprotein a levels were not affected with soy protein diet (table).

<table>
<thead>
<tr>
<th>Before diet</th>
<th>After diet</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>268 ± 35</td>
<td>223 ± 37</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>244 ± 104</td>
<td>205 ± 61</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>42 ± 8</td>
<td>41 ± 6</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>176 ± 35</td>
<td>139 ± 35</td>
</tr>
<tr>
<td>Apo A (mg/dL)</td>
<td>137 ± 36</td>
<td>132 ± 21</td>
</tr>
<tr>
<td>Apo B (mg/dL)</td>
<td>153 ± 47</td>
<td>141 ± 36</td>
</tr>
<tr>
<td>Lipoprotein a (mg/dL)</td>
<td>22 ± 26</td>
<td>22 ± 28</td>
</tr>
</tbody>
</table>
Conclusion: Soy protein diet significantly decreases the cholesterol and LDL levels in patients with hypercholesterolemia.

TuT10:W12 Effects of different forms of edible fats on serum lipoprotein cholesterol levels in young men
M. Kozlowska-Wojciechowska1, M. Naruszewicz2, 1Nat. Food and Nutrition Inst., Warsaw; 2Pomeranian Academy of Medicine. Szczecin, Poland
Objective: In Poland, coronary heart disease is the most important cause of death. One of the risk factors associated with coronary heart disease is a diet high in saturated fat. In a typical polish diet 38–41% of energy intake comes from fats near 15% from SAFA. This was the diet reason for seeking the method of lowering SAFA content in everyday diet, pending consumer acceptance, to improve their lipid profile.
Methods: The study was conducted on a group of 83 young healthy men, of an average 24.6 years of age. The content of their typical diet provided them with 2016 kcal (±300), 416 mg of edible cholesterol, 21.5 g of dietary fiber, and the P:S ratio equaled 0.3. The research was “blind” study character, which means the butter or soft margarine was given to tested persons in unmarked single-use containers in two 15.0 g doses in the morning and evening – total 30 g per day. The study period was 10 weeks divided into three phases: first phase of two weeks for observing the examined parameters over time to account for biological variation. Afterwards, within a 4 week period 2 groups of subjects consumed butter (content 55.1 g/100 g SAFA: 1.28 g/100 g PUFA) or soft margarine (12.9 g/100 g SAFA: 33.7 g/100 g PUFA) in a crossover system, and for a next 4 weeks the other way round. The group that ate butter was given margarine and the group fed with margarine eats butter.
Results: The composition of the diet changed. The intake of saturated fats in the butter diet decreased from 38.5 g to 27.3 g in the margarine diet. Similary the level of cholesterol intake decreased by 74 mg while using margarine. As a result of the diet containing margarine, which is rich in PUFA, the average total cholesterol level decreased by 10–15%, LDL cholesterol decreased by 12%. No significant changes were noted in the HDL cholesterol and triglyceride levels. As results of the diet, a decrease in the homocysteine level was noted in some men.
Conclusions: Our results suggest that when we changed only kind of spread (from butter to margarine) in typical, nonhealthy diet, we observe beneficial effects on serum lipoprotein cholesterol levels.

TuT11:W12 Reduction of triacylglycerols, free radicals and total cholesterol by low protein diet containing ketosterol
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Objective: Ketosterol, if used in LPD of patients with renal insufficiency decreases the parameters mentioned above. The aim of our paper is to explain possible way of it’s effect.
Methods: A group of 42 patients, men and women in age 28–72 y, was selected as acceptor of LPD in combination of Ketosterol (0.1 g/kg/day). Patients treated by LPD without Ketosterol formed the control group. The condition of both groups were similar (renal insuf. Cr: 15–36 ml/min). All lipid parameters were estimated by use of common kits, automatically, on Hitachi 908. The concentration of free radicals (FR) only was measured directly by our original spectrophotometric method.
Results: After 6 months of treatment following significant changes of all measured parameters were observed in patients obtained Ketosterol.

TAG (mmol/l): 4.26 ± 1.92 vs 2.24 ± 1.28 (p 0.001)
TC (mmol/l): 7.31 ± 4.2 vs 5.82 ± 2.29 (p 0.05)
HDL-CH (mmol/l): 0.92 ± 0.24 vs 1.34 ± 0.45 (p 0.01)
FR (% of elevation): 112 ± 10 vs 100 ± 3 (p 0.001)
Apo A1 (mmol/l): 0.88 ± 0.26 vs 1.34 ± 0.45 (p 0.05)

No significant changes were found in control group. Small decline of TAG or TC corresponds to the LPD. We have two working hypothesis how to explain the Ketosterol effect on lipid profile: A: Ketosterol can change or inhibit homeostatic mechanisms of cholesterol biosynthesis; B: Ketosterol can inhibit cholesterol biosynthesis by competition with cholesterogenous sterols. B: The metabolic cycle of ketosterol can induce endogenous TAG and apolipoprotein metabolism and by this manner to inhibit their absorption.
Conclusion: Ketosterol causes observed changes in measured values by the two possible ways. Both systems are depending on properties of branched AmAc metabolism.

TuT12:W12 Oolong tea increases energy expenditure
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1Applied Nutrition, School of Medicine, The University of Tokushima, Tokushima, 2School of Social Health, Fukauka Prefecture University, Fukuoka; 3Suntory Research Center, Osaka, Japan
Objective: Oolong tea is a traditional Chinese tea and familiar in Japan, too. Oolong tea has been believed to be good for health in China. We were interested in one of their beliefs that oolong tea decreases body fat. We tried to confirm it by two energy expenditure (EE) studies.
Methods and Results: Experiment 1) Young female subjects had a common Japanese style dinner previous night. Only water was allowed after dinner. Next morning they woke up at 6:00, unarumed and sat in the reclining chair for 30 min. Then the expired gas was collected for 5 min and resting energy expenditure (REE) was calculated. Subjects took 300 ml of water, oolong tea or powdered green tea within 5 min on different 3 days. The expired gas was collected for 5 min in every 30 min up to 2 hrs and EE was calculated. Caffeine concentration of green tea was higher than that of oolong tea. Energy expenditure in oolong tea group was significantly higher than that in the composition of water (p < 0.05) but that in green tea group was not (p > 0.05).
Experiment 2) Effect of oolong tea on energy expenditure was studied when they had breakfast. Study design was similar as that of Experiment 1 except they had breakfast (464 kcal: including 18.6 g of protein and 19 energy percent of lipids), gas was collected until 3.5 hrs and beverages were water, oolong tea and caffeine (19 mg; same level as that of oolong tea). Energy expenditure in oolong tea group was significantly higher than that in the control group (P < 0.05) but that in caffeine was not (p > 0.05).
Conclusions: The results indicated that oolong tea increased EE and may be useful for weight control and the prevention of CVD.

TuT13:W12 Potencies of postprandial chylomicrons produced by a meal rich in saturated fat (SF) or polyunsaturated fat (PUF) to promote the foam cell formation in cultured macrophages
Byung-Hong Chung1, Karen Hart1, B.H. Simons Cho1, 1Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294; 2Moore Heart Res. Foundation, University of Illinois, Champagne, IL 61820, USA
Dietary fat is an exclusive precursor of postprandial chylomicrons. We have determined the effect of the fat composition of a single meal on the lipid composition of postprandial chylomicrons and the potential of chylomicrons to induce the neutral lipid accumulation in cultured macrophages. Chylomicrons were obtained from normal lipidemic subjects 4 hr after a meal rich in SF (P/S = 0.4) and another meal rich in PUF (P/S = 2.49); the mean P/S of triglyceride (TG) in chylomicrons obtained after a meal rich in SF and PUF was 0.39 and 1.62, respectively. PP chylomicrons of either SF or PUF, when added into cholesterenium lipid medium of macrophages, caused a marked (≥3X) increase in cellular TG level but had little or no effect on the cellular cholesterol level. PP chylomicrons of PUF were significantly more potent than PP chylomicrons of SF in increasing cellular TG level (p < 0.05). The P/S ratio of TG associated with macrophages cultured in control medium, control medium + SF chylomicrons and control medium + PUF chylomicrons were 0.16, 0.23 and 0.96 respectively, indicating that the composition of chylomicrons-TG influences cellular TG composition. Incorporation of macrophages foam cells induced by the chylomicrons of SF or PUF with fatty acid poor albumin for 24 hrs resulted in 28% and 42% reduction of cellular TG levels and reduction of the P/S of cellular TG from 0.23 to 0.20 and 0.96 to 0.49, respectively. The above data indicates that dietary PUF entering into circulating blood as a component of chylomicrons is more readily incorporatable into macrophages but is more readily regressive from cells than that of dietary SF.

TuT14:W12 Ramipril effect under various fat diet on serum lipid parameters of rats
H. Cangoeorgiou, G. Zoulomnis, J. Kostara, H. Parasevkaou, A. Margeli, C. Kaboroukis, D. Sakelariou, C. Tessouras. Pharmacology Department, Medical School, Athens University, Greece
Objective: To investigate the role of ramipril (ACEI) on the biochemical and histological profile of rats in relation to different lipid diet.
Methods: One hundred male Wistar rats (b.w. 200 ± 20 g) were divided in 2 groups (n = 50) were used (group-1 = control, group-2 = ramipril 1 mg/kg/48 h
by gavage). Both groups were subdivided in 5 subgroups (A, B, C, D, E), each being fed with low fat diet (b), medium fat diet (c), high fat diet (d), total cholesterol (TC), triglycerides (TG), and TC/HDL were measured. Histological specimens obtained from the vesiess of heart, kidneys and the aorta were also examined. Statistical analysis was performed by t-test.

**Results:**

- **Groups:**
  - A1
  - A2
  - B1
  - B2
  - C1
  - C2
  - D1
  - D2
  - E1
  - E2

- **Variables:**
  - HDL mg/dl
  - TC mg/dl
  - TG mg/dl
  - TC/HDL

- **Values:**
  - A1: 48 52 45 42 44 42 52 49 35 35
  - A2: 64 78 64 68 70 65 65 62 60 80
  - B1: 50 59 88 112 98 115 52 57 88 89
  - B2: 1.73 1.5 1.42 1.61 1.59 1.54 1.25 1.26 1.71 2.28

**Conclusions:** It is concluded that there is no statistically significant difference between the groups. Ramipril induces a slight improvement on the atherogenic index TC/HDL only in the controls A2/A1. In fat-treated and control animals similar histological findings for intimal thickening, endothelial injury and smooth muscle proliferation were observed. This may be due to the insufficient amount of lipid administration.

**TuT15:W12**

**Content of stearic fatty acid (SFA) in adipose tissue and a process of atherosclerosis**

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**The aim of our study was to determine the percentage of content of SFA in adipose tissue of persons suffering from some illnesses in conjunction with preterm development of atherosclerosis and its complications.**

**Methods:** The method of gas-liquid chromatography was used to evaluate the percentage of content of SFA adipose tissue of the anterior abdominal wall. The investigated group was formed of 250 persons (both sexes) divided in 8 subgroups and the control group was made of 93 metabolic completely healthy and normally nourished persons.

**Results:** For persons with and with phenotype picture of hyperlipoproteinemia (HLP) type IIa, in all persons significant lower values of SFA compared to the control group are determined: obese persons (BMI = 30–30.9) for 16% (P < 0.005), extremely obese persons (BMI = over that 40) for 41% (P < 0.001), NIDDM for 15% (P < 0.005), patients with sudden death from myocardial infarction for 28% (P < 0.001), type IIb HLP for 21% (P < 0.005), type IV HLP for 26% (P < 0.001). A significant connection of its decrease with the degree of nourishedness in obese persons is also discovered (r = 0.062, P < 0.001). In opposite to that, undernourished persons had some higher values of SFA statistically insignificantly.

**Conclusions:** The fact that the results are completely in accordance with our earlier findings in total serum as well as in some isolated serum lipid fractions, our investigation represents the confirmation of a new knowledge about the antiatherogenic effects of the SFA in contrast with other long chain saturated fatty acids.

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**TuT16:W12**

**Phenolic content of Merlot wines from several countries**

R.S. Faustino, S. Sobratte, A.L. Edel, G.N. Pierce. Division of Stroke and Vascular Disease, St. Boniface Hospital Research Centre, Winnipeg, MB, Canada

**Objective:** To compare quantities of catechin, epicatechin, rutin, transresveratrol and quercetin in Merlot wines from Chile, Canada, and the United States.

**Methods:** Catechin, epicatechin, rutin, transresveratrol and quercetin standards were separated by HPLC to determine time of elution from the column and ii) establish a standard calibration curve. 10 ul aliquots of red wines were analyzed for polyphenolic content. Concentration in mg/L was determined for the phenolic species.

**Results:** Catechin possesses the highest content in all Merlot wines sampled (66% of total flavonoid content). The remaining are epicatechin (26%), quercetin (5%), rutin (2%) and transresveratrol (1%). Catechin content was highest in Chilean and Canadian wines. American wines had the highest epicatechin and quercetin content. Rutin content was greatest in Chilean wines and transresveratrol occurred equally in wines from all three countries. Catechin had the highest correlation with price (R = 0.5, P < 0.05). Generally, there was a very poor correlation between the price of the wine and polyphenolic content.

**Conclusions:** Phenolic content of the wines varies and reflects differences in processing, harvesting, or environment in which the grapes are grown. Catechin and epicatechin were the largest flavonoid components with the remaining three occurring at small percentages. Pricing of Merlot wines and polyphenolic content were poorly correlated.

This work was supported by the Medical Research Council of Canada.

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**TuT17:W12**

**Effects of diacylglycerols on serum lipids and adipocytokines in patients with hypertriglyceridaemia**

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**Objective:** To investigate the effects of diacylglycerols (DG) on lipid metabolism and adipocytokines such as PAI-1 and leptin released from the adipose tissue in patients with hypertriglyceridaemia.

**Methods:** Eight subjects (2 males and 6 females) with hypertriglyceridaemia having an average age of 61 ± 11 years participated in the study. We prepared cookies, dressing, short bread and soup containing 10 g of DG. They were asked to replace one item of their regular meals with one of these prepared foods. Blood samples were taken every 4 weeks in the fasting state for measurement of serum lipids, apoproteins, insulin, leptin and PAI-1. The average observation period was 11 ± 2 weeks.

**Results:** After DG ingestion serum levels of TC and TG significantly decreased (TC: 262 ± 42 mg/dl to 218 ± 32 mg/dl, TG: 259 ± 102 mg/dl to 158 ± 64 mg/dl, p < 0.05), while HDL levels did not change. Serum levels of RLP-TC decreased, although not significant (12.5 ± 7.5 mg/dl vs 8.6 ± 2.9 mg/dl). Serum leptin levels did not differ, while plasma levels of PAI-1 antigen decreased, though not significant (30.5 ± 8.9 mg/ml vs 21.7 ± 10.5 mg/ml).

**Conclusions:** DG improved lipid metabolism and fibrinolysis system in patients with hypertriglyceridaemia.

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**TuT18:W12**

**Effects of green tea on oxidizability of LDL and plasma in healthy volunteers**

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**Objective:** To investigate the effects of green tea on the oxidizability of LDL and plasma in healthy volunteers.

**Methods:** We administered powdered green tea of 4.5 g/day (total catechins 180 ± 13 mg/kg) to 8 healthy volunteers (2 males and 6 females, 28 ± 10 years old, 56 ± 10 kg of body weight) for 14 days. Green tea drinking was prohibited for 1 week before and after test period of tea drinking and during entire test period food intake were not changed as much as possible. Blood samples were collected after a 12-hour fasting. Plasma lipids and antioxidants were measured. The oxidizability of LDL was measured by the method of Esberich using 2.2'-Azobis-(2-amidinopropano) dithydrochloride and CuSO4. The oxidizability of plasma was measured by Kontush's method using CuSO4.

**Results:** After green tea drinking, plasma levels of total and LDL cholesterol were decreased but the levels of α-tocopherol were not changed. The lag time of plasma diene formation was prolonged from 114 ± 48 min to 240 ± 70 min significantly (P < 0.001). The lag time of LDL diene formation was not changed during tea drinking but shortened after the cessation of tea drinking.

**Conclusions:** Drinking of green tea decreased plasma cholesterol and reduced oxidizability of plasma and might be antiatherogenic.

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**TuT19:W12**

**Inhibition of lipoxgenase activity prevents development of alimentary atherosclerosis**

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**Objective:** The role of lipoxgenase activity in atherosclerosis development is not clear. The aim of our study was to investigate the effect of long-term intravenous administration of lipoxgenase inhibitor, linoleoyl hydroxamic acid (LHA, 1.5 mg/kg) on atherogenesis in rabbits.

**Results:** Morphological examination of aorta arc in animals treated during 1.5 month with cholesterol-enriched diet (G2 group) has shown that the mean area of lipid plaques was 15.1 ± 1.4% of the arc wall surface, while the mean area of cholesterol + LHA-treated animals (G3 group) was only 6.1 ± 1.0%. Comparison of biochemical parameters showed that in the G1 (control), G2...
and G3 groups cholesterol levels were 30 ± 3, 520 ± 63 and 318 ± 24 mg %, respectively. The amount of triacylglycerides was almost similar in all groups. In
Alterations of lipid profile during acute ischaemic syndromes

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Objective: Alterations in plasma lipid levels during acute myocardial infarction (AMI) are observed. In this study we investigated alterations during all acute ischaemic syndromes with an equal pathogenesis, using the following categories: AAMI, non-Q-AMI and unstable angina (UA).

Methods: We studied 23 patients (pts) with AMI (ST segment elevation and high levels of myocardial injury markers CK, LDH) – group I, 7 pts with non-Q-AMI (ST depression with low negative T waves and high levels of CK, LDH) – group II and 12 pts with UA (ST depression without CK, LDH increase) – group III. Samples were taken the first day after admission and before discharge. A decrease of 18 ± 8 mg/dl (5 ± SD) in total cholesterol at the admission in comparison to discharge value there was in group I (p < 0.001), 12.2 ± 7.9 mg/dl in group II (p < 0.05) and –8 ± 12 mg/dl in group III (NS). The LDL-cholesterol was also reduced in comparison to discharge values at the admission by 15.5 ± 7.2 mg/dl in group I (p < 0.001) and 8 ± 5.2 mg/dl in group II (p < 0.05) while there were no significant changes in group III. There were no significant changes in levels of HDL-cholesterol and triglycerides in any of the three groups. No differences in these results are observed when the patients of group I were subdivided in those who received thrombolysis and those who did not.

Conclusions: During acute ischaemic syndromes alterations in lipid profile were observed in admission only when infection necrosis occurred as shown by the high levels of myocardial injury markers independently of development or no of a Q wave. The decrease appeared in total and LDL-cholesterol.

Coronary disease model in patients with ventricular arrhythmias induced with exercise testing

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Purpose: To evaluate whether the presence of frequent ventricular premature beats (VPB) and no sustained ventricular tachycardia (NSVT) during the exercise testing is associated with a greater quantity of myocardial ischemia especially in the territory of the anterior descending artery (AD), and the clinical differences.

Methods: Consecutively we studied 74 patients (pts) who underwent a treadmill exercise testing and a cardiac catheterism as a result of a history of angina. Two groups were established: Group A made up of 43 pts with VPB or NSVT and Group B made up of 31 pts without ventricular arrhythmias. Seventeen clinical and invasive variables were evaluated.

Results:

<table>
<thead>
<tr>
<th>Group A (n = 43)</th>
<th>Group B (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Diabetes</td>
<td>6%</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number vessels</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td>AD</td>
<td>80%</td>
</tr>
<tr>
<td>Circumflex</td>
<td>5%</td>
</tr>
<tr>
<td>Multisegmant</td>
<td>4%</td>
</tr>
<tr>
<td>Mammography</td>
<td>59%</td>
</tr>
<tr>
<td>Coronary disease</td>
<td>22%</td>
</tr>
</tbody>
</table>

Conclusions: The presence of VPB or NSVT during the stress testing is associated with a smaller number of coronary risk factors, worse ejection fraction with a similar number of vessels affected, similar lesion of DA but with lesser multisegment pathology in the affected vessel.

Characteristics of patients with early-onset coronary disease and normal coronaryangiography

A. Batalla1, G.I. Cubero2, J.J.R. Reguero2, S. Hevia2, E. Merino2, S. Braga2, E. Bustillo2, J.C. Sammartini2, A. Cortina2. Department of Cardiology; 1Hospital de Cabueñas (Gijón); 2Hospital Central de Asturias (Oviedo), Spain

Purpose: To determine common characteristics of patients under 50 years of age with coronary disease and normal coronaryangiography.

Methods: Consecutively, 230 male patients before 50 years of age (mean age 43 ± 5 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease, were prospectively studied. Cardiac catheterism was made in 140 patients, and normal coronaryangiography was seen in 13 of these. Smoking habits, arterial hypertension, diabetes and dyslipidemia were determined during the acute phase by means of a questionnaire, physical examen and fasting analysis. Levels of Total cholesterol (Tcho), HDL cholesterol (HDL), triglycerides (TG), apolipoprotein AI (Apo AI), apolipoprotein B (Apo B), Lp(a), LDL cholesterol (LDL) and total corrected cholesterol (TCC) were analysed after patients had fasted for more than 12 hours. In order to determine new coronary events a mean follow-up of 32 ± 13 months was carried out.

Results: Differences were found in the levels of HDL: 32 ± 9 mg/dl in abnormal coronaryangiography compared with 38 ± 10 mg/dl in normal coronaryangiography (p < 0.05); Lp(a): 37 mg/dl (16-65) compared with 12 mg/dl (6-34) (p = 0.006); TG: 152 mg/dl (124-209) compared with 115 mg/dl (95-184) (p < 0.05). No differences were found in the levels of Apol AI, Apo B, Tcho, LDL and TCC nor in the prevalence of hypertension, diabetes and smoking habits.

No differences were seen during follow-up in relation to the number of coronary events, mortality and prevalence of angina, myocardial infarction and heart failure.

Conclusions: Patients with early-onset coronary disease under the age of 50 years and with normal coronaryangiography present a more favourable lipid profile with higher levels of HDL and lower levels of TG and Lp(a) than those patients with the same characteristics and with coronary stenosis shown on coronaryangiography.

Angiographic findings in non-Q wave myocardial infarction: Differences between sexes

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Purpose: To determine the angiographic findings in non-Q wave myocardial infarction according to the gender.

Methods: From a cohort of 48 patients who were consecutively admitted to the hospital with a diagnosis of non-Q wave myocardial infarction and underwent a coronarographic study a group of 37 males (Group M) and a group of 11 females (Group F) were made. The age, tobacco consumption and significative lesions in the three major coronary vessels were analyzed.

Results: The significant data are presented

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Tobacco consumption</th>
<th>Left anterior descending</th>
<th>Proximal lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>58 ± 11</td>
<td>75%</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>68 ± 10</td>
<td>9%</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>64%</td>
<td>90%</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>50%</td>
<td>27%</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Conclusions: Females with non-Q wave myocardial infarction resulted to be older than males and the left anterior descending artery was the most frequently affected. However, proximal lesions and tobacco consumption were significantly more frequent in males.

Changes of lipid values in the acute phase of coronary artery disease

A. Batalla1, J.J.R. Reguero2, G.I. Cubero2, J.C. Sammartini2, S. Hevia2, M. Sieres1, T. Ravina1, A. Cortina2. Department of Cardiology; 1Hospital de Cabueñas; Gijón; 2Hospital Central de Asturias, Oviedo, Spain

Purpose: To determine which changes of the lipid profile exist between the acute phase of the coronary event and after a follow-up.

Methods: We choose 96 patients (mean age 42 ± 5 years) from a cohort of 230 patients (pts) admitted to our hospital for an acute coronary event and who were not under hypolipidemic treatment nor at the admission neither during the follow-up. The diagnosis was acute myocardial infarction in 60 pts (62.5%) and unstable angina in 36 pts (37.5%). After 12 hours of fasten, determinations of total cholesterol (Tcho), HDL cholesterol (HDLcho), triglycerides (TG), Apo AI, and Apo B were carried out by the same laboratory in the seventh day of hospitalization and after a follow-up of 31 ± 12 months. Data are presented in mg/dl for variables of gaussian distribution.

<table>
<thead>
<tr>
<th>mg/dl</th>
<th>Acute Phase</th>
<th>Chronic Phase</th>
<th>Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>149 (74-324)</td>
<td>136 (54-410)</td>
<td>-9</td>
</tr>
<tr>
<td>ApoB</td>
<td>91 ± 22</td>
<td>124 ± 23</td>
<td>+33</td>
</tr>
<tr>
<td>ApoAI</td>
<td>95 ± 19</td>
<td>136 ± 27</td>
<td>+43</td>
</tr>
<tr>
<td>Tcho</td>
<td>203 ± 39</td>
<td>232 ± 46</td>
<td>+14</td>
</tr>
<tr>
<td>HDLcho</td>
<td>34 ± 9</td>
<td>43 ± 12</td>
<td>+26</td>
</tr>
</tbody>
</table>

Note: The values are presented as median (range) for continuous variables, and as counts (proportion) for categorical variables. The comparisons were made using the non-parametric Mann-Whitney U test for continuous variables and the chi-square test for categorical variables. The p values were corrected for multiple comparisons using the Bonferroni correction.
as mean ± SD and for TG as the median and P5–P95. To analyze differences between parametric variables the Student t-test was used. For non-parametric variables, we used the Wilcoxon test.

**Results**: A significant decrease of the lipid profile in the acute phase of a coronary event is found.

**Conclusions**: A significant decrease of the lipid profile in the acute phase of a coronary event is found.

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**Tu7T:W14** Characteristics of coronary heart disease in women older than 65 years

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**Purpose**: To determine the characteristics of coronary heart disease in women older than 65 years.

**Methods**: From prospective form we have studied a group of 100 women older than 65 years, admitted to our hospital with a diagnosis of coronary heart disease. The following variables were studied: age, clinical presentation, tobacco consumption, hypercholesterolemia, arterial hypertension, diabetes, complications, thrombolytic therapy and acute mortality.

**Results**: The results are shown in the following table:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>74 ± 6 years</td>
</tr>
<tr>
<td>Clinical presentation:</td>
<td></td>
</tr>
<tr>
<td>Angina</td>
<td>45%</td>
</tr>
<tr>
<td>Acute myocardial infarction</td>
<td>55%</td>
</tr>
<tr>
<td>Tobacco consumption</td>
<td>4%</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>33%</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>50%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>25%</td>
</tr>
<tr>
<td>Heart failure</td>
<td>21%</td>
</tr>
<tr>
<td>Arhythmias</td>
<td>17%</td>
</tr>
<tr>
<td>Acute mortality</td>
<td>13%</td>
</tr>
<tr>
<td>Thrombolytic therapy</td>
<td>17%</td>
</tr>
</tbody>
</table>

**Conclusions**: In our study arterial hypertension was the most prevalent coronary risk factor in women older than 65 years, the predominant complication was heart failure and thrombolytic therapy was given less than 20%.

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**Tu8T:W14** Unstable angina and persistent T wave inversion. Prospective study

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**Purpose**: To determine the clinical implications of T wave inversion in unstable angina.

**Methods**: We prospectively studied 90 patients (pts) admitted to the coronary care unit for prolonged chest pain (≥20 minutes) while at rest with T wave inversion in more than two precordial electrocardiogram (ECG) leads, during hospital stay, without previous myocardial infarction, enzymatic arise and all patients were given a daily ECG and hemodynamic study and echocardiogram on the seventh day of their hospital stay.

**Results**: The results are shown in the following table:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>67 ± 8 years</td>
</tr>
<tr>
<td>Hypertension</td>
<td>45%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>17%</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>39%</td>
</tr>
<tr>
<td>Tobacco consumption</td>
<td>43%</td>
</tr>
<tr>
<td>Apical dyskinesy</td>
<td>55%</td>
</tr>
<tr>
<td>Normal coronary angiographic</td>
<td>6%</td>
</tr>
<tr>
<td>Critical lesion in main coronary artery</td>
<td>2%</td>
</tr>
<tr>
<td>Lesion in left anterior descending</td>
<td>93%</td>
</tr>
<tr>
<td>– Complex lesion</td>
<td>89%</td>
</tr>
<tr>
<td>– Proximal</td>
<td>73%</td>
</tr>
<tr>
<td>Lesion in right coronary</td>
<td>1%</td>
</tr>
<tr>
<td>Total occlusion</td>
<td>7%</td>
</tr>
<tr>
<td>Appropriate collateral</td>
<td>8%</td>
</tr>
</tbody>
</table>

**Conclusions**: Patients with unstable angina with persistent T wave inversion, usually are male, with several risk factors, high frequency of apical dyskinesy and proximal complex lesion in left anterior descending. They have low frequency of previous angina, critical lesion in left main coronary artery, total occlusion and appropriate collateral circulation.

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**Tu9T:W14** Coronary angiographic findings in the early coronary artery disease

A. Batalla1, J.R. Reguero2, G.I. Cubero2, S. Hevia2, E. Merino2, E. Bustillo2, M. Rodríguez1, A. Cortina2, Department of Cardiology, 1Hospital de Cabueñas, Gijón; 2Hospital Central de Asturias, Oviedo, Spain

**Purpose**: To determine the angiographic characteristics in the early symptomatic coronary artery disease.

**Methods**: Two-hundred and thirty patients (pts) admitted to the coronary care unit by acute myocardial infarction and unstable angina with electrocardiographic ischaemic changes were prospectively evaluated (mean age 43 ± 5). One-hundred and forty-two pts (62%) underwent a coronary angiographic study. The 43% and the 87% of coronary angiographies were done in case of myocardial infarction and unstable angina respectively. We decide a score as follow: 1 = one vessel stenosis > 70%; 2 = two vessels stenosis > 70%; 3 = three vessels stenosis > 70%; 4 = milking; 5 = isolated left main coronary artery disease; 6 = left main coronary artery disease and someone more vessel; 7 = isolated vessel stenosis ≤ 70%; 8 = stenosis ≤ 70% and someone more lesion > 70%; 9 = normal coronary angiogram.

**Results**: Score 1 = 14 (32%), 35% of infection of (I) an, 29% of angiina (A). Score 2 = 31 (22%), 26% of I, 19% of A. Score 3 = 23 (16%), 11% of I, 20% of A. Score 4 = 2 (1.5%), 0% of I, 25% of A. Score 5 = 3 (2%), 0% of I, 35% of A. Score 6 = 5 (3.5%), 0% of I, 6% of A. Score 7 = 5 (3.5%), 5% of I, 2.5% of A. Score 8 = 15 (10.5%), 13% of I, 9.5% of A. Score 9 = 13 (9%), 10% of I, 8% of A.

In 87 pts (61%) the lesion was located in the left anterior descending artery. The circumflex showed lesions in 60 pts (42%) and the right coronary in 79 pts (55%). We diagnosed left main coronary artery disease in 8 pts (5%).

**Conclusions**: In our study, we found normal coronary angiogram in 10% of the cases. The left anterior descending was the artery more frequently affected. Isolated left main coronary disease was an infrequent finding and angina was the predominant symptom. Isolated lesions ≤ 70% were not related with the clinical presentation.

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**Tu10T:W14** Therapeutic strategy in coronary artery occlusion according ejection fraction

A. Batalla1, J. Mayordomo1, J.C. Sammartín2, S. Hevia3, T. Ravilla1, Department of Cardiology, 1Hospital de Cabueñas, Gijón; 2Hospital Central de Asturias, Oviedo, Spain

**Purpose**: To show the importance of ejection fraction on the therapeutic strategy in coronary artery occlusion.

**Methods**: The study was done in 317 consecutive patients (pts) undergoing coronary angiography for suspected coronary artery disease (CAD) yield ≥ one coronary artery narrowed ≥70% in diameter assessed visually in terms of diameter narrowing. The patients were separated into two groups according to ejection fraction (<40% and >40%). Differences between frequencies were assessed using the chi-square test.

**Results**: F, FE < 40% (39) FE > 40% (262) p

<table>
<thead>
<tr>
<th>Category</th>
<th>FE &lt; 40%</th>
<th>FE &gt; 40%</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTCRA</td>
<td>5%</td>
<td>30%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Bypass</td>
<td>21%</td>
<td>31%</td>
<td>ns</td>
</tr>
<tr>
<td>Medical</td>
<td>74%</td>
<td>38%</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Conclusions**: The ejection fraction is a major factor determinant of the difference in the management of patients with coronary artery occlusions.

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**Tu11T:W14** Severity of early coronary disease and cardiovascular risk factors

A. Batalla1, J.J.R. Reguero2, G.I. Cubero2, J.C. Sammartín2, S. Hevia2, M. Sieres1, T. Ravilla1, Department of Cardiology, 1Hospital de Cabueñas, Gijón; 2Hospital Central de Asturias, Oviedo, Spain

**Purpose**: To determine whether the principal cardiovascular risk factors have a relationship with the number of significantly stenosed coronary arteries in subjects with early onset of coronary disease.

**Methods**: A prospective study was made of 132 males less than 50 years of age (25–49) having coronary disease (acute myocardial infarction or unstable angina) and who had undergone cardiac catheterization. Coronary lesions with >50% luminal stenosis were considered as significantly. We studied the prevalence of principal coronary risk factors: smoking habit, hyperension, diabetes and ratio Total cholesterol/HDL cholesterol > 5 (Tchol/HDL > 5).
5. Differences between frequencies were assessed using the chi-square test. During hospitalization and after 12 hours of fasting, blood samples were drawn and total cholesterol (TC), HDL cholesterol (HDL), triglycerides (TG), apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB) and Lp(a) were determined in the same laboratory. The total corrected cholesterol (TCC) was calculated employing the formula TCC = TC-chol - Lp(a)/3. LDL cholesterol (LDL) was calculated using the formula of Friedewald, when the TG did not exceed 300 mg/dL. ANOVA was used for the parametric variables and Kruskal-Wallis for the non-parametric ones.

**Results:** Significant differences were found in hypertension (p = 0.04), ratio Tchol/HDL > 5 (p = 0.003) and in the levels of Tchol (p < 0.03), LDLChol (p < 0.05), ApoB (p < 0.03), TG (p < 0.02) and Lp(a) (p < 0.005) according to the number of vessels showing significant stenosis. No significant differences were obtained in smoking habit, diabetes nor in the levels of HDL, Apo AI and TCC.

**Conclusions:** In our study of young coronary patients, significant differences were found in prevalence of hypertension, ratio Tchol/HDL > 5 and in the levels of TG, LDLChol, ApoB and Lp(a) according to the number of vessels showing lesions. In spite of differences found in Tchol levels, these disappeared once the levels were corrected.

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**TuT12/W14**

**Is the different composition of wall in the proximal left main coronary artery responsible of different sign to distal lesion?**

A. Batalla1, I. Mayordomo2, J.C. Sanmartín3, J. Gutiérrez1, T. Raviña1.

1. Department of Cardiology; 2. Hospital de Cabueñas, Gijón; 3. Hospital Central de Asturias, Oviedo, Spain

**Purpose:** The left main coronary artery (LMCA) in its first 2-4 mm are within the aortic wall and are subject to conditions that affect the aorta and has considerable smooth muscle and elastic tissue. We try to know if these anatomic and histologic factors of the proximal lesion could produce different signs to distal lesion.

**Methods:** We studied 132 patients (pts) with LMCA obstructive disease and were divided in two groups: 36 with proximal lesion (Group P) and 96 with distal lesion (Group D).

**Results:**

<table>
<thead>
<tr>
<th>Group</th>
<th>P</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTA</td>
<td>69%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Complete</td>
<td>23%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>FE</td>
<td>63 ± 9%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>N Vessels</td>
<td>1.8 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Collateral</td>
<td>53%</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*HTA = Arterial hypertension, N. Vessels = Number of vessels

**Conclusion:** The proximal lesion in LMCA is more frequent in hypertension patients, is less complex and is associated to left ventricular disfunction and more developed collateral circulation than lesion in distal LMCA.

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**TuT13/W14**

**Association of risk factors in early coronary disease**

A. Batalla1, G.I. Cubero0, J.J.R. Reguero0, S. Hevia2, S. Braga2, E. Bustillo2, A. Cortina0.

1. Department of Cardiology; 2. Hospital de Cabueñas, Gijón; 0. Hospital Central de Asturias, Oviedo, Spain

**Purpose:** To determine the association of the principal risk factors in patients with early-onset coronary disease before 50 years.

**Methods:** Consecutively, 230 male patients before 50 years of age (mean age 43 ± 5 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease, were prospectively studied. By means of a structured questionnaire the presence of smoking habits, hypertension, diabetes and dyslipidemia were determined. A physical exam and fasting analysis were also made. Patients were grouped according to the number of risk factors.

**Results:** Only 1 patient (0.5%) showed no risk factors; 39 patients (17%) showed 1 factor; 105 patients (45%) showed 2 factors; 79 patients (34.5%) showed 3 factors and 6 patients (3%) four factors.

The most frequent association were: smoking habits and dyslipidemia in 92 patients (40%) and smoking habits, hypertension and dyslipidemia in 66 patients (29%).

**Conclusions:** Most of males with early-onset coronary disease (under 50 years) present more than one coronary risk factor, being the association smoking habits-dyslipidemia the more frequent combination.

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**TuT14/W14**

**Are non-significant coronary lesions related to clinical events?**

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**Purpose:** To determine the angiographic characteristics of the early symptomatic coronary artery disease.

**Methods:** Ninety-eight patients (pts) admitted to the coronary care unit presenting unstable angina with electrocardiogram (ECG) changes were evaluated (mean age 43 ± 5). Eighty-five patients (87%) underwent a coronaryangiographic study. Scoring was as follow: 1 = one vessel stenosis > 70%; 2 = two vessels stenosis > 70%; 3 = three vessels stenosis > 70%; 4 = milking; 5 = isolated left main coronary artery disease; 6 = left main coronary artery disease and other vessel; 7 = isolated vessel stenosis ≤ 70%; 8 = stenosis ≤ 70% and other lesion > 70%; 9 = normal coronary angiogram. The frequency of the lesions and the ischaemia related artery was studied.

**Results:**

<table>
<thead>
<tr>
<th>Score</th>
<th>n</th>
<th>%</th>
<th>Related</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>2.5</td>
<td>(100%)</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>9.5</td>
<td>3 (37%)</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions:** In our study non significant lesions were poorly related with coronary events. Most of the anginas and infarctions showed some lesions greater than 70%.

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**TuT15/W14**

**Characteristics of hypertensive patients with early coronary disease**

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**Purpose:** To determine the characteristics of patients under 50 years of age with coronary disease and arterial hypertension.

**Methods:** Consecutively, 229 male patients (pts) before 50 years of age (mean age 42 ± 4 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease were prospectively studied. Smoking habits, arterial hypertension, diabetes and dyslipidemia were determined during the acute phase by means of a questionnaire, physical examen and fasting analysis. In order to determine new coronary events a mean follow-up of 32 ± 13 months was carried out.

**Results:**

<table>
<thead>
<tr>
<th>Hypertension (84)</th>
<th>No Hypertension (145)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tchol/HDL &gt; 5</td>
<td>77 (92%)</td>
<td>113 (98%)</td>
</tr>
<tr>
<td>Mortality during follow-up</td>
<td>1 (1%)</td>
<td>12 (8%)</td>
</tr>
</tbody>
</table>

No differences were found in the prevalence of diabetes or smoking habits in either group. No differences were seen during follow-up in the presence of anger, myocardial infarction, heart failure and the need for revascularisation.

**Conclusions:** Patients with clinical onset of coronary heart disease before 50 years and with arterial hypertension present a worse lipid profile than those without hypertension. However, they present a better prognosis with less mortality in the follow-up.

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**TuT16/W14**

**Therapeutic approach in the acute phase in early-onset coronary disease**

A. Batalla1, G.I. Cubero0, J.J.R. Reguero0, S. Hevia1.

1. Department of Cardiology; 2. Hospital de Cabueñas, Gijón; 0. Hospital Central de Asturias, Oviedo, Spain

**Purpose:** To determine which are the treatments employed during acute phase in male patients under 50 years of age with coronary disease.

**Methods:** Consecutively, 230 male patients before 50 years of age (mean age 43 ± 5 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease (166 myocardial infarction, 64 unstable...
angina), were prospectively studied. The treatment employed at discharge and the need for revascularisation were determined.

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>Total Group</th>
<th>Infarction</th>
<th>Angina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Revascularisation</td>
<td>76 (33%)</td>
<td>44 (27%)</td>
<td>32 (30%)</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>126 (55%)</td>
<td>100 (61%)</td>
<td>26 (41%)</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>82 (36%)</td>
<td>43 (26%)</td>
<td>39 (61%)</td>
</tr>
<tr>
<td>Antithrombotic treatment</td>
<td>230 (87%)</td>
<td>145 (88%)</td>
<td>55 (58%)</td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>24 (10.5%)</td>
<td>24 (14.5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Nitrates</td>
<td>134 (58.5%)</td>
<td>92 (56%)</td>
<td>42 (66%)</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>33 (14%)</td>
<td>29 (18%)</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>Diuretics</td>
<td>20 (9%)</td>
<td>16 (10%)</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>Lipid lowering treatment</td>
<td>84 (37%)</td>
<td>62 (38%)</td>
<td>22 (34%)</td>
</tr>
</tbody>
</table>

**Conclusions:** One third of male patients under 50 years of age underwent cardiac revascularisation. A poor treatment with betablockers and excessive use of nitrates are the most notable findings in patients with myocardial infarction. Finally, a 37% of the patients is undergoing lipid lowering treatment at the time of discharge.

**TuT17W14 Elevation of cardiac troponin I as a prognostic factor after coronary bypass surgery**

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**Objective:** To compare prognostic significance of Troponin I (TnI) and Creatine kinase MB mass (CK-MB) elevation and effectiveness of measurement of TnI for detection of perioperative myocardial infarction (PMI) after coronary bypass surgery (CABS).

**Methods:** 42 patients admitted for elective CABS were studied. Blood samples were drawn exactly before surgery and 1, 2, 12, 24, 36 h after aortic cross-clamp release. Serum levels of CK-activity (Hitachi 912), CK-MB mass and TnI (MIAA, Abbott, normal value < 0.4 and < 2.3 ng/mL for non-AMI) were measured. During 7 day postoperative period clinical ECG, echocardiographic evidence of PMI were checked.

**Results:** Almost all patients (40 of 42) had elevated TnI value > 2.3 ng/mL. All patients were divided into two groups according to the TnI levels. 16 patients were included into group I with highly increased Tnl > 25 ng/mL. Adverse outcome for these patients was significantly higher: 3 died in postoperative period, in 4 of them PMI was diagnosed and new ischemic ECG changes or new area of hypokinesia on echocardiography was detected in 5 patients. 26 patients were included into group II with TnI < 25 ng/mL in which no death or PMI were noted.

**Conclusions:** Measurement of TnI may be used as a good prognostic factor after coronary artery bypass surgery.

**TuT18W14 Inflammatory indexes and acute myocardial infarct size**

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**Objective:** We explored the strict relationship between inflammation and myocardial lesion extension.

**Methods:** The study was carried out on 87 patients (23% women, 26% with non-insulin diabetes), admitted in our Coronary Unit for a first Q-wave myocardial infarction. The study provided analysis of family anamnesis, clinical evolution, serum inflammatory indexes (CRP, WBC and Fibronogen), myocardial necrosis enzymes (Creatine Kinase, CK) and intercurrent complications.

**Results:** We divided patients in two groups:

<table>
<thead>
<tr>
<th>CK peak value (U/L)</th>
<th>Fibrogen on admission</th>
<th>Fibrogen peak value</th>
<th>CRP peak value</th>
<th>CRP on admission</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&gt;1000 Group A)</td>
<td>378.7 mg/dl</td>
<td>513.96</td>
<td>11.03</td>
<td>4.045</td>
</tr>
<tr>
<td>(&lt;1000 Group B)</td>
<td>269.7 mg/dl</td>
<td>303.21</td>
<td>3.05</td>
<td>0.631</td>
</tr>
<tr>
<td>P value</td>
<td>0.007</td>
<td>0.00066</td>
<td>0.025</td>
<td>0.035</td>
</tr>
</tbody>
</table>

**Conclusions:** Fibrogen concentration on admission > 500 mg/dl was related with later arrival at hospital (45–60 min), higher CK levels on admission (762.1 ± 235 IU p = 0.016), greater incidence of complication (47% vs 33%), in particular ventricular aneurysm formation (22% vs 8%) and worse prognosis. CRP peak value > 5 mg/dl was instead associated with myocardial lesion extension (1897 vs 957.4 U/L p = 0.026) and left ventricular EF (45.6 vs 55.71% p = 0.01).

**TuT19W15 Clusters in patients with cardiovascular disease**

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**Introduction:** An elevated percentage of patients with hypertension have several risk factors, which could lead them a high cardiovascular risk. On another hand it is also well known the phenomena of "clustering", that is the family prevalence.

**Objective:** The authors evaluated in obese hypertensive of a referred hypertension clinic, the prevalence of other cardiovascular risk factors in their parents.

**Material and Methods:** There were studied 350 patients of both sexes, with essential hypertension and obesity. The patients were submitted to an inquiry, after a previously established protocol that included the determination of the body mass index, cholesterol, blood pressure (BP), coronary heart disease (CHD), strokes (S), and diabetes (DMNID). Information of the existence of these same cardiovascular risk factors and concomitant diseases were picked up in their progenitors. With exception of 8 fathers and 1 mother, it was possible to take a sample database of the remaining progenitors.

**Results:**

<table>
<thead>
<tr>
<th>(&gt;65 Years)</th>
<th>(&lt;65 Years)</th>
<th>DMNID</th>
<th>High cholesterol</th>
<th>Obesity</th>
<th>BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Father</td>
<td>30</td>
<td>30</td>
<td>27</td>
<td>24</td>
<td>261</td>
</tr>
<tr>
<td>(n = 342)</td>
<td>8.8%</td>
<td>8.8%</td>
<td>7.9%</td>
<td>7.6%</td>
<td>5.2</td>
</tr>
<tr>
<td>Mother</td>
<td>48</td>
<td>42</td>
<td>25</td>
<td>24</td>
<td>17</td>
</tr>
<tr>
<td>(n = 349)</td>
<td>13.8%</td>
<td>12.9%</td>
<td>7.1%</td>
<td>7.1%</td>
<td>6.9%</td>
</tr>
</tbody>
</table>

**Conclusion:** The data suggest family clustering of obesity, high blood pressure, stroke and coronary heart disease in this sample of hypertensive and obese patients.

*XIIth International Symposium on Atherosclerosis, Stockholm, Sweden, June 25–29, 2000*
TuT3W15  Association between apolipoprotein E phenotype and lipid profile and adiposity in Portuguese children

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Objective: The main objective of the study was to examine the effect of apolipoprotein E (apoE) phenotype on tracking of serum cholesterol, and also the association between nutritional status and body composition according to apoE phenotypes.

Population and Methods: 45 randomly selected children living in a small rural village in north-east Portugal were prospectively evaluated regarding nutritional status, body composition and lipid profile. The apoE phenotype was determined by the polymerase chain reaction (PCR) followed by restriction enzyme digestion.

Results: The frequencies of the apoE alleles *2, *3 and *4 were 0.08, 0.78 and 0.14, respectively. No differences between gender were found. ApoE phenotypes did not influence total, LDL or HDL-cholesterol. However, apoE genotypes influences the tracking of lipids. Children with 3/3 and 3/4 had significant correlations between total and LDL cholesterol prospectively observed with the highest values for those with apoE 3/4 (LDL-cholesterol observed at 2–5 years and 9–12 years, p < 0.001). A significant association was found between adiposity and ApoA1 levels in apoE*2 carriers (subcapsular plus tricipital skinfolds: r = -0.821, p < 0.01; submandibular skinfold: r = -0.858, p < 0.01).

Conclusions: contrary to our previous estimates in the urban Porto district (NW Portugal), the frequencies of apoE*2 and apoE*4 alleles now observed are higher than those currently reported in other populations from southern Europe. As apoE phenotype seems to influence cholesterol tracking from childhood, and also risk factors aggregation, this genotype must be considered when predicting future cardiovascular risk factors, namely cholesterol levels and adiposity.

TuT4W15  Evidence for angiotensin 1 converting enzyme gene (ACE) polymorphism as modulator of factor VII activity in families of men with coronary heart disease

T. Wesołowska, M. Jastrzębska, A. Ciechanowicz, K. Chełstowski, M. Naruszewicz. Pomeranian Medical University, Szczecin, Poland

Objective: To assess the role of ACE gene polymorphism in determining factor VII activity in members of a family with a positive history of ischaemic heart disease (IHD).

Methods: Activities of ACE and factor VII (FVII), levels of fibrinogen (FB), triglycerides (TG) and total cholesterol concentrations (TC), ACE genotypes and body mass index (BMI) were analyzed in 67 families (134 parents, mean age 43.7 ± 5.3 yrs and 171 children, mean age 16.6 ± 4.0 yrs).

Results: Significant differences in the frequency of ACE genotypes were found between fathers and mothers (ID 26% vs. 24%, ID 57% vs. 53%, ID 17% vs.23%) and sons and daughters (ID 17% vs. 32%, ID 64% vs. 58%, ID 19% vs. 10%, respectively). Activities of ACE and factor VII differed according to ACE genotype and were highest in subjects with allele D. FVII activities were higher in daughters than sons. ACE activity correlated with FVII activity and FB values in mothers. FVII correlated with TG levels in mother with ID genotype and with ACE in daughters. ACE activity correlated with TG, BMI and diastolic blood pressure values in daughters.

Conclusions: Our results suggest that subjects with the D allele are at risk of prothrombotic state. A change in life-style is advisable for daughters of fathers with IHD.

TuT5W15  Risk factors in young patients with peripheral atherosclerosis

K. Kröger, C. Buss, F. Santos, G. Rudowsky. Department of Angiology. University of Essen, Germany

Objective: Especially the combination of multiple risk factors is associated with the development of atherosclerosis. Patients with an early manifestation of atherosclerotic disease are likely to show an extraordinary risk profile. Therefore we analyzed different risk factors in young patients with peripheral arterial occlusive disease (PAOD) compared to old patients.

Methods: We did an analysis of the risk profiles in 303 patients, who were send for interventional treatment of PAOD. The risk profiles were described for different age groups (54 patients ≤ 50 years, 194 patients 51–74 years, 55 patients ≥ 75 years). Multiple linear regression analysis and analysis of variance was performed to look for age-dependent effects.

Results: Elevated total cholesterol, triglyceride and nicotine abuse were more frequent in patients younger than 50 years. Diabetes mellitus (DM) and hypertension were more frequent in patients older than 75 years. The different frequencies for smoking, DM and hypertension were age-related (p < 0.05). Concerning HDL- and LDL-cholesterol, fibrinogen, lipoprotein a and homocysteine there were no relevant age-related differences with the exception of homocysteine and uric acid. The coincidence with clinical manifest myocardial infarction (MI) was 11.5% in the patients ≤ 50 years, 20.6% in the patients aged 51–74 years and 16.4% in the patients ≥ 75 years, for cerebral stroke it was 5.6%, 17.5% and 14.5%. Patients ≤ 50 years with PAOD and a history of MI were characterized by high levels of total cholesterol, triglyceride and lipoprotein a. Excluding patients with prior MI there were no differences in risk profiles between the 3 age groups.

Conclusions: In patients with PAOD the risk profile in those ≤50 years is not different from that in older patients. In contrast an additional MI in such a population is associated with pathologic lipid profiles.

TuT6W15  Segmental manifestation of peripheral atherosclerosis and 1st association to risk factors

C. Buss, K. Kröger, K. Renzing-Köbler1, F. Santos, G. Rudowsky. Department of Angiology; 1Institute for Medical Informatics, Biometrics and Epidemiology. University of Essen, Germany

Objective: Peripheral atherosclerosis (PASCL) often begins with segmental manifestation. There is some evidence for an association between aortoiliac manifestations and younger age of patients. More specific risk profiles for isolated aortoiliac, femoropopliteal or crural manifestations have not been described. To prove for possible associations we did a statistical analysis of segmental PASCL and risk factors.

Methods: In 132 patients (mean age: 61 ± 13 years) with PASCL the arterial segments with occlusion or stenosis were angiographically documented: 17 aortoiliac, 45 femoropopliteal and 25 crural arteries and 45 multiple manifestations. We analysed total-, HDL- and LDL-cholesterol, triglyceride, lipoprotein a, fibrinogen, uric acid, homocysteine, hematocrit, erythrocyte sedimentation rate, HbA1, IgG- and IgM-antibodies vs. Cytomegalovirus, Herpes simplex-virus, Chlamydia pneumoniae and Helicobacter pylory and smoking, hypertension and obesity were evaluated.

Results: Patients' age had the strongest correlation with isolated segmental manifestation (p < 0.0001). With isolated aortoiliac manifestation patients were younger than without (54 ± 9 vs. 62 ± 13 years). Patients with isolated femoropopliteal manifestation were older than patients without (66 ± 11 vs. 58 ± 13 years). None of the investigated risk factors showed a correlation with these age related differences. Independent from the age related differences for the nicotine abuse a p-value of 0.08 was estimated, but in smokers a diffuse manifestation was most frequent.

Conclusions: There are age dependent differences of the prevalence of isolated aortoiliac or femoropopliteal atherosclerotic occlusions or stenosis. An association of these differences to a specific risk profile, as it has been described in the literature, was not found.

TuT7W15  Risk factors for atherosclerosis in survivors of myocardial infarction and their spouses: Comparison with spousal pairs without personal and family history of atherosclerosis

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Objective: We compared the risk factors for atherosclerosis (AS) among 71 survivors of myocardial infarction (MI), their Spouses (n = 54) and 38 Control spousal pairs with no personal and family history of AS in three generations.

Methods: Clinical status, life-style risk factors, plasma lipoprotein and homocysteine (Hcy by HPLC) levels were examined. Apo-E polymorphisms, mutations of C677T of MTHFR and lipoprotein lipase (ApoA1, ApoB100 and LPL) were assessed using the PCR method.

Results: MI-survivors and their spouses had significantly higher blood pressure, BMI, WHR and more pathalogical plasma lipid levels compared to control men and women, respectively. They also had higher Hcy levels than...
Association between genetic polymorphisms (ACE genotype, PAI-1 genotype and MTHFR genotype) and coronary artery stenosis

E. Okada, K. Oda, K. Asazuma, K. Eguchi, S. Kosaka, G. Tohda, S. Takahashi, H. Kimura, H. Yoshida, I. Miyamoto. Third Department of Internal Medicine; Department of Clinical and Laboratory Medicine; Fukushima Medical University, Fukushima, Japan

Objective: To assess the relationship between genetic polymorphisms (ACE gene, PAI-1 gene, and MTHFR gene) and coronary artery disease.

Methods: ACE genotype controls, PAI-1 genotype (4G/5G), and MTHFR genotype (A/V) were investigated in 61 cases with 75% ≤ coronary stenosis by CAG (patients group) and 258 controls (patients without 75% ≤ stenosis, or normal volunteers having normal ECG and no past history of heart disease).

Results: Age (63.5 vs 48.9, P < 0.0001), HbA1c (6.0 vs 5.5%, P = 0.01), fasting plasma glucose (103.4 vs 96.4 mg/dl, P = 0.049) and fibrinogen (340.4 vs 309.9 mg/dl, P = 0.01) were higher in patients group than control group. HDL-C (43.5 vs 51.5 mg/dl, P < 0.0001) and serum creatinine (0.89 vs 0.94 mg/dl, P = 0.02) were lower in patients group than control group. Odds ratio (OR) for coronary artery stenosis with ACE II genotype was 2.06 (95% CI 1.36 to 3.11) and that with PAI-1 5G/5G genotype was 3.73 (95% CI 2.03 to 6.84). But OR of MTHFR A/V genotype was not high (0.98, 95% CI 0.63 to 1.52). OR for coronary artery stenosis both with ACE II genotype and PAI-1 5G/5G genotype was 6.50 (95% CI 3.95 to 29.04).

Conclusions: These results suggest that ACE II genotype and PAI-1 5G/5G genotype, but not MTHFR A/V genotype may be a predictor of coronary artery disease.

THE X–X/–/E+E+ genotype of the XbaI/EcoRI polymorphism at the apo B gene is a marker of coronary artery disease

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Objective: To investigate the associations between two DNA restriction length polymorphisms (XbaI and EcoRI) of the apolipoprotein B gene and Coronary Artery Disease (CAD).

Methods: We investigated in Brazil among 116 patients (92 men and 24 women) with CAD diagnosed by angiography (CAD+), compared with 78 control patients (26 men and 52 women) without ischemia or arterial damage as determined by angiography (CAD–). Mean age was 44 ± 7 years for both groups (ascertainment below 56 years). The genomic DNA was extracted from leucocytes of total blood collected with EDTA and the PCR methodology was used for the amplification of the chosen segments. The final amplification products were submitted to digestion with the respective restriction enzymes (XbaI and EcoRI) and the variations were visualized after electrophoresis on 1.5% agarose gel and 6% polyacrylamide gel, respectively, with ethidium bromide under UV light, followed by photographic documentation.

Results: The allele frequencies of the polymorphic sites in the apo B were not different between the two groups. A statistically significant difference appeared when the distributions of CAD+ and CAD– patients, classified into two genotype classes (X–X/–/E+E+ and the remaining XbaI/EcoRI genotypes), were compared (χ² = 6.27; p < 0.02). The odds ratio estimated for the X–X/–/E+E+ genotype and CAD was 3.2. Within a 95% confidence interval the X–X/–/E+E+ genotype may indicate from 1.24 to 8.25 higher chance of developing CAD than the other XbaI/EcoRI genotypes of the apo B gene.

Conclusions: The presence of the X–X/–/E+E+ genotype of the XbaI/EcoRI polymorphism at the apolipoprotein B gene is a marker of coronary artery disease.

Homocysteine and the 5’10’ methylene tetrahydrofolate reductase C677T polymorphism and risk of cardiovascular disease in familial hypercholesterolemia


Objective: The role of homocysteine and the 5’10’ methylene tetrahydrofolate reductase (MTHFR) C677T polymorphism as risk factors for coronary heart disease (CHD) is controversial. This study investigated their role in patients with heterogeneous familial hypercholesterolemia (FH).

Methods: Fasting plasma homocysteine and the C677T MTHFR polymorphism were determined in 112 patients with FH and 72 patients with polygenic hypercholesterolemia of whom 26.7% and 41.6% respectively had established CHD.

Results: Neither plasma homocysteine nor the MTHFR C677T polymorphisms were associated with risk of CHD in patients with polygenic hypercholesterolemia. Logistic regression analysis of risk factors for CHD in patients with FH identified male sex (OR = 3.03), smoking (OR = 2.91), diastolic blood pressure (OR = 3.70), plasma glucose (OR = 3.51) but again neither plasma homocysteine nor the MTHFR C677T polymorphisms were associated as risk factors for CHD in patients with FH.


Efficacy and safety of NK-104 (Ivatavatin) in hyperlipidemic patients – A double-blind comparative study

Y. Saito, Y. Goto. Japan Iatavatin Clinical Study Group; Department of Internal Medicine, University of Chiba, Japan

The efficacy and safety of ivatavatin, a novel potent HMG-CoA reductase inhibitor, were compared with a reference drug of pravastatin.

In the observation period for 4 weeks or more, hyperlipidemic patients meeting the conditions of 220 mg/dl or more in TC and less than 400 mg/dl in TG were randomized, and 2 mg of ivatavatin and 10 mg of pravastatin were administered for 12 weeks in a double-blind manner. The numbers of cases subjected to analysis were 125 cases for ivatavatin and 111 cases for pravastatin. The results at assessment showed the following TC change rates (%), LDL-C change rates (%), TG change rates (%)) in cases with TG value of 150 mg/dl or more at the start of administration, and HDL-C change value (mg/dl).

<table>
<thead>
<tr>
<th></th>
<th>TC</th>
<th>LDL-C</th>
<th>TG</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivatavatin</td>
<td>–28(284)</td>
<td>–38(195)</td>
<td>–23(228)</td>
<td>+4.2(56.0)</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>–14(276)</td>
<td>–18(191)</td>
<td>–20(240)</td>
<td>+4.9(52.5)</td>
</tr>
</tbody>
</table>

As for the LDL-C decrease rate and TC decrease rate, it was proved that ivatavatin was significantly superior to pravastatin. As for the TG value, it was proved that ivatavatin was not statistically inferior to pravastatin. With respect to the HDL value, it was shown that both groups exhibited a statistically significant increase from the value at the start of administration.

The incidence rates of adverse reactions in ivatavatin and pravastatin were 26.6% and 18.3%, respectively, and no statistically significant difference was observed. There was no case developing a serious adverse reaction in both groups.
TuT2W16 Medium term therapeutic approach in early-onset coronary disease
A. Batala1, G.I. Cubero2, J.J.R. Regueró2, S. Hevia2
1Hospital de Cabueñes, Gijón; 2Hospital Central de Asturias, Oviedo, Spain

Purpose: To determine which are the treatments employed during medium term follow-up of male patients under 50 years of age with coronary disease.

Methods: Consecutive, 230 male patients before 50 years of age (mean age 43 ± 5 years), who were admitted to our Coranary Care Unit as result of an episode of coronary disease (166 myocardial infarction, 64 unstable angina), were prospectively studied. A mean follow-up of 32 ± 13 months was made. The treatment employed at the follow-up and the need for revascularisation were determined.

Results:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Group</th>
<th>Infarction</th>
<th>Angina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Revascularisation</td>
<td>21 (9%)</td>
<td>13 (8%)</td>
<td>8 (13%)</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>39 (39%)</td>
<td>71 (43%)</td>
<td>18 (25%)</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>84 (37%)</td>
<td>52 (32%)</td>
<td>32 (51%)</td>
</tr>
<tr>
<td>Antiprotein treatment</td>
<td>179 (79%)</td>
<td>134 (62%)</td>
<td>45 (71%)</td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>14 (6%)</td>
<td>13 (6%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Nitrates</td>
<td>104 (46%)</td>
<td>74 (45%)</td>
<td>30 (48%)</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>50 (22%)</td>
<td>40 (26%)</td>
<td>10 (16%)</td>
</tr>
<tr>
<td>Diuretics</td>
<td>23 (10%)</td>
<td>17 (9%)</td>
<td>6 (9.5%)</td>
</tr>
<tr>
<td>Lipid lowering treatment</td>
<td>110 (48.5%)</td>
<td>86 (52%)</td>
<td>24 (39%)</td>
</tr>
</tbody>
</table>

Conclusions: During medium term follow-up of male patients under 50 years of age with coronary disease, 10% underwent cardiac revascularisation. It can be seen that only one third of the patients were treated with the different treatments employed.

TuT3W16 The potential cholesterol absorption inhibitor, ezetimibe, is glucuronidated in the intestine, localizes to the intestine, and circulates enterohepatically
M. van Heek, C. Farley, D. Compton, L. Hoos, K. Alton, E. Sybertz, H. Davis. Schering-Plough Research Institute, Kenilworth, New Jersey, USA

Objective: Ezetimibe (SCH58235) is glucuronidated in the intestine and, once glucuronidated, appears to be more potent in inhibiting cholesterol absorption than the parent compound. To understand the increased potency of the glucuronide, the metabolism and distribution of ezetimibe and its glucuronidated form (SCH60663) were studied.

Methods: Bile duct-cannulated rats were used to study the potency, metabolism and distribution of ezetimibe and SCH60663.

Results: One minute after intraduodenal delivery of ezetimibe, significant levels of compound were detected in portal plasma; >95% was glucuronidated, indicating that the intestine was metabolizing ezetimibe to its glucuronide. When intraduodenally delivered as ezetimibe, the compound was glucuronidated, moved through the intestinal wall, into portal plasma, through the liver, and into bile. However, when delivered as SCH60663, >95% of the compound remained in the intestinal lumen and wall, which may explain its increased potency. Significant cholesterol absorption inhibition and glucuronidation occurred when ezetimibe was intravenously injected into bile duct-cannulated rats. Autoradiographic analysis demonstrated that compound was located throughout the intestinal villi, but concentrated in the villus tip.

TuT4W16 The novel cholesterol absorption inhibitor, ezetimibe, selectively inhibits the intestinal absorption of free cholesterol in the presence and absence of exocrine pancreatic function
M. van Heek, C. Farley, D. Compton, L. Hoos, H. Davis. Schering Plough Research Institute, Kenilworth, NJ, USA

Objective: To determine whether exocrine pancreatic function is involved in the mechanism of action of the novel cholesterol absorption inhibitor, ezetimibe (SCH58235). To determine whether ezetimibe would affect the absorption of molecules other than free cholesterol, namely triglyceride, vitamins A and D, and taurocholate.

Methods: Intact hamster and rat animal models were used to study the intestinal absorption of free cholesterol, cholesteryl ester, triglyceride, vitamin A, vitamin D and taurocholate in the absence and presence of ezetimibe. A biliary anastomosis model, which eliminates exocrine pancreatic function from the intestine while maintaining bile flow, was developed in the rat to determine whether pancreatic function was involved in the activity of ezetimibe.

Results: Utilizing cholesteryl esters labelled on either the cholesterol or the fatty acid moiety, we demonstrated that ezetimibe did not affect the cleavage of cholesteryl ester and the absorption of fatty acid thus generated. The free cholesterol from this cleavage, however, was not absorbed (>95% inhibition) in the presence of ezetimibe (1 mg/kg). Eliminating pancreatic function abolished cleavage of cholesteryl esters, but did not affect the ability of ezetimibe (3 mg/kg) to block absorption of free cholesterol (>95%). Ezetimibe did not affect the absorption of triglyceride, vitamins A or D, or taurocholate.

Conclusions: Ezetimibe is a potent inhibitor of intestinal free cholesterol absorption that does not require exocrine pancreatic function for activity. Ezetimibe does not affect the absorption of triglyceride as a pancreatic lipase inhibitor (Orlistat) would, nor does it affect the absorption of vitamin A, D or taurocholate, as a bile acid sequestrant (cholestyramine) would.

TuT5W16 Ciprofibrate-statin combination as third line therapy for severe resistant combined hyperlipidaemia patients
D. Yeshrunj, H. Hamnrud, D. Keren, J.E. Naschitz. Bnai Zion Medical Center, Haifa, Israel

Objectives: To evaluate the ciprofibrate statin combination therapy in patients whom previous medical regimes have failed to achieve the recommended lipid levels.

Methods: Based on the experience of numerous studies that statin-fibrate combination is an accepted combination in certain patients, we evaluated 11 patients (8 males and 5 females), mean age 58, who were all severe resistant combined hyperlipidaemia patients, in very high risk. 8 of them were diabetics, 6- with known coronary artery disease, 6- hypertensives, 5-obese, (BMI > 30) and 2 post CVA. All tried different previous dietary and drug regimes. We started them on "ciprofibrate" in combination with statins (6 simvastatin, 5-20 mg/d and 5 pravastatin 20-40 mg/d).

Results: The ciprofibrate-statin combination resulted in a further decrease of 23% in total cholesterol, 26% in LDL Cholesterol, and 32% triglycerides as compared with the pre-combination levels, already much modified from the original lipid levels. All statistically significant. HDL levels increased mildly (1.6%). All patients suffered the combination very well except one, who was after renal transplant receiving cyclosporine, who had a mild transient CPK elevation.

Conclusion: The ciprofibrate statin combination is a potent and relatively safe method to treat resistant combined hyperlipidaemia patients.

TuT5W16 Effect of the potent aromatase inhibitor letrozole on lipid parameters in postmenopausal women with breast cancer
E. Baiakari1, M. Elisa2, K. Nikolaides2, V. Kakadi2, A. Katsaraki2, N. Pavlides2. 1Biochemistry Lab; 2Dept of Internal Medicine, University of Ioanna, Greece

Objective: Hormonal therapy plays a central role in the overall treatment of breast cancer. Aromatase inhibitors can inhibit the aromatase enzyme system resulting in a reduction of estrogens. Letrozole is a non steroidal aromatase inhibitor that effectively blocks aromatase activity without interfering with adrenal steroid biosynthesis. The drug can significantly reduce the levels of plasma estrogens, which remain suppressed throughout the treatment. There are scarce data concerning the influence of these drugs on serum lipid parameters.

Methods: In the present study we evaluated the effects of letrozole on serum lipid profile in postmenopausal women with breast cancer. A total of 20 patients with breast cancer were treated with letrozole, 2.5 mg once daily. Serum lipid parameters (T CHOL, HDL CHOL, LDL CHOL, triglycerides, apolipoproteins A1, B and E and lipoprotein (a)) were measured after an overnight fast before treatment and at 8 and 16 weeks afterwards.

Results: A significant decrease in serum estradiol levels followed letrozole administration. Furthermore, a significant increase in serum T CHOL, LDL CHOL, and apolipoprotein B levels as well as in the atherogenic risk ratios T CHOL/HDL CHOL and Apo A1/Apo B was noticed after letrozole treatment.
Conclusion: Letrozole administration in postmenopausal women with breast cancer has an unfavorable effect on serum lipid profile possibly owing to the drug’s estrogen lowering activity.

TuT7:W16 Serum uric acid levels: A useful indicator of fenofibrate treatment compliance

D.N. Kiotis, M.S. Elisaf. Department of Internal Medicine, University of Ioannina, Ioannina, Greece

Objective: To evaluate whether measurement of serum uric acid levels is a reliable method to assess adherence to fenofibrate therapy.

Methods: Seventy-five hyperlipidemic patients aged 28–71 years were studied. All the subjects were under fenofibrate therapy for at least 3 months. Serum lipid profile and plasma uric acid concentrations were measured. The same day compliance was assessed using a clinical interview which included non-threatening, non-accusative and non-embarrassing questions. Patients who took less than 75% of the prescribed pills during the last month, were considered as poor compliers.

Results: The patients who exhibited good compliance with fenofibrate treatment had lower serum uric acid concentrations (3.8 ± 1.2 mg/dl vs 5.1 ± 1.4 mg/dl, p < 0.01). Moreover, they demonstrated lower levels of triglycerides (175 ± 65 mg/dl vs 205 ± 42 mg/dl, p < 0.05), and higher HDL-cholesterol concentrations (44 ± 8 mg/dl vs 40 ± 5 mg/dl, p < 0.05). The body mass index was not different between the two groups.

Conclusions: Serum uric acid concentrations may be used as a useful tool to assess compliance with fenofibrate therapy.

TuT8:W16 Fluvastatin in the treatment hyperlipoproteinemiaes in the elderly

Edita Stojic, M. Djeric, A. Stojic. Dpt of Endocrinology; 1 Dpt of Pathophysiology; 2 Dpt of Operative Oncology, Medical Faculty, NOVI SAD, Yugoslavia

The aim of the study was to evaluate the role of fluvastatin therapy in elderly men and women with serious forms of hyperlipoproteinemia (HLP) type IIa and IIb. The study was carried out as an open study in a group of 30 patients (age 65.32 ± 7.83 years). None of patients had clinical or laboratory evidence of thyroid, hepatic or renal disorders. The body weight was within 10% of the ideal weight and remain constant within 1.5 kg during the study. Patients were treated by fluvastatin 40 mg/day for 12 weeks. Before and at the end of this period total, LDL-, HDL- cholesterol and triglycerides were evaluated. During the treatment the decrease of total cholesterol, LDL-cholesterol and triglycerides levels were 24.54%, 23% and 17.65%. Furthermore at the end of 12th week, the increase of HDL-cholesterol levels was 13.6%. Relative patients risk for coronary heart disease reduced for 22.52%. The safety and tolerability profile was good, there were no serious clinical and laboratory adverse effects over 3 months of treatment.

In conclusion, fluvastatin has been found to be safe and effective in reducing total cholesterol, LDL-cholesterol in elderly subjects with HLP.

TuT1:H3 Why advisable or prescribed regimes are not followed?

Richard Monin. Dept. of Cardiology, Clinique La Paristiere, Av Antonin Vallon, 26300 Bourg de Peage, France

Each Year the obesity and its complications costs in Cares 102 Billions (NH, 6–98)
Each Week appear about twenty advice articles of Regime in various weekly’s destined for women.
We assist helpless the inexorable climbing of the obesity: 10% of the population in France, 25% in the USA.

WHY?:
• Socio-economic differences: the obesity is more present in disfavoured population enable to understand councils.
• Influence of advertising: fatter products generally are presented as best.
• All the vegetables are more expensive than floury meal.
• Affair of taste: the absence of diversity in the feeding make childhood fat.
The diversity of feeding is a factor of french paradox

Conclusions: The war against the bad grub must be: to learn to childhood and adolescents a big diversity of taste, to learn him a culinary culture and not see him became obese and more cardiovascular sickness than their parents.
Wednesday June 28, 2000: Workshop Abstracts

**W:17 GENETIC ANIMAL MODELS OF LIPOPROTEIN METABOLISM**

**WeW1:17 Multifunctionality of apolipoprotein E in lipoprotein metabolism as studied in transgenic mice**
Louis M. Havlik, Bart J.M. van Vlijmen, Mick C. Jongs, Marten H. Hofker, Ko Willems van Dijk, TNO-PG, Gaubius Laboratories, Leiden; Leiden University Medical Center, Leiden, The Netherlands

Studies with apolipoprotein E (ApoE) deficient and ApoE transgenic mice has revealed that ApoE has various major functions in lipoprotein metabolism.

**Ligand for lipoprotein receptors:** ApoE is a ligand for receptor-mediated uptake of ApoB-containing lipoproteins by the liver. ApoE2 and ApoE3 Leiden proteins are both defective in binding to the LDL receptor. However, in ApoE2- and ApoE4 Leiden transgenic mice, the LDL receptor still constitutes the predominant route for clearance of VLDL remnants. These ApoE variants can also still bind to the LRP and VLDL receptor. However, as compared to binding to the LDL receptor, for binding to the LRP a relatively high amount of apoE per particle is needed.

**Inhibitor of LPL-mediated VLDL-triglyceride lipolysis:** Above a certain amount per particle apoE leads to a dose-dependent inhibition of LPL-mediated lipolysis, whereas an apoE variant-specific inhibition of VLDL lipolysis has also been found. If lipolysis of VLDL is inhibited, binding of VLDL to the LRP is severely hampered.

**Stimulator of hepatic VLDL assembly and secretion:** In apoE-deficient mice the newly secreted VLDL particles are smaller in size due to less triglyceride in the core. The hampered VLDL-triglyceride secretion in these mice could be enhanced upon introducing physiologically relevant ApoE gene expression in the liver by APOE-adenovirus transduction, indicating a regulating role for apoE in hepatic VLDL secretion.

**WeW2:17 The multiple roles of macrophage scavenger receptors in vivo**
Hiroshi Suzuki, Youichiro Wada, Takao Hamakubo, Tatsukiko Kodama.
Molecular Biology and Medicine, RCAST#55, The University of Tokyo, Japan

Macrophage scavenger receptors (MSR) are implicated in the pathologic disposition of cholesterol during atherogenesis. More than 10 types of MSRs are reported and their roles in vivo have been studied using several knockout mice strains and human genetic disorders. The targeted disruption of the type I and II class A MSR gene (SRA-KO) results in a reduction in the size of atherosclerotic lesions in apo E deficient mice (60%) and in LDL receptor deficient mice with high fat diet (about 20%). de Witther reported that in ApoE3 Leiden mice, SRA deficiency caused development of more severe lesions. We found that SRA-A KO in C57BL6 mice with high cholesterol diet developed smaller atherosclerotic lesion, supporting the hypothesis that SRA-A mediates the development of atherosclerosis in vivo. Davignon reported a Canadian Family with increased SRA expression indicated xanthomatosus. SRA KO macrophages exhibit a marked decrease in modified LDL uptake in vitro, whereas modified LDL clearance from plasma occurs at a normal rate, suggesting that there are alternative mechanisms for the uptake of mL DL from the circulation. SRA-KO macrophages had reduced phagocytic activity and impaired adhesion function. Oxidized LDL can induce macrophage growth stimulation, but the activity is weak in SRA-KO. In addition, MSR-A knockout mice show increased susceptibility to Listeria monocytogenes infection and herpes simplex virus type-1 (HSV-1) infection, indicating a role for MSR-A in host defense against various pathogens. Krieger et al. reported that the targeted disruption of SR-B1 resulted in impaired HDL metabolism and enhanced atherosclerosis. Matsuzawa et al. reported that human CD36 deficiency caused higher rate of atherosclerotic events. These results suggest that the protective role of class B scavenger receptors against atherosclerosis.

**WeW3:17 VLDL or LDL cholesterol: Which is more atherogenic?**
Stephen G. Young, Gladstone Inst. of Cardion. Disease, San Francisco, CA 94141-9100, USA

Apo-E deficient "apo-B100-only" mice (ApoE/- ApoB100) and LDL receptor deficient apo-B100-only mice (ApoE/- ApoB100) have similar total plasma cholesterol levels on a chow diet (~300 mg/dl) and virtually identical HDL cholesterol levels. However, nearly all of the cholesterol in the ApoE/- ApoB100 plasma is contained in VLDL particles, while nearly all of the cholesterol in the LDL/- ApoB100 plasma is contained in smaller LDL particles. In this study, we sought to compare the sizes and numbers of apo-B100-containing lipoproteins in these mice, and then determine whether the differences were associated with different susceptibilities to atherosclerosis. The mean size of the apo-B100-containing lipoprotein particles in ApoE/- ApoB100 plasma was 53.4 nm. while it was 22.1 nm in LDL/- ApoB100 plasma; the smallest 10% of particles in the ApoE/- ApoB100 plasma was larger than the largest 10% of particles in LDL/- ApoB100 plasma. The plasma levels of apo-B100 were ~450% higher in the LDL/- ApoB100 mice, compared with the ApoE/- ApoB100 mice. After 40 weeks on a chow diet, 14.0 ± 2.9% of the aortic surface in LDL/- ApoB100 mice was covered with atherosclerotic lesions, versus only 4.8 ± 2.5% in the ApoE/- ApoB100 mice (p < 0.0001; n = 40 mice in each group). We conclude that large numbers of small apo-B100-containing lipoproteins (the LDL/- ApoB100 phenotype) are much more atherogenic than smaller numbers of large lipoproteins (the ApoE/- ApoB100 phenotype).

**WeW4:17 Creation and initial characterization of apo B48 receptor knockout mouse**
J. Smith, 1, M. Brown, 2, W. Bradley, 2, S. Giarusuro, 2. Rockefeller Univ., New York; 2University of Alabama, Birmingham, USA

**Objective:** To determine the in vivo and ex vivo effects of knocking out the monocyte, macrophage, and endothelial cell specific apoB48 receptor (B48r) gene.

**Methods and Results:** The human B48r cDNA was cloned from a THP-1 cell library using a probe derived from peptide sequence of the purified protein. The mRNA codes for a polypeptide of 1088 amino acid residues with 2 hydrophobic domains. The cDNA and protein sequence are not closely related to previously identified genes. Transfection of full-length cDNA into CHO cells results in uptake of remnant lipoproteins. A mouse genomic clone was isolated from a phage library, and a targeting vector was constructed which deleted the promoter, first exon, first intron, and a portion of the second exon. Transfection into ES cells resulted in 3 cell lines with gene disruption via homologous recombination, 2 of which yielded germline transmitting chimeric mice after blastocyst microinjection. Heterozygous mice were bred and yielded expected ratio of wildtype, heterozygous, and knockout mice. The knockout mice appear healthy and are being bred back to C57BL6 stock and with apoE-deficient mice. Bone marrow derived macrophages derived from the wildtype, heterozygous, and knockout mice were treated with Dil labeled apoE-deficient B48 LDL alone and in the presence of excess human LDL as competitor. B48 LDL uptake was observed by all cells, with the notable exception that in the presence of excess LDL, the B48r knockout macrophages failed to take up any B48 LDL.

**Conclusion:** The B48r may play an important role in macrophage uptake of triglyceride rich and remnant lipoproteins, and may play a role in macrophage foam cell formation during atherogenesis, especially when apoE is compromised.

**WeW5:17 Disturbances of fatty acid and cholesterol metabolism in peroxisome proliferator-activated receptor-alpha (PPARa) deficient mice**
D. Patel, 1 B. Knight, 1 S. Humphreys, 2 G. Gibbens, 1 Lipoprotein Group, MRC Clinical Sciences Centre, London; 2Oxford Lipid Metabolism Group; 3Metabolic Research Laboratory, Oxford, UK

**Objectives:** To establish whether PPARα deficiency affects the co-ordinate regulation by food intake and the normal diurnal cycles of hepatic fatty acid oxidation, fatty acid synthesis (FAS) and cholesterol synthesis (CS).

**Methods:** PPARα null mice and their respective controls were maintained on a 12 h dark/12 h light cycle with free access to food. Plasma lipids were determined at 4 h intervals and livers removed for measurement of mRNA.
In vitro rates of hepatic FAS and CS were determined by incorporation of tritiated water.

Result: Plasma levels of free fatty acids (NEFA), cholesterol, ketone bodies and triacylglycerol were increased in the (+/--) mice and did not follow the circadian variation seen in (+/+)/ mice. In the (+/+)/ mice, the rates of FAS and CS were significantly higher during the dark phase (D6) than during the light phase (L6). These differences were abolished in the (+/--)/ mice. The lower rate of FAS at D6 in the (+/--)/ mice was associated with a decreased expression of acetyl CoA carboxylase (ACC) mRNA and could be related to the increased level of plasma NEFA. Surprisingly, the increased rate of hepatic CS in the (+/--)/ mice was associated with a marked decline in the expression of hydroxymethylglutaryl CoA reductase (HMGR) mRNA. PPARα deletion did not appear to have any effect on the expression of LDL-receptor or cholesterol 7α-hydroxylase mRNAs.

Conclusion: Deletion of PPARα attenuates or abolishes the circadian rhythm of hepatic and plasma lipid metabolism. The rate of hepatic CS is increased whilst that of FAS is diminished. The FAS change may be explained by a decreased expression of ACC mRNA. The effects on CS do not appear to result from changes in HMGR mRNA but may be related to changes in the demand for a common substrate, acetyl-CoA.

WeW6:17 Generation of adult LPL-deficient mice
Juliane G. Strauss1, Sasu Frank2, Gabrielle Knipping2, Rudolf Zechner1.  
1Institutes of Biochemistry; 2Medical Biochemistry, Karl-Franzens University, A-8010 Graz, Austria

Objective: The transient adiponovirus-mediated expression of LPL during the suckling period to rescue LPL knock-out mice (Lo) from neonatal death and generate adult LPL deficient mice.

Methods: A total of 5 × 10^7 plaque forming units (pfu) of LPL-expressing adenovirus was injected intraperitoneally into mouse littermates from matings of heterozygous LPL knock-out mice immediately after birth.

Results: Over 90% of treated L0-mice survived the first days of life. In 3% of all cases L0-mice survived the suckling period and stayed alive for at least one year. Rescued L0-mice were smaller than their control littersmates until 3 months of age and had strongly elevated triglyceride levels in the fed (5000 mg/dl) and in the fasted state (2000 mg/dl). Total cholesterol levels were also increased in both conditions due to increased VLDL-cholesterol. L0-mice lacked HDL-cholesterol indicating that LPL is essential for the HDL biogenesis. Glucose levels of L0-mice were normal, whereas ketone bodies and FFA were elevated.

Conclusion: Adult L0-mice will provide a valuable model to study the role of LPL deficiency in rodents.

WeW7:17 Apolipoprotein (apo) E modulates hepatic very low density lipoprotein (VLDL) assembly and secretion
Y. Huang1, 2, W.J. Brechtl1, X.Q. Liu1, Y. Wang1, 2, J.M. Taylor1, 2, S.C. Rall, Jr.1, R.W. Mahley1, 2. 1Gladstone Institute of Cardiovascular Disease; 2University of California, San Francisco, CA 94114-9100, USA

Objective: To investigate apoE's regulatory effect on hepatic VLDL assembly and secretion in transgenic mice (apoE-null background) and rabbits expressing medium (10-20 mg/dl) and high (20-200 mg/dl) levels of human apoE3 in the liver, hepatic triglyceride (TG) and VLDL apoB production rates were determined with the Triton-WR1339 method. Compared with wild-type mice, apoE3 medium- and high-expressing mice had increases of 29% and 56% in VLDL-TG production and 20% and 42% in VLDL-apoB production, respectively; apoE3-null mice had decreases of 47% in VLDL-TG and 53% in VLDL-apoB production. Compared with nontransgenic rabbits, apoE3 medium- and high-expressing rabbits had increases of 2-fold and 4-fold in VLDL-TG production and 1.5- and 3-fold in VLDL-apoB production, respectively. The increased hepatic VLDL production contributed largely to hypertriglyceridemia in high-expressing mice and combined hyperlipidemia in high-expressing rabbits. After 2 h of incubation with 1H-glycerol, 35S-methionine, and 0.4 mM oleic acid, stably transfected Mc-A-RH7777 cells overexpressing apoE3 (1.2 μg/mg cell protein/h) secreted 2.5-fold more VLDL-1H-TG and twofold more VLDL-35S-apoB into the medium than nontransfected cells; transfected cells also had 75% higher 1H-TG content, most of which accumulated in the lumen of the endoplasmic reticulum. At each VLDL assembly occurs. Micromolar TG transfer protein activity was about twofold higher in transfected than nontransfected cells and may have helped to mobilize TG to the endoplasmic reticulum.

Conclusion: ApoE overexpression stimulates the synthesis and mobilization of TG to the endoplasmic reticulum and increases hepatic assembly/secretion ofTG-rich VLDL. Thus, apoE appears to be a powerful modulator of hepatic VLDL assembly and secretion.

WeW8:18 Divergent roles of extracellular proteases in plaque stabilization and rupture; what are the molecular switches?
A. Newby, T. Izard, M. Bond, S. Hussein, A. Chase. Bristol Heart Institute, Bristol Royal Infirmary, Bristol BS2 8HW, UK

Baseline membrane degrading metalloproteinases (MMPs-2 and -9) may be essential to allow vascular smooth muscle cells (VSMC) to migrate and proliferate in response to injury and inflammation. The molecular events which need to take place before quiescent VSMC in the artery wall can migrate and proliferate are however ill-defined. The presentation will identify the important events taking place during the G1 phase of the cell cycle, which have been compared in isolated rat VSMC and segments of rat aorta. The studies identify molecular switches that may be controlled by injury and extracellular proteolysis, which permit proliferation.

MMPs with activity against intestinal matrix, MMPs-1, -3, -7, -12 and -13, for example, have been associated with plaque rupture rather than stabilisation. We have sought to identify common and divergent features of the regulation of the MMP genes in VSMC. These studies may identify whether plagues prone to rupture produce an unfavourable complement of MMPs, and, if so, by what mechanisms. We also hope to identify potential inhibitors so as to stabilise plaques.

WeW2:18 Why is it that some plaques rupture and others don't?  
A. E. Becker, A.C. van der Wal, O.J. de Boer, C.M. van der Loos, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Plaque rupture is defined as the condition in which the fibrous cap of an atherosclerotic (AS) plaque is torn apart producing a fissure into the lipid core, associated with intraplaque hemorrhage and adherent mural thrombosis. This is the most common pathology underlying acute myocardial infarction (AMI). Over the past decade it has been shown that plaques prone to rupture are characterized by a large lipid core, an attenuated fibrous cap and a dense intraplaque inflammatory infiltrate composed of macrophages and T lymphocytes and, to a lesser extent, mast cells.

It is presently acknowledged that the immune system is involved; AS plaques contain an inflammatory cell-mediated (Th1)-like response, responsible for the release of a variety of cytokines, growth factors and enzymes. It appears as if the balance between these products may be responsible for the eventual effects on the tissues. The release of a cascade of matrix metalloproteinases, for instance, may lead to excessive collagen breakdown and this process, in the presence of high tissue tensile forces and rheological factors, may produce plaque rupture.

Why then is it that some AS plaques with characteristics alluded to above remain intact and others rupture?

Atherectomy specimens from patients with AMI show recent onset activation of T lymphocytes. This fits with clinical observations of increased inflammatory markers in peripheral blood. We have isolated T cells from AS plaques, which were tested against particular antigens, such as C. pneumoniae. Our preliminary results have shown that in some patients T cells cloned from plaques respond to C. pneumoniae. This boosts our belief that an additional stimulus serves as the last straw that breaks the camel's back.

WeW3:18 A novel adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class a scavenger receptor expression in human macrophages
N. Ouchi, S. Kihara, Y. Artia, A. Matsuyama, M. Nishida, Y. Okamoto, T. Nakamura, S. Yamashita, T. Funahashi, Y. Masuzawa. Department of Internal Medicine and Molecular Science, Graduate School of Medicine, Osaka University, Suita. Osaka, Japan

Objectives: Obesity is the most common nutritional disorder and one of the major risk factors of atherosclerosis. However the molecular basis for the link between obesity and atherosclerosis has not been fully elucidated. We found
an adipocyte-specific secretory protein, adiponectin, and developed an ELISA system to determine adiponectin concentrations. Plasma adiponectin level negatively correlated with body mass index (BMI) (Arita et al. BBRC 1999) and was significantly low in patients with coronary artery disease compared with BMI-adjusted control subjects (Ouchi et al. Circulation 1999). Here we showed the effects of adiponectin on lipid accumulation and macrophage scavenger receptor (MSR) expression in human macrophages.

**Methods:** Human monocytes were differentiated in human type AB serum for 7 days and then studied with adiponectin. The intracellular cholesteryl ester content was determined by the enzymatic, fluorimetric method. Lipid droplets were stained with oil red O. The expression of class A MSR was analyzed by immunoblotting and northern blotting. The expression of CD36 was quantified by flow cytometry. The class A MSR ligand binding and uptake activities were examined by flow cytometry using Dil-ACLDL.

**Results:** Treatment with physiological concentration of adiponectin induced intracellular cholesteryl ester content. Adiponectin-treated macrophages contained smaller lipid droplets. Adiponectin suppressed the expression of class A MSR at both mRNA and protein level without affecting the expression of CD36. Adiponectin treatment dose-dependently decreased both binding and uptake of Dil-ACLDL.

**Conclusions:** Adiponectin suppresses lipid accumulation in macrophages through inhibition of class A MSR expression.

**Macrophage P53-deficiency leads to enhanced atherosclerosis in APOE-3-Leiden mice**

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Cell proliferation and cell death (either necrosis or apoptosis) are key processes in the progression of atherosclerotic plaques. The tumor suppressor gene p53 is an essential gene in cell proliferation and death, and is upregulated in human plaques. We investigated the importance of macrophage p53 in the progression of atherosclerotic lesions using bone marrow (BM) transplantation in APOE-3-Leiden transgenic mice, an animal model for human-like atherosclerosis. Reconstitution of APOE-3-Leiden mice with p53 deficient BM (p53-/-) did not cause any blood or splenic abnormalities within the period studied (i.e. a total of 16 weeks). Mice reconstituted with p53-/- BM showed a strong exacerbation of the progression of atherosclerotic lesions as compared to mice reconstituted with p53+/+ BM. Plaques in APOE-3-Leiden mice reconstituted with p53-/- BM had significantly larger total lesion area as compared to mice reconstituted with p53+/+ BM (186 ± 127 versus 82 ± 72 µm², P = 0.006); in addition, these plaques contained more necrosis (necrotic index: 1.1 ± 1.3 versus 0.2 ± 0.7, P = 0.04) and more lipid-loaded macrophages (macrophage area: 113 ± 56 versus 54 ± 68 µm², P = 0.05). Importantly, these observations coincided with a decrease in apoptosis (TUNEL-positive nuclei going from 0.42 ± 0.39 to 0.14 ± 0.15%, P = 0.06), while the number of proliferating cells (BrdU-positive nuclei) was not affected. These studies indicate that p53 is important in the progression of atherosclerotic plaques due to its effect on macrophage cell death.

**Functional 5A/6A polymorphism in human stromelysin-1 gene is in age-dependent relation to autopsy-verified calcified coronary lesions**

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**Objective:** We studied the association between human stromelysin-1 promoter 5A/6A genotype and autopsy-verified coronary atherosclerotic lesions in a cohort of 30 men, average age 53 years, followed for 70 years.

**Methods:** The allelic variation was detected from purified DNA by PCR and by automated minisequencing method. The right and left anterior descending coronary arteries (RCA and LAD) were stained for fat, and areas covered with fatty streaks, fibrinous plaques and complicated lesions were measured.

**Results:** A significant stromelysin promoter genotype-age association was observed with areas of calcified lesions of the coronary arteries (P = 0.0395). In the RCA and LCX arteries of men > 53 years, carriers of the 5A/5A, 5A/6A had on average a 101% and a 79% increase, respectively, in the area of calcified lesions, when compared with the carriers of 6A/6A genotype.

**Conclusions:** In men, the stromelysin 5A allele is a significant genetic risk factor for calcified atherosclerotic lesion rupture at late middle age.

**Genetic regulation of the MMP-7 and MMP-12 gene expression**

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Degradation of extracellular matrix within the aortic wall by matrix metalloproteinases has been implicated in several pathological conditions such as development of atherosclerosis, plaque rupture, high blood pressure and aneurysm formation. To elucidate the role that elastolytic enzymes may play in these conditions, we studied the metalloelastase (MMP-12) and the matrixin (MMP-7) gene for polymorphisms by single strand conformational polymorphism (SSCP) analysis. Two common polymorphic consisting of an A to G substitution were found in the metalloelastase gene, one located in exon 6 and one in the promoter region. The exon 6 mutation was a silent mutation. The promoter A/G polymorphism was located 82 bp upstream from the transcription start site adjacent to an activator protein-1 (AP-1) consensus sequence. The allele frequency of the G allele was 0.19 and in linkage disequilibrium with the exon 6 polymorphism. By EMSA studies we established that the promoter polymorphism influences the binding of transcription factor AP-1 with a higher affinity for the A allele. Furthermore, transfection studies showed that the polymorphism influences the transcriptional activity of the MMP-12 promoter and that there is an allele-specific effect on arterial luminal dimensions in a cohort of patients undergoing PTCa with stent implantation.

At present, we have found two promoter polymorphisms in the MMP-7 gene and studies are ongoing in order to analyse the functional significance of these mutations.

**Strategies to prevent stroke: Lessons for developing countries**

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Cerebrovascular disease (stroke) is the second leading cause of death worldwide, responsible for approximately 9.5% of all deaths. Although we have access to almost 50 years of epidemiological research and knowledge about appropriate interventions, the global burden of stroke is still increasing, with most of the stroke deaths now occurring in the poorer regions of the world. Although many lessons have been learnt, the strategies adopted to date have been only partially effective in preventing and controlling the stroke epidemic. Given the increasingly global nature of the stroke epidemics, a global response is required. This presentation describes the dimensions of the global burden of stroke, the evolution of the global stroke epidemic and its several patterns, summarizes current understanding of the determinants of the stroke epidemics at both the individual and population levels, and identifies appropriate policies for the primary prevention and control of stroke with a specific focus on developing countries.

**Neuroprotection in acute brain infarction – Where do we stand?**

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Severe cerebral ischaemia rapidly leads to cell death but moderate ischaemia can be withstood for several hours. Reperfusion with alteplase or anecrod improves clinical outcome and specialist care is of proven value. Experimental data suggest that infarct size can be limited by early administration of NMDA antagonists, ion channel blockers or anti-inflammatory measures. Unfortunately, neuroprotective strategies have so far failed to live up to their initial promise. Saffron and a range of other agents with antioxidant and antiplatelet properties have been tested in various sites of the NMDA receptor, and each has been unsuccessful in clinical trials. Free radical scavenging with tiron/lazed, an anti-inflammatory

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approach with enlimomab and ion channel blockade with lubeluzole fared no better. Closer analysis of the preclinical data and the trial results reveals many potential explanations for the disappointing results so far. These include a lack of robust animal data to support neuroprotective efficacy, a low therapeutic index that precluded adequate dosing in man, and failures of trial design. A major factor may be the discrepancy between the effective time window in animals and the delay to treatment in man. More rigorous guidelines for development have recently been established, and the lessons learned are now being applied. These include strategies to enhance dose selection, to "enrich" the trial population with potential responders, to control for confounding factors, to optimize trial endpoints or to use surrogate imaging measures. Drugs presently in development include the GABA agonist, clomethiazole, a potassium channel opener (BMS 204532), magnesium, a neuophili inhibitor factor (NIF), and a nitrore, NXY-059. Inhibitors of the proteasome, such as PS-519, and of PARP will shortly be available. Finally, experimental approaches are being revisited to consider if generic brain protection rather than specific neuroprotection may improve outcome.

**Conclusions:** 1. HCY levels correlate with severity of ICS; 2. In families with a history of ICS the levels of risk factors in children are determined by levels in parents.

### WeW5:19 Functional Significance Of G-33A mutation in the promoter region of thrombomodulin gene and its association with carotid atherosclerosis


**Objective:** We have previously reported that G-33A promoter mutation of thrombomodulin (TM) gene is associated with coronary atherosclerosis. This study was conducted to determine if the G-33A mutation is a genetic risk factor for ischemic stroke or carotid atherosclerosis. The functional significance of this mutation was also examined.

**Methods:** We investigated 333 patients (mean age 65 years, 59% male) with ischemic stroke and 257 age and sex-matched control subjects. In all studies the carotid atherosclerosis was assessed by Duplex scanning and TM G-33A promoter mutation was detected by single-strand conformation polymorphism. To assess the influence of this mutation on TM promoter activity, TM promoter/luciferase reporter gene plasmids containing normal sequence or G-33A mutation were constructed and transfected into endothelial cell culture.

**Results:** There was no significant difference (18.3% vs 24.1%, p = 0.105) in the TM G-33A mutation frequency (GA + AA genotypes) between stroke and control group, even only in younger (age ≤ 60 years) subjects (20.9% vs 22.0%, p = 0.991). All study participants were reclassified into 2 groups according to the presence or absence of significant carotid atherosclerosis. The TM G-33A mutation frequency was similar between the subjects with and without carotid atherosclerosis (22.2% vs 19.8%, p = 0.550). But when only younger subjects were included, the mutation occurred more frequently in carotid atherosclerosis group (33.3% vs 17.3%, odds ratio [OR] = 2.38, p = 0.027). Logistic regression analysis demonstrated that only diabetes mellitus (OR = 3.11, 95% confidence interval [CI] = 1.33-7.30, p = 0.009) and G-33A mutation (OR = 2.46, 95% CI = 1.14-5.29, p = 0.021) were independently associated with carotid atherosclerosis in younger subjects. As assessed by luciferase reporter gene assays, the activity of the constructs bearing the G-33A mutation showed a significant decrease (~36 ± 12%) in transcriptional activity compared with the wild type constructs.

**Conclusions:** These findings suggest that the TM G-33A promoter mutation reduces the endothelial TM synthesis and is related to carotid atherosclerosis in younger subjects.

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### WeW4:19 A possible role of thermolabile methyltetrahydrofolate reductase in the occurrence of vascular dementia

**Jin-Hyun Yoo, Gyu-Dong Choi, Soo-Sang Kang.**

**Objective:** To investigate the relationship of the TT genotype of MTHFR and hyperhomocysteineemia in the development of cerebral infarction with and without cognitive impairment.

**Methods:** We assessed the status of hyperhomocysteineemia and the C677T genotypes of MTHFR in 143 patients with vascular dementia, 122 non-demented patients with cerebral infarction and 306 healthy subjects matched for age and gender, in a hospital-based setting.

**Results:** Proportion of moderate hyperhomocysteineemia (plasma homocysteine ≥ 15 μmol/L) as higher in patients with vascular dementia or cerebral infarction than in normal controls (42.6%, 20%, 10.1%, p = 0.001). In contrast, a higher frequency of the TT genotype of MTHFR was found only in demented patients compared to non-demented patients or controls (25.3%, 9.8%, 12.1%, p = 0.01), with odds ratios of 2.70 (95% CI, 2.05-3.53, p = 0.0002) adjusted for hypertension, smoking, diabetes mellitus, age, and gender. Demented patients with multiple infarct had a higher frequency of TT
TRIGLYCERIDES AND CVD

Hypertriglyceridemia and the metabolic syndrome
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Most patients with hypertriglyceridemia have other cardiovascular risk factors that are typical of the metabolic syndrome. These include elevations of apolipoprotein B, remnant lipoproteins and small LDL particles; reduced HDL cholesterol; elevated blood pressure; insulin resistance and glucose intolerance; and coagulation abnormalities. Thus, elevated serum triglycerides are a marker for the metabolic syndrome. This is especially the case when patients have abdominal obesity. Rarely patients will have hypertriglyceridemia in the absence of abdominal obesity and insulin resistance, but such cases are an exception, and not the rule. Hypertriglyceridemia in patients with insulin resistance is usually secondary to fatty liver, which in turn is the result of elevated plasma nonesterified fatty acids (NEFA). Elevations of NEFA can be due either to obesity or to insulin resistance in adipose tissue. In addition to being a marker for the metabolic syndrome, elevated triglycerides may be directly or indirectly atherogenic. Some triglyceride rich lipoproteins, particularly VLDL remnants, appear to be directly atherogenic; also elevated triglycerides give rise to small LDL particles and low HDL levels, both of which may be atherogenic. The primary treatment of hypertriglyceridemia is weight control plus physical activity; both reduce insulin resistance and mitigate the metabolic syndrome. A question of great importance is whether triglyceride-lowering drugs will also reduce risk for coronary heart disease. Several clinical trials give strong supportive evidence of risk reduction from these triglyceride-lowering drugs.

Twenty-year cardiovascular disease mortality in the familial forms of hypertriglyceridemia

Background: Familial combined hyperlipidemia (FCHL) and familial hypertriglyceridemia (FHTG) are two of the most common familial forms of hyperlipidemia. The purposes of this study were to estimate 20-year cardiovascular disease (CVD) mortality among relatives in these families, and to evaluate plasma triglyceride as a predictor of CVD mortality.

Methods: The study was based on lipid and medical history data from 101 families ascertained in two studies conducted in the early 1970s. Vital status and cause-of-death was determined during 1993-97 for 685 family members, including first degree relatives of the probands and spouse controls.

Results: Compared to spouse controls, CVD mortality was increased among sibs and offspring in FCHL (relative risk = 1.7, P = 0.02) after adjustment for baseline covariates. Baseline triglyceride was associated with increased CVD mortality independent of total cholesterol among relatives in FHTG families, (relative risk = 2.7, P = 0.02), but not in FCHL families (relative risk = 1.5, P = 0.16), after adjustment for baseline covariates.

Conclusions: This prospective study establishes that relatives in FCHL families are at increased risk for CVD mortality and that triglyceride predicts CVD mortality among relatives in FHTG families. The findings add to the growing evidence for the importance of hypertriglyceridemia as a risk factor for CVD.

Diurnal triglyceridemia: A surrogate of postprandial lipemia?

Background: Postprandial hypertriglyceridemia is regarded as an independent risk factor for atherosclerosis. Large prospective studies have not been performed due to the workload and the costs involved in performing oral fat loading tests. We evaluated the feasibility of determining ambulatory diurnal capillary TG (TGC) profiles compared to standardized oral fat loading tests (OFLT) in 18 subjects and we determined the variability of diurnal triglyceridemia in a larger cohort of 106 subjects.

Methods: In 18 subjects with a wide range of fasting plasma TG, results of OFLT (50 g/m2, 10 hrs) were compared to diurnal TGC profiles measured in an out-patient clinic setting. In an observational study, 106 healthy volunteers (54 females and 52 males) measured TGC. Food intake was recorded in a diary and fasting blood was drawn once at inclusion. Diurnal TGC profiles were estimated as the mean area under the TGC curve of 6 time-point measurements on 3 different days.

Results: Plasma TG clearance after the acute OFLT correlated well with the diurnal TGC-AUC (r = 0.77; P < 0.01; n = 18). In addition, hyperTG subjects (plasma TG > 2.0 mmol/m) had a higher diurnal triglyceridemia (49.8 ± 15.4 h.m/m) as well as a higher response of plasma TG to the OFLT (42.1 ± 15.4 h.m/m), than the subjects with normal fasting plasma TG (29.8 ± 11.8 h.m/m [P < 0.05] and 20.8 ± 5.9 h.m/m [P < 0.01]), respectively. In the cohort of 106 subjects, repeated measurements of diurnal triglyceridemia tended to be less variable than fasting capillary TG (mean coefficients of variation 15% [range: 0.60-46%] and 25% [range: 1.4-73%], respectively; P = 0.09) for the whole group and in males (19% [0.60-46%] and 24% [1.4-58%], respectively; P = 0.07). Stepwise multiple regression analysis with TGC-AUC as dependent variable showed that the best predictors were fasting TG, gender, systolic blood pressure and mean daily energy intake, explaining 72% of the variation of diurnal triglyceridemia.

Conclusion: Diurnal capillary TG profiles may be used to estimate the total daily load of potential atherogenic triglyceride rich particles to which individuals are subjected during the day without the need for metabolic ward studies.

Application of a sandwich ELISA for apo B48
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Object: Chylomicron (CM) remnants are assumed to play important roles in atherogenesis. However, there have been no appropriate methods to sensitively evaluate the fasting levels of CM remnants. The object of the current study was to raise monoclonal antibodies (mAbs) against human apo B-48 and to establish a sensitive ELISA for measuring serum apo B-48 levels in normolipidemic and hyperlipidemic subjects.

Methods and Results: mAbs were raised against human apo B-48 by immunizing synthetic peptides. These mAbs specifically reacted with the C-terminal of apo B-48, but not B-100. Using these mAbs, a sandwich ELISA was established to measure serum apo B-48 levels, using recombinant apo B-48 as a standard (kindly provided by Dr. Yao). The CV of the assay was approximately 10%. After oral fat loading in normolipidemic subjects, serum apo B-48 increased with a peak at 3-4 h. In the normolipidemic subjects (n = 369), fasting serum apo B-48 levels were 41 ± 28 (Mean ± SD) arbitrary units (AU/ml) (100 AU/ml ~ 1 mg/dl). Serum apo B-48 concentration positively correlated with serum triglyceride levels (r = 0.72, p < 0.05), while there was no significant correlation between serum apo B-48 and cholesterol levels. Apo B-48 was detected in ultracentrifugally separated lipoproteins including CM, VLDL, IDL and even LDL. In dyslipidemic subjects with apo E2/2, apo B-48 was extremely increased even if they were normolipidemic. In patients with diabetes mellitus, apo B-48 was also increased, suggesting the impairment of CM metabolism. Furthermore, we used the mAbs to examine the presence or absence of apo B-48 in human aortic tissues. Immunohistochemical analysis demonstrated a strong positive staining of apo B-48 mainly in the extracellular matrices of atherosclerotic aorta.

Conclusion: These data suggest that this apo B-48 ELISA provides a useful tool to estimate the impairment of CM metabolism and that the deposition of CM remnants could contribute partly to the pathogenesis of atherosclerosis.
**WeW5.20**  
**Metabolic risk factors for CHD in the elderly**  
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**Objective:** To identify metabolic risk factors for coronary heart disease (CHD) in the elderly.

**Methods:** A 10 year follow-up in a prospective study of cardiovascular disease in the Australian elderly. The cohort, first examined in 1988–89, was comprised of 2805 men and women 60 years and older. The prediction of CHD was examined in a Cox proportional hazards model.

**Results:** CHD outcomes (ICD-9 410–414) occurred in 448 men (36%) and 435 women (28%). The hazard ratios (95% CI) for men and women respectively were: total cholesterol 1.09 (1.01–1.18) and 1.09 (1.02–1.17); HDL cholesterol 0.64 (0.46–0.90) and 0.62 (0.46–0.83); total/HDL cholesterol 1.05 (1.01–1.09) and 1.04 (1.01–1.09); log triglycerides 1.07 (0.89–1.29) and 1.42 (1.14–1.76); LDL cholesterol 1.14 (1.04–1.25) and 1.08 (0.99–1.17); serum apo-B 1.64 (1.15–2.34) and 1.60 (1.16–2.21); apo-A1 0.74 (0.53–1.03) and 0.81 (0.59–1.10) [per 1 unit change in each continuous variable]; Lp[a] 1.52 (1.13–2.02) and 1.25 (0.91–1.71) [Quintile V vs Quintile 1]; diabetes 1.18 (0.87–1.59) and 1.95 (1.44–2.63). Hazard ratios by age for total cholesterol (sexes combined) were: 60–69 y, 1.17 (1.08–1.27); 70–79 y, 1.02 (0.94–1.11); 80+ y, 1.05 (0.88–1.25).

**Conclusions:** Lipids and lipoproteins still predict CHD in the elderly, but with some attenuation as the population ages.

**WeW6.20**  
**Paraoxonase activity in primary hypertriglyceridaemia**  
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**Objective:** The aim of the present study was to evaluate paraoxonase (PON) activity in primary hypertriglyceridaemia (HTG), whose independent relationship with atherosclerosis still remains controversial.

**Methods:** We studied a group of well-characterised men with primary hypertriglyceridaemia (Triglycerides, TG ≥ 200 mg/dl) and low HDL-cholesterol levels (HDL-C ≤ 35 mg/dl) (n = 12) in comparison to 60 normal controls (NTG) subjects with TG ≤ 200 mg/dl (n = 12) or without low HDL-C concentration (n = 12). The lipid, lipoprotein and apolipoprotein profiles were evaluated by standardised methods and previously reported (Brites et al. Atherosclerosis, in press). PON was evaluated following the original method of Furlong et al. and employing two different substrates: paraoxon (PON activity) and phenylacetate (aryl esterase, ARE activity), the latter being a better indicator of the enzyme mass. PON activity was also measured in the presence and in the absence of 1 M NaCl (PON 1 M).

**Results:** PON, PON 1 M and ARE activities were significantly reduced only in patients who combined HTG and low HDL-C levels in comparison to NTG subjects with normal HDL-C (PON = 166 ± 63 vs. 247 ± 69 μmol/min, p < 0.01; PON 1 M = 201 ± 115 vs. 376 ± 180 μmol/min, p < 0.05; ARE = 760 ± 199 vs. 950 ± 216 μmol/min, p < 0.01, respectively). All the three activities showed positive and significant correlations with total HDL-C, HDL-C:LDL-C, apo A-I and LpA1-A-I-A-I-I levels.

**Conclusions:** HDL antiatherogenic capacity is not only limited to its role in reverse cholesterol transport, but also to its antioxidant potential, mainly attributed to PON. In patients with primary HTG and low HDL-C, the low PON activity, evaluated by different ways, could reflect a reduced antioxidant capacity, thus contributing to the association between hypertriglyceridaemia and atherosclerosis.

**W:21**  
**CELLULAR STRESS AND GENE REGULATION**  

**WeW1.21**  
**Hemodynamic forces, endothelial gene regulation and atherogenesis: An overview**  
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The localization of atherosclerotic lesions to arterial geometries associated with disturbed flow patterns suggests an important role for hemodynamic forces in atherogenesis. There is increasing evidence that the endothelium can discriminate among different biomechanical forces and transduce these stimuli into genetic regulatory events. At the level of individual genes, this regulation is accomplished via the interaction of transcription factors such as NF-kB with promoter elements such as the shear-stress response element (SSRE), in the EDRG α gene. Several such biomechanical force-sensitive transcriptional mechanisms appear to exist. At the level of multiple genes, distinct patterns of up- and down-regulation appear to be elicited by exposure to physiological levels of steady laminar shear stress (LSS), compared with similar magnitudes of turbulent shear stress (TSS), versus cytokine (IL-1) stimulation. LSS appears to elicit a distinct pattern of endothelial gene expression, not observed with TSS or IL-1. Certain of the genes upregulated by steady LSS, such as eNOS, MnSOD, COX-2, support vaso-protective (anti-inflammatory, anti-thrombogenic, anti-oxidant, anti-apoptotic) functions in endothelium. The selective and sustained upregulation of these “atheroprotective genes” in the endothelial lining of lesion-protected areas may be a mechanism by which hemodynamic forces can influence lesion formation and progression. The application of these genome-wide, high-throughput technologies for the analysis of endothelial phenotypic modulation by biomechanical stimuli promises to afford new insights into the signaling networks that orchestrate endothelial gene regulation in atherogenesis. These new insights may enable novel strategies for early diagnosis, treatment and prevention of atherosclerotic vascular disease.

**WeW2.21**  
**Focal atherogenesis, hemodynamics, and heterogeneous endothelial gene expression**  

Atherosclerosis originates at predictable focal loci that have long been known to be associated with regions of disturbed blood flow. Improved precision in experimental models of spatially defined flow has recently been combined with regional and single-cell gene-expression (“transcriptional profiling”) to investigate the relationships linking haemodynamics to vessel wall pathology. We have proposed that a limited number of pro-atherosclerotic genes expressed in only a few endothelial cells may play a dominant role in focal pathogenesis and vascular regulation. However, their identity may be masked because of message dilution in mRNAs isolated from a larger pool of cells. To begin to address the hypothesis, we have applied an amplified cDNA microarray technique to the cardiovascular system. Quantitative profiles of gene expression, including the use of high-throughput hybridization to screen many genes simultaneously, now allow endothelial heterogeneity to be addressed in a detailed (single cell) yet comprehensive (multiple genes, high-throughput) approach that increases the probability of finding new therapeutic targets. Supported by NIH grants HL62250, HL36491, and a grant from Astrazeneca Pharmaceuticals.

**WeW3.21**  
**Gene regulation by hypoxia**  

Tissue hypoxia underlies the pathophysiology of much human disease. Erythropoietin gene expression has provided a paradigm for transcriptional regulation in response to hypoxia. The erythropoietin 3’ enhancer is activated in hypoxia in specialised cells by the binding of an inducible DNA binding complex, hypoxia inducible factor-1 (HIF). HIF is widely expressed and has a key role in other cellular responses to hypoxia, binding the DNA consensus sequence BRGCTGV of genes involved in a variety of processes including energy metabolism, angiogenesis, vasomotor tone and apoptosis and regulating their expression. Furthermore, tumour transplantation experiments indicate that HIF is activated by hypoxia within solid tumours and that this activation has a critical bearing on tumour angiogenesis and growth. HIF is a heterodimer of basic-helix-loop-helix PAS domain containing alpha and beta chains, each of which exist as gene families. We have mapped functional domains within the HIF-1 alpha and HIF-2 alpha chains. Two mechanisms of regulated transactivation are revealed, one involving the carboxyl terminus, which interacts with the co-activator P300, and the other involving an internal domain responsible for rapid degradation of the molecule by the proteasome in normoxia. Degradation is prevented by hypoxia, cobaltous ions or iron chelators.

Experiments with mutant cell lines and model organisms have allowed molecular dissection of the HIF alpha degradative pathway. We have demonstrated a critical role for the von Hippel-Lindau tumour suppressor protein, pVHL, in the degradation process. The interaction between these molecules
requires part of the internal oxygen dependent degradation domain of HIF alpha chains and is disrupted by tumour associated mutations in the beta domain of pVHL. Loss of this interaction is associated with a failure to ubiquitinate HIF alpha chains and consequently, a failure to regulate HIF and HIF-dependent genes.

**WeVW4.21**
Mechanical stress-induced HSF1 expression in SMC via RAS/RAC G-proteins, but not MAPK
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**Objective:** Previous reports documented that acute elevation in blood pressure results in heat shock protein 70 (hsp70) mRNA expression followed by hsp70 protein production in rat aortas. The present study was designed to study whether mechanical stress per se induces HSF1 expression in smooth muscle cells (SMCs).

**Methods and Results:** Western blot analysis demonstrated that hsp70 protein induction peaked between 6 and 12 h after treatment with cyclic strain stress (60 cycles/min, 5-30% elongation). Elevated protein levels were preceded by hsp70 mRNA transcription, which was associated with HSF1 phosphorylation and activation stimulated by mechanical forces, suggesting that the response was regulated at the transcriptional level. Conditioned medium from cyclic strain-stressed SMCs did not result in HSF1 DNA-binding activation. Furthermore, mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinases, c-Jun NH2-terminal protein kinases or stress-activated protein kinases and p38 MAPKs, were also highly activated in response to cyclic strain stress. Inhibition of ERK and p38 MAPK activation by their specific inhibitors (PD98059 and SB 202190) did not influence HSF1 activation.

Interestingly, SMC lines stably expressing dominant negative rac (rac N17) abolished hsp production and HSF1 activation induced by cyclic strain stress, while a significant reduction of hsp70 expression was seen in ras N17 transfect SMC lines.

**Conclusions:** Our findings demonstrate that cyclic strain stress-induced-hsp70 expression is mediated by HSF1 and regulated by rac/ras GTP-binding proteins. Induction of hsp70 could be important in maintaining SMC homeostasis during vascular remodeling stimulated by hemodynamic stress.

**WeW5.21**
Genotype dependent and environmental effects on expression of nicotinamide synthase (eNOS) gene expression and enzyme activity
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**Objective:** We have shown that the rare 427bp repeat allele at intron 4 of the eNOS is associated with an elevated risk for coronary artery disease (CAD) in smokers but higher basal NO levels in healthy non-smokers. In the present study, we explored the interactive effects of eNOS genotypes and cigarette smoking on the eNOS gene expression and enzyme activity.

**Methods:** We measured levels of the eNOS mRNA (quantitative RT-PCR) and protein (Western blotting), and eNOS enzyme activity (L-arginine conversion) in 33 postpartum placenta.

**Results:** We found that the eNOS protein levels were significantly lower among those with the rare 4 repeat allele (0.48 ± 0.11, n = 9) compared to the common allele (1.05 ± 0.10, n = 24, p < 0.01). In contrast, the eNOS enzyme activity was about 7 fold higher in the rare allele (455.62 ± 255.4 cpm/mg/min) than that in the common allele (621.8 ± 180.5 cpm/mg/min).

**Conclusion:** Smoking reduced the eNOS activities for more than half of the value in the rare allele placenta (non-smokers: 1.09 ± 0.12 vs 0.90 ± 0.27) and the rare allele (0.42 ± 0.27 vs 0.27 ± 0.28), it had an interactive effect on eNOS activities. Cigarette smoking reduced the eNOS activities for more than half of the value in the rare allele placenta (non-smokers: 614.38 ± 251.2, n = 5, smokers: 2968.5 ± 259.4, n = 4). But the enzyme activities for the common allele placentas were even modestly elevated (non-smokers: 521.3 ± 110.8, n = 19, smokers: 722.2 ± 251.1, n = 5).

**Conclusion:** Although the rare allele may generate more NO when not exposed to smoking, the capacity of NO production by eNOS is seriously compromised when the rare allele carriers smoke, which does not occur for the common allele. We established a genotype dependent and environmental specific model in eNOS regulation.

**WeW6.21**
25-hydroxycholesterol modulates an inflammatory response in human macrophages
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**Objective:** Several different oxysterols and Tumor Necrosis Factor-α (TNFα) have been found in atherosclerotic plaques. Oxysterols have in earlier studies been shown to have regulatory effects on expression of different genes. The aim of this study was to examine the impact of these oxysterols on the TNFα secretion in human monocyte-derived macrophages (HMDM) and to investigate the regulatory pathway.

**Methods:** Binding of transcription factors to DNA was studied by Electrophoretic Mobility Shift Assay (EMSA) and TNFα protein secretion was assayed by Enzyme-Linked Immuno-Sorbent Assay (ELISA). Intracellular levels of H2O2 were determined and Western Blot was used to analyze activity of Stress Activated Protein Kinase/Jun N-terminal Kinase (SAPK/JNK).

**Results:** An increased TNFα secretion was found when mHDM were treated with 25-OH (5 µg/ml) in combination with IFNY (500 U) for 24 h. Other oxysterols assayed did not increase the TNFα secretion in combination with IFNY. The DNA-binding of the transcription factor complex Activating Protein-1 (AP-1) was increased when treating cells with 25-OH. However, no NF-kB was induced to bind to the TNFα promoter. The AP-1 complex is activated by JNK/SAPK. We found that 25-OH caused an increase in SAPK/JNK activity, in contrast to 7-ketocholesterol (7-keto), which did not activate SAPK/JNK. 25-OH, but not 7-keto, increased the intracellular levels of H2O2. Transfection studies showed that the primary action of IFNY is on the 3′ UTR of the TNFα gene.

**Conclusion:** 25-OH might influence the expression of TNFα through activation of the SAPK/JNK pathway.

**WeW7.21**
Gene expression in atherosclerotic lesions analyzed by DNA array
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**Objective:** DNA arrays are revolutionizing the analysis of gene expression. Currently, the expression of 10-15% of human genes can be analyzed simultaneously in a single experiment. With the aid of DNA array it is possible to identify multiple, simultaneous, transcriptional events that ameliorate or contribute to atherogenesis.

**Methods and Results:** We analyzed the gene expression patterns of vessel wall during atherogenesis using DNA array techniques. GenomeSystem’s DNA microarrays of 18 000 DNA clones were used. Atrial samples were collected from vascular surgery operations (n = 3) or immediately after death (n = 2). Normalization of the data was carried out using the expression intensity of all genes. A large number of activation and inactivation in gene expression patterns was identified in atherosclerotic lesions which were previously unknown or not connected to the pathogenesis of atherosclerosis. Changes in gene expression of selected identified genes were confirmed using in situ hybridization.

**Conclusions:** Many of the novel genes, which were activated in atherosclerotic lesions, have a role in monocyte differentiation.

**W.22**
REVERSE CHOLESTEROL TRANSPORT

**WeW1.22**
Synthesis of HDL by apolipoprotein-cell interaction: Its molecular mechanism and physiological importance
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Helical apoproteins interact with various types of cells to generate new HDL particles with cellular lipids. This is an important reaction as one of the two major pathways for the release of cellular cholesterol to maintain cholesterol homeostasis both in a cell and for a whole body and also as a major source of plasma HDL.
Two major molecular mechanisms are involved in this reaction. 1) An apolipoprotein-interaction site of the cell surface. This site involves a protein component(s) and is inducible by CAMP and other compounds in certain types of cells. Progesterone inhibits its function and ABC1 is not absolutely required to compose this site. 2) Intracellular cholesterol mobilization specific for its incorporation into the HDL. This is triggered by the apolipoprotein-cell interaction, perhaps mediated by a signal transduction system. Newly synthesized cholesterol is preferentially mobilized, and caveolin-1 is responsible for its trafficking. Cholesterol-poor HDL is generated when this part is lacking or insufficient in the cells. A sphingomyelin-rich domain of plasma membrane seems a site for cholesterol incorporation into the HDL.

This reaction seems a major source of plasma HDL. Impairment of the apolipoprotein-cell interaction causes the decrease of plasma HDL. This is suggested not only by Tangier disease, but also the effect of progesterone in reduction of HDL. In vitro, it completely inhibits apolipoprotein-cell interaction. In mice, it rapidly reduce the HDL level without changing the major HDL-regulatory parameters such as the messages of apoA-I, LCAT, SRB1 and PLTP or CETP activity and plasma HDL-CE clearance rate, except for the reduction of cellular interaction with apolipoprotein and the increase of HDL-apoB clearance rate, both being consistent with the findings with Tangier disease.

**WeW2-22** Lipid transfer proteins and receptors in HDL in metabolism

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The reverse cholesterol transport pathway is initiated in the arterial wall, by the interaction of HDL or apoA-I with cholesterol-loaded macrophages. The latter is facilitated by ABC1. Cholesterol may be directly transported in HDL to the liver and removed from the circulation by interaction with scavenger receptor BI (SRBI), or transferred to other lipoproteins by cholesteryl ester transfer protein (CETP). Recent studies on the mechanism of sterol-mediated up-regulation of CETP gene transcription indicate that this involves the hydroxysterol-regulated transcription factors, the LXR1s. LXR1s may co-ordinately induce several different molecules involved in reverse cholesterol transport, including ABC1, CETP and cyp7a. In the circulation HDL is also modified by transfer of phospholipids derived from triglyceride-rich lipoproteins. This process is mediated by the phospholipid transfer protein (PLTP); knock-out of PLTP results in reduced HDL levels, but also has unexpected effects on the metabolism of apoB-lipoproteins and atherosclerosis. The final step of RCT involves the excretion of HDL cholesterol into bile. Although SRBI has been thought to primarily mediate selective uptake of HDL CE at the cell surface, recent studies indicate that SRBI also functions as an endocytic receptor. In hepatocytes, SRBI mediates internalization and recycling or transcytosis of HDL particles across the liver cell to bile canalicus. Thus, SRBI mediates selective transcytosis of HDL cholesterol across the hepatocyte.

**WeW3-22** Mechanisms of cholesterol movement between high density lipoproteins (HDL) and cells

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HDL plays a central role in reverse cholesterol transport because it is capable of mediating both the delivery to and removal from cells of cholesterol. Scavenger receptor class B, type I (SR-BI) plays a central role in the mechanisms of these processes. The presence of SR-BI in the plasma membrane (PM) is essential for the phenomenon of cholesterol ester (CE) selective uptake. The binding of CE-containing HDL particles to SR-BI allows diffusion of CE molecules down a concentration gradient along a non-aqueous channel, created by the extracellular domains of SR-BI, between the bound HDL and the PM. The apolipoprotein (apo) A-I in HDL is a ligand for the SR-BI and the structural motif recognized by receptor is the amphiphatic α-helix. Binding of HDL to SR-BI can also facilitate the bidirectional flux of unesterified cholesterol (C) molecules between HDL and the cell PM. The direction of net flux is determined by the C concentration gradient. Independent of HDL binding, SR-BI also enhances efflux of C from cells by causing a reorganization of the PM so that C can desorb more readily into the extracellular aqueous phase and be absorbed by HDL particles.

**WeW4-22** The dual role of HDL in macrophage lipid processing and immune regulation

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Activation of CD14 has been shown to be modulated by a complex interaction of endotoxin and plasma components including HDL, LBP and CETP while ceramide (Cer) which structurally resembles LPS has been postulated as a naturally occurring ligand. As CD14 is a GPI-anchored protein, signal transduction is assumed to occur within cholesterol and sphingolipid rich microdomains (rafts) via clustering with proteins such as the β2-integrin CD11b/CD18. Using fluorescence resonance energy transfer (FRET) we were able to demonstrate no significant co-association between CD14 and CD11b in resting monocytes. Both LPS and Cer, in contrast, induced a significant co-association between CD14 and CD11b suggesting an activation-dependent conformational change of the receptor complex due lipid binding. Integron Associated Proteins (IAP, CD47 and CD101) and the FCγRII (CD16) were identified as further components of the multimeric functional receptor complex. LPS and Cer have been shown to be associated to HDL lipoproteins in a process mediated via lipid transfer proteins including LBP and PLTP. Recently, an apo-AI/LBP containing particle has been demonstrated, facilitating cellular responses to LPS. Based on our data we propose CD14 to be a raft receptor for LPS/Cer enriched apo-AI/LBP-containing HDL-particles in the acute phase response. The increase in LBP and PLTP activities in inflammation may further enhance the transfer of LPS to HDL. Together with the recent report of an increased expression of CD14 on monocytes in myocardial infarction, this suggests the targeting of proinflammatory HDL particles to rafts and a signaling through an innate immunity receptor complex as important cellular activation processes in the acute phase response.

**WeW5-22** Age-related decline of the expression of a Rho GTPases family, CDC42Hs, in human skin fibroblasts in association with reduction of HDL-mediated cholesterol efflux

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**Objective:** Reverse cholesterol transport (RCT) is one of the major protective system against atherosclerosis. The initial step of RCT is named "cholesterol efflux", where HDL particles take up cholesterol from the lipid-laden cells. Recently, we have found that the expression of a member of RhoGTPases, Cdc42Hs, is decreased in association with the abnormal actin cytoskeleton, slow cell proliferation, and cholesterol efflux, in cells from patients with Tangier disease, a model for the impairment of RCT (Hirano K. et al.). Although it is obvious that senescence is the unescapable risk factor for atherosclerosis, the effect of aging on the RCT has not been fully clarified yet. The aim of the present study is to know whether or not aging affects the initial step of RCT, cholesterol efflux.

**Method:** We have analyzed HDL-mediated cholesterol efflux as well as the expression levels of RhoGTPases in passaged human skin fibroblasts obtained from seven subjects whose age are ranging from 24 to 86 years old.

**Results:** The expression levels of Cdc42Hs were decreased in proportion to aging, which was associated with altered actin cytoskeletons and slow cell proliferation. HDL-mediated cholesterol was reduced in proportion to aging. There was a close correlation between the expression levels of Cdc42Hs and cholesterol efflux.

**Conclusion:** The present study demonstrates that aging may decrease HDL-mediated cholesterol efflux in conjunction with the decreased expression of the small G protein, suggesting that aging affects the reverse cholesterol transport system. The present data support our recent finding that the small G protein may play an important role as one of the physiological key components for cholesterol efflux.

**WeW6-22** CAMP regulates apolipoprotein-mediated cholesterol efflux by induction of expression of the tangier protein ABC1

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The ATP-binding cassette transporter ABC1 was identified as the defective gene in Tangier Disease and a key component of the regulation of cellular cholesterol efflux. This was accomplished by a comparison of gene expression in Tangier and normal fibroblasts by hybridisation to microarray chips, and biochemical studies that demonstrated that over-expression of the gene
increased, and ABC1 antisense oligonucleotide treatment decreased specific cellular efflux of cholesterol to apolipoprotein A-I. In previous studies apoA-I mediated cholesterol efflux from RAW 264.7 macrophages was shown to be absolutely dependent on cAMP analogs. Here we show by quantitative RT-PCR of ABC1 mRNA that transcription of the gene in RAW cells is rapidly induced from very low levels by cAMP treatment, in parallel with the cells’ capacity to efflux cholesterol to apoA-I. Immunoprecipitation of ABC1 protein from pulse-chase labelled cells also showed that cAMP confers a marked increase in protein half-life compared to unstimulated cells. The acquisition of the cells ability to efflux cholesterol is therefore dependent on the induction of ABC1 expression, and stabilisation of the protein. Assays of reporter gene activity in RAW cells transfected with wild-type and mutated ABC1-promoter-luciferase fusion constructs have enabled us to identify important regulatory elements in the promoter including those responsible for the transcriptional response to cAMP.

**WeW7:22** Oxidation of specific Met residues does not decrease anti-atherogenic activities of apolipoprotein A-I

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In human lesions, lipids in high- (HDL) and low-density lipoproteins (LDL) are oxidized to comparably extent (ATVB 1999; 19: 708). HDL may become oxidized directly or accept preformed lipid hydroperoxides (LOOH) from oxidized LDL via cholesteryl ester transfer protein (JLR 1995; 36: 2017). Once present in HDL, LOOH are detoxified to the corresponding alcohols (FRBM 1995; 18: 421), a process associated with the oxidation of two Met residues of apolipoprotein A-I (apoA-I) to Met sulfoxides (MetO; JBC 1998; 273: 6080; ibid 273: 6088). Formation of such specifically oxidized apoA-I (apoA-I-MetO) may affect reverse cholesterol transport as Met residues are implicated in cholesteryl efflux and activation of lecithin:cholesterol acyltransferase (LCAT). Mass spectrometry of intact and proteolytic fragments revealed that Met93 and Met112 are present as MetO in apoA-I-MetO, circular dichroism showed that the alpha helical content of lipid-free and lipid-associated apoA-I and apoA-I-MetO were comparable, as was the affinity for LCAT for reconstituted HDL containing apoA-I-MetO or apoA-I. By contrast, lipoprotein MetO displays a greater affinity for liposomal phospholipid and cholesterol, phospholipids, and vitamin E present in macrophage HDL, and in HDL isolated from human atherosclerotic lesions. Such lesion HDL remained capable of promoting cellular cholesterol efflux, independent of the severity of atherosclerosis. Together, these results suggest that mild oxidation does not diminish known anti-atherogenic activities of apoA-I, consistent with our hypothesis that detoxification of pro-atherogenic LOOH by HDL may be anti-atherogenic.

**W:23 IMMUNE AND INFLAMMATORY MECHANISMS INATHEROSCLEROSIS**

**WeW1:23** Biological significance of autoantibodies to oxidative neoepitopes

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Adducts of reactive aldehydes or oxidized phospholipids formed during lipid peroxidation with (apolipo) proteins or other phospholipids trigger extensive humoral and cellular immune responses in vivo. Tiers of autoantibodies against "oxidation-specific epitopes" generally correlate with the extent and rate of progression of atherosclerosis in humans and murine models. However, antigen formation is not limited to atherosclerotic lesions, but also occurs in other chronic inflammatory diseases or aging cells. Immunization of rabbits and mice with oxidized LDL reduces the progression of atherosclerosis, suggesting that modulation of specific humoral or cellular immune responses may be beneficial. Naturally occurring antibodies cloned from non-immunized atherosclerotic apoE-/- mice ("EO"-antibodies) provide insights into the nature of antigens formed in vivo and on biological effects of selected antibody populations. For example, EO antibodies binding to oxidized phospholipids adducts inhibit macrophage uptake of oxidized LDL by blocking scavenger receptors. Similar antibodies in human subjects with "anti-oxidized phospholipid-antibody syndrome" may also affect thrombosis. Cloning of the VH and Vκ genes of four EO antibodies to oxidized phospholipids that had been independently selected for binding to copper-oxidized LDL showed that these antibodies were identical to classical T-15 antibodies, evolutionarily selected, B1 lymphocyte dependent antibodies that protect against pneumococcal infection. Antibodies against oxidation-specific epitopes can also be used to quantify atherosclerosis. Murine studies indicated that the in vivo uptake of 125I-labeled antibodies correlated with several conventional measures of atherosclerosis but was more sensitive to regression of lesions rich in oxidation-specific epitopes. An oxidation-specific human Fab antibody cloned from a phage display library, IX17, may be particularly useful for diagnostic purposes, because it mainly recognizes epitopes in lipid-rich core regions of human and animal atherosomas.

**WeW2:23** Autoimmunity to heat shock protein 60/65 as an initiating mechanism in the atherosclerosis development

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In the last decade, we have developed a new "autoimmune" concept for the pathogenesis of atherosclerosis that is supported by solid experimental and clinical data. In essence, we have shown that heat shock protein 60 (HSP60) expressed by stressed arterial endothelial cells at known predilection sites for the development of atherosclerotic lesions (i.e. branching points of arteries) form the target for cellular and humoral immunity against highly conserved HSP60 epitopes in various forms of microbes (viruses, bacteria, parasites). We are thus "paying" for protective immunity in younger age by the development of atherosclerosis in older age in case vascular endothelial cells are subjected to classical risk factors for atherosclerosis, notably hypertension, oxidized low-density lipoproteins (oxLDL), toxins, etc. We now show that the life-long exposure of arterial endothelial cells to the high arterial blood pressure makes these cells more vulnerable to the effect of other stressors that lead to a simultaneous expression of HSP60 and certain adhesion molecules (ICAM-1, VCAM-1, ELAM-1). In this context, the question will be addressed which are the cellular sensors for mechanical stress that may be responsive for inflammatory arterial changes and even advanced severe vascular lesions in older age. Furthermore, results of a prospective clinical study will be presented to show that immunization to HSP60 is not only a parameter for morbidity but also for mortality.

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**Extra Content**

Atherosclerosis has been recognized as an inflammatory disease. Proinflammatory cytokines, including TNFα, IL-1, IL-8, IL-12, oncostatin M and IFNγ are all present within the human atherosclerotic plaque, and participate in the activation of macrophages, lymphocytes, and vascular cells. However, the inflammatory response is known to be balanced by anti-inflammatory cytokines, including IL-4, IL-10 and IL-13. Among the anti-inflammatory cytokines produced by Th2 cells, IL-10 is also produced by macrophages and has potent deactivating properties. Whereas IL-4 and IL-13 can be barely found in human atherosclerotic plaques, IL-10 mRNA and protein are highly expressed, mainly by macrophages. IL-10 expression is associated with a marked decrease in NOS II expression and low levels of apoptosis, suggesting that locally produced IL-10 has an anti-inflammatory role and protects from excessive cell damage and death in the plaque. Indeed, endothelial NF-κB activation and expression of VCAM-1 and ICAM-1 that are markedly increased in C57BL/6J mice after a short period of time (10 days) on an atherogenic diet, can be totally prevented by in vivo transfer of murine IL-10 cDNA. On the other hand, IL-10-deficient C57BL/6J mice fed an atherogenic diet and raised under specific-pathogen-free conditions exhibit a significant 3-fold increase in atherosclerotic lesions compared with wild type mice. Interestingly, the susceptibility of IL-10-deficient mice to atherosclerosis is exceedingly high (30-fold increase) when the mice are housed under conventional conditions. Moreover, atherosclerotic lesions of IL-10-deficient mice show increased T-cell infiltration, abundant IFNγ expression, and decreased collagen content. Therefore, IL-10 appears to have critical roles in both atherosclerotic lesion formation and stability, and might be crucial as a protective factor against the effect of environmental pathogens on atherosclerosis.
Wednesday June 28, 2000: Workshop Abstracts
W24 Hormones and Cardiovascular Disease

WeW4:23 Human vascular smooth muscle cells express an endogenous inhibitor of Caspase-1
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Objective: Caspase-1 regulates key steps in inflammation and immunity, by either activating the pro-inflammatory cytokines IL-1beta and IL-18 or mediating apoptosis, processes associated with the chronic inflammatory disease, atherosclerosis. We recently provided evidence for the regulation of Caspase-1 activity via an endogenous inhibitor expressed by human vascular smooth muscle cells (SMC). However, the molecular identity of this endogenous inhibitor remained undefined, rendering Caspase-1 inhibitors restricted to either synthetic peptides or viral proteins.

Methods and Results: We report here, that the serine protease inhibitor Serpin (P)I-9 accounts for the endogenous Caspase-1 inhibitory activity in human SMC and prevents processing of the natural substrates, IL-1beta and IL-18 precursor, as determined by Western blot analysis. Treatment of SMC lysates with anti-PI-9 antibody abrogated the Caspase-1 inhibitory activity and co-precipitated the enzyme, demonstrating protein-protein interaction. Furthermore, PI-9 antisense oligonucleotides coordinately reduced PI-9 expression and promoted IL-1beta release. SMC comprise the majority of cells in the vascular wall, and Caspase-1 and IL-1 are firmly implicated in atherogenesis, we tested the biological validity of our findings within human atheroma in situ. The unabstracted arterial wall contains abundant, homogeneously distributed, PI-9. In human atherosclerotic lesions, however, PI-9 expression correlated inversely with immunoreactive IL-1beta, supporting a potential role of the endogenous Caspase-1 inhibitor in this chronic inflammatory disease.

Conclusions: Our results provide new insights into the regulation of Caspase-1, an enzyme involved in immune and inflammatory processes of chronic inflammatory diseases, and point to an endogenous anti-inflammatory action of PI-9, dysregulated in the human prevalent disease, atherosclerosis.

WeW7:23 Adaptope transfer of b2-glycoprotein I (b2GPI)-reactive lymphocyte enhances atherosclerosis in LDL receptor deficient mice
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Background: It has been proposed that autoimmune factors may influence the progression of atherosclerosis. We have previously shown that immunization of LDL receptor deficient (LDL-RD) mice with b2 glycoprotein I (b2GPI; a principal target of ‘autoimmune’ anti-phospholipid antibodies) enhances early atherosclerosis. In the current study we tested the hypothesis that adoptive transfer of b2GPI reactive T-cells can accelerate atherogenesis in LDL-RD mice.

Methods and Results: LDL-RD mice were immunized with human b2GPI. An additional group of mice were immunized with b2GPI and boosted with the same antigen 3 weeks later. Control mice with immunized with human serum albumin (HSA). Lymphocytes obtained from the draining lymph-node cells or from splenocytes of b2GPI or HSA immunized mice were stimulated in vitro with b2GPI or with the mitogen Concanavalin A, respectively. The cultured lymphocytes were transferred intraperitoneally to syngenic LDL-RD mice and fed for 3 weeks a high-fat ‘Western’ diet until sacrifice. Mice injected with lymphocytes from draining lymph nodes or spleens of b2GPI-immunized animals displayed larger atherosclerotic lesions as compared to those induced by control treated animals. T-cell depleted splenocytes from b2GPI were unable to promote lesion formation in the mice. Lymphocytes that mediated lesion enhancement displayed a predominant THelper 1 phenotype evident by increased secretion of g interferon in vitro priming with b2GPI.

Conclusion: This is the first direct evidence for a role of antigen (b2GPI)-reactive T-cells in promoting atherosclerotic lesions in mice.

WeW5:23 Contrastinig effects of AT1 and AT2 antagonon angiotensin II induced atherosclerosis and abdominal aortic aneurysms
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Objective: Previously, we have demonstrated that infusion of angiotensin II (AngII) into apolipoprotein E--/-- mice leads to the rapid formation of atherosclerotic lesions and development of abdominal aortic aneurysms (AAA). The aim of the present study was to define the effects of specific AngII receptor antagonists on the development of murine pathology.

Methods: Mature apoe--/-- mice were infused via osmotic pumps for 28 days with either an AT1 (losartan, 30 mg/kg/day) or AT2 (PD 123319, 3 mg/kg/day) antagonist, either alone, or with combined administration of AngII (1 mg/kg/min).

Results: AngII infusion increased the extent of atherosclerosis and generated AAA in 70% of mice. Losartan totally ablated the AngII induced atherosclerosis and AAA. PD123319 failed to influence the AngII induced development of atherosclerosis. However, it increased the incidence of aneurysms to 100%. Furthermore, the aneurysms formed were larger and had a more complex appearance. Administration of losartan or PD123319, in the absence of AngII infusion, had no effect on the development of atherosclerotic lesions or AAA.

Conclusion: AT1 receptor antagonism profoundly decreased AngII induced vascular pathology, while blockade of AT2 receptors unexpectedly promoted effects on AAA.

WeW4:23 Anti-atherosclerotic effect of SB-244323, a lipoprotein associated phospholipase A2 inhibitor, in WHHL rabbits
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Lipoprotein associated phospholipase A2 (Lp-PLA2) activity is expressed by macrophages in atherosclerotic lesions and is responsible for generating significant quantities of the pro-inflammatory mediator lyso-PC during the oxidation of LDL. We therefore investigated the potential anti-atherogenic properties of the potent Lp-PLA2 inhibitor SB-244323 (ethyl ester pro-drug of SB-245713, IC50 = 8 nM) in WHHL rabbits. A dose of compound was selected that reduced aortic Lp-PLA2 activity to that observed in aortas of normal NZW rabbits. Male WHHL rabbits (n = 21/group) were treated with 20 umol/kg/d of SB-244323 in the diet or diet alone for 12 weeks. The rabbits were then killed, their aortas were removed and the atherosclerotic lesions were measured and characterised. SB-244323 reduced Lp-PLA2 activity in rabbit plasma and aortas by 99% and 54%, respectively, and there were no adverse reactions to treatment. Whereas plasma lipids were unaffected by treatment, concentrations of plasma PAI-1 were significantly reduced by 41% by SB-244323 after 6 weeks of treatment. The total cross-sectional area, thickness and macrophage content of aortic atherosclerotic lesions were measured in histological sections. Treatment reduced all parameters measured with larger lesions; SB 244323 reduced median lesion cross-sectional area and thickness significantly by 38% and 24% respectively in sections taken from just below the coeliac artery.

Conclusion: These results support the view that Lp-PLA2 plays a significant role in the development of atherosclerotic plaque.

WeW1:24 A clinical overview with focus on growth hormone
K.J. Osterziel. Franz-Volhard-Klinik/Charité, Humboldt University of Berlin, Germany

Ischemic and dilated cardiomyopathies are characterized by ventricular dilatation, thin ventricular walls and impaired systolic function. Recently, it could be shown that the activity of the somatotrophic system is decreased in heart failure. Cardiac cachexia is characterized by low IGFI. The decrease of insulin-like growth factor 1 (IGF-I), one of the mediators of growth hormone (GH) effects, correlates to left ventricular ejection fraction and inversely to left ventricular size. Subtle, yet unknown alterations of the somatotropic system may lead to the decrease of serum IGF-I levels. Several pilot studies have shown hemodynamic and clinical improvement after treatment with human recombinant growth hormone (GH) for 3 months. Larger randomized, double-blind and placebo-controlled trials could not confirm the expected clinical improvement. A subgroup of patients showed hemodynamic improvement, most likely due to an increased NO-formation. The significant increase of myocardial mass by GH was related to the increase of IGF-I. When IGF-I increased by more than 80 pg/ml a significant increase of ejection fraction could be shown.

Summary: The somatotrophic axis is altered in patients with heart failure. Short-term treatment with GH leads in a subgroup of patients to an improvement of left ventricular function.

**Cellular and molecular mechanisms of the atheroprotective effects of estrogens**

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**Objective:** The mechanism(s) whereby the atheroprotective effect of estrogens is mediated has been thought to be due to potentially favorable changes in blood lipids and lipoproteins but a number of animal studies strongly suggest a direct effect on the vascular wall.

**Methods:** Estradiol-17β (E2) prevents fatty streak formation in apolipoprotein E-deficient (apoEKO) mice. We used this animal model under variable experimental conditions of diet, pharmacological and genetic manipulations, to characterize the mechanisms which mediate E2 effects in this process.

**Results:** Studies of the lesion-serum cholesterol relationships confirm that the main action of E2 is on cells of the vascular wall. Endothelial cell turn-over and production of EDRF are under estrogen control but do not constitute a main target. Using apoEKO mice also deficient in other cell populations of the inflammatory-immune system, we show that lymphocytes are involved, possibly by conditioning the behavior of monocytes/macrophages. However, contrary to our expectation, E2 increases the macrophage production of pro-inflammatory cytokines such as IL-12 and INF-γ and decreases the production of IL-10. The relationships between development of this pro-inflammatory profile and the atheroprotective effect, together with the respective roles of estrogen receptor α (ERα) and ERβ as mediators of these effects, are under current investigation.

**Conclusion:** The apoEKO mouse model enabled us to determine the target cell populations which mediate the atheroprotective effect of E2.

**The role of the estrogen receptor-β for the vascular effects of estrogen**

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The recent discovery of a second estrogen receptor, ERβ, has radically changed our view on how estrogens act. It appears that ERβ in many contexts counteracts ERα so that the two receptors can be said to have a yin/yang relationship. Interestingly, this ERα controlling activity of ERβ seems to be exerted not only by ERα but also by at least one variant form of ERα, namely ERαβ. This ERβ isoform has a different last exon than wild-type ERα making it incapable of binding steroid but still able to heterodimerize with ERα thereby somehow inactivating this receptor. These fundamental properties of ERα and its isoform ERαβ are of vital physiological importance as judged from some phenotypical characteristics of ERβ (−/−) mice, namely hyperproliferative tendencies in prostate as well as uterus. Indeed, ERβ seems to exert antiproliferative actions in these tissues. In other contexts ERβ may mediate other estrogenic effects than ERα, such as in the immune system and bone, where we also use ERβ (−/−) as well as ERα (−/−) mice to dissect ERβ and ERα controlled signal transduction pathways. Also, it turns out that the issue of estrogen receptors in breast needs to be thoroughly revisited in view of these new concepts. Finally, ERα/ERβ mediated signal transduction may be involved in some of the estrogenic effects on vessels.

**Estrogen receptor-β expression in male coronary arteries**

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**Objective:** The purpose of this study was to examine the expression of the novel estrogen receptor beta (ERβ) in normal and diseased human and porcine coronary arteries in both sexes.

**Methods:** ERβ mRNA and protein expression was assessed using reverse transcription PCR and immunocytochemistry, respectively. Pairs of normal (internal mammary artery) and diseased (coronary endarterectomy specimens) human vascular tissues from patients undergoing coronary artery bypass surgery (5 males, 1 female), as well as porcine coronary arteries (n = 24 pigs) subjected to balloon injury were studied.

**Results:** There was no difference in the abundance of ERβ mRNA expression amongst the pairs of normal and diseased human arteries. ERβ protein was immunodetected in endothelial cells, smooth muscle cells and adventitial cells of normal human and porcine coronary arteries. For 14 days post-balloon injury, there was transient over-expression of ERβ in porcine coronary arteries, despite very low levels of circulating estradiol (<100 pg/mL). Human coronary arteries with complex atherosclerotic lesions, also expressed ERβ protein, however, at low abundance. Overall, there was no apparent gender difference in ERβ expression. To extend these findings we tested the ability of smooth muscle cells from male and female pigs to respond to estradiol in vitro. Both populations of cells responded to estradiol administration by showing decreased levels of proliferation, as assessed by tritiated thymidine incorporation.

**Conclusion:** ERβ mRNA and protein are expressed in normal and disease arteries. The abundance of ERβ expression does not appear to be sex-related, and smooth muscle cells of both males and females are responsive to estradiol.

Taken together, these data suggest that strategies to develop estrogen therapies should be considered for, not only female but also male populations that are at risk for coronary artery disease.

**Free plasma testosterone and estradiol and the extent of coronary artery disease in males**

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**Objective:** Male sex may be an important risk factor of coronary artery disease. It has been suggested that sexual hormones may influence the development and progression of coronary artery disease (CAD). The objective of the present study was to investigate the relationship of free plasma testosterone, estradiol and coronary atherosclerosis.

**Methods:** 237 consecutive male patients who underwent elective coronary angiography were included. Exclusion criteria were previous angioplasty, coronary artery bypass grafting or diabetes mellitus in the history. Blood samples were taken in a fasting state prior to the procedure. The extent of coronary atherosclerosis was determined by the number of affected vessels (vessel score) (stenosis > 50% in a vessel area) and the number of stenoses > 50% (stenosis score) (0 = 0; 1 = 1-2; 2 = 3-4; 3 > 4).

**Results:** 49 (21%) patients had one vessel disease, 37 (16%) two vessel disease, 50 (21%) three vessel disease and 101 (42%) showed normal coronary angiograms. Free plasma testosterone significantly differed between the groups with lowest values in patients with severe CAD (vessel score p < 0.03; stenosis score p < 0.04; ANOVA). No significant differences were observed regarding estradiol (p = 0.69; p = 0.73).

![Mean values of free testosterone (pg/ml) depending on the different atherosclerosis scores](image-url)

**Conclusions:** Our results indicate that the extent of angiographically documented atherosclerotic lesions defined by two different scores is significantly associated with free plasma testosterone levels. Estradiol, however, had no influence on the atherosclerotic process in this cohort. In conclusion, low plasma testosterone levels might be an additional risk factor in the development and extent of CAD.

**Sexual steroids upregulate thrombin receptor expression in cultured vascular smooth muscle cells: Role of the glucocorticoid receptor**

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**Objective:** Both the estrogen and the progesteron component of oral contraceptives are thought to be involved in the development of thromboembolic diseases in women. This study examines whether sexual steroids affect the expression of thrombin receptors which mediate numerous proinflammatory responses in vascular smooth muscle cells (SMC).

**Methods:** Experiments were performed with confluent cultures of serum-deprived rat aortic SMCs. Thrombin receptor expression in SMC was assessed by Northern blot analysis and thrombin receptor function by the thrombin-induced release of 6-keto-prostaglandin F1α.

**Results:** 3-keto-desogestrel time-dependently increased the steady state level of thrombin receptor mRNA after a delay of 4 h and thrombin
receptor mRNA levels remained elevated for at least 48 h. Increased thrombin receptor mRNA levels were also obtained with medroxyprogesterone acetate (MPA), gestodene and progesterone and with the glucocorticoid dexamethasone, whereas levonorgestrel, norethisterone, norgestimate and 17 alpha-ethyl-norestradiol had no effect. Increased thrombin receptor mRNA levels were associated with a significant greater thrombin-induced release of 6-keto-prostaglandin F1alpha. The stimulatory effect of MPA and dexamethasone was reduced by the glucocorticoid and progesterone receptor antagonist RU 38486.

**Conclusions:** These findings indicate that several progestogens increase the expression of thrombin receptors in cultured vascular SMCs through activation of the glucocorticoid and/or progesterone receptor whereas estrogen were inactive. The upregulation of thrombin receptors in the vascular wall may represent a mechanism which contributes to the increased risk of vascular diseases in users of oral contraceptives.

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**WeW2:25**  
**Stress and coronary disease in Swedish women**

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Coronary heart disease (CHD) is almost as common in women as in men. Our knowledge about risk factors in women is surprisingly scarce. This is particularly true for psychosocial factors.

The Stockholm Female Coronary Risk Factor Study (Fem Cor Risk) was designed to study both standard and psychosocial risk factors, with an emphasis on mechanisms which mediate psychosocial influences on the heart. It was designed as a population based case control study including all female cases of acute events of CHD (acute myocardial infarction and unstable angina pectoris) who were 65 years or under, who were hospitalised in the greater Stockholm area during a three year period. For each case an age matched control woman was obtained from the City Census Register. Around 600 women were examined for standard and psychosocial risk factors by means of questionnaires, interviews, laboratory mental stress testing, blood samples, cardiological examination, exercise stress testing, and (in a subsample) quantitative coronary angiography. All but two women were employed, and only two house wives were found. All women were followed for five years. Among clinical factors, a history of diabetes and dyslipidemia were the strongest predictors of poor outcome. Of psychosocial factors, perceived mental stress from family life, but not stress from working life, was a strong predictor of risk. In addition, depressive symptoms, social isolation, and low socio-economic status (SES) further increased the risk in Stockholm women. Furthermore, low SES and social isolation were strongly associated with the Metabolic Syndrome, suggesting a major pathway of psychosocial influences on CHD in women.

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**WeW3:25**  
**Social stress, behavioral mechanisms, and atherosclerosis**

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**Objective:** To review mechanisms through which social stress influences progression of atherosclerosis.

**Methods:** Research review of mechanisms through which acute psychological stress promotes atherosclerosis. Acute vasoconstriction begins to be evident in the early, subclinical stages of atherosclerotic disease. Chronic stress ac-
WeW4:25  Trigginger of acute coronary syndrome at work in people free of coronary heart disease. Final results of a case-crossover Israeli experience

N. Lipovetsky, H. Hod, A. Rot, Y. Kishon, Sh. Sclarovsky, M.S. Green. Department of Epidemiology and Preventive Medicine, Sackler Faculty of Medicine, Tel-Aviv University, Israel

Objective: To study extreme episodes at work as triggers for the onset of an acute coronary syndrome (ACS).

Methods: A case-crossover study design was used where each patient serves as his/her own control. We interviewed 209 patients (194 men and 15 women) an average of two days after an acute ischaemic event that occurred at work. Emotional events were assessed by the positive and negative affect scale questionnaire (PANAS), comprising two nine-item, three-level, mood scales. Physical exertion was measured by an eight-item metabolic equivalents (MET) scale. Anger was measured by the Onset Anger Scale. Intellectual activity, overeating and lack of sleep were assessed by a standard 5 level scale. Patients were asked about the occurrence of these activities in the hours preceding the onset of ACS (hazard period) and for two types of control data: (1) the same period during the previous day, (2) the estimated usual frequency during the previous year.

Results: 24% of the study population report one trigger at least in the hour who preceded the symptoms of ACS. The odds ratio for an ACS in the first hour after the exposure to a potential trigger compared to the same period in the day before the ACS was 3.5 (95% CI 0.7-16.8) for positive emotions, 14 (95% CI 1.8-106.5) for negative emotions, 14 (95% CI 1.6-30.8) for physical exertion, 9 (95% CI 1.1-71) for anger, 2.5 (95% CI 0.5-12.9) for intellectual activity, 7 (95% CI 0.75-65.8) for overeating and 1.85 (95% CI 0.97-3.56) for lack of sleep. By conditional logistic regression negative emotions and physical exertion were the only two significant triggers for ACS.

Conclusions: Episodes of extreme negative emotions and heavy physical exertion at work can trigger the onset of an ACS. Possible preventive strategies in the workplace should be explored.

WeW6:25  Socioeconomic status and superoxide dismutase levels in a Spanish female population


Objective: To explore whether levels of the antioxidant scavenger enzyme superoxide dismutase were associated with socioeconomic and educational status in a female Spanish population.

Methods: Cross-sectional study in 434 Spanish women aged 18 to 60 years. Superoxide dismutase in erythrocytes (E-SOD) activity levels were measured as described previously (Covas M.I. et al. Clin Chem 1997; 43: 562-8). Socioeconomic status was assessed on the basis of occupation. Occupation was recorded into five categories based on the British Classification of occupations. Educational level was classified into five categories from "primary school" to "tertiary education". In a subsample of 150 women lipid peroxidation was assessed by the thiobarbituric acid reactive substances (TBARS) method (Vasanak T. et al. Clin Chim Acta 1995; 234: 63-9).

Results: In bivariate analysis a negative association was found between TBARS and E-SOD (r = -0.317, p = 0.001). TBARS were directly related to the socioeconomic status (r = 0.288, P = 0.002) and educational status (r = 0.295, P = 0.001). Multiple regression analysis showed that higher socioeconomic (P = 0.005) and educational status (P = 0.003) were associated with low E-SOD activity levels after adjustment for age, body mass index, tobacco consumption, and physical activity.

Conclusions: Socioeconomic status appear as a determinant of E-SOD activity levels. From our results a high-ranking position in work is a factor which could promote oxidative stress in our female population.

W:26 REGULATION OF ENDOTHELIAL FUNCTION

WeW1:26  Phosphorylation of NO synthase. Impact on endothelial function

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The endothelial NO synthase (eNOS) was, until recently, thought to be regulated solely by changes in the intracellular Ca2+ concentration ([Ca2+]i). It is now clear that eNOS can be phosphorylated on serine, threonine and tyrosine residues and that a battery of protein kinases is involved in the regulation of eNOS activity. In both, the presence and the absence of an increase in [Ca2+]i.

Tyrosine kinases modulate endothelial NO production, especially in response to mechanical stimuli and growth factors. However, the tyrosine kinases responsible and the residues targeted remain to be identified. Of the potential phosphorylation sites within the eNOS sequence, most is known about the CaM-binding domain (CBD) and the carboxy terminal region of the reducease domain. Phosphorylation of serine by PKC, or threonine by the AMP-activated protein kinase (AMPK) within the CBD decreases eNOS activity, presumably by interfering with the binding of Ca2+/CaM.

Phosphorylation of the second eNOS regulatory site (Ser1177) by AMPK, CaM kinase II, or Akt/PKB enhances NO production. AMPK and CaM kinase II phosphorylate eNOS only following an increase in [Ca2+]i, but Akt can phosphorylate eNOS in the absence of Ca2+. Indeed, the physiologically most important endothelial stimulus, fluid shear stress, known to elicit the activation of eNOS in an apparently Ca2+-independent manner, achieves this effect by activating phosphorylidyinositol 3-kinase which subsequently activates Akt. The phosphorylation of Ser1177 by Akt is sufficient to enhance enzyme activity at sub-physiological levels of [Ca2+]. This Akt-induced activation of eNOS, which results in the maintained production of NO, is implicated not only in the adjustment of local vascular tone, but also in the regulation of gene expression, angiogenesis and endothelial cell migration.

WeW2:26  Regulation of endothelial function by cardiovascular risk factors and race

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Endothelial dysfunction is an important early event in atherosclerosis, preceding discrete plaque formation and also determining vascular reactivity at the sites of established stenosis. The influence of various risk factors on endothelial function, particularly
release of nitric oxide by the vessel wall, is now being explored, with potential diagnostic and therapeutic implications. We have recently described the effects of cholesterol, active and passive cigarette smoking, diabetes mellitus and hyperhomocysteinemia amongst others on endothelial function, and also elucidated some racial differences in the susceptibility of the vessel wall to these various risk factors, in different populations. For example, Chinese subjects appear less prone to the deleterious effects of aging and smoking on endothelial function, compared to white populations.

In vivo endothelial function testing is useful for the assessment of preventive strategies, when applied to serial studies. Strategies such as L-arginine therapy, antioxidants, ACE inhibition and folic therapy (for high homocysteine levels) have been assessed in clinical trials, and these results will be reviewed.

**WeW3:26**

**Differential regulation of ET-1 and eNOS expression in human vessels exposed to complex mechanical forces**

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**Objectives:** To investigate how combined shear and pressure modulate the opposing vascular factors, NO and ET-1 in intact conduit vessels.

**Methods:** In a novel computerized perfusion system, human umbilical veins were perfused with high vs low shear stress (25 vs <4 dyn/cm²) at identical intraluminal pressure (20 mmHg) or high vs low pressure (40 vs 20 mmHg) at identical shear stress (10 dyn/cm²) for 1.5, 3 or 6 hours. Endothelial eNOS and ET-1 gene expressions were quantified by real-time RT-PCR with b-actin as endogenous control. Semi-quantification of the protein expression by immunohistochemistry. The vascular NO producing capacity was measured with an amperometric NO probe. The vascactivity was measured continuously as vascular resistance (pressure drop/flow) during the perfusion experiments.

**Results:** After a transient slight 28% down-regulation, ET-1 gene expression returned to base-line level after 6 h high shear perfusion, while high pressure up-regulated ET-1 expression by 111 ± 54% after 6 h (p = 0.02). The temporal regulation patterns of eNOS gene by shear and pressure was significantly different (p < 0.05). High shear up-regulated eNOS gene expression gradually by 114 ± 56% (p = 0.035) over 6 h, while high pressure induced only a transient up-regulation of eNOS by 205 ± 90% (p < 0.05) after 3 h perfusion. These transcriptional events were accompanied by increased protein expressions, enhanced enzymatic activity and synchronous changes in vascular tone.

**Conclusions:** Vascular production of NO and ET-1 is differentially regulated by shear and pressure and the balance between these two pathways appears to be important for the regulation of the vascular tone.

**WeW4:26**

**Lyrophosphatidylcholine induces early growth response factor-1 and activates the core promoter of PDGF-A chain in cultured vascular endothelial cells**

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**Objectives:** Lyrophosphatidylcholine (Lyso-PC), a bioactive phospholipid component increased in atherogenic oxidized low density lipoprotein, has been shown to induce various genes relevant to atherogenesis including PDGF-A chain. We therefore sought to define whether lyso-PC can induce expression of Egr-1, an inducible transcription factor which regulates gene expression including PDGF-A chain in cultured vascular endothelial cells.

**Methods & Results:** Northern blot analyses showed that lyso-PC (10-20 μmol/L) transiently (30 minutes-1 hour) induced expression of Egr-1 mRNA. Induced expression of Egr-1 mRNA by lyso-PC was associated with increased amounts of Egr-1 protein in nuclei as shown by western blot analyses. Transient transfection of the oligonucleotide corresponding to the proximal core promoter of the PDGF-A chain (oligo A) linked to the luciferase reporter gene revealed that lyso-PC activates the core promoter of the PDGF-A chain by 5-fold. Mutation in the nucleotide sequence of oligo A abolished the lyso-PC-induced increases in luciferase activities. Electricphoretic mobility shift assay using radiolabeled oligo A showed a lyso-PC-inducible shift band, which was suppressed by excess amounts of unlabeled oligo A and an anti-Egr-1 antibody. Induced expression of Egr-1 by lyso-PC appeared to precede the lyso-PC-induced expression of the PDGF-A chain mRNA by Northern blot analysis.

**Conclusion:** Induced expression of Egr-1 by lyso-PC may be a key regulator in the transcription of various endothelial genes relevant to atherogenesis.
Wednesday June 28, 2000: How-to Sessions Abstracts

**H:4 HEALTH ECONOMY**

WeH1:4 Prospective study of pravastatin in the elderly at risk: Vascular risk profile of the prospective recruits

James Shepherd, Michael Murphy, Gerard J. Blauw. On behalf of the PROSPER Study Group; 1 University of Glasgow, Scotland; 2 University College Cork, Ireland, UK; 3 Leiden University Medical Center, The Netherlands

**Objective:** Arterial disease is the leading cause of death and disability and a major contributor to cognitive decline and dementia in the elderly. The PROSPER study, a randomised, double-blind, placebo controlled trial is designed to test whether treatment with pravastatin will diminish or delay these events in a cohort of 70-82-year-old men and women with existing vascular disease or at significant risk of developing this condition. Patients in the PROSPER study include what is probably the largest female cohort ever enrolled in a statin trial. This study will contribute significantly to our understanding of cardiovascular disease in women - a population in which arterial disease previously has been under treated and under researched.

**Results:** The vascular risk factor profile of the 5804 study recruits is presented in the Table.

<table>
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<tr>
<th>Baseline risk factor profile in PROSPER</th>
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<tr>
<td><strong>Cohort</strong></td>
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<td>TOTAL</td>
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45% of the cohort had established vascular disease, 13% having had a myocardial infarction, 3.9% a stroke and 7.4% a history of transient ischaemic attacks. One quarter of the total cohort was being treated for angina pectoris and 62% for hypertension. By design, the baseline Mini-mental Score of the cohort was greater than 24 (mean value ± SD = 28.0 ± 1.5).

**Conclusion:** The expectation from the above risk factor profile is that there will be a rapid accrual of vascular events over the remaining life of the project which is scheduled to terminate in 2002.

WeH2:4 Statistical issues in evaluating the cost-effectiveness of lipid lowering therapy: Application to 4S

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Randomized clinical trials are increasingly used to evaluate the cost-effectiveness of pharmaceutical products. Due to sampling variability in the trials, there is uncertainty associated with these trial-based cost-effectiveness evaluations. Recently, alternative methods for assessing the statistical uncertainty associated with the estimated cost-effectiveness ratio have been developed.

In this presentation we briefly explore issues associated with estimating the cost-effectiveness ratio and describe alternative methods for constructing confidence intervals for the cost-effectiveness ratio. We will also show how to develop a cost-effectiveness acceptability curve to aid the decision makers interpret the results.

These issues will be developed using data from the Scandinavian Simvastatin Survival Study (4S) to assess the cost-effectiveness of lipid lowering therapy in secondary prevention patients with elevated cholesterol.

**H:5 GENETIC SCREENING**

WeH1:5 Identification of susceptibility genes for cardiovascular diseases: Which strategy to develop?

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Whole-genome association studies have recently been proposed as an efficient approach for the identification of susceptibility genes underlying common diseases. There are two different strategies: direct or indirect. The direct strategy is to characterize all common variants of human genes and to test directly their association with disease. The indirect strategy is to develop very dense maps of neutral single nucleotide polymorphisms (SNPs) and to detect susceptibility genes through linkage disequilibrium with unidentified functional variants. The choice of a strategy is dependent on a number of critical elements, including the number of functional polymorphisms within a gene, the combination of their effects, their allele frequencies and the extent of linkage disequilibrium among them. We performed an extensive molecular screening of the coding and flanking regions of 36 candidate genes for cardiovascular diseases. All polymorphisms identified by this screening were further genotyped in 750 subjects of European descent. This study provides new insights into the type and amount of DNA sequence variation that might be expected in human genes, as well as on the extent of linkage disequilibrium within candidate regions.

The results suggest that genome-wide SNPs maps might be efficient for identifying new susceptibility genes, provided they are sufficiently dense. However, a number of important genes will be missed by this approach. On the other hand, the relatively large number of functional polymorphisms within coding and regulatory regions of candidate genes raises the possibility that several of them might functional and that the pattern of genotype-phenotype association might be more complex than initially envisaged, as actually observed in some well-characterized genes.

WeH2:5 Complex problems with a simple quantitative trait

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Lipoprotein (a) (Lp(a)) is a quantitative trait in human plasma which is associated with atherothrombotic disease. The covalent Lp(a) complex is assembled in human plasma from LDL and the plasminogen-related apolipoprotein (a). The enormous within and between population variation in Lp(a) plasma concentrations is controlled by variation at the apo(a) gene locus. Three types of variation related to apo (a) levels have been detected in the apo (a) gene i) a variable number of identical 5.6 kb tandem repeats which contain the exons coding for the kringle IV-2 domain in apo (a). This variation is causal as it has a direct effect on apo (a) secretion from liver cells ii) Polymorphisms in the gene including a PNR in the promoter which are in alleleic association with causal mutations in some populations. Identification of the linkage disequilibrium has allowed to identify such a mutation and iii) Population specific mutations in coding sequences and splice-site mutations (including null alleles) which directly effect Lp(a) levels. Such effects may however be masked by linkage disequilbria. Together our data indicate that a combination of KIV-2 repeat variation, point mutations, and intragenic linkage disequilibria determine some or all of the wide range of Lp(a) concentrations in the general population. In addition rare genetic conditions e.g. FH may effect both Lp(a) levels and Lp(a) level variability.
Wednesday June 28, 2000: Poster Abstracts

**P:W17 GENETIC ANIMAL MODELS OF LIPOPROTEIN METABOLISM**

**WeP1:W17 New HMG-CoA reductase inhibitor ZD4522 lowers plasma lipids and VLDL production in APOE3-Leiden transgenic mice**

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**Objective:** To study the efficacy and underlying mechanisms of the lipid-lowering effect of ZD4522 (rosuvastatin) in an animal model of hyperlipidaemia.

**Methods:** Female APOE3-Leiden transgenic mice, were fed a Western-type diet to induce a "human-like" lipoprotein profile with total plasma cholesterol levels of approximately 12 mmol/L. After 2 weeks, ZD4522 was added to the diet at levels of 0.005%, 0.0025% and 0.00125% by weight with no addition or 0.05% lovastatin as the controls. After 4 weeks of treatment, blood samples were taken for lipid analyses. Intestinal cholesterol absorption and hepatic VLDL triglyceride and VLDL apoB production were then measured in vivo. After sacrifice, liver samples were taken for quantification of mRNAs.

**Results:** ZD4522 dose-dependently lowered plasma cholesterol and triglyceride (39% and 42%, respectively, at 0.005%) compared with 19% and 12%, respectively, at the 10-fold higher level of lovastatin. These decreases with ZD4522 were mainly confined to the VLDL fraction. Administration of ZD4522 tended to lower intestinal cholesterol absorption but without statistical significance. VLDL-triglyceride and VLDL-apoB production were strongly reduced after ZD4522 treatment (50% and 43%, respectively, at 0.005%), whereas there was no significant effect of lovastatin. Surprisingly, using a Western-type diet, both LDL receptor and HMG-CoA synthesize mRNA levels were dose-dependently decreased by ZD4522 treatment (50% to 60%, respectively, at 0.005%). By contrast, on chow diet, ZD4522 treatment resulted in an increase in the level of these mRNAs (1.5 and 3 fold, respectively, at 0.005%).

**Conclusions:** In APOE3-Leiden transgenic mice, plasma cholesterol and triglycerides are effectively lowered by ZD4522 primarily due to a reduction in VLDL production rate.

**WeP2:W17 Distinct patterns of apo B lipoproteins defined by apo CII or E in hyperlipidemias**

**H. Campos, D. Perlov, C. Khoo, F. Sacks. Harvard School of Public Health, Boston, MA, USA**

**Objective:** To study the relationship between apolipoprotein (apo) CII and apoE VLDL, IDL and LDL containing lipoproteins in hyperlipidemias.

**Methods:** 10 hypercholesterolemic (HC) and 13 hypertriglyceridemic (HTG) were compared to 12 normolipidemias (N). 16 apoB lipoprotein particle types were separated by first using anti-apoE and anti-apoCIII immunoaffinity chromatography in sequence and then ultracentrifugation (light VLDL, dense VLDL, IDL and LDL, with apoCIII with or without apoE (C+E, C+E–), or without apoCIII with or without apoE (C–E, C–E–)).

**Results:** HTG had 2-fold higher concentrations of C+E+ and C+E–. In HTG, most of the C+ particles were in VLDL (75-80%), while in N and HC were in IDL and LDL. The VLDL C+E+ and C+E– were the most triglyceridich and cholesterol-rich of the particles, and had 1.5-2-fold higher lipid content in HTG than in other patients. HTG had similar concentration of E–C– particles to N, while in E–C– particles were distributed more to light VLDL than to denser fractions as in N and HC. HC compared to the others had 2-fold higher concentrations of C–E– in each density fraction. C–E+ particles were the least prevalent, comprising <10% of particles in all groups.

**Conclusion:** Thus, HTG are distinguished from N and HC by increased apoCIII-containing light VLDL (C+E+, C+E–) that are rich in cholesterol and triglyceride. In contrast, HC are characterized by high concentrations of E–C– particles distributed to denser lipoproteins, particularly LDL; the overall particle type distribution is similar to that of N. The presence or absence of apoE on apoB lipoproteins does not discriminate the lipoprotein pattern among the patients up to 10 as well as present in the intima, 2) regular fatty streak: more than 10 foam cells present in the intima, 3) mild plaque: foam cells in the intima with a fibrotic cap, 4) moderate plaque: media is affected without loss of architecture, 5) severe plaque: media is severely affected, elastic fibers are broken, cholesterol clefts, calcification and necrosis are frequently observed. A weight factor was assigned to each category based on size and composition of the lesion. Degree of atherosclerosis in individual mice was determined by multiplying the number of each lesion with the accompanying weight factor divided by the number of sections screened. This method was used in the quantification of atherosclerosis in a large mapping study concerning identification of new quantitative trait loci in the lipid metabolism and susceptibility to atherosclerosis. We compared this new classification system in female APOE3Leiden and (129 X 129 X APOE3Leiden) backcross mice and in ApoE–/– mice with the common area measurements of the plaque.

**Results and Conclusion:** This classification system is in agreement with the common method of area measurement. Interestingly, the (129 X 129 X APOE3Leiden) backcross mice are less susceptible to atherosclerosis compared to the APOE3Leiden strain while the cholesterol levels are similar. This new classification system based on size and composition of individual lesions is a very useful, fast and convenient method to quantify and qualify atherosclerosis in mice.

**WeP3:W17 Asparagine inhibition of aortic acyl-coenzyme A: Cholesterol acyl transferase detected in vivo with pyrenionedecarboxylic acid in LDL-receptor (–/–) mice**


**Objective:** Detect direct inhibition of ACAT in atherosclerotic lesions by an orally administered ACAT inhibitor.

**Methods:** LDL-receptor (–/–) mice with atherosclerotic lesions due to 90 days feeding on a cholesterol-rich diet were orally gavaged with 0, 10 or 30 mg/kg of the ACAT inhibitor asparagine (AVM) in a carboxymethyl-cellulose/Tween 20 vehicle. Four hours later, a bolus of the metabolically active and fluorescent fatty acid analog 9-(1-pyrenyl) nonanoyc acid (PNA), complexed to albumin, was injected into the tail veins. Tissues were collected 1 hour after PNA injection and lipids extracted for analysis of cholesteryl pyrennononoic acid (PNA) formation by HPLC.

**Results:** C-PNA was detected in the liver, small intestine, spleen, adrenal gland, aorta and plasma. C-PNA was decreased in tissues from mice treated with AVM. Significant decreases occurred in the liver (~82%) at 10 mg/kg AVM and in the intestine (~75%), liver (~87%), plasma (~94%), and aorta (~68%) at 30 mg/kg AVM. AVM was detected in the plasma at 5.1 and 22.5 μg/ml in the low and high dose groups, respectively. The decrease in aortic C-PNA content in AVM-treated mice was not due to the loss of plasma C-PNA content since mice that were injected (IP) with brefeldin A (40 mg/kg) one hour before PNA/albumin injection to block lipoprotein secretion from the liver and intestine during PNA exposure, exhibited a ~94% decrease in plasma levels of C-PNA relative to control mice without significant changes in the C-PNA contents of the other tissues, including the aorta.

**Conclusions:** Orally administered AVM directly inhibits cholesteryl ester synthesis by ACAT in atherosclerotic lesions.

**WeP4:W17 Alternative method for quantification of atherosclerosis**

**M.J.J. Gibbels 1, C.J.A. Moen 1, P.J.J. van Gorp 1, R.R. Frants 1, L.M. Havekes 2, M.H. Hofker 1, 1Leiden University Medical Center; 2TNO-FG, Leiden, The Netherlands**

**Objective:** Evaluation of a semi-quantitative method for measuring the susceptibility to atherosclerosis based on size and plaque composition.

**Methods:** We setup a mouse classification system for atherosclerosis. Arterial lesions were classified into five categories: 1) early fatty streak: per section up to 10 foam cells present in the intima, 2) regular fatty streak: more than 10 foam cells present in the intima, 3) mild plaque: foam cells in the intima with a fibrotic cap, 4) moderate plaque: media is affected without loss of architecture, 5) severe plaque: media is severely affected, elastic fibers are broken, cholesterol clefts, calcification and necrosis are frequently observed. A weight factor was assigned to each category based on size and composition of the lesion. Degree of atherosclerosis in individual mice was determined by multiplying the number of each lesion with the accompanying weight factor divided by the number of sections screened. This method was used in the quantification of atherosclerosis in a large mapping study concerning identification of new quantitative trait loci involved in lipid metabolism and susceptibility to atherosclerosis. We compared this new classification system in female APOE3Leiden and (129 X 129 X APOE3Leiden) backcross mice and in ApoE–/– mice with the common area measurements of the plaque.
**WeP5:W17** The effect of scavenger receptor A1 (SRA1) on arteriosclerosis


**Objectives:** Arteriosclerosis is a multifactorial condition in which macrophages play a role during the development of plaques. To get more insight in the role of the macrophage scavenger receptor A1 in plaque formation bone marrow transfer experiments were performed using bone marrow from SRA1 knockout mice and APOE3/Leiden mice as recipient.

**Methods:** One of the early stages in the development of arteriosclerosis is the uptake of ox-LDL by macrophages through the scavenger receptor. The scavenger receptor A plays a role in foam cell formation, cellular adhesion and host defense. There are two isoforms type 1 and 2 and the differences between type 1 and 2 lie in the cystein rich domain of type 1 scavenger receptor. We generated mice deficient in the SRA1 gene expression by inserting a neomycin resistance gene in exon 10 of the SRA1 gene. APOE3/Leiden mice which are highly susceptible for arteriosclerosis after high fat diet were used as recipient for bone marrow from either SRA1 +/− or wildtype mice. After reconstitution the mice were fed a high fat diet to induce arteriosclerotic lesions. They were sacrificed after two months and heart sections and serum samples were analyzed.

**Results:** SRA1 knockout mice were generated and the absence of SRA1 mRNA was confirmed by RT-PCR. The in vitro association and degradation of both acetylated and oxidized LDL by macrophages was not changed in SRA1 knockout mice compared to wildtype. We are currently analyzing the hearts and serum samples of the SRA1 bone marrow transfers in APOE3/Leiden mice to determine the extent of arteriosclerotic lesion formation and differences in triglycerides.

**WeP6:W17** Liver-specific overexpression of a constitutive active estrogen receptor alpha (ERα)-variant induces hypolipidemia in male APOE3-leiden mice

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Estrogens protect against cardiovascular disease via effects on the vascular wall and liver. We are investigating the role of estrogens in the liver by manipulating ERα-signalling. This is achieved by expressing the hERα wildtype (wt) and a constitutive active hERα mutant (mt) in the liver of hyperlipidemic mouse models using adenovirus-mediated gene transfer.

To assay the activity of the hERα adeno-noviral vectors in vitro, mouse hepatoctye-like (mHA1T3) cells were transiently co-transfected with an estrogen responsive luciferase reporter plasmid. Cells were treated with estradiol (E2) or antiestrogen (ICI 164,384). The activity of wt with E2 was blocked using the antiestrogen compound. The mt receptor was able to induce transcription independently of E2 and its activity was of the same magnitude as that of wt with E2.

To investigate the effect of estrogens on lipid metabolism, the hERα adenoviral vectors were injected into APOE3-Leiden mice fed a high fat diet. The total plasma cholesterol in the female mice was not lowered when either the wt or mt ER was expressed. Likewise, no difference was observed when male mice expressed the wt ER. Surprisingly, in male mice expressing the mt, plasma cholesterol levels were decreased significantly on day 5 as compared to pre-injection values (from 7.4 ± 0.3 to 4.9 ± 0.2 mmol/l; p < 0.0001). We are currently in the process of analyzing the expression of genes involved in lipid metabolism to define the mechanism by which estrogen exerts this hypolipidemic effect in APOE3-Leiden mice.

**WeP7:W17** Transgenic rabbits expressing human lipoprotein lipase

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**Objective:** To study the functions of lipoprotein lipase (LPL) in lipid and lipoprotein metabolism and its relationship with atherosclerosis, we generated two kinds of transgenic rabbits which express the human LPL under the control of system specific and macrophage-specific promoters.

**Methods:** A total of 5159 zygotes from Japanese White rabbits was micromanipulated with two constructs: Cal-HPLP construct containing the chicken β-actin promoter, the human LPL cDNA and rabbit β-globin sequence with poly(A) signals and pA1-HLPL construct containing the human macrophage scavenger receptor promoter and the human LPL cDNA. Of the 200 pups, 3 transgenic rabbits (L01, L04, L17) with Cal-HLPL construct and 1 transgenic rabbit (L05) with pA1-HLPL construct were identified by Southern blot analysis.

**Results:** (1) Northern blot analysis showed that Cal-HLPL transgenic rabbits had a multiple tissue expression of human LPL transgene, including aorta, stomach, brain, intestine, heart, lung, spleen, kidney, adrenal, ovary, muscle, bone marrow whereas endogenous rabbit LPL was mainly expressed in adipose, heart, and muscle. In post-heparinized plasma, human LPL protein levels analyzed by ELISA were 395 (L01), 157 (L04) and 654 (L17) ng/ml, which is comparable to human values (135-321 ng/ml). Expression of human LPL in high expressor transgenic rabbit (L17) resulted in a marked reduction of plasma lipids: ~2-fold in total cholesterol, ~5-fold in triglycerides, ~2-fold in phospholipids due to decreased VLDL contents. (2) In pA1-HLPL transgenic rabbit (L05), there was an increased expression of LPL in peritoneal thioglycollate-elicited macrophages and alveolar macrophages compared to control rabbits. (3) Both lines of transgenic rabbits were established and their susceptibility to atherosclerosis is currently under investigation.

**Conclusion:** Transgenic rabbits expressing human LPL with different tissue specificity have been generated. They will be valuable to investigate systemic increased LPL and macrophage-derived LPL activity in atherosclerosis.

**WeP8:W17** Role of lipoprotein lipase and apolipoprotein E in macrophages in modulating VLDL degradation and triglyceride accumulation

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Lipoprotein lipase (LPL) is bound to heparan sulfate proteoglycans (HSPG) on the luminal surface of endothelial cells and is the key enzyme in the hydrolysis of plasma triglycerides (TG). Apolipoprotein E (ApoE) is thought to stabilize the lipoprotein-LPL-HSPG complex, thereby facilitating the lipolysis of triglyceride-rich lipoproteins. To investigate the role of endogenously expressed LPL and ApoE in macrophages we isolated peritoneal macrophages from LPL/ApoE double-knockout mice (LOE0) and compared them to macrophages from wildtype mice (L2E2) and single knockout mice (LOE2, L2E0). The TG accumulation in macrophages was quantitated after a 6-hour exposure to VLDL. Compared to L2E2 macrophages the TG accumulation of L2E0, LOE2 and LOE0 cells was decreased by 50%, 84% and 87%, respectively. Inhibition of lipase activity with Orlistat decreased TG accumulation in L2E2 and L2E0 macrophages by 74% and 57% but not in LOE2 and LOE0 macrophages. Degradation of 125I-labeled VLDL in L2E2, LOE2 and LOE0 macrophages was decreased by 45%, 38% and 74% compared to L2E2 macrophages, respectively. Orlistat had no effect on VLDL degradation. Our results support the hypothesis that ApoE stimulates LPL-mediated triglyceride lipolysis possibly by enhancing the association of the lipoprotein-LPL-HSPG complex. The effects of LPL and ApoE on VLDL degradation are additive and do not depend on the enzymatic activity of LPL.

**WeP8:W17** Lipid ester hydrolyzing enzymes in retinoid (vitamin A) metabolism


**Objective:** To study the role of lipid ester hydrolyases in the absorption and tissue uptake of dietary vitamin A (retinoids).

**Methods:** Absorption studies using 3H-retinyl ester (RE)/3H-cholesterol administered by stomach feeding) were carried out in wild type (WT) and carboxyl ester lipase (CEL) deficient mice. Tissue uptake studies were performed in WT, CEL-deficient and lipoprotein lipase (LPL) deficient mice (using rat chylomicrons (CM) containing 3H-RE injected intravenously). Tissue enzyme activities were carried out using pancreati obtained from WT and CEL-deficient mice, and rats. Pancreatic homogenates (0.25 M sucrose/g tissue) were assayed under either CEL-assy conditions, or pancreatic triglyceride lipase (PTL) assay conditions using triglyceride (TG), cholesteryl ester or RE as a substrate. Soluble fractions of these homogenates were also chromatographed on a DEAE-column in order to separate PTL-related from CEL-related en...
zyme activities. Also, purified human PTL was assayed for hydrolyase activity using TG or RE as a substrate.

**Results:** Tissue uptake studies indicated that LPL modulates uptake of dietary CM-RE in heart and skeletal muscle. CEL-decificiency, however, affected neither tissue uptake nor intestinal absorption of RE. It appears that PTL is a major pancreatic RE-hydrolase in mice, as well as rats.

**Conclusions:** PTL plays a role in the intestinal absorption of RE, whereas LPL is involved in the uptake of CM-RE in muscle. Knowledge of factors regulating RE absorption and tissue uptake is important in view of the established effects of retinoid metabolites on the expression of genes involved in lipid metabolism and atherosclerosis.

**WeP10:W17** Apolipoprotein E Sendai (R145P): Its characterization and affect to lipoprotein glomerulopathy


**Objective:** Lipoprotein glomerulopathy (LPG) is a novel renal disease characterized by nephropathy, lipoprotein thrombi in the glomeruli, and increased concentration of plasma apolipoprotein E (apoE). Previously, we reported a novel apoE variant, apoE Sendai (R145P), which was identified from LPD patient. We analyzed a biological characterization of apoE Sendai and tried to make an animal model of LPG by adenoviral gene transfer.

**Methods:** Lipoprotein receptor-binding activities of the recombinant apoEls were determined in an in vitro competition assay. We constructed recombinant adenoviruses encoding each of apoE isoforms included apoE Sendai and injected to apoe deficient mice.

**Results:** ApoE Sendai had a low binding affinity to LDL and VLDL receptor compared with apoE3. Plasma TC levels and atherosclerosis area of aorta of mice with apoE expression dramatically decreased, respectively. In analysis of kidney, mice with apoE Sendai showed a lipid accumulation in capillary lumina which was very similar to lipoprotein thrombi of LPG.

**Conclusions:** ApoE Sendai showed poor affinity to lipoprotein receptors and it may cause of abnormal lipoprotein profile in LPG. In examination of gene transfer, apoE Sendai presented similar effects to apoE2 on lipoprotein metabolism and reduction of atherosclerosis. Only mice with apoE Sendai expression developed glomerulopathy as like as human LPG. It was concluded that apoE Sendai was closely associated with LPG.

**WeP11:W17** Increased renal catabolism of endogenous apo A-I in transgenic mice overexpressing human apo A-II

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**Objective:** Overexpression of human (h) apo A-I in mice resulted in a dose-dependent decrease of endogenous mouse (m) apo A-I in HDL and total plasma. We hypothesize that mapo A-I was displaced from HDL by the more hydrophobic hapo A-I in excess, and cleared rapidly in kidney.

**Methods:** Renal tissue from control and two lines of hAII-transgenic mice was studied by immunohistochemical/cocktail techniques using fluorescein and rhodamine probes to detect antibodies against mapo A-I, mapo A-II, hapo A-II, rat cubilin (a recently published apo A-I endocytic receptor) and rat megalin (its putative coreceptor).

**Results:** In transgenic mice, mapo A-I was abundantly present in the apical surface of proximal tubules, particularly in the first (S1) segment, whereas considerably less apo A-I was detected in the same location in control mice. Human apo A-II was also present in the same segment and was more abundant than mapo A-II. Furthermore, both mapo A-I and hapo A-II colocalized with cubilin and megalin.

**Conclusions:** The low plasma apo A-I of hAII-transgenic mice is probably due to increased apo A-I clearance in the kidney. Our results show for the first time, to our knowledge, that the kidney is also a site of apo A-II catabolism. The abundance of hapo A-II in the proximal tubule may result from increased HDL holoprotein uptake. Greater HDL uptake in the kidney would be consistent with the low plasma HDL content of hAII-transgenic mice.

**WeP12:W17** In vivo production of VLDL-apo-B and VLDL-triglycerides are not affected by the LDL receptor


**Objective:** In vitro, the absence of the LDL receptor (LDLr) in mouse hepatocytes leads to an increase in VLDL-apoB secretion [Twisk et al. (1998), Circulation 98, S-178, abst. 919]. Deletion of the LDL receptor in mice leads to severe accumulation of LDL in plasma. This rise in LDL is caused by impaired clearance, but also by increased production of LDL [Osmon et al. (1995), J. Clin. Invest. 95, 1124]. VLDL being the precursor for LDL, the observed increase in VLDL production by LDLr−/− hepatocytes may explain the higher LDL production rates in LDLr−/− mice. Therefore, we tested whether also in vivo hepatic VLDL production is increased in the absence of the LDL receptor.

**Methods:** In LDL receptor knockout mice and wild type controls the hepatic VLDL production rates were measured by the Triton WR1359 method. Accumulation of VLDL-triglycerides were followed in plasma, apoB production and secretion in liver as measured in the VLDL after injection of 33S-methylionine/cysteine prior to Triton administration.

**Results:** Although there was a trend towards a higher production in the knock-out mice, the production of VLDL in vivo was not significantly higher in LDL receptor knockout mice than in controls (triglycerides, 105 ± 14 and 119 ± 11 mmol h−1 kg body weight−1; apoB, 100% ± 31% and 125% ± 43% for wild type and LDLr−/−, respectively).

**Conclusions:** In contrast to the in vitro results, in vivo hepatic VLDL production in LDL receptor knockout mice was not significantly higher than in controls. Thus, the increased production rate of LDL in the knock-out mice must be caused by a larger proportion of the secreted VLDL being converted into LDL, rather than by an increased VLDL production rate.

**WeP13:W17** A 315-bp enhancer, located more than 55 KB 5′ of the apolipoprotein B gene, confers intestinal expression in transgenic mice

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**Objective:** To identify and characterize the intestinal regulatory elements of the human apoB gene (which is not expressed in mice).

**Methods and Results:** Using transgenic mice, we initially localized the apoB intestinal control region (ICR) to a 3-kb segment (−54 to −57 kb), DNasel hypersensitivity (DH) studies uncovered a DH site, within a 315-bp fragment at the 5′ end of the 3-kb segment, in apoB-expressing CaCo-2 cells but not in non-expressing HeLa cells. Transient transfection experiments with CaCo-2 and HepG2 cells indicated that the 315-bp fragment was an intestine-specific enhancer. Binding of the tissue-specific transcription factors HNF-3β, HNF-4, and C/EBPβ to the 315-bp enhancer was demonstrated in gel retardation experiments. Co-transfection experiments with expression plasmids for these factors demonstrated that they act synergistically and are responsible for the activity of the apoB intestinal enhancer (IE). The mouse apoB IE (located −40 to −83 kb 5′ of the structural gene) also exhibited enhancer activity in transient transfection assays with CaCo-2 cells. Its sequence was highly conserved in the binding sites for the key transcriptional activators. In transgenic mouse expression studies, the 315-bp enhancer conferred intestinal expression to the apoB transgenes.

**Conclusions:** Intestinal expression of the apoB gene requires a 315-bp enhancer situated over 55kb 5′ of the transcriptional start site. A chromatin loop model depicting the three-dimensional organization of the ICR has been developed.

**WeP14:W17** apoE-induced hypertriglyceridemia is modulated by deleting the carboxy-terminal region of the apoE molecule

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**Objective:** To dissect the domains of apoE required for cholesterol and triglyceride...
clearence in vivo, we have utilized adenovirus mediated gene transfer of wild type and mutated forms of apoE in apoE-deficient mice. A dose of 2 x 10^9 pfu of apoE-expressing adenovirus reduced cholesterol levels by up to 25% and resulted in severe hypertriglyceridemia, due to the accumulation of apoE-rich and triglyceride-rich lipoproteins in the VLDL region. In contrast, apoE truncated at position 202 (apoE-420), at a dose of 2 x 10^9 pfu or even at much a higher dose of 1 x 10^10 pfu, resulted in a 90% reduction in the cholesterol levels, without causing any increase in plasma triglyceride levels. Analysis of total RNA from the livers of the infected mice for apoE mRNA expression, showed comparable levels of apoE4 and apoE4-202 mRNA in vivo. These findings suggest that the amino-terminal 1-202 region of apoE contains the domains required for the association of apoE with lipoproteins and their subsequent clearance via hepatic receptors in vivo. Furthermore, the carboxy-terminal 203-299 residues of apoE contribute to the apoE-induced hypertriglyceridemia. Thus, in apoE deficient mice, the negative effect of apoE expression on plasma triglyceride levels can be dissociated from the positive effect on plasma cholesterol levels by deletion of a specific region of apoE.

P.W18 PROTEOLYSIS AND PLAQUE RUPTURE

P.WP1W18 | Enhanced expression of tissue transglutaminase and elastin in human atherosclerotic coronary artery. Potential role of TNF-alpha


Objective: Extracellular matrix (ECM) is an important determinant for atherosclerotic plaque instability. Elastin is the proteinase inhibitor and it contains a transglutaminase substrate domain which moiety is readily cross-linked to ECM by tissue transglutaminase (TG). The purpose of the present study is to examine the expression of TG and elastin in human coronary artery using autopsy samples. Furthermore, we investigated the potential role of cytokines in the expression of TG in cultured vascular smooth muscle cells (SMC).

Methods & Results: The expression of elastin and TG in human coronary arteries by immunohistochemistry was examined using autopsy samples. In non-atherosclerotic coronary arteries, the expression of elastin and TG was slightly detected in endothelium, medial SMC, fibroblast and the ECM around these cells. In atherosclerotic coronary arteries, the expression of elastin and TG was increased. Especially, the intensive immunoreactivity was observed in medial SMC and ECM. The expression of elastin and TG was not observed at accumulating macrophages in the shoulder region. Interestingly, double staining demonstrated the co-localization of TG and TNF-alpha in atherosclerotic coronary arteries. This finding prompted us to investigate the role of TNF-alpha in the expression of TG. Treatment of rat aortic SMC with TNF-alpha increased TG activity and its protein expression in dose- and time-dependent manner.

Conclusion: The expression of TG and elastin in human coronary arteries was enhanced as atherosclerosis progressed. TNF-alpha might mediate the upregulation of TG. Our findings suggest the possibility that activation of elastin and TG might stabilize the atherosclerotic plaques via binding ECM and protect atherosclerotic erosion or rupture.

P.WP2W18 | Mast cell chymase induces apoptosis of vascular smooth muscle cells

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Objectives: In human coronary atherosom, the numbers of degranulated mast cells and of apoptotic smooth muscle cells (SMCs) are increased. Accordingly, the possibility exists that mast cells participate in the regulation of SMC apoptosis in the lesion.

Methods and Results: Mast cells isolated from the serosal cavities of rats were stimulated to release their secretory granules. The neutral protease chymase, present in the exocytosed granules, was found to induce apoptosis when added to rat aortic SMCs in culture. The chymase-induced apoptosis of SMCs was detected by flow cytometry, microscopic analysis of cellular morphology, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL), and electrophoretic demonstration of DNA laddering. Re-combiant human chymase also induced apoptosis of human coronary artery SMCs in culture. The proteolytic activity of chymase was found to be essential for the apoptotic effect.

Conclusions: Chymase, secreted by activated mast cells is capable of inducing apoptosis of SMCs in vivo. This suggests that mast cells may participate in the apoptotic regulation of SMCs in atherosclerotic lesions.

P.W19 CEREBROVASCULAR DISEASE

P.WP2W19 | Immunohistologic features of cerebral arteriosclerosis in vascular dementia with special reference to fibrosis

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Objective: Cerebral arteriosclerosis is regarded as a major contributing factor for diffuse white matter lesions of the brains in patients with vascular dementia. The pathogenesis of the vascular lesions is still unknown.

Methods and Results: We investigated 17 autopsy brains of patients with vascular dementia immunohistologically. The deep white matter diffusely revealed edema and irregularly loosened glia tissue with demyelination. The small vessels in the white matter were thickened with the increase of collagen fibers consisting largely of type I and V, and partially of type VI and IV. The proximal small arteries supplying the white matter showed medial necrosis or atrophy without stenosis, which is characteristic of hypertensive arterial changes. Perivascular CD68 positive cells in the white matter were much sparser than in the cortex (p < 0.05).

Conclusions: The insufficiency of blood flow-regulation of the proximal small arteries and the decreased clearance of macrophages may play important roles in the pathogenesis of the deep white matter lesions associated with small-vessel fibrosis.

P.WP3W19 | Erythrocyte band 3 damage and leukocyte activation in ischemic stroke

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Objective: Activated white blood cells (WBC) release oxygen metabolites, cationic proteins and proteases, all of which may induce oxidative and proteolytic lesions to red blood cells (RBC). Aggregation of RBC membrane band 3 protein (involved in aging and required for removal) occurs in vivo after incubation of RBCs with activated neutrophils and in vivo in patients at risk for cardiovascular events. Looking for new markers of risk, we evaluated WBC activation, oxidative and proteolytic lesions in band 3, and the lipid risk profile (which predict only about one third of cardiovascular events) in a group including 21 ischemic stroke cases, evaluated in the first 24 hours, and in a control group including 29 individuals with normal hematological and lipid values. As markers of WBC activation we measured the concentration of neutrophil elastase and lactoferrin; as markers of RBC damage we evaluated band 3 profile (aggregates, monomers and fragments) and membrane bound hemoglobin (MBH). We also evaluated the concentration of total and differential WBCs, concentration of RBC and hemoglobin, hematocrit, and hematometric indexes. The lipid profile included tryglycerides, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, apolipoproteins A1 and B, and lipoprotein (a). (a).

When compared to the control, the pathologic group presented significant higher values of MBH, of both WBC activation products - elastase and lactoferrin – and a different band 3 profile (a significant rise in aggregation and in the reduction of fragments).

Our data suggest that cerebrovascular events is associated with increased WBC activation, which may underlie oxidative and proteolytic changes in RBCs. Moreover, we suggest that further studies should focus on WBC activation products and band 3 profile as potential markers of risk for cardiovascular events.
**WeP4:W19**

**Systemic mechanisms of formation of hereditary disposition to stroke**

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**Objective:** Study the state of cerebral hemodynamics and lipid metabolism in subjects with a hereditary disposition to stroke.

**Methods:** Relatives of stroke patients 1st and 2nd kinship degree aged 20–79 years (RSP, n = 210) and control subjects without cerebrovascular pathology in their genealogy (C, n = 150) were examined by ultrasound dopplergraphy for cerebral hemodynamics, and lipid and lipoprotein contents.

**Results:** There were structural-functional changes of extracranial (ECA) and intracranial sinus carotid arteries in 27% and 12%, respectively, of RSP and only in 10% of C (ECA case). In RSP of all age groups (20 to 79), linear blood flow velocity (LBFV) in ECA and medial cerebral artery was lower compared to C, while at 20–59 years it was less pronounced than in LBFV characteristic of stroke patients (54.0 ± 6.7 and 51.2 ± 8.8 cm/s, respectively). In RSP, LBFV asymmetry (more than 30%) was highest at 40–49 and 50–59 years, i.e. 25% and 3% (8% and 7% in C). At 50–59 years, marked atherosclerotic changes of cerebral vessels were diagnosed in 25% of RSP (10% in C). Blood cholesterol level exceeded the norm (>6.6 mEq/L) in 38% of RSP (in 15.3% of C). After 20–29 years, apoAI concentration was lower in RSP than in C (1.4 ± 0.2 vs. 2.28 ± 0.1 mg/mL). Correlation coefficients between apoAI and HDL contents of stroke patients and their relatives were 0.87 and 0.75.

**Conclusion:** The specifics of cerebral hemodynamics and lipid metabolism should be considered as systemic manifestations of hereditary disposition to stroke.

**WeP5:W19**

**α1-acid glycoprotein but not C-reactive protein discriminates late restenosis after carotid endarterectomy**

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**Objective:** To search among acute-phase reactants and lipids for a discriminant of carotid restenosis after carotid endarterectomy.

**Material and Methods:** Patients with internal carotid stenosis ≥ 70% as assessed by color Doppler USG, and DSA when necessary, underwent endarterectomy during the past six years. Postoperative assessment (USG/DSA) was done 30 days after surgery, every three months during the first year, and every six months thereafter. Two groups were subsequently formed: with (n = 17, mean age 61.9 ± 5.8) or without (n = 16, mean age 59.9 ± 9.1) restenosis.

**Lipids:** Total, LDL and HDL cholesterol (Ch), LDL-Ch, HDL-Ch, and triglycerides (Tg) were measured using commercial kits. Acute phase reactants C-reactive protein (CRP), α1-acid glycoprotein (AAG), prealbumin (PAP), complement C-3 and factor B, were measured with an immunonephelometric method (Array 360 instrument from Beckman).

**Results:** Lipids Ch, LDL-Ch, HDL-Ch and Tg levels were elevated in both groups (Ch: 262.8 ± 39.0 mg/dL vs 263.6 ± 47.7 mg/dL; LDL-Ch: 168.3 ± 39.8 mg/dL vs 170.6 ± 44.3 mg/dL; HDL-Ch: 42.7 ± 14.2 mg/dL vs 38.4 ± 12.1 mg/dL; Tg: 206.7 ± 157.1 mg/dL vs 280.8 ± 215.1 mg/dL — with vs without restenosis). No statistically significant differences were found.

**Acute phase reactants** AAG level was significantly higher in the restenosis group (114.7 ± 18.9 mg/dL vs 99.5 ± 18.1 mg/dL; p < 0.02). CRP was increased in both groups (4.7 ± 3.7 mg/L vs 3.4 ± 1.9 mg/L) but without statistical significance.

**Conclusion:** α1-acid glycoprotein seems to be superior to CRP in discriminating patients with late restenosis after carotid endarterectomy.

**P:W20**

**TRIGLYCERIDES AND CVD**

**WeP1:20**

**Effect of increasing doses of micronized fenofibrate on post-prandial triglycerides and fasting plasma lipid levels and lipoproteins in hypertriglyceridemic patients**


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**Objective:** To evaluate increasing doses of micronized fenofibrate (mF) on post-prandial (PP) triglycerides (Tg) identified as a predictor of Coronary Heart Disease (CHD).

**Methods:** We undertook an open forced-titration dose-response study of increasing doses of mF (67, 134 & 201 mg/day) on PP Tg values, in 38 patients (pts) with fasting Tg > 250 mg/dL. Determinations of lipids following an oral fat load (1g/kg) were performed on four occasions: after a 4-wk NCEP Step I diet and after each 4-wk treatment with mF.

**Results:** A linear cumulative dose-effect was demonstrated on fasting values: Tg: 450 ± 235 to 196 ± 83 mg/dL; TC: 234 ± 49 to 208 ± 40 mg/dL; HDL: 38 ± 13 to 44 ± 14 mg/dL; Apo B: 144 ± 43 to 126 ± 40 mg/dL; TCHDL-C ratio: 6.8 ± 2.6 to 5.2 ± 2.0 (n = 33 pts, all p < 0.05). PP determinations (n = 28 pts without any missing data) revealed a similar dose-related reduction of Tg Cmax (p < 0.0001; ANOVA), and the median Tg Tmax was reduced from 6 to 4.5 hrs (p < 0.0001; sign test). (See figure).

Two pts discontinued prematurely due to adverse events: one with increased transaminases (<3 x UNL) on 67 mg/d, and one with constipation.

**Conclusions:** mF dose-dependently reduces PP Tg, and favorably alters other plasma lipid risk factors for CHD in hypertriglyceridemic pts.

**WeP2:20**

**Association of hypertriglyceridemia and metabolic syndrome abnormalities in adolescents**


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**Objective:** To examine the association of hypertriglyceridemia with the metabolic syndrome coronary risk factors in adolescents.

**Methods:** Sampling design: Of 889 junior high school students, 455 were stratified as non-obese (NOB) (2nd quartile of BMI) and obese (OBE) (4th quartiles of BMI); as hypertriglyceridemic (HTG) (Tg ≥ 150 mg/dL) and normotriglyceridemic (NTG) (Tg < 150 mg/dL); and as boys and girls. Risk factors: Anthropometry and blood pressure were measured. Lipids, lipoproteins, apolipoprotein A-I and B, insulin and glucose were quantified in a venous blood sample after a 12-hour fast.

**Results:** We studied 171 boys and 284 girls with a mean age of 13.6 ± 1. OB HTG had higher prevalence of HDL-C < 35, fasting insulin ≥ 75, and waist circumference ≥ p75. Both OB and NOB HTG had higher proportion of small LDL particles (estimated as LDL-Ch/apolipop < p25). Multiple regression analysis showed that Tg explained insulin (10.4%), waist circumference (11.5%) and HDL-C (14.2%) in boys; and insulin (1.2%), LDL size (8.9%) and HDL-C (4.8%) in girls.

**Conclusions:** Our results confirm that in the adolescent population, HTG when accompanied with OB is associated with a higher prevalence of metabolic syndrome risk factors. However, Tg can independently explain, some of these factors.

**WeP3:20**

**An enzyme-linked immunosorbent assay for the measurement of activated factor XII in human plasma**

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**Background:** Factor XII is activated to FXIIa on the surface of triglyceride-rich lipoproteins and it has been reported that FXIIa is associated with cardiovascular risk. The aim of this study was to investigate the characteristics of an ELISA for the measurement of FXIIa.

**Methods:** Citrated plasma samples were collected from a total of 339 apparently healthy asymptomatic volunteers. The age range of this population was 18–68 years, and comprised a 50% male to female distribution.

**Results:** The mean FXIIa value of this asymptomatic population was 1.53 ng/ml ± 0.73 ng/ml. The range of the values obtained was 0.03–4.8 ng/ml. The within assay imprecision was <5% and between assay imprecision <12%. The lower limit of detection of the assay was 0.11 ng/ml. Within-day, within-person variation of FXIIa levels was assessed by taking 7 citrated blood samples...
samples from volunteers (n = 8) over a 24 hour period, and assaying for XIIa. No significant within-day, within-person variation in XIIa was observed. No observable differences in FXIIa with gender were observed. FXIIa values increased with age (n = 262) and in female users of contraceptive pill (OC users). Recovery of FXIIa added to normal plasma was, as expected, less than 10%. This observation is explained by the presence of protease inhibitors that rapidly inactivate exogenous FXIIa when added to plasma. No assay interference was observed by bilirubin (<10 mg/dL) Intralipid (<375 mg/dL), salicylate (<300 mg/dL), Coughadin (<5 mg/L) or Cyclosporin (1 mg/L).

Conclusion: It is concluded that the ELISA is a precise reproducible assay that can be used to investigate the role of FXIIa in health and disease.

**WeP4:20**

**Comparison between retinyl esters and RLP-cholesterol in studying postprandial lipoprotein metabolism in normolipidemic subjects**


Abnormal postprandial lipoproteins are associated with an increased cardiovascular risk. Postprandial remnant lipoproteins were typically measured indirectly using retinyl esters (RE) as a chylomicron core label. VLDL remnants in addition to chylomicron remnants can also be directly quantified using the rLP-Cholesterol Immunosuppression Reagent from Japan Immunoresearch Laboratories (IMRL, Chino, Japan; 1998; 44: 2000-9), using monoclonal antibodies to apo A-I and apo B-100 to remove non-remnant lipoproteins and quantifies cholesterol in the remaining apo E-rich remnants. In this study we compared the RE method with the RLP-Cholesterol (RLP-C) Reagent in measuring postprandial remnant lipoproteins. After a ten-hour fast, 16 healthy normolipidemic subjects (8 males/8 females) ingested cream in a single dose (50 g fat (40% w/w) plus Vitamin A) per m² body surface. Venous blood samples were drawn in fasting and hourly between 1 and 8 hours after fat load. Postprandial plasma RE and RLP-C peaked at 4.31 ± 1.14 hr and 3.56 ± 0.63 hr, respectively (P < 0.01). In comparison, postprandial plasma TG peaked at 3.25 ± 1.13 hr (P < 0.005 and P > 0.05 compared to RE and RLP-C, resp.). TG/Apo-B ratio, a measure of postprandial lipoprotein particle size, peaked at 3.38 ± 1.59 hr (P = 0.05 and P > 0.05 compared to RE and RLP-C, resp.). Delay in peak PE may be explained by quantitatively more PE incorporated in chylomicrons with a larger size in enterocytes in late postprandial period. In conclusion, postprandial RLP-C changed in parallel with plasma TG and FFA concentrations and peaked before RE. Although both PE and RLP-C can be used to study postprandial lipoprotein metabolism, they measure different remnant lipoprotein fractions.

**WeP5:20**

**Severe hypertriglyceridemia (SHTG) and atherosclerosis**

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While there is common agreement that pancreatitis may be caused by severe hypertriglyceridemia (SHTG), its role in atherogenesis remains controversial. We therefore set out to evaluate a large number of patients with SHTG for clinical risk.

306 patients (229 male = 75%, 77 female = 25%) with SHTG (TG > 750 mg/dl) were recruited from 12 participating centers with lipid research units in Germany and Austria. Mean value for age was 49 years (21–80 y), BMI: 28 (19–31), maximum TG: 2461 (741–15678) mg/dl, total Cholesterol: 459 (157–1550) mg/dl, fasting glucose: 128 (51–318) mg/dl. Concomitant conditions and diseases: Hypertension was found in 41%, hyperuricemia in 42%, diabetes mell. in 31%, pancreasitis in 19%, gallstone disease in 12%. Atherosclerosis was present in 24%. Vascular disease manifestations included PVD in 12%, angina pectoris in 8%, CHD 11%, myocard. infarction in 8%, CABG 4%.

Almost 25% (n = 72) of the patients with SHTG (TG > 750 mg/dl) suffered from some kind of atherosclerotic vascular disease. CHD was more frequent than PVD. Our results show that severe hypertriglyceridemia does not only induce pancreatitis but seems to play a major role in atherogenesis, possibly in association with comorbid conditions.

**WeP6:20**

**Abnormal postprandial plasma triglyceride levels in normotriglyceridemic children of familial combined hyperlipidemic parents**

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**Objective:** We have analyzed the postprandial triglyceride levels during the fat loading test in the hyperlipidemic parents and their normotriglyceridemic children in four familial combined hyperlipidemic (FCHL) families.

**Methods:** The study included 12 young (age: 21 ± 4 years) subjects (fasting triglycerides: 1.4 ± 0.5 mmol/L (G2), and 12 hyperlipidemic relatives (age: 57 ± 9 years, fasting triglycerides: 2.7 ± 1.6 mmol/L, total-cholesterol: 7.5 ± 1.2 mmol/L) (G1) and 12 normolipidemic, healthy control (C). Blood samples of lipid parameters have taken fasting and 2, 4, 6, 8, 10 hours postprandially after the standard fat rich test meal. We determined also the apo-polymorphisms.

**Results:** The maximum triglyceride values in G2 appeared 2 hours later (4 vs. 2) than in C, but 2 hours earlier than in G1. The area under curve (AUC) was significant (p < 0.001) higher in G2 vs. C, but it was by 50% lowered than in G1. The subjects of FCHL families with apo4 5/4 (n = 14) had been significant (p < 0.001) higher and extended postprandial lipemia.

**Conclusion:** Our results suggest that young normotriglyceridemic subjects of FCHL families have already abnormal postprandial state.

**WeP7:20**

**Improved assessment of LDL cholesterol and LDL/HDL ratio in patients with hypertriglyceridemia, using a direct assay**

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**Objective:** To evaluate the clinical accuracy of a new method for the direct assessment of LDL cholesterol in patients with hypertriglyceridemia. This method combines automated agarose gel electrophoresis, with enzymatic staining for cholesterol within gels, followed by automatic scanning of gels.

**Methods:** The new method (e) was compared with ultracentrifugation (u) (Beckman TL-100) and calculation with the Friedewald formula (c) in 442 outpatients (M/F = 1.14), age 11–85 y (53 ± 15), including 136 pts with moderate TG (150–400 mg/dl) and 31 pts with high TG (>400).

**Results:** Plasma lipids ranged within (mg/dl) 78–596 for TC (235 ± 55), and 16–1840 for TG (165 ± 181). A 94% correlation was observed between (e) and (u). Bias was within recommended goals (+2.9%) for (e) whereas (c) underestimated LDLc (−6.7%) especially when TG > 200 (−12%). Sensitivity to detect NCEP LDLc cut-points was higher 92% (e) vs 82% (c), remaining unchanged with TG (95 vs 78%) even above 400 mg/dl. In moderate TG pts, underestimation was divided by 5 with (e) (c) at all cut-points. In high TG pts excluded by (c), (e) detected 11 with 1/3 with LDLc above 130 and 160 mg/dl, respectively. HDLc was obtained simultaneously, giving a sensitivity of 78% (e) vs 53% (c), to detect pts with a high LDLc/HDLc ratio.

**Conclusions:** Direct assessment of LDLc with (e) provides a risk level estimation closer to the reference method (u) than calculation, in patients with hypertriglyceridemia.

**WeP8:20**

**Postprandial remnant-like particles cholesterol in patients with acute myocardial infarction**

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**Objectives:** To determine the usefulness of postprandial remnant-like particles cholesterol (RLP-C/HDL-C ratio) as an index for detecting the severity of coronary stenoses in patients with acute myocardial infarction.

**Methods:** Twenty-nine normolipidemic and non-diabetic patients with acute myocardial infarction were selected for this study. All the patients were...
fat loaded (30 g/m² of fat for body surface area) and bloods were withdrawn in 0, 2 and 4 hours for TC, TG, LDL-C, HDL-C and RLP-C analysis.

Results: Twenty-four patients had significant coronary stenoses (>75%) and five had no significant stenoses (<50%). Those two groups showed no significant differences in patient characteristics. Before fat loading, RLP-C/HDL-C ratio index had no significant differences between the two groups (p = 0.71). Whereas, 2 hours after fat loading, RLP-C/HDL-C ratio showed significant differences in the two groups. Namely the patients with significant stenoses showed marked increase in the ratio (p = 0.03) of the ratio index. The significant difference of the RLP-C/HDL-C ratio remained significantly higher in 4 hours (p = 0.04) after fat loading.

Conclusions: The elevated RLP-C/HDL-C ratio after fat loading predicted the severity of atherosclerosis in normolipidemic patients with acute myocardial infarction.

P.W21 CELLULAR STRESS AND GENE REGULATION

WeP1.W21 Inhibition of stress-activated protein kinase in ischemic/reperfused heart: Role of magnesium tanshinoate B in preventing apoptosis
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Objective: The activation of stress-activated protein (SAP) kinase may lead to an induction of apoptosis that is responsible for part of the cardiomyocyte death in reperfusion injury. The objective of the present study was to investigate the role of magnesium tanshinoate B (MTB), a bioactive compound isolated from Dansheng, in the regulation of SAP kinase in the ischemia/reperfused heart.

Methods: Isolated adult Sprague-Dawley rat hearts were perfused by Langendorff mode with media containing MTB prior to the induction of global ischemia for 30 min, followed by 20 min reperfusion. SAP kinase activity and its nuclear localization were determined in the MTB-treated and untreated ischemia/reperfused heart. Apoptosis of cardiomyocytes was detected by the TUNEL method.

Results: SAP kinase activity was elevated 2-fold in ischemic/reperfused rat hearts. Treatment with MTB abolished this elevation in SAP kinase activity, which was also decreased by 40% in the nucleus. MTB also directly inhibited the phosphotransferase reaction of SAP kinase while at the same time enhanced the binding of SAP kinase to c-jun. This resulted in an overall inhibition of the kinase activity. The number of apoptotic nuclei in MTB-treated ischemic/reperfused heart was also significantly reduced by 4.2-fold in comparison to that in untreated ischemic/reperfused controls.

Conclusion: MTB was shown to have cardioprotective activity against apoptosis through the inhibition of SAP kinase activity.

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WeP2.W21 Expression of candidate genes of atherosclerosis. During macrophage differentiation
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Objective: The emigration of circulating monocytes from the blood into the arterial wall with subsequent transformation into macrophages is a key event in atherogenesis. However, there is increasing evidence, that the state of activation of circulating monocytes and macrophages may be associated with a particular risk of atherosclerosis. The aim of our study was to follow the expression of macrophage genes, which are relevant in intracellular cholesterol homeostasis and procoagulatory activity during the monocyte/macrophage differentiation process.

Methods: Mononuclear cells from healthy donors were allowed to adhere in culture dishes and RNA was isolated from the adherent cells after 2, 4, 24, 48 h, 96 h, 8 d and 14 d. RNA was also isolated from monocytes directly after purification by magnetic cell sorting using anti CD-14 coated microbeads. Gene expression of ApoE, SR-A, SR-BI, SR-E, LDL-receptor, HMG-CoA reductase and tissue factor was quantified using a fluorogenic RT-PCR method (TaqMan). The gene expression was normalized to beta-actin to account for any differences of RNA concentration.

Results: There was a selective activation of the investigated genes during monocyte/macrophage differentiation. A very early increase of mRNA (2 h) was observed for SR-E and TF, whereas ApoE and SR-A mRNA showed a strong increase after 48 h and 8 d, respectively. LDL-R and HMG-CoA reductase mRNA showed no significant changes.

Conclusion: Cell adhesion and time dependent differentiation of monocytes/macrophages resulted in selective patterns of gene expression, which may play a central role in cellular cholesterol homeostasis and "house-cleaning" macrophage functions.

WeP3.W21 Hypernuclear acetylation of the vascular smooth muscle cells in atherosclerotic lesion
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Objective: To investigate the involvement of coactivator, CBP/p300 in the pathomechanism of atherosclerosis.

Methods: Recent studies have implicated acetylation of several nuclear proteins such as histones and p33 on their epison-por-tyion of lysine residues in eukaryotic transcription. To investigate the involvement of the acetylation in atherosclerosis/thrombosis, we raised a specific polyclonal antibody against epison-acetylated lysine. Using the antibody, we examined the nuclear acetylase in atherosclerotic vascular smooth muscle cells (VSMCs) in culture and in surgical specimens.

Results: We showed acetylated proteins are accumulated in the nucleus of the VSMs in atherosclerotic lesions. Thrombin caused activation and proliferation of VSMCs with marked nuclear acetylation in culture. MAP kinase pathway and a signal coactivator CREB binding protein (CBP) were involved in thrombin-induced nuclear acetylation of VSMCs.

Conclusions: Our results suggest that coactivator CBP/p300, cooperating with signal-dependent transcription activators, plays an important role in atherosclerosis via nuclear acetylation in VSMCs.

WeP4.W21 The role of nuclear receptor NGFI-B in vascular smooth muscle cell apoptosis
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Objective: NGFI-B is one of the orphan nuclear receptors as well as immediate early genes. It has been reported that the gene is implicated in T cell apoptosis. However, there is no report demonstrating the expression and the role of NGFI-B in vascular smooth muscle cells (VSMC). In this study, we investigated the expression of NGFI-B in VSMC and its role in VSMC apoptosis in response to pyrrolidine dithiocarbamate (PDTC), an antioxidant agent.

Methods: VSMC apoptosis was examined using Hoechst 33258 dye under fluorescent microscopy, and quantitatively evaluated by ELISA detecting DNA fragmentation. The expression of NGFI-B was analyzed by Northern blotting and Western blotting.

Results: Addition of PDTC caused VSMC apoptosis only when cell density was low (10,000 cells/cm²). In this experimental condition, NGFI-B mRNA was induced at one hour after addition, peaking at 6 hours and persisted for more than 12 hours. Consequently, NGFI-B protein was induced at 4 hours after, peaking at 8 hours and persisted for more than 12 hours. The degree of apoptosis and the expression of NGFI-B stimulated by PDTC were dose-dependent. In contrast, when the cell density was high (50,000 cells/cm²), VSMC apoptosis was not induced and the expression of NGFI-B was transient; peaking at 2 hours and disappearing at 4 hours after addition of PDTC.

Conclusions: This study, for the first time, demonstrated NGFI-B expression in VSMC, and suggests that NGFI-B may play a role in VSMC apoptosis in response to PDTC.

WeP5.W21 A nuclear orphan receptor identified in human atherosclerotic plaques is induced by LDL
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Smooth muscle cells (SMC) play a key role in the development of atherosclerosis and restenosis. Recently, we have cloned a new gene (NOR-1) involved in SMC activation. NOR-1 is a nuclear orphan receptor that is transiently induced in SMC by several mitogens (PDGF, α-thrombin, serum) and is overexpressed in atherosclerotic plaques.
Objective: Our objective was to analyze the ability of LDL and cytokines to induce NOR-1 and to characterize the signaling mechanisms involved in NOR-1 induction.

Methods: SMC cultures were obtained from coronary arteries by the explant method. Quiescent SMC were treated with native LDL (nLDL, 50 to 600 μg protein/mL), oxidized LDL (oxLDL, 25 to 100 μg protein/mL), cytokines (TGFβ, IL-1β, TNFα) or increasing concentrations of activators or inhibitors of intracellular signaling pathways and NOR-1 mRNA levels were analyzed by Northern blot 1 h after of stimuli.

Results: nLDL significantly induced NOR-1 mRNA levels (in contrast the effect of oxLDL and cytokines was negligible). PMA, an activator of PKC and A23187, an activator of intracellular calcium levels ([Ca^{2+}]_i) significantly induced NOR-1. In contrast, GF-19023X, an inhibitor of PKC, and EGTA, an inhibitor of [Ca^{2+}]_i, reduced serum-induced NOR-1 mRNA levels. Two products that increase cAMP ( forskolin, an adenosine cyclase activator and isoproterenol, a β-agonist), and 8-Br-cAMP, a cAMP analogue, stimulated NOR-1 expression.

Conclusions: NOR is highly induced by nLDL, but not by oxLDL and cytokines involved in inflammation. The activation of NOR-1 is dependent on PKC activation, and requires the increase of cAMP and [Ca^{2+}]_i levels.

WT6P2W21 Transcriptional repression of the human CYP7A1 gene by a nuclear matrix protein
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Objective: To identify and characterize hepatic regulatory elements of the human CYP7A1 gene.

Methods and Results: Although significant progress has recently been made in identifying regulatory elements and transcription factors implicated in the regulation of the CYP7A1 gene in rodents, very little knowledge is available regarding regulation of the human CYP7A1 gene. We recently reported that the human promoter alone is not capable of conferring expression of CYP7 transgenes in the livers of mice. As a first approach to identify key liver-specific elements in other segments of the human gene, DNeat hypersensitivity ( DHS) studies were performed with HepG2 cells. Three DHS sites were detected within the first intron of the human CYP7A1 gene, but only in hepatic cells. Transient transfection experiments with HepG2 cells revealed a transcriptional repressor within intron 1. Intron 1 was also found to be anchored to the nuclear matrix. Gel retardation experiments and cell transfection studies provided evidence for the repression mechanism. Repression is achieved by the nuclear matrix-bound CAAT-displacement protein (CDP). CDP binds to and interferes with binding of two key hepatic activators, namely, HNF-1 and C/EBP within intron 1. The relative ratios of the activators versus that of the repressor CDP at different stages of development may regulate the levels of hepatic CYP7A1 transcription.

Conclusions: The nuclear matrix protein CDP represses transcription by displacement of 2 key hepatic activators, HNF-1 and C/EBP, that bind within intron 1 of the human CYP7A1 gene.

WT7P2W21 Arsenic and atherosclerosis
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Objective: Epidemiological studies have marked a correlation between environmental or occupational arsenic exposure and development of atherosclerosis and increased mortality from cardio-vascular diseases. Since arsenic has been characterized to have strong affinity for cysteine groups of the protein molecules and to modulate the redox stage through depletion of reduced glutathione, we hypothesized that arsenic can accelerate the atherosclerotic process through direct effect on endothelial cells and/or through oxidation of lipids.

Methods: In vivo: in vitro systems; patho-morphometric analysis; RT-PCR; RNA-ase protection assay; DNA-shift assay, lipid peroxidation.

Results: ApoE-/- mice exposed to arsenic in drinking water for 12 weeks, compared to mice on control water, exhibited a substantial increase in atherosclerotic lesion size. The effect was more prominent when a high fat diet was used. Arsenic exposure was not associated with significant differences in the serum lipid profiles. Arsenic induced a dose-dependent increased gene expression of tissue factor, interleukine-8, c-jun and heme oxygenase in human endothelial cells. The gene expression was associated with activation of transcription factors such as NF-kB and AP-1. Arsenic injection in rats did not modulate LDL oxidation in the presence of endothelial cells.

Conclusions: We demonstrate for the first time in an animal model that arsenic stimulates atherosclerotic formation and suggest that arsenic-induced gene expression in endothelial cells contributes to this process.
**WeP2:** Deletion of the propeptide of apolipoprotein A-I reduces protein expression, but stimulates effective conversion of prebeta-HDL to alpha-HDL.

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The properties of the mature and pro-forms of recombinant apolipoprotein A-I (apoA-I) were compared with those of apoA-I isolated from human plasma. When the synthesis and secretion of pro- and mature forms of apoA-I from a baculovirus/insect cell expression system were compared in parallel experiments, the amount of the pro-form of apoA-I synthesized and secreted was several fold higher than that for the mature form of apoA-I. A comparison of the properties of the pro- and mature forms of recombinant apoA-I and human apoA-I showed a clear distinction between all four in their secondary structure, their ability for self-association, lipid binding and lecithin-cholesterol acyltransferase activation. The properties of reconstituted HDL particles formed from the proteins and their ability to promote cholesteryl and phospholipid efflux from human skin fibroblasts were also similar. When the ability of the protein to bind to plasma HDL subfractions was studied, twice as much preapoA-I was found in prebeta-HDL and prebeta-HDL subfractions compared with both mature recombinant and plasma apoA-I. Correspondingly, the amount of preapoA-I in alpha-HDL subfractions, especially in alpha1-HDL and alpha2-HDL, was decreased. We conclude that while pro-peptide of apoA-I is required for the effective synthesis and secretion of apoA-I, cleavage of this peptide is then requisite for the effective inter-conversion of HDL subfractions.

**WeP3:** A clinical study on hyper-HDL-cholesterolemia in Japan

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**Objective:** Hyper-HDL-cholesterolemia has been considered to be anti-atherogenic and called as longevity syndrome. However, hyper-HDL-cholesterolemia induced by cholesteryl ester transfer protein (CETP) deficiency may not be athero-protective, and rather atherogenic in nature. In a rural area in central Japan, the incidence of hyper-HDL-cholesterolemia is rather high (3.1% of healthy people). In this area, we studied Japanese with hyper-HDL-cholesterolemia, particularly in relation to CETP.

**Methods:** 122 Japanese (37 males and 85 females) with HDL > 100 mg/dl were examined. Serum HDL, TG, HDL-C, LDL-C (Friedewald's formula), Apo A-I, A-II, B, C-II, C-III, E, Lp(a), Lp(a)-I and CETP were measured.

**Results:** Serum CETP mass in hyper-HDL-cholesterolemia was distributed in a wide range. Less than one fourth was estimated to be heterozygous of CETP deficiency. Two persons were homozygous of CETP deficiency. Less than one fourth was estimated to be heterozygous of CETP deficiency. Serum CETP mass positively correlated with LDL-C, Apo B, and LDL-C/HDL-C, with statistical significance. CETP also positively correlated with LDL-C/Apo B.

**Conclusion:** These results suggest that hyper-HDL-cholesterolemia is not a single clinical entity, but a mixture of various pathophysiological conditions. CETP positively correlated with LDL-C/HDL-C and LDL-C/Apo B, suggesting that the ratio of LDL-C to HDL-C and the size of LDL may be important factors to differentiate these conditions.

**WeP4:** Why are C3H mice resistant to diet induced atherosclerosis?

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**Objective:** C3H mice are resistant to diet induced atherosclerosis in comparison to C57Bl/6 mice. In this research we tested whether C3H mice might have a more efficient reverse cholesterol transport which would contribute towards resistance to atherosclerosis.

**Methods:** Female mice from C57Bl/6 and C3H strains, fed chow or cholate containing atherogenic diet (A-diet) for 1 month were studied. To create a lipoprotein cholesterol depot, cationized LDL (200 μg cholesterol) labeled with 3H-cholesterol was injected under ketamine anesthesia into the rectus femoris muscle. Retention of exogenous cholesterol mass (ECM) in the muscle was determined by HPLC 8 and 12 days after injection. Endogenous cholesterol mass/mg wet weight determined on the contralateral muscle, was subtracted from the total cholesterol in the injected muscle, to obtain retention of ECM.

**Results:** HDL cholesterol (HDL-C) on chow was 59 and 88 mg/dl in C57Bl/6 and C3H mice, respectively, and HDL phospholipids (PL) were 189 and 227 mg/dl. On A-diet, HDL-C was 36 and 75 mg/dl and HDL-PL was 78 and 156 mg/dl for C57Bl/6 and C3H mice, respectively. In C57Bl/6, on chow, retention of ECM in the muscle 8 and 12 days after injection was 71 ± 3 and 35 ± 2% of injected dose; in C3H it was 46 ± 3 and 20 ± 2%, respectively. On atherogenic diet, the retention of ECM was 82 ± 3 and 76 ± 5% in C57Bl/6; it was 70 ± 5 and 28 ± 4% in C3H on days 8 and 12 respectively. Similar results were obtained in male mice (data not shown).

**Conclusions:** The more efficient cholesteryl clearance from the muscle depot in C3H mice, which in some respects mimics an atheroma, appears to be related to higher HDL phospholipids especially on the A-diet, and could contribute towards resistance of this strain to diet induced atherosclerosis.

**WeP5:** A familial massive tendon xanthomatosis in association with decreased cholesteryl efflux in monocyte-derived macrophages

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**Objective:** Cholesterol efflux (CE) is the important and initial step of reverse cholesterol transport. Although it is well known that fibroblasts from familial high density lipoprotein (HDL) deficiency syndrome including Tangier disease display impaired CE, any other clinical disorder with impaired CE has still never been reported. In the current study, we identified a family with massive tendon xanthomatosis with decreased CE in the patient’s monocyte-derived macrophages (MF).

**Methods and Results:** The proband was a 62-year-old male suffering from massive xanthomatosis with pleuriphoneum. Similar xanthomatosis was also observed in his two brothers. Known xanthomatosis were excluded. HDL showed a marked thickening of Achilles tendons (50 mm in thickness). To clarify the mechanism of xanthoma formation in this family, we examined oxidized low density lipoprotein (ox-LDL) uptake and CE in the proband’s MF. Although there was no significant difference in the ox-LDL uptake between the proband’s and control MF, his MF displayed a marked reduction in CE compared with control MF. We cloned differentially expressed genes in the proband’s and control MF by using cDNA subtraction technique. The mRNA level of annexin II (AXII), which plays an important role in vesicular transport, was decreased in his MF compared with control MF.

**Conclusions:** These observations suggest that the impaired CE with decreased expression of AXII in MF may be, at least in part, related to the appearance of severe xanthomatosis.

**WeP6:** Triglyceride (TG) enrichment of HDL does not alter HDL selective cholesteryl ester (CE) clearance in rabbits

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**Objective:** TG enrichment of HDL, which occurs in hypertriglycerideremic states, significantly enhances the rate at which HDL apo A-I is cleared from the circulation of healthy humans. In the NZW rabbit, a species naturally deficient in the lipoprotein enzyme, hepatic lipase (HL), TG enrichment of HDL requires prior lipolytic modification to enhance apo A-I clearance. However, the effect of TG enrichment of HDL on the subsequent clearance of HDL CE has not previously been examined in vivo. Therefore, we investigated, in the NZW rabbit, the effect of ex vivo TG enrichment of rabbit HDL on the clearance of HDL CE and apo A-I.

**Methods:** Serum from donor rabbits was incubated ex-vivo with human VLDL to enrich the HDL particles with TG. Fasting and TG-rich HDL were radioiodinated with 125I and enriched with 3H cholesteryl oleyl ether. The TG-rich and fasting HDL tracers were then each injected into a separate recipient rabbit to determine the fractional catabolic rates (FCR) of the HDL apo A-I and CE respectively, by multicompartamental modeling.

**Results:** In 6 experiments TG enrichment of rabbit HDL resulted in a 76% average increase in HDL TG and a corresponding 29% reduction in HDL CE content. The calculated apo A-I and CE FCRs associated with TG-rich versus fasting HDL tracers were not significantly different (apo A-I: 0.11 ± 0.01 vs. 0.10 ± 0.01 pools/hr; P = 0.70; CE: 0.24 ± 0.02 vs. 0.18 ± 0.03 pools/hr, P = 0.10).

**Conclusions:** In an animal model naturally deficient in HL, TG enrichment of HDL does not alter the rate of HDL apo A-I metabolic clearance, nor the rate of selective HDL CE uptake. Further studies are needed to determine the exact mechanism of TG enrichment of HDL on HDL CE clearance.

Intravascular hemolysis after intralipid infusion indicates that the reverse cholesterol transport is optimal in the postprandial phase.

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Objectives: We studied whether thermodynamic factors and the law of mass action regulate 1) concentration and composition of the various lipoproteins and 2) lipid composition of the erythrocyte membrane.

Methods: 500 ml 20% Intralipid were infused at a rate of 100 ml/h in 10 controls and 6 patients on parenteral nutrition. Blood samples were obtained at regular intervals. The phytosterols (mainly sitosterol) were measured as markers of the sterol transfer from the fat particles.

Results: VLDL-cholesterol increased parallel with a decrease in LDL- and HDL-cholesterol. The LDL- and HDL-fractions became relatively richer in triglycerides at the expense of cholesterol esters, ascribable to transfer.

In all subjects intravascular hemolysis was detectable: free hemoglobin concentrations significantly increased with about 48 and 23 μmol/l in 5 h; haptoglobin concentrations decreased with 0.3 and 0.04 g/l in the controls and the patients, respectively. Hemolysis was maximal at the peak of hypertriglyceridemia.

Both in the controls and in the patients, sitosterol contents in the VLDL-fraction increased parallel to cholesterol. In the LDL- and HDL-fractions however, sitosterol contents increased, but cholesterol contents in these fractions decreased (not ascribable to transfer).

Conclusions: Intralipid infusion introduces a large thermodynamic concentration gradient. Following the law of mass action, the system strives to reach a new equilibrium. This explains the temporary changes in lipoprotein concentration/composition and cellular membrane lipids. The latter is reflected by the degree of hemolysis and may be regarded as a measure of the reverse cholesterol transport.

The influence of phospholipid acyl chain composition on the hepatic lipase-mediated hydrolysis of HDL phospholipids

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Hepatic lipase (HL) is an enzyme that is bound to sinusoidal endothelial cells in the liver. It hydrolyses triglycerides and the sn-1 acyl chains of phospholipids (PL). This study shows that the rate of HL-mediated hydrolysis of the phospholipid sn-1 acyl chains in HDL is regulated by the phospholipid sn-2 acyl chain composition. This was achieved using spherical, reconstituted HDL (rHDL) that varied only in the length and unsaturation of their sn-2 acyl chains. The spherical rHDL were prepared by incubating discoidal rHDL with lecithin:cholesterol acyl transferase and unesterified cholesterol. The phospholipids in the discoidal rHDL all contained palmitic acid in the sn-1 position and either oleic acid (POPC), linoleic acid (PLPC), arachidonic acid (PAPC) or docosahexaenoic acid (PDPC) in the sn-2 position. APO-A-I was the sole apolipoprotein in all the discoidal rHDL preparations. The resulting spherical rHDL were comparable in size and composition. The PL/Unesterified cholesterol/cholesterol ester/apo-A-I molar ratios of the (POPC) rHDL, (PLPC) rHDL, (PAPC) rHDL and (PDPC) rHDL were 26/1/29/1, 30/1/31/1, 15/1/28/1 and 25/2/25/1 respectively. Their respective Stokes’ diameters were 10.1 nm, 8.9 nm, 9.5 nm and 8.9 nm. The spherical rHDL were incubated with HL up to 3 h. Phospholipid hydrolysis was monitored by the release of nonesterified fatty acid (NEFA) mass from the rHDL. It was also determined spectrophotometrically by the binding of NEFA to the fluorescent probe ADIFAB (AcryloxyDAn-derivatized Intestinal Fatty Acid Binding Protein). The results showed that the rate of phospholipid hydrolysis was greatest in spherical (PLPC) rHDL, followed by (POPC) rHDL and (PAPC) rHDL. (PDPC) rHDL was the least effective substrate for HL-mediated phospholipid hydrolysis. In conclusion, we have shown that the ability of HL to hydrolyse phospholipid sn-1 fatty acyl chains in rHDL is regulated by the sn-2 acyl chain composition of the particles.

Influence of triglyceride enrichment on the remodelling of high density lipoproteins by PLTP

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Phospholipid transfer protein (PLTP) converts high density lipoproteins (HDL) into larger and smaller particles and transfers phospholipids between different HDL particles. The conversion into larger and smaller particles is markedly enhanced in triglyceride (TG)-containing HDL. In this study the conversion products are characterised. The influence of TG enrichment of HDL on PLTP-mediated phospholipid transfers, and on the binding of PLTP to HDL are also studied. Spherical reconstituted HDL (rHDL) containing cholesteryl esters in their core (CE-rHDL) were enriched with TG by incubation with CETP and Intralipid. When the CE-rHDL (PL:UC:CE:TG:apo-A-I molar ratio 35:5:17:0:1; diameter 9.3 nm) were incubated with PLTP, larger (11.1 nm) and smaller (7.7 nm) particles were formed. Approximately 24% of the CE-rHDL did not change in size. When the TG-rHDL (PL:UC:CE:TG:apo-A-I molar ratio 36:7:14:6:1; diameter 9.5 nm) were incubated with PLTP, they were completely converted into larger (11.3 nm) and smaller (7.7 nm) particles. The large and small conversion products contained 4 and 2 apo-A-I particle, respectively. The PL:UC:CE:TG:apo-A-I molar ratio of the large CE-rHDL and TG-rHDL conversion products was 29:1:45:0:1 and 33:1:56:18:1, respectively, while the small conversion products had PL:UC:CE:TG:apo-A-I molar ratios of 14:1:22:0:1 and 12:2:19:8:1. The CE-rHDL and TG-rHDL were incubated with [14C]-DPPC-labelled small unilamellar vesicles and PLTP. PLTP transferred phospholipids between the vesicles and TG-rHDL 5 times faster than between the vesicles and CE-rHDL. Association constants (ka) for the binding of CE-rHDL and TG-rHDL to PLTP showed that PLTP had a comparable affinity for CE-rHDL and TG-rHDL. In conclusion, these studies show that TG enrichment of rHDL enhances the PLTP-mediated conversion of HDL, and phospholipid transfers, but does not affect the affinity of PLTP for HDL.

The influence of apolipoproteins on hepatic lipase-mediated phospholipid hydrolysis in recomconstituted high density lipoproteins

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Hepatic lipase (HL) is a lipolytic enzyme which hydrolyses phospholipids (PL) and triacylglycerols in high density lipoproteins. We have shown previously that HL has a much higher affinity (Kd) for, spherical reconstituted HDL which contain apolipoprotein (apo) A-II as the sole apolipoprotein, (A-I) rHDL, than for (A-I) rHDL, which contain apo-A-I as the sole apolipoprotein. This study also showed that the maximal rate of PL and triacylglycerol hydrolysis (Vmax) is greater in (A-I) rHDL than in (A-II) rHDL. The first aim of the present study was to determine whether the PL hydrolysis was additive when (A-I) rHDL and (A-II) rHDL were both present in the incubation mixture. This was achieved by incubating mixtures of (A-I) rHDL and (A-II) rHDL with HL. PL hydrolysis was measured as the release of nonesterified fatty acid from the rHDL. The results showed that PL hydrolysis is greater in a mixture of (A-I) rHDL and (A-II) rHDL than for equivalent concentrations of either (A-I) rHDL alone or (A-II) rHDL alone. This was due to an increase in PL hydrolysis by the (A-II) rHDL. In the second part of the study we determined whether HL-mediated PL hydrolysis in rHDL containing both apo-A-I and apo-A-II, (A-I/A-II) rHDL, resembles that of (A-I) rHDL or (A-II) rHDL. (A-I/A-II) (A-I) rHDL and (A-II) rHDL were incubated individually with HL. Kinetic analysis showed that the affinity of HL for (A-I/A-II) rHDL (Kd = 0.05 mmol L^-1) was between that of (A-I) rHDL (Kd = 0.15 mmol L^-1) and (A-II) rHDL (Kd = 0.5 mmol L^-1). The Vmax for PL hydrolysis in (A-I/A-II) rHDL (778.3 mmol NEFA formed/ml HLA) was between that of (A-I) rHDL (1159.4 mmol NEFA formed/ml HLA) and (A-II) rHDL (395.1 mmol NEFA formed/ml HLA). In conclusion, these results show that apolipoproteins are major determinants of HL-mediated PL hydrolysis in rHDL.
**Phosphatidylinositol promotes sterol transport in vivo in rabbits**

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**Objective:** To examine the role that lipoprotein charge plays in cholesterol transport in vivo.

**Methods:** A small bolus (40 μmol) of an uncharged phospholipid (phosphatidylcholine, PC) or an anionic phospholipid (phosphatidylinositole) was injected into fasted rabbits and plasma lipids/intravascular cholesterol flux were evaluated.

**Results:** PC injection had a negligible effect on lipoprotein charge and composition, similar to that observed in a saline injected animal. In contrast, PI injection caused a significant increase in the net negative surface charge of all lipoproteins after only 10 min, followed by a gradual return to normal by 24 h. Lipoprotein compositional analysis showed a 3–5-fold increase of cholesteryl ester (CE) and cholesterol (FC) in the VLDL pool by 3 h, with no changes in VLDL-triglyceride content. While the bulk of the plasma CE was located in the HDL pool in the PC injected animals, in the PI animals, VLDL became the major CE storage compartment. No changes in the levels or composition of HDL or LDL were evident over the 24 h turnover period. Co-injection of [3H]-FC revealed a 30-fold greater rate of clearance of the labelled cholesterol from the PC injected rabbit plasma. In addition, the rate of cholesterol esterification by lecithin:cholesterol acyltransferase was almost completely inhibited in the PC animals.

**Conclusion:** A bolus injection of PI into rabbits appears to promote rapid sterol flux through the plasma compartment and enhance the production of cholesterol enriched VLDL particles. These data show that lipoprotein charge can affect cholesterol transport and that this process can be selectively manipulated.

**Determination of plasma pre-β high density lipoprotein (HDL) concentration in healthy humans**

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Pre-βHDLs include the initial acceptors of cholesterol from cultured cells. The metabolism of pre-β-HDL is influenced by several transfer proteins and enzymes, including CETP, PLTP and LCAT. To study the extent to which plasma pre-βHDL concentration is associated with this component of the apolipoprotein A-Ia subfamily, we have examined their associations before and after incubation of plasma samples at 37 °C. Blood was collected from 131 apparently healthy Japanese subjects aged 46.7 ± 11.8 years (mean ± SD). Plasma TC and TG were 204 ± 25 mg/dl and 101 ± 39 mg/dl, respectively. Pre-βHDL conc was assayed before and after a 16 h incubation by quantitative crossed immunoelectrophoresis, using an anti-apolipoprotein A-I antisem. Before incubation pre-βHDL conc was 18.7 ± 4.7% of total apo A-I (absolute conc, 13.6 ± 5.2 mg apo A-I/dl). Positive correlations were observed between preβ-HDL (mg/dl) and total apoA-I (r = 0.66), HDL-C (r = 0.56), LCAT activity (r = 0.54) and PLTP conc (r = 0.39). There was no significant correlation with plasma CETP. During incubation of plasma, pre-βHDL conc decreased during the first 1 to 2 h, and thereafter (in 105/131 subjects) increased. After 16 h incubation, plasma pre-βHDL had increased to 28.0 ± 6.9% of apo A-I. Absolute increments were 1.52 ± 0.29 mg apoA-I/dl. The magnitudes of the increments were not significantly correlated with any of the other measured variables. These results suggest: (1) That plasma pre-βHDL conc in normal humans is associated more closely with PLTP than with CETP; and (2) That other factors must operate in vivo to determine the steady state conc and production rate of pre-βHDL.

The association between pre-βHDL conc and plasma LCAT activity is consistent with the observation that pre-βHDLs are preferred substrate for LCAT.

**Presence of two PLTP particles in human plasma**

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We have developed a sandwich ELISA to determine the concentration of PLTP in human plasma. Our studies revealed that in normolipidemic subjects, there is a discrepancy between PLTP mass and PC transfer activity (PLTP activity). To undertaken this phenomenon, we analyzed the expression of PLTP in human plasma.

**Human plasma was separated by gel filtration chromatography. PLTP activity was measured by a PC liposome-HDL system, and PLTP was detected by sandwich ELISA or immunoblotting. In the fractions obtained by gel filtration chromatography, PLTP mass was distributed in molecular size of 400 kDa at the peak size, whereas PLTP activity was distributed in molecular size of 110 kDa at the peak size. The size of PLTP particles in the fractions containing maximal PLTP protein or activity analyzed by native-PAGE and immunoblotting were 12–14 nm and 9–11 nm, respectively. The different distribution between PLTP mass and activity in human plasma was detected. These results are suggested that human plasma contains two types of PLTP, the one is in active form and the other in inactive form. This is the reason for the discrepancy between PLTP mass and activity in human plasma.

**Gamma-LpE (γ-LpE) concentrations were regulated by cell membranes and phospholipids**

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Gamma-LpE, a spongimyelin-rich lipoprotein that contains apolipoprotein (apo) E as its only protein component, has been proposed to play a role in cellular cholesterol efflux. In order to further characterize the effect of cell membranes and phospholipids on the plasma γ-LpE concentration, we have separated γ-LpE by two-dimensional non-denaturing polyacylamide-gradient gel electrophoresis and detected its presence by immunoblotting with anti-apoE antibody. Incubation of native plasma for 30 minutes at 37°C decreased the concentration of γ-LpE by 80%. However the level of plasma γ-LpE is fully protected in the presence of fibroblasts or cultured human monocyte-macrophages but not erythrocytes. The loss of γ-LpE observed in isolated plasma was not dependent on lecithin: cholesterol acyltransferase (LCAT) activity.

To investigate the importance of phospholipids in the regulation of this particle, dimyristoyl phosphatidylcholine (DMPC) and bovine brain spongimyelin (BBSM) multilamellar vesicles were incubated with normal human plasma from subjects with apoE3/E3 phenotype. DMPC (0.5 mg/ml plasma), increased the concentration of plasma γ-LpE by +80%, in a dose-dependent manner, whereas addition of BBSM had no effect on γ-LpE. DMPC plasma showed an increased efflux (~50%). HDL-deficient plasma from a subject carrying an ABC1 gene mutation, enriched with DMPC showed no increase.
in γ-LpE level. These results demonstrate that: 1) in vitro concentration of γ-LpE is directly dependent on its interaction with competent peripheral cells; 2) enrichment of plasma with DMPC increased the level of plasma γ-LpE; 3) DMPC vesicles displace apoE from apoE-containing apoA-I to HDL-LpE particles. Enrichment of plasma with phospholipids to form new γ-LpE particles and promote reverse cholesterol transport, may have potential therapeutic applications in the treatment of atherosclerosis.

**WeP16:W22**
Effects of fenofibrate on the HDL system in hyperpaliproteinemia

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**Objectives:** Low plasma levels of high density lipoproteins (HDL) are associated with increased risk of cardiovascular disease. The protective effect of HDL seems mainly due to their major functions in reverse cholesterol transport (RCT), the process by which excess cholesterol in peripheral tissues is transported to the liver for excretion. In this study, we evaluated the effects of fenofibrate on RCT in patients with hyperpaliproteinemia (HA) (HDL < 10 g/dL).

**Methods:** The study was a randomized double-blind crossover trial: 28 HA patients, 14 hypertriglyceridemic (HTG) and 14 normotriglyceridemic (NTG) were randomized to receive comirazonized fenofibrate (200 mg/die) or placebo for 8 weeks in a different sequence.

**Results:** Fenofibrate significantly lowered plasma TG levels, by 45% in the HTG group and by 13% in the NTG group. Total and LDL cholesterol decreased in both groups, with a more significant reduction in the NTG. HDL-cholesterol levels increased remarkably, by 25% in HTG and by 15% in NTG, mainly because of an increase of the HDL3 fraction. ApoA-II concentrations increased by 16% in both groups with parallel changes in LpA:A-II levels. No changes in apoA-I and LpA-I levels were observed. The cholesterol esterification rate and plasma CAT levels did not change in both groups; the cholesteryl ester transfer rate decreased by 30% in the HTG, and did not change in the NTG.

**Conclusions:** Fenofibrate significantly increases plasma HDL in HA patients. The effect is direct, due to an increased production of apoA-II; in the HTG an additional rise of HDL due to enhanced calcium binding of TG-rich particles is observed. Fenofibrate is therefore indicated to raise plasma HDL in individuals with isolated hyperpaliproteinemia.

**WeP17:W22**
Low apolipoprotein A-IV plasma concentrations in men with coronary artery disease

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**Objective:** Experimental in vitro and in vivo studies favor apoA-IV to be protective against the development of atherosclerosis. Mice that overexpress either human or mouse apoA-IV demonstrated a significant reduction of aortic atherosclerotic lesions compared to control mice. Data on apoA-IV plasma concentrations and coronary artery disease (CAD) in humans are lacking.

**Methods:** We determined in two independent case-control studies of a Caucasian and an Asian Indian population whether apoA-IV plasma concentrations are related to the presence of angiographically assessed CAD.

**Results:** Plasma apoA-IV levels were significantly lower in 114 male Caucasian subjects with angiographically defined CAD when compared to 114 age-adjusted male controls (10.2 ± 3.8 mg/dL vs. 15.1 ± 4.0 mg/dL, p < 0.001). Logistic regression analysis indicated that the association between apoA-IV levels and CAD was independent of the HDL cholesterol and triglyceride concentrations. The inverse relationship between plasma levels of apoA-IV and the presence of CAD was confirmed in an independent sample of 58 male Asian Indians with angiographically documented CAD and 68 age-matched controls.

**Conclusions:** The results of this cross-sectional study demonstrate for the first time an association between low apoA-IV concentrations and CAD in humans and suggest that apoA-IV may play an anti-atherogenic role also in humans.

**WeP18:W22**
Oxidized HDL reduces human THP-1 macrophage membrane fluidity contributing to an impaired free cholesterol efflux

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It is known that oxidized HDL (ox-HDL) reduces the efflux of free cholesterol from cells, but which mechanisms are implicated is not completely known. In this study we investigate whether this effect is due to a decrease in cell membrane fluidity and if products of PUFA oxidation such as aldehydes could play a role in the reduction of free cholesterol efflux from cells. Oxidation of HDL was assessed by conjugated dienes formation. HDL in the maximum rate (MaR) and maximum diene production (MaD) phases became a less fluid particles compared to that of the lag phase (LaP) and native HDL (nHDL) (p < 0.05). THP-1 macrophages incubated with HDL in the MaR and MaD phases presented less fluid membranes than those incubated with HDL in LaP and native HDL (p < 0.05). Conversely, fluidity was reduced not only in ox-HDL but also in the cell membranes exposed to ox-HDL. A decrease in free cholesterol efflux from THP-1 macrophages was observed in the MaR and MaD phases (16% and 13%, respectively) compared to that of the LaP and native HDL (nHDL) (p < 0.05). On the other hand, incubation with apoA-IV increased the fluidity of membranes, such as 2-hexanoyl, hexanoyl, 2-octanoyl and 2,4-decadienoyl resulted in a decrease in free cholesterol efflux when compared to non treated cells (p < 0.05). In conclusion, the oxidation process of HDL leads to a loss of the HDL fluidity associated with a reduction in macrophage membrane fluidity that contributes to a reduced cholesterol efflux from cells to HDL.

**WeP19:W22**
Involvement of decreased expression of a member of Rho GTPases family, CDC42Hs, in the pathophysiology of tangier disease

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**Objective:** Tangier disease (TD) is a rare, autosomal recessive disorder of lipid and lipoprotein metabolism, characterized by the severe reduction of plasma high density lipoprotein (HDL)-cholesterol. Because many previous studies showed impaired cholesterol efflux and abnormal intracellular trafficking of HDL in TD cells, TD is thought to be a model for the impairment of the first step of reverse cholesterol transport, a major protective system against atherosclerosis.

**Methods:** To know the molecular basis of TD, we performed the approach of cDNA subtraction technique using cells from two unrelated TD patients. Results: cDNA subtraction technique has revealed a decreased expression of a member of Rho GTPases family, Cdc42Hs, in TD cells. We have found that the expression of this molecule was decreased in both macrophages and fibroblasts from TD patients and that abnormal actin cytoskeletons and low DNA synthesis were observed in these cells. In order to correlate these observations as well as impaired cholesterol efflux, we have done the transfection experiments using dominant active or dominant negative mutant of Cdc42. Transient transfection of dominant negative form of Cdc42Hs (N17Cdc42Hs) induced TD-like morphology in normal fibroblasts. The introduction of dominant negative Cdc42Hs into MDCK and COS-7 cells caused the decrease in HDL-mediated cholesterol efflux, irrespective of the expression of scavenger receptor class B type I (SR-BI) in the cells tested.

**Conclusions:** These findings clearly show that the decreased expression of Cdc42Hs is involved in the pathophysiology of TD. The present study has raised the possibility that the small G protein, Cdc42Hs, may play a role in cholesterol efflux.

**WeP20:W22**
Phospholipid transfer protein (PLTP) and coronary artery calcification in type 1 diabetic and non-diabetic subjects

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**Objectives:** i) Plasma PLTP activity was compared in type 1 patients (DM) and non-diabetic subjects (non-DM) without renal failure and ii) the association between coronary artery calcification (CAC), a measure of atherosclerosis, and PLTP activity was examined.
**Methods:** Electron beam CT scanning was used to quantify CAC in 198 DM patients and 198 non-DM subjects (age 30–55 yrs; 50% female). PLTP activity was measured as an estimate of its mass using an exogenous substrate assay. Five non-diabetic subjects with triglyceridem (TG) > 6 mmol/L were excluded. All analyses were adjusted for age and sex.

**Results:** TG were positively associated with PLTP activity (partial correlation coefficient \( r = 0.11, p = 0.04, \) adjusted for diabetes). TG were 0.2 mmol/L lower in DM than non-DM subjects (\( p = 0.001 \)). Despite this, DM patients had significantly elevated PLTP activity (+15 arbitrary units [AU], SD 19, \( p < 0.001 \)). In those with TG above the median (1.1 mmol/L), PLTP activity was not associated with calcification (Odds ratio [OR] for any CAC per 10 AU increase in PLTP = 0.94, 95% CI 0.8–1.1, \( p = 0.5, \) adjusted for age sex and DM). However, in those with below median TG, higher PLTP activity was associated with increased CAC (OR = 1.3, 95% CI 1.1–1.6, \( p = 0.004 \) for the TG-PLTP interaction). This association between PLTP activity and CAC was independent of HDL-cholesterol and LDL-cholesterol (OR = 1.3 on adjustment, \( p = 0.004 \) for the interaction).

**Conclusions:** Compared to non-DM subjects, DM patients have elevated PLTP activity despite having lower TG. In the absence of raised TG, elevated PLTP activity is associated with coronary artery calcification. These data suggest that PLTP activity could be involved in atherosclerosis in diabetes.

**WeP21:W22** Relationship between phospholipid transfer and HDL conversion activities of human plasma PLTP

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Phospholipid transfer protein (PLTP) is an important factor participating in HDL metabolism. It transfers phospholipids between lipoprotein particles and mediates conversion of HDL into larger and smaller particles. Our objective was to determine whether the phospholipid transfer and conversion are two distinct activities of PLTP, or whether PL transfer is a prerequisite for the conversion process.

Two separate approaches were used to resolve the relationship between the PL transfer and conversion activities: Chemical modification of purified PLTP and the use of recombinant PLTP containing point mutations. The conversion activity was assessed using native gradient gel electrophoresis and the release of apo-AI upon incubation of PLTP with radioactively labelled HDL.

Chemical modification of PLTP with either diethylpyrocarbonate (DEPC) or Thimerosal resulted in dose-dependent inhibition of PL transfer. In parallel, the ability to cause HDL conversion was diminished. Furthermore, heat inactivation of PLTP at +58°C resulted in inhibition of both activities. Two of the previously described PLTP mutants (J. Huuskoenen et al. J Lipid Res. 1999, 6: 1123–1130, L1906W and F464E, which affect the negative-charge lipid-binding pocket, were produced in a baculovirus/insect cell system as histidine-tagged fusion proteins. The purified proteins exhibited 25% of the PL transfer activity compared to wild-type PLTP. Both of the mutant proteins also displayed severely impaired HDL conversion activity.

The results suggest that the phospholipid transfer and conversion activities reside in the same structural motifs of the PLTP molecule. The data are in agreement with the model where the phospholipid transfer precedes, and is required for the HDL conversion process.

**WeP22:W22** Lipoprotein lipase and hepatic lipase mediate an increase in HDL selective cholesterol ester uptake in BHK cells independent of scavenger receptor BI (SR-BI)

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**Objective:** We have shown that lipoprotein lipase (LPL) and hepatic lipase (HL) mediate an increase in HDL selective cholesterol ester (CE) uptake in cultured cells which is independent of lipolysis. Now we investigated whether SR-BI is involved in this lipase-mediated increase in selective CE uptake.

**Methods:** Baby hamster kidney (BHK) cells were stably transfected with pBS.C-3-BRI in the full-length bidirectional DNA or pBK-CMV (control). In addition, both cell types were stably transfected with pCDNA 3.1-HL containing HL-cDNA. HDL, \( d = 1.25–1.21 \) g/ml was labeled with \([^{14}C] \) cholesterylester (CE). HDL was isolated from milk and HDL was prepared from cells secrete HL. Cells incubated (37°C, 4 h) in medium containing HDL and liposomes were absent or present.

**Results:** BHK were cotransfected with no detectable or high SR-BI expression as determined by specific immunoblot. HDL selective CE uptake (1\( ^{14} \)C)CE,\( ^{125} \)I) was 10-fold increased due to SR-BI expression. Addition of exogenous LPL or HL to the medium containing HDL stimulated selective uptake in both cell types. However, the absolute increase in selective CE uptake mediated by 1\( ^{4} \)C-labeled CE was quantitatively very similar in cells with no or high SR-BI expression. This was observed over a wide range of LPL, HL, and HDL concentrations. PCNA 3.1-HL transfected cells with no or high SR-BI expression secreted HL in the medium. Endogenous expression and secretion of HL in the medium stimulated selective CE uptake to an identical extent in cells with no or high SR-BI expression.

**Conclusions:** The LPL- and HL-mediated increase in selective CE uptake in BHK cells is independent from SR-BI. Therefore besides SR-BI, alternative cellular mechanisms may contribute to the lipase-induced increase in HDL selective CE uptake.

**WeP23:W22** Dynamic changes in mouse serum lipoprotein profile induced by transiently expressed human phospholipid transfer protein

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The human plasma phospholipid transfer protein (PLTP) plays an important role in the regulation of plasma HDL levels and governs the distribution of HDL subclasses. PLTP facilitates the transfer of phospholipids among lipoproteins and it is capable of inducing HDL conversion into large and small particles. In the present study adenosine mediated overexpression of human PLTP in mice was employed to investigate i) the distribution of human PLTP mass and activity in serum and ii) the correlation between PLTP activity and the generation of preβ-mobil HDL particles. Fractionation of mouse sera by size-exclusion chromatography indicated that PLTP exists as two distinct forms with high or low specific activity. PLTP overexpression at day 5 post-injection causes a drastic depletion of the HDL fraction followed by a replenishment when PLTP activity declines. During the replenishment process the lipoprotein profile displayed transient populations of apoA-IV and apoE-rich particles in the LDL size range as well as small apoA-II only particles. Analysis of serum preβ-HDL by crossed immunoelectrophoresis demonstrated a clear positive correlation (\( r = 0.95, p < 0.001 \)) between serum PLTP activity and the ability to form preβ-HDL. In conclusion, the present results provide novel evidence that PLTP is an important regulator of HDL metabolism and suggest that one function of PLTP in vivo is to generate preβ-HDL during the HDL conversion process.

**WeP24:W22** Decreased SR-BI expression by CETP antisense treatment in HepG2 cells

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**Objective:** To examine a possibility of coordinated changes in SR-BI during CETP inhibition, we determined the changes of SR-BI by CETP antisense treatment in HepG2 cells.

**Methods:** Antisense treatment was done with compounds of CETP antisense or sense oligonucleotides (ODNs) and a polycationic liposome carrier (THF-20) for 24 hours in HepG2 cells. The RNA and proteins from cells were collected. Total RNA was reverse transcribed into cDNA and amplified by PCR. The change of protein was determined using western blotting.

**Results:** The amount of CETP and SR-BI mRNA were measured by RT-PCR and expressed as a ratio CETP or SR-BI RT-PCR products to G3PDH RT-PCR products using densitometry, the mean values were (0.400 ± 0.009 (mean ± SD), 1.110 ± 0.042 in the sense group, 0.375 ± 0.007, 1.099 ± 0.044 in the control group, and 0.121 ± 0.001, 0.542 ± 0.019 (p < 0.0001)) in the antisense group, respectively (n = 3). The SR-BI western blotting showed a significant reduction (52%) in antisense CETP cells compared with sense and control cells.

**Conclusions:** Inhibition of CETP expression significantly decreased SR-BI expression in HepG2 cells. These findings suggest a role of SR-BI down-regulation as a cause of elevated HDL in patients with CETP deficiency.
WeP25:W22 Release of cholesteryl esters from macrophage foam cells in culture

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Thioglycollate-elicted peritoneal macrophages from cholesterol-fed, atherosclerosis-susceptible White Carneau pigeons contain high concentrations of free (FC) and esterified (EC) cholesterol (200+ ~ 1000 μg/mg cell protein), similar to what has been reported for macrophage foam cells from atherosclerotic plaques. When incubated in culture medium containing apo HDL (phosphophytidylinositol) (APC) vesicles for 48 hrs. there was a stimulation of cholesteryl efflux with up to 50% of the cellular cholesterol appearing in the culture medium. Of the cholesterol in the medium, 20-40% was EC. Both the FC and EC in the medium appeared to be associated with the APC vesicles based on their ability to pass through a 0.45 um filter. The appearance of EC in the medium could not be explained by gross cell death, based on changes in cell protein, or by esterification of FC in the culture medium via LCAT secreted by the macrophages. These findings were unexpected since most studies of cholesteryl efflux from cells show that EC must first be hydrolyzed to FC prior to efflux. The majority of the published studies, however, have been done with cells either not loaded with EC or loaded to levels much less than seen in these macrophage foam cells. Although the mechanism by which macrophages release EC is unclear, it may represent a unique property of macrophage foam cells that could play an important role in the pathogenesis of atherosclerosis.

WeP26:W22 Cyclodextrins acting as sinks and shuttles differentially mobilise free and esterified cholesteryl from human foam cell macrophages

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Objective: To investigate whether cyclodextrins (CD) with high cholesterol acceptor activity as sinks, or low cholesterol acceptor activity as shuttles to phospholipid vesicles (PV), deplete esterified cholesterol (EC) and free cholesterol (C) from primary human foam cell macrophages (HMFC) with equivalent efficiency.

Methods: HMFC were generated by incubating monocyte derived macrophages with acetylated low density lipoprotein radiolabelled with [3H]-C. HMFC were incubated for up to 24 hrs in medium containing PV (200 μg/ml), CD (1.0 mg/ml) with differing affinity for C (low affinity hydroxpropyl-CD, hp-CD, or high affinity trimethyl-CD, tm-CD), and hp-CD or tm-CD with PV. Cells and media were analysed for C and EC by HPLC, TLC and radiometric detection.

Results: Within 2 hrs, tm-CD depleted C to 42.3 ± 10% of HMFC in control medium RPMI, but did not deplete even after 24 hrs (95.0 ± 6.1% of control), hp-CD caused minimal C efflux on its own, but with PV it depleted C to 36.7 ± 5.0% and CE to 35.2 ± 4.9% of control at 24 hrs, tm-CD with PV was also ineffective at depleting EC (102 ± 5.0% of control). In the absence of CE, tm-CD depleted C and CE to <0.1%.

Conclusions: In HMFC, synergistic efflux with CD and PV achieves superior depletion of CE than does efflux to high affinity CD alone.

WeP27:W22 Stimulation of apo E secretion from human foam cell macrophages by apolipoprotein A-I mutants

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Objective: We have recently identified that apolipoprotein A-I (apo A-I) induces secretion of apo E from foam cell macrophages (J. Biol. Chem. 1999, 274, 27925), and that proapo A-I mutants achieve differential cholesterol (C) and phospholipid efflux (J. Biol. Chem. 1999, 271, 33277). This study has investigated whether specific sequences of apo A-I mediate apo E secretion from human foam cell macrophages.

Methods: Primary human monocyte-derived macrophages were incubated with acetylated low density lipoprotein radiolabelled with [3H]-C to generate HMFC, and HMFC then incubated in RPMI and delipidated proapo A-I mutants (25 μg/ml). APO-A-I mutants were expressed as fusion proteins in an E. Coli/pGEX vector system with whole pro apo A-I (~243) and truncated forms (~222) and (~6-150) used herein. Lipid analyses in cells and media were performed by HPLC, TLC and radiometric detection of [3H]-C, and apo E quantified by Western blotting.

Results: Mutants ~6-150, ~243, ~222 respectively achieved 3.5-fold, 2.8-fold and 2.0-fold the C efflux achieved in controls at 8 hrs. All 3 mutants stimulated time-dependent apo E secretion, respectively 5.0-fold, 3.0-fold and 3.0-fold the apo E secretion of controls at 8 hrs. Interestingly, ~6-243 stimulated the initial secretion of a lower MW form of apo E which did not accumulate further after 2 hrs.

Conclusions: In HMFC, apo A-I mutants induce C efflux and overall apo E secretion approximately in parallel. Residues 222-243 may specifically stimulate the secretion of lower MW, presumably less glycosylated, apo E.

WeP28:W22 Mechanisms of 7-ketocholesterol-mediated inhibition of cholesteryl efflux from human foam cell macrophages


Macrophage foam cells are early and characteristic components of atherosclerotic lesions. They contain large cytoplasmic deposits of esterified cholesterol, which presumably results from a disparity between the rates of uptake, synthesis and export of cholesterol. Generally cells are able to closely control their cholesterol content through regulation of lipoprotein uptake and of cholesterol synthesis. In addition, most cells efficiently export excess free cholesterol to appropriate extracellular acceptors such as high-density lipoproteins (HDL), or its major protein component, apolipoprotein A-I (apo A-I). Addition of such acceptors to cells in vitro leads to a progressive release of free cholesterol and concomitant hydrolysis and depletion of intracellular cholesteryl esters. Human foam cells contain a range of oxidised cholesterol (oxysterols), of which 7-ketocholesterol (7KC) is a major component. The effect of 7KC on sterol efflux to apolipoprotein A-I (apo A-I) was examined in human monocyte-derived macrophages (HMDM) loaded with cholesterol only or with a combination of cholesterol and 7KC. The amount of 7KC released was within the range found in human lesion foam cells. Efflux of cholesterol from HMDMs was stimulated by apoA-1, but was inhibited to basal levels when 7KC was present. Only 7% of cell phospholipid was exported to apoA-I from cells loaded with 7KC compared with 25% from AcL DL-loaded cells. ApoA-I did not stimulate efflux of 7KC. Similar but less profound effects were also found in comparable murine foam cells. These data suggest that 7KC may contribute to atherogenesis by its ability to inhibit reverse cholesterol transport in human macrophage foam cells. Likely mechanisms under investigation include inhibition of plasma membrane cholesterol and phospholipid desorption to apoA-I and inhibition of phospholipid synthesis by 7KC.

WeP29:W22 Clinical and molecular characteristics of homozygous CETP deficiency

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Objective: Relationship between CETP deficiency and atherosclerosis remains controversial and appears dependent on serum HDL-C levels.

Methods: By PCR genotyping and/or plasma CETP measurement by ELISA in hyperalphaproteinemic subjects (n = 1000, HDL-C >> 80 mg/dl), 44 cases (18 men and 26 women) with homozygous CETP deficiency from 35 unrelated families (age 22-89 yrs, 61 yr ± 13 [SD]) were identified, and 11 cases of them were followed-up.

Results: Subjects with homozygous CETP deficiency had BMI 21.4 kg/m² ± 3.1; CHOL 267 mg/dl ± 40; TG 99 ± 63; HDL-C 147 ± 42, apo A-I 228 ± 53, A-I 44 ± 15, B 74 ± 53, CII 7.5 ± 5.5, C-III 20.9 ± 11.5, E 10.7 ± 5.2, and Lp(a) 11.9 ± 9.1. There were 30 complete CETP deficiency caused by 27 true homozygotes of interon 14 G+(+)-to-A (interon14A) mutation (mean HDL-C 167 mg/dl), 1 compound heterozygote of interon 14A and interon 14 T (+) insertion (HDL-C 106), 1 compound heterozygote of interon 14A and unknown mutation (HDL-C 223), and 1 unknown mutation (HDL-C 130). There were 14 partial CETP deficiency caused by 7 compound heterozygotes of interon 14A and D442G (HDL-C 124), and 7 true homozygotes of D442G (HDL-C 93). Plasma CETP levels in interon 14A/D442G and D442G homozygotes were 0.7 μm/l and 0.9, respectively. Hypertension (SBP ≥ 160 and/or DBP ≥ 95 mmHg or drug treatment) was found in 8 cases. Cerebral infarction was found in 2 cases (ages of 50 and 66), polycystic kidney disease.
WeP30:W22
Inhibition of lecithin: Cholesterol acyltransferase by cholesterol oxides
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Objective: To investigate the effect of cholesterol oxides on esterification of cholesterol by LCAT.

Methods: HDL particles enriched with increasing concentration of cholesterol oxides were incubated with fresh plasma as source of LCAT. The esterification of cholesterol and cholesterol oxides was followed by measuring the consumption of the respective free steroid and oxysterols. Measurements of cholesterol and cholesterol oxides were done by gas chromatography with flame ionization detection.

Results: All the studied cholesterol oxides were esterified by LCAT after incorporation into HDL particle, competing with cholesterol by LCAT-mediated esterification. Cholesterol esterification by LCAT was inversely related to the cholesterol oxide concentration in HDL particles. Kinetic studies with cholesterol, 3β, 5α, 6β-triol and 5-cholesten-3β-25dion (25-hydroxycholesterol) showed a non-competitive inhibition with a Ki of 103 and 15.02 ng/ml, respectively. This study shows marked differences for inhibition parameters and apparent K among the different cholesterol oxides.

Conclusions: Data suggest that cholesterol esterification by LCAT is inhibited in the cholesterol oxide-enriched HDL particles which could disturb the reverse cholesterol transport. In contrast, the esterification of cholesterol oxides by LCAT may have an anti-atherogenic effect considering that this step may increase theatabolism of these atherogenic compounds by enhancing their liver uptake either by direct HDL removal or by the previous transfer of the cholesteryl oxide esters to apo, B-containing lipoproteins followed by their hepatic catabolism. Supported by FAPESP

WeP31:W22
Lipases and transfer proteins in patients with hyperalphalipoproteinemia
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Objective: To determine in Brazilian hyperalphalipoproteinemic (HALP) patients the activities of factors that modulate the metabolism of HDL: lipoprotein lipase (LPL), hepatic lipase (HL), cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP).

Methods: Ninety-five volunteers were defined by their HDL-chol as controls (CTRL = 50, below 68 mg/dl) and HALP (n = 60, equal and above 68 mg/dl), the 90th percentile for a local population. HL. (post-heparin plasma), CETP and PLTP were measured by exogenous radiometric methods using respectively triolein emulsion, 14C-CE-HDL and phospholipid liposomes as substrates.

Results: HL was significantly lower (p = 0.04) in HALP, but no differences were found for LPL, CETP and PLTP (Mann-Whitney). The scatterplots are shown below:

Conclusions: The hyperalphalipoproteinemia was explained, at least in part, by the lower plasma levels of HL in this population.

WeP32:W22
LCAT-dependent cholesterol esterification rate is slower in in vitro glycolyzed HDL but not altered in plasma HDL drawn from NIDDM patients

Objective: Alterations in the reverse cholesterol transport has been related to glycemia of plasma lipoproteins. Present study investigates the influence of HDL glycation on the in vitro activity of the enzyme lecithin cholesterol acyltransferase (LCAT).

Methods: HDL obtained from normal controls (N) and poorly controlled NIDDM (D) patients was separated by ultracentrifugation, and plasma d > 1.14 g/ml from N were used as the source of LCAT. Reconstituted HDL (rec HDL) from N were prepared by sonication of delipidated HDL previously submitted to selective glycation of the lipid and protein components separately, or both together, by exposure to a concentrated glucose solution. RecHDL as well as intact HDL from N and D were then labeled with 14C-unesterified cholesterol (14C-C) and incubated with LCAT at 37°C along time. 14C-ester and 14C-C were isolated after TLC and the percent of esterification was calculated.

Results: In vitro glycation of the intact HDL N particle, as well as of its protein component, impaired the activity of plasma LCAT. However, plasma LCAT esterified equally well 14C-C HDL that had been drawn from N (n = 11) and from D patients (n = 12). In spite of the elevated level of HBAlc in D, there was no difference in the level of HDL glycation between N and D subjects.

Conclusion: In vitro HDL particle glycation rate impairs the LCAT-dependent 14C-esterification rate. However, this rate is not modified in HDL D as compared to HDL N plasma, possibly because the faster removal rate of HDL D brings on a low plasma glyced HDL level.

WeP33:W22
The stimulation of cholesterol efflux by cpt-cAMP and free apo A-I from various cell lines
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Studies have shown that unassociated, lipid-free apo A-I stimulates the release of cholesterol and phospholipid from cells. APT-Binding Cassette I (ABC1) is implicated in this release, providing evidence that it is critical in the formation of HDL. Specific binding of apo A-I is upregulated by cAMP or enrichment with cholesterol. In this study, we determined the kinetics of cholesterol efflux from J774 mouse macrophages with and without exposure to cAMP. Uptake is correlated to increased cholesterol efflux in a dose- and time-dependent manner with efflux first detectable 2-4 hours after treatment with cAMP. Efflux is upregulated by cholesterol enrichment of the same cell line. Stimulated efflux exhibits specificity for apo A-I, HDL, apo E and apo C as acceptors, but not for small unilamellar vesicles, bile acid micelles, or cyclodextrins, demonstrating that a protein-specific interaction is required. We have extended this study to 13 other cell lines under a standardized protocol, including fibroblasts (normal, transformed, and stiitheterocellular), human and murine macrophages, hepatocytes, kidney cells, ovarian cells, and human enterocytes. Only J774 land elicited mouse macrophages show a significant increase in efflux with treatment. Apo A-I-stimulated efflux was detected from the majority of cell lines examined, independent of treatment. Apo A-I-stimulated efflux varied greatly among cell types, both in the percent and calculated mass of sterol released. Other data are consistent with upregulation of ABC1 by cAMP and cholesterol enrichment.

WeP34:W22
Cholesteryl ester transfer protein activity and hyperalphalipoproteinemia in Chinese
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Objective: Cholesteryl ester transfer protein (CETP) mediates the transfer of neutral lipids between lipoproteins and plays a significant role in HDL metabo-
lism. CETP deficiency is an important cause of hyperalphalipoproteinaemia in Japanese. We have measured CETP activity in healthy subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study (a territory-wide community-based health survey) whose HDL was >90th percentile to determine whether changes in CETP activity played a role in hyperalphalipoproteinaemia in Chinese.

Methods: Plasma CETP activity of 60 male and 70 female subjects with hyperalphalipoproteinaemia was compared with that of controls with normal HDL matched for age, sex, and body mass index. All subjects were non-smokers. Plasma CETP activity was determined by an isotopic assay measuring the transfer of [1H]cholesterol oleate from radiolabelled HDL to LDL/VLDL fraction.

Results: The mean HDL level was 1.95 ± 0.18 and 1.99 ± 0.21 mmol/L in male and female subjects with hyperalphalipoproteinaemia and 1.23 ± 0.22 and 1.30 ± 0.22 mmol/L in male and female controls respectively. Plasma CETP activity was significantly lower in subjects with hyperalphalipoproteinaemia than that of their matched controls (male: 10.2 ± 4.8 vs 15.8 ± 5.4% transferred/μg C/4 h, p < 0.01; female: 10.9 ± 4.3 vs 17.7 ± 5.2, p < 0.01). Plasma CETP activity correlated with HDL level in both male and female subjects (r = -0.47, p < 0.01) and r = -0.55, p < 0.01 respectively).

Conclusion: Hyperalphalipoproteinaemia in Chinese subjects is associated with a reduction in plasma CETP activity.

WeP35:W22
Tyrosyl radical-oxyxidized HDL elevates plasma HDL in APOE-deficient mice
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Objectives: Tyrosyl radical-mediated cross-linking of apolipoprotein (apo) A1 to apoAII in HDL (TyroHDL) markedly enhances cholesterol removal from cultured cells by HDL, by increasing translocation of cholesterol available for esterification to a cell-surface pool available for efflux. We tested the hypothesis that TyroHDL would raise HDL by increasing the availability of lipids for removal by nascent HDL in vivo.

Methods: Ten-week old, chow-fed, apoE-deficient mice were injected twice weekly for 8 weeks with 0.15 mg apoE-free mouse HDL or TyroHDL. Blood for plasma total and HDL cholesterol was withdrawn when injected HDL levels were less than 1% of initial dose.

Results: ApoE-free mouse HDL oxidized by tyrosyl radical exhibited apoAII-AIIS crosslinks, and a markedly enhanced ability to deplete cellular cholesterol available for esterification by cultured fibroblasts. The residence times of intravenously injected mouse HDL and TyroHDL in apoE-deficient mice were identical. Endogenous HDL as percent of total plasma cholesterol increased by 54% at Day 10 of the study in TyroHDL-treated mice, compared to no change in HDL-treated mice. At Day 56, endogenous HDL levels were 150% higher than baseline levels in TyroHDL-treated mice, versus 60% higher in HDL-treated mice.

Conclusions: These results suggest the increase in efflux-available cholesterol induced by TyroHDL (and to a lesser extent HDL) also occurs in vivo, and results in a striking increase in endogenous HDL cholesterol levels in this animal model of atherothrombosis. This elevation in plasma HDL would be expected to increase all of the cardioprotective actions of HDL, including reverse cholesterol transport.

WeP36:W22
Inhibition of ACAT enhances APOA-I-Mediated Cellular cholesterol efflux by increasing the APOA-I/CELL interaction
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Objective: Removal of cellular cholesterol by HDL particles occurs by two distinct pathways: a non-specific diffusion-mediated mechanism, and a specific pathway by lipid-free apolipoprotein (apo) A-I to remove cell lipid to generate HDL. To characterize this specific pathway, we examined the effect of ACAT inhibition on the apoAI-mediated free cholesterol (FC) and phosphatidyl (PL) efflux.

Methods: Acetylated-cholesterol-induced mouse foam macrophages were incubated with apoA-I in the presence or absence of an ACAT inhibitor. The medium was collected to measure the mass of FC and PL.

Results: Dose-dependent and time-dependent increase of FC and PL efflux by lipid-free apoA-I (a specific pathway) was observed by the ACAT inhibition. It caused the increase of intracellular FC content and enhanced the apoAI-mediated FC and PL efflux by 1.9-fold, 1.8-fold, respectively. Simultaneous treatment of the macrophages with a protein synthesis inhibitor, cycloheximide, cancelled the ACAT inhibitor-induced enhancement of the FC and PL efflux. On the other hand, inhibition of ACAT un influenced the non-specific FC efflux.

Conclusion: These results demonstrated that the increase of the intracellular FC pool by the ACAT inhibition enhanced only the apoAI-mediated specific pathway. The possible mechanism of the enhancement may involve the expression of an unidentified protein(s) to mediate apoAI-cell interaction to generate HDL.

WeP37:W22
Effects of apoE polymorphism on its stability and interactions with lipids
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Apolipoprotein (apo) E is present on plasma lipoprotein particles and can bind with high affinity to the low-density lipoprotein (LDL) receptor thereby modulating cholesterol transport. The single polypeptide chain of apoE (299 residues, Mr = 34,200) contains two independently folded domains that are approximated by 2 thrombolytic fragments (residues 1-191 and 216-299). The 22kDa N-terminal fragment adopts a 4-helix bundle structural motif and contains the LDL receptor-binding domain (residues 136-150) of the protein, while the C-terminal 12kDa fragment represents the major lipid-binding region of the protein. Examination of the crystal structures of the three common isoforms suggest that the presence of Cys at positions 112 and 158 in apoE2 allows tighter packing and stronger Van der Waals interactions in the vicinity of these residues. As these Cys residues are replaced by Arg residues (at position 158 in the case of apoE3 at both positions 158 and 112 in apoE4), defects appear in the packing of the helical bundle reducing the stability of the protein in an isoform-specific manner; this destabilization is consistent with results of thermal and Ghn HCl denaturation experiments. Rates of apoE association with DMPC were measured spectrophotometrically at 325 nm as a function of decrease in turbidity. In agreement with the results above, measured rates of solubilization of DMPC liposomes by the isoform N-terminal domain fragments, an event shown to be dependent on opening of the helical bundle, are in the order apoE2 < E3 < E4 at low concentration where self-association of the protein is avoided. Full-length apoE isoforms do not show any significant differences in rate of DMPC association, supporting the role of the C-terminal domain in mediating initial interaction. The variations in stabilities among the N-terminal domains of apoE2, 3 and 4 underlie some of the functional differences exhibited by these isoforms.
not of A-I rHDL to promote cholesterol efflux from macrophage foam cells. Moreover, incubation with chymase did abolish the high-affinity component of cholesterol efflux induced by blood plasma derived from C57BL/6 (background) or the A-I-KO mice. We found that chymase degrades apoA-I from the background plasma and depleted apoE from both the background and A-I-KO plasma. Interestingly, chymase modified the lipoprotein profile of the background plasma and made it to resemble that of the A-I-KO mouse plasma. Addition of either A-I rHDL or A-II rHDL to the background plasma or to the A-I-KO mouse plasma reduced the cholesterol-induced potential of the plasmas whether the plasmas had been pretreated with chymase or not. The cholesterol efflux-increasing effect of A-I rHDL was smaller if the particles had been pretreated with chymase. We conclude that proteolysis of plasma and A-I HDLs by mast cell chymase reduces their cholesterol acceptor function by degrading apoA-I and apoE but not apo-II contained in HDL particles. These results support the hypothesis that mast cells may block the initial steps of reverse cholesterol transport.

Objective: To determine if the HMG-CoA reductase inhibitor simvastatin has anti-inflammatory and anti-atherosclerotic activities. Methods: Simvastatin was tested in a classic model of inflammation, carrageenan-induced foot edema. To determine whether the anti-inflammatory activity of simvastatin might affect atherogenesis, we tested simvastatin using a minimal model that is resistant to lipid-lowering by this class of drugs, apoE deficient mice. Results: Simvastatin (10 mg/kg) administered orally to mice 1 hour prior to carrageenan injection significantly reduced the extent of foot edema by 48%, which was comparable to the effect of 3 mg/kg indomethacin. These results confirm previous reports that simvastatin has anti-inflammatory activity. In apoE-deficient mice, simvastatin (10 or 100 mg/kg for 6 weeks) did not alter plasma lipids or lipoprotein profile. Atherosclerosis was quantified by measuring aortic cholesterol content, expressed as mmol/mg wet weight of aorta. Aortas from control mice (n = 20) contained 56 ± 4 mmol of total cholesterol/mg, 38 ± 2 of free cholesterol, and 17 ± 2 of cholesteryl ester. Simvastatin at 100 mgp (n = 22) decreased these three parameters by 23%, 19% and 34% respectively. All of these effects were statistically significant (p < 0.02). Aortic cholesterol content in the 10 mg/kg group was intermediate between the control group and the 100 mg/kg group. Histology of the atherosclerotic lesions showed that simvastatin did not dramatically alter lesion composition. Conclusions: These data confirm the anti-inflammatory activity of simvastatin after oral dosing in mice and support the hypothesis that simvastatin has anti-atherosclerotic activity beyond its plasma cholesterol-lowering activity.

Objective: To examine the effects of minimally modified and native low density lipoproteins (MM-LDL and n-LDL) on adhesion to and migration across human endothelial cells in vitro of human mononuclear cells. Methods: Endothelial cells were treated with MM-LDL either prior to adhesion and transendothelial migration, or added simultaneously with mononuclear cells at the commencement of migration. Mononuclear cells were identified as monocytes (CD14+), T-cell (CD3+) subpopulations (CD4+ and CD8+), B-cells (CD19+), natural killer (NK) cells (CD3–CD16/CD56+) and CD4+ cells (NK cells and T-cell subsets). Expression of activation markers (CD25, CD45R0, HLA-DR) on T-cell subpopulations was also determined.

Objective: To study endothelial function and exposure to oxidized low density lipoproteins (LDL) in male lifelong smokers (n = 128) and nonsmokers (n = 33). In addition, we evaluated effects of α-tocopherol treatment on these variables in the smokers. Methods: As measures of endothelial function and exposure to oxidized LDL, we determined plasma levels of soluble intercellular adhesion molecule-1 (sICAM-1) and autoantibodies against oxidized LDL, respectively. The smokers received either capsules containing a daily dose of 400 IU (268 mg) dl-α-tocopherol or placebo capsules for 2 years. Results: Compared to non-smokers, plasma concentrations of total cholesterol, total triglycerides and LDL cholesterol in smokers were higher, and HDL cholesterol lower. Plasma levels of IgG but not IgM autoantibodies against oxidized LDL, and concentrations of sICAM-1 in smokers were elevated (27% and 40%, respectively). Autoantibody titers and sICAM-1 concentrations did not correlate with age or plasma lipids or lipoproteins. After treatment with α-tocopherol but not placebo in vitro oxidizability of LDL decreased, but plasma levels of autoantibodies and sICAM-1 did not change.

Conclusion: Male lifelong smokers, with only minor abnormalities in plasma lipids and lipoproteins, have elevated plasma levels of sICAM-1 and IgG autoantibodies against oxidized LDL, which do not normalize follow-
WeP4/W23

Comparison between incidence of helicobacter pylori infection in patients with and without coronary artery disease

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Objectives: The inflammatory genesis of atherosclerosis has been an object of discussion. The correlation between Chlamydia infection and development of atherosclerosis has been described. Aim of our study was to compare the frequency of Helicobacter pylori (HP) infection in patients with and without coronary artery disease (CAD).

Methods: 150 patients were included in the study: 75 in group A (45 male, 30 female, age 47 ± 14 years) with CAD and 75 in group B without CAD. All patients of group A were diagnosed invasively; 31 patients received coronary bypass surgery, 25 patients coronary balloon angioplasty and 19 patients were treated conservatively.

In patients of group B (49 male, 26 female, age 49 ± 12 years) CAD was excluded by coronary angiography. All patients were tested for HP-antibody-titers in serum (Westernblot); especially for vacuolating cytotoxicity antibody (Vac A) and cytotoxins associated Protease A-antibody (Cag A).

Results: 73% of the patients in group A were tested positive for HP-antibodies, 27% negative. 85% of the HP-positive patients showed antibodies for Vac A and Cag A. 34% of the patients in group B were tested positive for HP-antibodies, 66% negative. Of those patients tested positive 44% showed antibodies for Vac A and Cag A. We found significant differences in incidence of HP-infection in patients with CAD and without (p < 0.01). Especially the incidence of Vac A-antibodies and Cag A-antibodies was significantly higher in patients with CAD compared to patients without CAD (p < 0.01).

Conclusion: HP-infection seems to have an influence on the development of coronary artery disease.

WeP5/W23

In vivo electrotransfer of interleukin-10-encoding plasmid prevents diet-induced endothelial activation

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Background: Induction of the endothelial cell adhesion molecules, particularly vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1, is triggered by a variety of pathophysiologically relevant stimuli. This induction is under inflammatory control and is largely modulated by the activation of the nuclear transcription factor NF-κB. Interleukin (IL)-10 is a potent anti-inflammatory cytokine that is expressed in the atherosclerotic lesion and we have recently shown that it exerts a major protective role against the development of diet-induced atherosclerosis in C57BL/6 mice.

Objectives: To examine the role of IL-10 in endothelial NF-κB activation and expression of VCAM-1 and ICAM-1 in vivo.

Methods and Results: In this study, we showed basal activation of NF-κB and expression of VCAM-1 and ICAM-1 in the endothelial lining of lesion-prone areas of the aortic sinus of C57BL/6 mice. This constitutive endothelial activation was not different between C57BL/6 IL-10/+/− and IL-10−/− mice. Endothelial NF-κB activation and expression of VCAM-1 and ICAM-1 were markedly increased after 10 days on the atherogenic diet (n = 5 to 9, P < 0.001), and were similarly upregulated in both IL-10+/+ and IL-10−/− mice. However, endothelial NF-κB activation was significantly increased in IL-10-deficient mice at day 21 on the atherogenic diet (P < 0.05). In vivo transfer of an IL-10-encoding plasmid completely prevented diet-induced endothelial NF-κB activation and expression of VCAM-1 and ICAM-1 in both IL-10+/+ and IL-10−/− mice (n = 5 to 9, P < 0.01).

Conclusions: Our results indicate that IL-10 has a profound impact on diet-induced endothelial cell activation in vivo.

WeP6/W23

IFN-γ induces type II secretary phospholipase A2 gene in human arterial smooth muscle cells by stat-3 activation.

Requirement of cell differentiation for gene expression

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Objective: Immunohistochemistry data show type II secretory non-pancreatic phospholipase A2 (sPLA2) associated with smooth muscle cells and mainly with extracellular matrix in human atherosclerotic lesions. In the present study we investigated the regulation of sPLA2 expression in human arterial smooth muscle cells (HASMC).

Methods and Results: HASMC were isolated from human uterine and aortic arteries. SnPNA gene was induced after 3 to 14 days of culture in non-proliferative conditions. SnPNA2 was co-expressed with heavy caldesmon, a cytoskeleton protein, and p27, a G1 cyclin inhibitor, both characteristically expressed in differentiated cells. IFN-γ 50-500 Units/ml for 4 or 24 h induced a 3 to 5 fold increase in snPNA2 mRNA and cell secretion sustained for 48 h. IFN-γ-induced snPNA2 enzyme activity was measured in cell media and associated with cell membrane proteoglycans. IL-1β, TNF-α IL-6 and IL-10 antagonized the IFN-γ-induced expression of snPNA2. IL-1β and TNF-α for 4 h induced a significant but transient increase in the secretion of SnPNA2 without changes in mRNA levels. IFN-γ-induced snPNA2 gene expression involved STAT-3 activation. Different from HASMC, in HepG2 liver cells IL-6 but not IFN-γ increased snPNA2 mRNA and protein secretion.

Summary: cell differentiation prompts snPNA2 gene expression in HASMC, and its transcription and secretion can be further up or down regulated by different pro- or anti-inflammatory cytokines in a cell specific manner.

WeP7/W23

Increased levels of soluble markers of endothelial dysfunction in patients with atherosclerotic heart disease

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Objective: To assess the circulating levels of cellular adhesion molecules (sCAMs), possibly reflecting the inflammatory state of the endothelium in patients with coronary heart disease, compared to healthy controls.

Methods: Fasting venous blood samples were obtained from 197 patients with documented coronary heart disease (CHD), sampled at least 10 days after acute events, and from age-and sex-matched healthy controls (mean age 55 years, 18% women). Levels of soluble VCAM-1, ICAM-1, E-selectin and P-selectin were measured using commercial ELISA methods (R & D Systems Europe, UK).

Results: The results are presented as mean values (SD) of the measured sCAMs (ng/ml) in patients with CHD and healthy controls. The level of significance is given as 2-tailed p-values for the difference:

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<th>sCAM</th>
<th>CHD patients</th>
<th>Healthy controls</th>
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<tbody>
<tr>
<td>VCAM-1</td>
<td>636.7 (205.5)</td>
<td>536.0 (159.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>322.9 (133.1)</td>
<td>250.6 (73.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E-selectin</td>
<td>47.5 (21.8)</td>
<td>42.3 (15.1)</td>
<td>0.006</td>
</tr>
<tr>
<td>P-selectin</td>
<td>46.9 (15.4)</td>
<td>38.4 (11.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

As can be seen, the levels of all the variables measured were significantly higher in the CHD group when compared to healthy controls.

Conclusion: The present results seem to strengthen the assumption that soluble markers of endothelial dysfunction are indicators of atherosclerosis.

WeP8/W23

Cellular and humoral immune responses to heat shock protein-65 are both involved in promoting fatty-streak formation in LDL-receptor deficient mice

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Inflammatory processes appear to influence the progression of atherosclerosis. Immunization with HSP65 was previously shown to induce atherosclerosis in rabbits and to enhance fatty-streak formation in mice. However, it has not been demonstrated directly whether HSP65-reactive antibodies and lymphocytes are separately capable of influencing lesion formation. LDL-receptor deficient (LDL-RD) mice were immunized with HSP65 or control bovine serum albumin (BSA). Lymph-node cells, splenocytes and IgG were obtained from the immunized mice and transferred separately to 6 groups of syngeneic LDL-RD mice. Adoptive-transfer of HSP65-reactive T-lymphocytes increased fatty-streak formation in comparison with mice treated with BSA-primed cells. Similarly, transfer of splenocytes reactive with HSP65 led to enhanced fatty-streak generation as compared with mice injected with BSA-sensitized splenocytes. Repeated, intraperitoneal administration of anti-HSP65 IgG (every 10 days) enhanced fatty-streak formation in mice in comparison to their anti-BSA-IgG injected littersmates. Thus, antibodies and lymphocytes reactive to HSP65 promote fatty streak formation in mice,
A requisite role for IL-4 in the acceleration of fatty-streaks induced by heat shock protein 65 or mycobacterium tuberculosis

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Atherosclerotic lesions can be induced in rabbits and mice immunized with HSP65. In the current study, we investigated the role of IL-4 in the HSP65 and MT induced models, which exhibit inflammatory phenotype. Fatty streak formation in IL-4KO mice immunized with HSP65 or MT was significantly reduced when compared with lesions in WT C57BL/6 mice. However, when injected with control (HSP-free) adjuvant, no differences were evident in the lesion size between the WT and the IL-4KO mice. Anti-HSP65 antibody levels were reduced in the HSP65-immunized IL-4KO mice as compared with their WT littermates, whereas no differences were evident between the groups with respect to the primary cellular immune response to HSP65. Other than the absence of IL-4 in the KO mice, cytokine pattern IFN-g and IL-10 in Con-A primed splenocytes was similar between the groups. HSP65-prime inuaginal lymphocytes from IL-4KO mice immunized with HSP65 secreted higher levels of IFN-γ as compared with their WT controls. 12F15-LO (a lipid peroxidizing enzyme upregulated by IL-4) activity was not influenced by the immunization protocol employed by IL-4 disruption.

Thus, IL-4 may prove a principal cytokine in the progression of early inflammatory atherosclerotic lesions and may serve as a target for immunomodulation.

Human genes induced during monocyte/macrophage differentiation

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Objectives: To study which genes are induced during monocyte/macrophage differentiation.

Methods: Peritoneal blood mononuclear cells (PBMC) were isolated from normal human healthy volunteers. Monocyte from PBMC were isolated by anti-CD14 mics obseads method. Monocytes were stimulated in the medium containing granzyme macrophage colony stimulating factor (GM-CSF) for 7 days. Total RNA from these cells was isolated by direct lysis in RNAzol B. Poly (A)++ RNA was isolated using the FastTrac mRNA purification kit. Genes induced during monocyte/macrophage differentiation were studied by using oligonucleotide microarray (Genechip, Affymetrix).

Results: Among 6410 human genes studied, the level of mRNA for apolipoprotein E was most profoundly induced (fold increase, X229.0) followed by type IV collagenase (X174.5), cartilage Gp-39 protein (X95.3), apolipoprotein C-1 (X84.9), and so on. These data were correlated to Serial Analysis Gene Expression (SAGE) using the identical mRNA. In addition to these high induced genes, LXR-alpha (X59), Lipoprotein Lipse (X25), Cholesterol ester hydrolase (X21), Fatty acid binding protein (X8), apo CII (X13), which are evolved in lipid metabolism, are induced during differentiation.

Conclusions: These results suggested that some important genes associated with lipid metabolism were significantly induced during monocyte/macrophage differentiation, therefore, macrophage may play more important role during atherogenesis than supposed before.

Genomic structure of human orphan receptor LXR alpha and its upregulation during monocyte to macrophage differentiation

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Objectives: To identify the genomic structure of human LXR alpha (LXαa) gene, and compare its mRNA expression levels among several different tissues.

Methods: We determined the full cDNA length of LXαa and determined the promoter region using PCR amplification and a 5'-RACE kit. Oligonucleotide microarray (GeneshipTM, Affymetrix) and relative quantitative RT-PCR were used to determine the expression levels of several different tissues such as liver, brain, human umbilical vein endothelial cells, monocytes, monocytes derived macrophages and the adrenal gland.

Results: DNA microarray analysis showed that LXαa is most abundantly expressed in monocytes-derived-macrophages which were stimulated with granulocyte macrophage colony stimulating factor (GM-CSF) and differentiated into macrophages. The mRNA level is about 10 fold higher than that in the liver. Non-stimulated monocytes did not express LXαa. We have verified the results by relative quantitative RT-PCR. The genomic structure of human LXαa consists of eleven exons and promoter analysis of human LXαa gene indicated the presence of conserved binding sites for myeloid zinc finger protein 1.

Conclusions: LXαa is induced during monocytes to macrophage differentiation and is most abundantly expressed in macrophages among the human tissues studied, which suggests it may be related to foam cell formation.

Inhibition of endothelial cell adhesion molecule expression by recombinant high-density lipoproteins: Determinants and characteristics


Objectives: To study, (i) the influence of recombinant high-density lipoprotein (rHDL) phosphatidylcholine (PC) composition on their ability to inhibit tumour necrosis factor (TNF-α) induced vascular cell adhesion molecule (VCAM-1) expression in human umbilical vein endothelial cells (HUVECs) and (ii) the time course of this rHDL-mediated inhibition.

Methods: rHDL containing apolipoprotein (apo) A-I as the sole protein and PC as the sole lipid were prepared (1:100 molar ratio respectively). The PC contained palmitoyl- in the sn-1 position and either palmitoyl-, oleoyl-, docosahexaenoyl-, arachidonyl- or linoleoyl- in the sn-2 position (DPPC, POPC, DPPC, POPC, PAPC or PLPC respectively). rHDL were also prepared with apoA-I and mixtures of PLPC and POPC. HUVECS were pre-incubated with the rHDL for 16 h (unless stated) before removal of the rHDL and stimulation of the cells with TNF-α. VCAM-1 expression was determined 5 h post-TNF-α.

Results: (i) rPLPC rHDL were more effective inhibitors than rHDL containing either PAPC, DPPC, POPC or DPPC (% inhibition at 16 μg apoA-I, 53, 33, 21, 10 and 0 respectively). (ii) PLPC and POPC rHDL had comparable binding to the cell surface. (iii) Increasing the molar ratio of POPC to PLPC in rHDL containing both species reduced their inhibitory activity. (iv) Pre-incubation of HUVECs with PLPC rHDL was necessary to achieve maximal inhibition which occurred after 8 h.

Conclusions: Inhibition of TNF-α induced VCAM-1 expression by rHDL is dependent on (i) rHDL PC composition and (ii) pre-incubation of the cells with rHDL. Differential inhibitory activity cannot be explained by differential binding of rHDL to HUVECs. These results suggest that transfer of rHDL lipid to endothelial cells is central to the inhibitory activity of rHDL. Moreover they may provide a link between dietary fat composition and the anti-atherogenic potential of HDL.

C5b-9 induce IL-6 synthesis in vascular smooth muscle cells via NF-κB activation

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Objective: Recent studies provide evidence that the complement system can contribute to the pathogenesis of arteriosclerosis. The terminal complement complex C5b-9 can induce proinflammatory activities. However, little is known about the transcriptional regulation by which complement-derived signals promote the induction of inflammatory mediators. Therefore we studied the link between complement activation and vascular inflammation.

Methods: Human smooth muscle cells (SMC) were stimulated with C5b-9. IL-6 mRNA and -synthesis were determined by Northern analysis and ELISA, respectively. Nuclear factor-kappa B (NF-κB) activation was investigated by electrophoretic mobility shift assay.

Results: Stimulation of SMC with C5b-9 resulted in a rapid increase in the level of IL-6 transcript, which was followed by enhanced production of the
proinflammatory cytokine IL-6. The effect was selective for the C5b-9 complex, whereas complement components without C7 had no effect. Pretreatment of cells with pertussis toxin or the NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC) inhibited complement dependent IL-6 mRNA expression and —release suggesting the involvement of Gi-proteins and NF-κB. Electrophoretic mobility shift assay revealed that C5b-9 induced NF-κB binding activity. To determine whether NF-κB is responsible for C5b-9-mediated IL-6 gene regulation, we used cis element double-stranded (decoy) oligonucleotides (ODN), corresponding to the NF-κB consensus sequence. Pretreatment with NF-κB decoy ODN, but not a control ODN inhibited the effect of C5b-9 induced IL-6 gene expression and secretion.

Conclusions: The results demonstrate that activation of the complement system induces IL-6 gene expression and release in human SMC largely via the activation of NF-κB. This may contribute to ongoing inflammation in atherosclerotic lesions. Our data support a new mechanism for the proatherogenic effect of complement.

VEGF synthesis in vascular smooth muscle cells is enhanced by nitric oxide and modified cholesterol

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Objective: Oxidised LDL are key determinants of atherosclerosis development, which is accompanied by inflammatory processes in the vascular wall. VEGF and inducible nitric oxide synthase (iNOS) are expressed in atherosclerotic plaques. OxLDL has been demonstrated to inhibit NO generation, however, the reciprocal relationship between VEGF, iNOS and oxLDL is not well known. Therefore, we investigated the effect of iNOS derived NO as well as some lipid components of oxLDL (7-ketocholesterol, 7β-hydroxycholesterol) on VEGF synthesis in SMC.

Materials and Methods: Confluent rat VSMC were exposed for 24 h to 7-Kcholesterol (10 μg/ml), or 7-flcholesterol (10 μg/ml). Other VSMC were treated with IL-1β (10 μg/ml) in the absence or presence of L-NAME (2 mM), a NOS inhibitor. Some were treated both by 7-Kcholesterol and IL-1β. NO generation was measured by Griess method, iNOS and VEGF gene expression was evaluated by RT-PCR, and VEGF protein synthesis was determined by ELISA.

Results: 7-Kcholesterol augmented VEGF expression, as evidenced by RT-PCR and ELISA determination of protein synthesis. Cells treated with IL-1β generated NO and produced several times more VEGF than control VSMC. Inhibition of NO generation by L-NAME resulted in diminished VEGF synthesis. 7-Kcholesterol also decreased IL-1β-induced iNOS expression and NO generation, but VEGF synthesis was increased in cells treated with 7-Kcholesterol and IL-1β.

Conclusions: The results demonstrate that both NO and modified cholesterol can increase VEGF synthesis in SMC. Inhibition of iNOS activity by oxLDL may decrease NO generation, but enhance superoxide radical formation. Thus, the concomitant activity of iNOS and oxLDL may aggregate inflammatory processes in the vessel wall, leading to enhanced production of VEGF. This might be of relevance for neovascularization of the plaque and its stability.

Atherosclerosis

Atherosclerosis

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WeP16:W23

Intimal infection with Chlamydia pneumoniae: relationship to loss of smooth muscle, gross necrosis and atherosclerosis

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Objective: Chlamydia pneumoniae is common in atherosclerotic lesions. It is controversial if it plays a role for the initiation and further development of the lesions. The aim of this study was to clarify if C. pneumoniae is spatially related to various expressions and stages of atherosclerosis.

Methods: Sections of carotid endarterectomy material with lesions of different severity were stained for cells (DNA) and immunohistochemically for C. pneumoniae, smooth muscle cells, macrophages and endothelial cells.

Results: C. pneumoniae was detected at sites in macrophages, smooth muscle cells and micro vascular endothelium. More inconspicuous foci with infection were infiltrates of varying size of C. pneumoniae-containing macrophages and with destruction of the smooth muscle cells. Spatial relation to very early expressions of atherosclerosis was not encountered. Conspicuous and more widespread infection appeared in relation to regions with gross necrosis, regarded as typical manifestations of mature atherosclerotic lesions.

Conclusions: The distribution of C. pneumoniae suggested that gross necrosis due to the infection could be responsible for or have contributed to the formation of at least some necrotic centres. Reduction of smooth muscle, increase of macrophages and of gross necrosis promote instability and rupture-proneness in the atheromatous plaque and, thereby, C. pneumoniae may be an important promoter for the serious and often mortal clinical effects of atherosclerosis.

WeP17:W23

25-hydroxycholesterol increases an LPS-induced IL-1β protein expression in human macrophages

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Objective: In the present study we investigated the effects of oxysterols (7β-hydroxycholesterol (7β-OH), 7-ketocholesterol (7-keto), 25-hydroxycholesterol (25-OH) and 27-hydroxycholesterol (27-OH)) on IL-1β protein and mRNA expression in human monocyte-derived macrophages (hMDM).

Methods: Early hMDM were allowed to adhere to the plate for one hour before being stimulated with increasing concentrations of oxysterols and LPS (1 μg/ml), for 4, 6, 8, 12 and 24 hours and the IL-1β protein expression was subsequently measured by ELISA and RT-PCR.

Results: A 20-fold increase of the IL-1β protein expression were obtained in hMDM, incubated with 25-OH 5 μg/ml together with LPS for 24 h, in comparison to cells incubated with only LPS. Only a slight increase of the IL-1β expression was found when 27-OH, 7-keto and 7-keto were used. No clear effect on the IL-1β expression was found in hMDM incubated for 24 h before the oxysterols and LPS were added for another 24 hours. No effect of the oxysterols on the LPS-induced IL-1β expression was found in the hMDM that were allowed to differentiate for 7 days. 25-OH in itself significantly increased the IL-1β mRNA expression, but not together with LPS. 27-OH did not increase the IL-1β mRNA expression.

Conclusions: These observations suggest that oxysterols may upregulate an inflammatory response in undifferentiated macrophages, while this effect decreases in differentiated macrophages. Oxysterols could therefore contribute to the inflammation found in atherosclerosis.

WeP18:W23

Cholesterol-independent effect of statins on the atherosclerotic lesions stability in primates

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Objective: We recently showed that pravastatin, but not simvastatin improves

coronary endothelium-mediated dilator responses and prevents myocardial ischemia in atherosclerotic monkeys independent of their effect on plasma cholesterol level. We hypothesized that statins similarly influence features of plaque vulnerability.

Methods: Adult male cynomolgus monkeys (n = 12/group) were fed an atherogenic diet for 12 months while receiving 1) no treatment (Ct); 2) pravastatin (40 mg/kg/day) (Prav); or 3) simvastatin (20 mg/kg/day) (Sim). Dietary cholesterol was adjusted to equalize total plasma cholesterol concentration among groups. Frozen sections of abdominal aorta were immunostained for SMC, macrophages, VCAM, IL-1 beta, lipids (oil red O), and collagen (sirius red). Staining was measured as percent positive area using computer-assisted color image analysis.

Results: While Intima/Media ratio was unchanged, all features of plaque vulnerability were reduced in drug-treated groups. Macrophage positive area decreased 2.4 ± 1.3 fold (p < 0.001) (Prav), and 1.3 ± 0.5 fold (p < 0.001) (Sim). Statin treatment similarly lowered expression of VCAM and IL-1 beta (~2 fold in Prav, and >1.2 fold in Sim). Prav (p < 0.001) but not Sim (p > 0.5) statistically decreased arterial lipid levels. Features associated with plaque stability improved in treated groups: SMC as well as collagen positive areas increased 2.1 ± 0.6 fold in Prav (p < 0.001), and 1.5 ± 0.4 fold (p < 0.001), and 1.5 ± 0.5 fold (p < 0.005) respectively in Sim.

Conclusions: These results provide further support in primates for a beneficial effect of statins on aspects of plaque stability independent of cholesterol lowering.

WeP19:W23 Effect of alpha tocopherol enrichment of human monocytes on tumor necrosis factor-alpha release

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Objective: Tumor Necrosis Factor alpha (TNF) plays a major role in insulin resistance and inflammation. The objective of this study was to test the effect of alpha tocopherol (AT) on the release of tumor necrosis factor-alpha (TNF) from human monocytes (Mo) in vivo and in vitro.

Methods: For the in vivo study, 21 healthy volunteers were supplemented with RRR-AT (1200 IU/day) for 3 months. TNF release from Mo activated with lipopolysaccharide was measured by ELISA, Messenger RNA (mRNA) for TNF was quantitated by RNase protection assay.

Results: AT supplementation resulted in a significant reduction in TNF release from activated human Mo (42%, p < 0.01). In vitro, there was a significant reduction in TNF-release with AT at concentrations ≥ 50 μM. Mechanisms of inhibition of TNF by AT that were explored included an antioxidant effect, inhibition of protein kinase C (PKC) and inhibition of 5-lipoxygenase (5-LO). While AT decreased TNF release, a similar antioxidant, beta tocopherol had no effect. Also, while AT decreased PKC activity in Mo, specific PKC inhibitors had no effect on TNF release. Leukotriene B4 (LTB4), a major product of 5-LO augments TNF release from human Mo. In presence of AT, there was a significant reduction in LTB4 and TNF levels, which was reversed by addition of LTB4. Similar observations were obtained with specific inhibitors of 5-LO. Co-incubation of activated human Mo with AT or a 5-LO inhibitor significantly decreased TNF mRNA; this was reversed by the addition of LTB4.

Conclusions: AT decreases TNF synthesis and secretion from activated human monocytes via 5-lipoxygenase. This could have major implications with regard to inflammation and atherosclerosis.

WeP20:W23 Inflammatory response in stable angina pectoris: The possible influence of statins

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Objective: The aim of this study was to investigate whether an increased inflammatory response could be detected in patients with stable angina pectoris.

Methods: 38 men (age ≤ 60 years) with stable angina and at least two coronary stenoses were included. 38 healthy but otherwise similar men served as controls. Lymphocyte subpopulations were assessed by three colour flow cytometry analysis.

Results: In patients the number of leukocytes, neutrophils, lymphocytes, CD4+ cells (p < 0.05) and activated CD4+ cells (p < 0.01) were higher than in controls. The patient group was divided in two groups according to statin therapy, treated (n = 23) and untreated (n = 15). The untreated group had significantly more leukocytes, neutrophils, CD4+ cells and activated CD4+ cells compared with controls. On the other hand, treated patients did not differ significantly in these respects compared with controls. The result for activated T cells is shown:

<table>
<thead>
<tr>
<th>CD3+ (cells/μl)</th>
<th>Treated</th>
<th>Untreated</th>
<th>Controls</th>
<th>p &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+/CD25+</td>
<td>185 ± 83</td>
<td>267 ± 144</td>
<td>170 ± 57</td>
<td>0.01</td>
</tr>
<tr>
<td>CD4+/HL-A2+</td>
<td>90 ± 50</td>
<td>106 ± 58</td>
<td>70 ± 31</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Conclusions: The inflammatory/immune response is increased in angina patients without statin therapy compared with healthy controls. However, in angina patients with statin therapy the number of activated T cells were significantly lower than in untreated patients. These findings may indicate that statins have an anti-inflammatory effect.

WeP21:W23 Cell adhesion molecules and secretory type II phospholipase A2 in relation to risk factors and carotid atherosclerosis

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Objective: Soluble cell-adhesion molecules and secretory type II phospholipase A2 (snPLA2) in plasma are markers for endothelial activation and inflammation, and have also been suggested to predict atherosclerosis. The aims of the present study were to investigate the relationship between atherosclerosis, cell-adhesion molecules and snPLA2 in hypercholersterolemic (total cholesterol ≥ 6.5 mmol/L) and normocholesterolemic subjects (total cholesterol ≤ 6.5 mmol/L); and also to investigate the relationship between conventional risk factors, cell-adhesion molecules and snPLA2.

Methods: Atherosclerosis was measured by ultrasound in the carotid artery. Cell-adhesion molecules and snPLA2 was measured by ELISA.

Results: Levels of sICAM was positively and significantly associated with plaque occurrence and size in the hypercholesterolemic group, but not in the normocholesterolemic group. There were no significant relationships between sVCAM, E-selectin and snPLA2 and atherosclerosis. However, sICAM was significantly associated with sVCAM, E-selectin and snPLA2 in both groups. In the hypercholesterolemic group sICAM was positively and significantly associated with triglyceride levels and also negatively associated with HDL levels.

Conclusions: The present study showed that sICAM-1 was significantly associated with atherosclerosis, as measured by ultrasound, in the carotid artery in subjects with hypercholesterolemia. Furthermore, sICAM-1 levels were associated with snPLA2 (r = 0.44, p < 0.001), which recently have been shown to predict coronary events. Finally, high sICAM-1 levels was associated with an atherogenic lipid profile including high triglyceride levels and low HDL levels.

WeP22:W23 Antibodies against oxidized LDL in relation to cell-adhesion molecules, secretory type II phospholipase A2 and carotid atherosclerosis

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Objective: To investigate the relationship between antibody titers against oxidized LDL and atherosclerosis in hypercholersterolemic (n = 102) and normocholesterolemic (n = 102) subjects; and also to analyze the relationship between antibody titers and other inflammatory markers of possible interest in atherosclerosis development, soluble cell-adhesion molecules and secretory type II phospholipase A2.

Methods: Atherosclerosis was measured by ultrasound in the carotid artery. Antibody titers, cell-adhesion molecules and snPLA2 were measured by ELISA.

Results: The results of the study showed that IgG titers against both Ox-LDL and MDA-LDL were significantly and positively associated with sICAM, E-selectin and secretory type II phospholipase A2 in the patient group, but not in the control group. In the patient group only weak associations were seen between antibody titers and plaque occurrence in the carotid artery. However, in the control group there was a highly significant and negative relationship between intima-media thickness of the carotid artery bulbi, plaque occurrence in the carotid artery and IgM titers against both Ox-LDL and MDA-LDL.
Conclusions: These data suggest that the humoral immune response (IgM) in early stages of atherosclerosis development may be protective rather than atherogenic. This is in agreement with recent experimental studies. In the present study atherosclerosis was related to IgM titers, but the role of the different classes and subclasses of antibodies needs further investigation.

Methods and Results: Hypercholesterolemia (0.5%, wt/wt diet) rabbits either received repeated intravenous injections of endotoxin (E. coli lipopolysaccharide 1.25-2.5 μg/kg, once per week) or a self-limiting cutaneous S. aureus infection was established with or without administration of a quinolone antibiotic. All measured laboratory parameters, including LDL cholesterol and HDL cholesterol, were similar on the different groups of hypercholesterolemic animals. Further, all endotoxin-treated animals developed transient fever episodes following administration of endotoxin. Atherosclerotic lesions were absent in all normocholesterolemic control animals. Atherosclerotic lesions were present in all hypercholesterolemic animals. The extent of atherosclerosis was evaluated by computer-assisted morphometry in the aortas en face (Sudan IV) and by histology at 8 weeks after start of the experiments. Endotoxin-treated animals exhibited significantly accelerated atherosclerosis when compared to control animals (72 ± 23 mm² total lesion load vs. 27 ± 9 mm², n = 5, each, P < 0.01).

Conclusion: It is concluded that non-specific stimulation of the innate immune system results in the acceleration of cholesterol-induced atherosclerosis. While consistent with the inflammatory concept of atherogenesis, these data render it improbable that a single infectious agent should assume particular importance in the initiation or acceleration of atherosclerosis.

Methods: We examined the ability of human monocyte/macrophages to inhibit the process of atherosclerosis progression from enrichment of VSMCs derived from human carotid plaques, aortic and coronary media. The methods: Macrophages, but not T-lymphocytes, induced a dose-dependent cessation of VSMCs, which required monocyte/macrophage contact via direct cell–cell contact/interaction. VSMCs were inhibited by neutralising antibodies to fibronectin and to the Fas-Fc fusion protein, indicating the requirement for membrane-bound Fas and Fas-L (data for plaque VSMCs, ANOVA, mean ± SEM, control apoptosis = 5 ± 0.9%; macrophage-induced apoptosis = 81 ± 2.9%; caspase inhibitor ω-VAD-fmk 11 ± 0.4% (reduction p < 0.01); neutralising anti-Fas-L 37.1 ± 4.3% (reduction, p < 0.01); Fas-Fc protein 31 ± 2% (reduction, p < 0.01); control IgG 83.1 ± 3.9% (NS)). Monocyte/macrophage contact was associated with increased surface expression of Fas-L, coincident with the onset of cytotoxicity. VSMCs expressed surface Fas, which was increased in plaque VSMCs, and plaque VSMCs also underwent Fas-induced apoptosis.

Conclusion: We conclude that human macrophages potently induce human VSMC apoptosis, which requires direct cell-cell interactions and is in part dependent upon Fas/Fas-L interactions. Macrophage-induced VSMC apoptosis may therefore directly promote plaque rupture.

WeP26:W23 Site-specific antiatherogenic effect of N,N'-diacyl-L-cystine in apoE-LDLr(-/-) mice
Annika Westin Eriksson, Knut Pettersson. Pharmacology CV, AstraZeneca R & D, SE-431 83 Mölndal, Sweden
N,N'-diacyl-L-cystine (DiNAC) has antiatherosclerotic effects in WHHL rabbits, and may represent a new treatment principle for atherosclerosis. Here we studied if DiNAC has an antiatherogenic effect also in mice. Moreover, we have shown that probucol enhances lesion formation in the aortic root in mice, but prevents atherogenesis in the thoracic aorta. We therefore studied if the DiNAC effects on atherogenesis are site specific. Ten weeks old apoE-LDLr(-/-) mice were administered 3 μmol DiNAC/kg/day (n = 10) or vehicle (n = 10) for 11 weeks (in the drinking water). At termination, mice were anaesthetized, the thorax was opened and a cardiac blood sample (for cholesterol analysis) was drawn. After perfusate injection, the heart and thoracic aorta were removed. Atherosclerosis in the aortic root region and around the third pair of intercostal arteries in the descending thoracic aorta was analysed histologically. The results are summarised in the table.

WeP27:W23 N,N'-Diacyl-L-cysteine reduces atherosclerosis in WHHL rabbits
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The immunomodulator N,N'-Diacyl-L-cystine (DiNAC) stimulates contact sensitivity/delayed type hypersensitivity reactions induced by oxazolone in mice (Sämmström et al. JPET 1999; 288: 1174-84). The maximal effect on CS/DTH was observed at an oral dose of 3 μmol/kg. Here we studied if DiNAC affected atherogenesis in atherosclerotic WHHL rabbits. Methods: WHHL rabbits (10 weeks old) were treated with 3 μmol DiNAC/kg/day for 12 weeks. Serum cholesterol (S-chol) was measured before and after treatment. Atherosclerosis was measured both as percentage fatty streak coverage of the thoracic aorta surface en face and histologically as intima volume (expressed as intima to media (I/M) ratio) in the thoracic aorta. Results: Basal S-chol was similar in the two groups, 11.2 ± 0.7 mM (controls) vs 10.2 ± 0.8 mM (DiNAC) (mean ± SEM). The table summarises the effects of DiNAC.

WeP28:W23 Endotoxin accelerates atherosclerosis in rabbits on hypercholesterolemic diet
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Objective: We report that non-specific stimulation of the innate immune system influences the progression of atherosclerosis.

WeP29:W23 Role of LOX-1 in inflammatory process
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2 Faculty of Medicine, Kyoto University, Kyoto, Japan
Objective: To clarify the role of a novel oxidized LDL receptor, LOX-1, in inflammatory processes that is possibly important in atherogenesis.

Methods: To induce inflammation in rats, lipopolysaccharide (LPS) was injected at footpad. A neutralizing antibody to LOX-1 or control IgG was administered to block the function of LOX-1. To evaluate the effects of the antibody on the endotoxin-induced inflammation, leukocyte count in blood, leukocyte dynamics in retinal vessels, and endotoxin-induced venules (EIVU) were analyzed.

Results: By the injection of high dose of LPS, 70% of rats were died after 24 hours. Preadministration of anti-LOX-1 antibody completely rescued the animals. Leukopenia occurred 1-6 hours after the injection was also blocked by the anti-LOX-1 antibody. Anti-LOX-1 antibody significantly reduced leukocyte infiltration and protein exudation into anterior chamber of eye, which is observed in EIU by the injection of low dose of LPS. LOX-1 expression in blood vessels were significantly up-regulated in endothelial cells in blood vessels after LPS injection. Furthermore, anti-LOX-1 antibody reduced the number of leukocyte rolling on retinal veins and increased the rolling velocity.

Conclusion: Involvement of LOX-1 in inflammatory process especially in leukocyte recruitment was suggested by the present results. This proinflammatory nature of LOX-1 might accelerate atherogenic process as well as mediating the action of oxidized LDL.
Conclusions: DiNAC reduced atherosclerosis in WHHL rabbits. This effect was not due to lipid lowering. DiNAC appeared more effective in preventing the growth of fatty streaks into more advanced lesions than in preventing their initial formation. DiNAC may represent a new treatment principle for atherosclerosis.

WeP28:W23 Iron chelation reduces the inflammatory response of endothelial cells upon infection

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Background: Infection of endothelial cells (EC) with Chlamydia pneumoniae (Cp) or influenza A leads to an inflammatory response (e.g., increased cytokine production, leukocyte adhesion).

Objective: To elucidate the mechanism of the endothelial inflammatory response after infection.

Methods: Cultured human ECs were infected with Cp strain AR39 or influenza A (H1N1). Increasing concentrations (microM) of Desferal (iron chelator), DMTU (hydroxyradical scavenger) and NAC ( scavenger of reactive oxygen species) was added to EC cultures. After 24 hrs' incubation the supernatants were harvested for interleukin-6 (IL-6) determination by ELISA.

Results: Increasing concentrations of Desferal or DMTU lead to decreased production of IL-6 (ng/ml) by EC after infection as well as at baseline. NAC did not affect the IL-6 response by ECs:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Uninfected</th>
<th>Cp</th>
<th>Inf A</th>
<th>DMTU</th>
<th>Uninfected</th>
<th>Cp</th>
<th>Inf A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>2863±385</td>
<td>447±60</td>
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<td>10</td>
<td>142±31</td>
<td>816±110</td>
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<td>7.5</td>
<td>131±29</td>
<td>2872±417</td>
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</tr>
<tr>
<td>100</td>
<td>106±5</td>
<td>542±751</td>
<td>273±54</td>
<td>10</td>
<td>90±22</td>
<td>1436±763</td>
<td>237±29</td>
</tr>
<tr>
<td>500</td>
<td>119±17</td>
<td>507±668</td>
<td>236±50</td>
<td>15</td>
<td>78±44</td>
<td>763±782</td>
<td>73±24</td>
</tr>
</tbody>
</table>

Conclusion: Iron chelation and scavenging of hydroxyradical resulted in a decreased IL-6 response of endothelial cells. This suggests that the Fenton reaction plays a major role in cytokine production by EC after infection.

WeP29:W23 Chlamydia pneumoniae or influenza A infection of human endothelial cells stimulate monocytes to express tissue factor in vitro

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Background: Microorganisms may play a role in atherogenesis either by direct endothelial cell infection or by indirect vascular effects. We previously showed that direct infection of endothelial cells leads to an inflammatory response. In the present study the effects of the supernatant of endothelial cells (EC) infected with Chlamydia pneumoniae (Cp) or influenza A on monocyte TF expression are determined.

Methods: Cultured human EC were infected with influenza A virus strain H1N1 or Cp strain AR39. Mononuclear cells (MC) were incubated with the supernatant of infected ECs for 24 hrs. Procoagulant activity of MCs was determined by adding citrated plasma. The time for fibrin to form was measured on MCs. Factor VII and Factor XI deficient plasma was used to investigate by which pathway this was taking place.

Results: Compared to uninfected EC (626±51 sec.) the ECs infected with Cp (349±56 sec.) or influenza A (516±58 sec.) caused a coagulation time reduction. As determined by experiments with factor deficient plasma, the extrinsic coagulation pathway (tissue factor expression) initiated the coagulation cascade.

Conclusion: Infection of EC with Cp or influenza A resulted in an increase of procoagulant activity of MCs. This coagulation time reduction was initiated by increased TF expression on MCs. Thus, direct infection of endothelial cells leads to indirect effects on MCs. It remains to be determined which cytokines (probably Interleukin-6; data not shown) are involved in the cross-talk between MCs and ECs.

WeP30:W23 Circulating immune complexes is a strong and independent risk factor for myocardial infarction

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Objective: The ability of circulating immune complexes (CIC) and autoantibodies against oxidatively modified low density lipoproteins (oxLDL) and cardiolipin to predict myocardial infarction was investigated in a prospective nested case-control study in which healthy 50-year-old men were followed for 20 years.

Methods: Two hundred and fifty seven men were included in the study and 119 developed MI (39 died) between 50 and 70 years of age. As controls served 138 randomly chosen healthy men.

Results: The prevalence and concentration of CIC at age 50 was associated with a marked increased risk for future MI and this risk was independent of other conventionally recognised risk factors (corrected odds ratio: 2.95 (95% confidence interval: 1.52 to 5.75; p < 0.0001)). There was a positive correlation between the levels of CIC and that of IgG antibodies to cardiolipin in the men who developed MI.

Conclusions: This prospective study shows that CIC alone or in combination with autoantibodies against cardiolipin in healthy males at 50 years of age predict subsequent MI between the age of 50–70 years.

WeP31:W23 Anticardiolipin antibodies are not an independent risk factor for stroke: An incident case referent study

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Background and Purpose: Anticardiolipin antibodies (aCL) have been proposed to be an independent/an increased risk factor for stroke.

Methods: World Health Organization Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) project and the Västerboten Intervention Program (VIP) health survey, 44 725 men and women were enrolled, and followed from January 1, 1985, to August 31, 1996. Individuals free from cardiovascular events were followed and 123 developed stroke (in average 34.1 months after blood sampling; 21 cerebral hemorrhage, 102 cerebral infarction) and compared with 241 age- and sex-matched controls. ELISA was used for the analyses of aCL of IgG, IgA and IgM.

Results: IgM-aCL were present in 11.4% (14/123) of the stroke patients and in 4.1% (10/241) of the healthy controls (p = 0.013, OR 2.97, 95% confidence interval 1.28–6.89). The odds ratio for the levels of IgM-aCL was 1.34 (p = 0.01, 95% confidence interval, 1.07 to 1.68) without adjustment for other risk factors and 1.24 when adjusted for hypertension, diabetes mellitus, cigarette smoking and use of smokeless tobacco (p = 0.077, 95% confidence interval 0.98 to 1.56). There was no difference between patients with cerebral hemorrhage and cerebral infarction for the prevalence of the 3 isoforms of aCL.

Conclusions: We conclude that aCL are associated to future stroke but do not constitute an independent risk factor.

WeP32:W23 Macrophage LPL production is increased in patients with heterozygous familial hypercholesterolemia

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Familial hypercholesterolemia (FH) is a genetic disorder leading to accelerated atherosclerosis. Uptregulation of plasma lipoprotein lipase (LPL) mass and activity has been observed in patients with FH and positive correlation between these parameters and the extent of calcific atherosclerosis has been documented. Based on the crucial role of macrophage (Mo) LPL in the development of atherosclerosis, we determined, in the present study, Mo LPL mRNA expression, immunoreactive mass and activity in patients with FH. The study group comprised 14 (8 women, 6 men) patients with FH. Their mean ± SEM age was 48 ± 5 years, total plasma cholesterol 7.65 ± 0.83 mmol/L, LDL cholesterol 5.93 ± 0.78 mmol/L, HDL cholesterol 1.05 ± 0.09 mmol/L, and triglycerides 2.48 ± 3.32 mmol/L. All patients were treated with HMG-CoA reductase inhibitors. Peripheral blood monocytes isolated from the patients and the control subjects (n = 14) were differentiated into Mo by culturing the cells for 9 days in medium supplemented with 20% autologous serum. Levels
of Mo LPL mRNA were measured by polymerase chain reaction. LPL mass and activity were measured in the culture medium using the Markit-F LPL and Confluent kits, respectively. Mo of patients with FH demonstrated a 3-fold increase in LPL mass as compared to those isolated from the control subjects (LPL mass (ng/mg prot.); controls: 96.2 ± 21.2; FH patients: 326.4 ± 68.7, P = 0.024). Mo of patients with FH also secreted significantly higher LPL activity than Mo of control subjects (LPL activity (pmol/mg prot.): controls: 149.9 ± 22.3; FH patients: 213.1 ± 34.8, P = 0.033). Induction of Mo LPL mass and activity in patients with FH was not associated with enhanced LPL mRNA levels, suggesting a post-transcriptional control of Mo LPL expression in FH. Overall, this study demonstrates that Mo of patients with FH overproduce LPL. Such alteration may promote atherogenesis in these patients.

WeP33:W23

Homocysteine upregulates macrophage lipoprotein lipase
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Hyperhomocysteinemia is an independent risk factor for atherosclerosis. Lipoprotein lipase (LPL), a key enzyme in lipid metabolism, is secreted by macrophages (Mo) in the vascular wall. It has been shown that Mo LPL has proatherogenic effects. The aim of the present study was to evaluate the in vitro effect of homocysteine (Hcy) on Mo LPL mRNA expression, immunoreactive mass and activity. J774 murine Mo were incubated with 5 and 50 mM Hcy for 0.5, 1.5, 3, 6, 18 and 24 h. At the end of these incubation periods, LPL mRNA expression was measured by Northern blot analysis. LPL mRNA levels in Mo and activity were determined in the supernatant after 24, 48, and 72 h exposure to Hcy using the Markit-F LPL and Confluent kits, respectively. Our results demonstrate that incubation of Mo with Hcys increases, in a time- and dose-dependent manner, LPL mRNA expression. Induction of LPL gene expression by 1 mM Hcy was biphasic, peaking at 1 and 6 h (LPL mRNA levels (% increase over control values): 1 h: 250 ± 14, P = 0.003; 3 h: 120 ± 12, P = 0.191; 6 h: 170 ± 24, P = 0.022). This effect declined towards baseline after 18 h of stimulation with Hcys. A similar kinetic pattern was documented when Mo were exposed to 5 mM Hcy, although a more dramatic effect on LPL gene expression was observed under these experimental conditions. A significant increase in extracellular LPL mass was also observed in Mo cultured with Hcys. Maximal effect occurred after a 72 h incubation period (LPL mass (% increase over control values): Hcys (1 mM): 247 ± 21, P < 0.001; Hcys (5 mM): 189 ± 1, P < 0.001). While no effect of 1 mM Hcy was found on Mo extracellular LPL activity, an increase in this parameter was observed in Mo treated with 5 mM Hcys for 24 to 72 h (LPL activity (% increase over control values): 24 h: 150 ± 3, P < 0.001; 72 h: 156 ± 39, P = 0.033). Overall, these results demonstrate a new role for Hcys, that of stimulating Mo LPL expression. These observations suggest a new mechanism by which Hcys may promote atherosclerosis.

WeP34:W23

Fatty acids regulate macrophage lipoprotein lipase
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The present study was aimed at evaluating the direct effect of fatty acids (FA) on macrophage (Mo) LPL mRNA expression, activity and immunoreactive mass. The involvement of peroxisome proliferator activated receptor (PPAR) in the regulation of Mo LPL by FA was also examined. J774 murine Mo were cultured for 24 h and 48 h with 0.2 mM unsaturated FA (arachidonic (AA), eicosapentaenoic (EPA), linoleic (LA)), monounsaturated FA (oleic (OA)) and saturated FA (palmitic (PA) and stearic (SA)) bound to bovine serum albumin. At the end of the incubation periods, Mo LPL and PPAR mRNA expressions were measured by Northern blot analysis. The amount of LPL mass in the culture medium and the levels of extracellular LPL activity were measured using the Markit-F-LPL and Confluent kits, respectively. The effect of FA on the binding of nuclear proteins to the regulatory peroxisome proliferator responsive element (PPRE) sequence of the human LPL gene promoter was analysed by electrophoretic mobility shift assay. Treatment of J774 cells for 24 h with LA, OA, PA and SA significantly increased Mo LPL mRNA expression. In contrast, exposure of Mo to AA and EPA significantly decreased this parameter. Except for EPA, all FA increased LPL secretion and activity at 24 h. The increase in Mo LPL mass and activity, induced by saturated fat, was still present after 48 h, whereas a significant decrease in LPL mass and activity was observed in Mo treated with EPA for 48 h. Although exposure of Mo to FA did not affect the levels of PPAR alpha and beta mRNA, an increase in the binding of nuclear proteins isolated from FA-treated Mo to the PPRE consensus sequence of the LPL promoter was observed. Incubation of nuclear extracts in the presence of anti-PPAR alpha and anti-PPAR gamma antibodies decreased saturated FA- and AA-stimulated binding activity to the PPRE, respectively. This study provides evidence for a direct regulatory effect of FA on Mo LPL secretion taking both place at the transcriptional and post-transcriptional levels. It supports a role of PPAR alpha and PPAR gamma in the regulation of Mo LPL expression by FA.

WeP35:W23

Peroxisome proliferator-activated receptor alpha activators upregulate macrophage lipoprotein lipase gene expression
L. Li, G. Renier. University of Montreal, Montreal, Canada

Peroxisome proliferator-activated receptors (PPARs) are transcriptional factors which mediate pleiotropic effects including regulation of genes involved in lipid metabolism and control of inflammation. In the present study, we measured the in vitro effects of PPAR alpha ligands on macrophage lipoprotein lipase (LPL) mRNA expression. J774 murine macrophages were cultured for 24, 48 and 72 hours with WY-14643 (20 nM) and ETYA (20 nM). At the end of these incubation periods, macrophage LPL mRNA expression was measured by Northern blot analysis. Cytoplasmic RNAs for use in polymerase chain reaction were also extracted from human control monocyte-derived macrophages exposed for 24 hours to PPAR alpha agonists. Incubation of murine macrophages with PPAR alpha ligands for 24 to 72 hours increased, in a time-dependent manner, LPL mRNA levels by these cells (LPL mRNA levels (% increase over control values): 24 h: WY-14643: 101 ± 5, P = 0.11; ETYA: 121 ± 8, P = 0.003; 48 h: WY-14643: 147 ± 18, P = 0.01; ETYA: 193 ± 9, P = 0.01; 72 h: WY-14643: 203 ± 26, P = 0.01; ETYA: 197 ± 31, P = 0.04). Treatment of human monocyte-derived macrophages with PPAR alpha ligands for 24 hours also significantly enhanced LPL mRNA expression (LPL mRNA levels (% increase over control values): WY-14643 (20 μM): 213 ± 38, P < 0.05; ETYA (20 nM): 177 ± 7, P < 0.001). Overall, these results demonstrate that PPARs alpha activators increase macrophage LPL gene expression. Given the proatherogenic effect of macrophage LPL in the vascular wall, better understanding of the biological role of PPARs in the regulation of macrophage LPL expression could lead to the development of new approaches in the prevention and treatment of atherosclerosis.

WeP36:W23

Transgenic mice (TG) expressing group IIA phospholipase A2 (sPLA2) show decreased plasma lipid and lipoprotein (LP) concentrations and enrichment of cholesterol in the liver
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Typical findings in systemic inflammatory diseases are low concentrations of cholesterol and LP as well as high activity of sPLA2 in plasma. Because phospholipid hydrolysis of LP results in an enhanced uptake of those particles by several cell types a decrease of blood cholesterol and an increased cholesterol accumulation in the liver could be the result because of an enhanced clearance from the plasma.

**Question:** Do TG expressing human sPLA2 show reduced LP serum concentration as well as increased cholesterol content in their livers?

**Methods:** 15 TG (DNX Transgenics, Princeton NJ, USA) expressing human sPLA2 and 15 nontransgenic littermates (C57BL/6) were fed a standard chow for 8 weeks. Subsequently 10 mice of each group received an atherogenic diet for 13 weeks. sPLA2 activity, TC, HDL, β-LP plasma concentration and composition were determined before and after the different diet periods and the contents of the main lipids were determined in organs after autopsy.

**Results:** TC, HDL and β-LP concentration were significantly decreased in TG. β-LP composition was changed. The cholesterol and cholesterol ester content in the liver was significantly increased.

**Conclusion:** Elevated sPLA2 activity in plasma increases HDL as well as β-LP clearance resulting in hepatic cholesterol accumulation.
WeP37:W23  Do changes in dietary and exercise habits in order to reduce cholesterol levels affect serum levels of inflammatory adhesion molecules?

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Objective: To investigate whether changes in dietary and exercise habits that reduced cholesterol levels affected inflammatory markers and adhesion molecules in serum.

Methods: From a six month controlled randomized diet and exercise intervention study on 158 healthy middle-aged men with moderately raised cholesterol levels, 23 men from the intervention group (I), who had reduced their cholesterol levels with ≥20%, were selected. Twelve men with unchanged cholesterol levels were selected from the control group (C). Before and after intervention interleukin-6 and the soluble adhesion molecules ICAM-1, VCAM-1, e-selectin and p-selectin were measured with ELISA technique.

Results: In the I group LDL cholesterol was reduced from 4.8 ± 0.8 to 3.6 ± 0.6 mmol/l (p < 0.001) and bodyweight, waist circumference and blood pressures were reduced as well, but not in the C group. At baseline significant correlations (r 0.3–0.6) were noted between adhesion molecules and cardiovascular risk factors as smoking, central obesity, LDL and HDL cholesterol, fibrinogen and PAI-1. The levels of ICAM-1 decreased -72.1% (29.7–74.9) and -44.0 (-14.8, -73.2) ng/ml and so did e-selectin -6.5 (-3.5, -9.5) and -4.4 (-1.3, -7.6) ng/ml after intervention in the I group and the C group, respectively. The changes in ICAM-1 and e-selectin were not correlated to changes in diet nor exercise or to changes in cardiovascular risk factors.

Conclusions: In healthy middle-aged men with moderately raised cholesterol levels the adhesion molecules ICAM-1, VCAM-1 and e-selectin were correlated to traditional cardiovascular risk factors. After six month of diet and exercise intervention the levels of ICAM-1 and e-selectin were significantly reduced in the intervention group as well as in the control group.

WeP38:W23  Transfer of CD4+ T cells aggravates atherosclerosis in immunodeficient apoE−/− mice

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Objective: Atherosclerosis is an immunological disease. T cells infiltrating lesions recognize epitopes on oxLDL and initiate an immune response. However, the effect of the T cell response on atherosclerosis has not been clear. The present study was performed to explore the role of immunity in atherosclerosis by using newly generated immunodeficient atherosclerotic SCID−/−/apoE−/− mice.

Methods and Results: The mice, generated by cross-breeding apoE-knockout mice with T and B cell deficient SCID mice on a C57BL/6 background, were fed with standard chow for 18 wks. A significant reduction of lesion formation was found in SCID−/−/apoE−/− mice compared with SCID+/+apoE−/− mice. Further, the disease was worsened dramatically in SCID−/−/apoE−/− mice after transplantation of CD4+ T cells enriched from aged SCID+/+apoE−/− mice. The acceleration of disease was paralleled by increased Interferon-γ levels in plasma.

Conclusion: The results demonstrate an accelerating role of T cells in the early stage of atherosclerosis. They suggest that interferon-γ-secreting Th1-type CD4 cells contribute to a proatherogenic T cell subset.

WeP39:W23  Enhanced IeBz degradation promotes NF-κB activation and inducible no synthase expression in intimal smooth muscle cells

Zhong-run Yan, Allan Sirjso, Göran K. Hansson. Center for Molecular Medicine, Karolinska Institute, Sweden

Objective: Intimal SMC formed after vascular injury participate in the inflammatory response by intensively expressing a series of inflammatory molecules. However, the mechanisms responsible for the overexpression of such genes by intimal SMC are largely undefined. The translocation factor nuclear factor kappa B (NF-κB) plays a pivotal role in the regulation of inflammatory genes. In the present study, we characterized this signal transduction pathway in vascular SMC.

Methods and Results: Compared with medial SMC, intimal SMC demonstrated intense constitutive NF-κB signal under resting conditions as determined by EMISA. Upon cytokine-stimulation, the transactivated signal for NF-κB was at least fourfold higher in intimal than in medial SMC. Associated with the activation of NF-κB, intimal SMC demonstrated a rapid degradation of IeBz, whereas the identical treatment caused an incomplete and transient degradation of IeBz in medial SMC. The half-life of IeBz was notably shorter in intimal than in medial SMC under both stimulated and resting conditions, although the abundance of the IeBz gene was higher in intimal SMC. Furthermore, inhibition of NF-κB activation effectively prevented iNOS expression in intimal SMC.

Conclusion: Our data indicate that the augmented degradation of IeBz accounts for the hyperactivation of NF-κB in intimal SMC. This may attribute to the enhanced expression of iNOS and other inflammatory genes by intimal SMC.

WeP40:W23  Cellular immunity in atherosclerosis: T cells specifically recognize oxidatively derived aldehyde adducts

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Objective: To determine the molecular basis for T cell recognition of neoaugments formed during oxidation of lipoproteins.

Methods: T cell clones were generated by clonage of mouse serum albumin (MSA), the fragments were isolated, modified and tested in antigen presentation assays using T cell hybridomas. The cell surface of antigen presenting cells (APC) where modified with malondialdehyde (MDA) and reactivity tested.

Results: Oxidation of low density lipoprotein (oxLDL) generates a number of reactive aldehydes, eg MDA, that bind to the protein component of the LDL. T cells recognizing oxLDL have been found in human atherosclerotic lesions. The T cell antigen receptor recognizes peptides bound to major histocompatibility complex class II (MHC-II), presented by specialized APCs after antigen uptake and processing. In the present study we show that the two T cell hybridoma lines M20 and M24 recognize MDA-MSA but not native MSA. This might be due to recognition of either native sequences or aldehyde adducts. However, addition of anti-MDA-antibodies to MDA-MSA-pulsed APC blocked antigen recognition. Moreover, modification of the APC cell surface with MDA, affecting peptides already bound to the MHC, resulted in recognition by the hybridoma M20. The results clearly indicate that the MDA adducts are pointing outwards from the peptide-binding groove of the MHC and is directly recognized by the T cell receptor.

Conclusions: In this study we provide, for the first time, evidence that T cell receptors can directly recognize lipid aldehyde adducts on self proteins rendering them immunogenic. This principle constitutes a key mechanism in T and B cell responses in atherosclerosis.

WeP41:W23  IL-8 receptor antagonist SB 256610 reduces atherosclerotic lesion areas in LDL receptor knockout mice

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There is growing evidence that the IL-8 receptor(s) (CXCR1 and or CXCR2) are involved in the development of atherosclerotic plaque. Therefore, antagonists of these receptors may inhibit plaque development. We investigated the effect of SB 256610, a selective antagonist for the human CXCR-2 receptor on the progression of atherosclerosis in LDL receptor deficient (LDLR−/−) mice. This compound antagonises the murine IL-8 (CXCR-2) receptor on peritoneal neutrophils with an IC50 of 3.6 nM against the natural ligand, KC. In the anti-atherosclerosis study male LDLR−/− mice were housed in groups of 5 and the cages of mice were randomly allocated to 2 treatment groups (n = 10/group) and a control group (n = 20). The mice were treated with either 20 or 100 mg/kg SB 256610 in high-fat diet or diet alone for 12 weeks. Blood samples were taken just prior to treatment and after 4, 8 and 12 weeks of treatment for the measurement of serum cholesterol and triglycerides. After 12 weeks of treatment, the hearts and aortas were perfuse-fixed in situ and were dissected out for the measurement of atherosclerotic lesion size. Lesion areas were measured in cross-sections of the aortic root using a colour image analysis system. SB 256610 had no effect on body weight or food intake throughout the treatment period. However, the compound reduced...
serum cholesterol levels by 18% at 100 mg/kg/d and serum triglyceride levels by 28% at 20 mg/kg/d. SB 265610 at the dose of 100 mg/kg/d reduced lesion areas in the aortic root and lipid stained areas within the lesions by 40% and 41% respectively. In conclusion, the data presented is consistent with a role for the CXCR2 receptor in the development of atherosclerotic plaque.

**WeP44:W23**

**Morphodynamic characteristics of normal and tumour cells degeneration induced by high doses of simvastatin**


**Objective:** To study the effect of simvastatin upon human normal (fibroblasts and monocytes) and tumour (malignant melanoma) cells.

**Material and Methods:** Tissue cultures: Fibroblasts (FA), monocytes (M), and malignant melanoma (ADLL) cells – Culture media: McCoy R5A supplemented with 15% serum, and antibiotics. – Statin: simvastatin (SV). – Growth curves. – Videointervalometry.

**Results:**
1. FA cells: 98 µg/ml SV produce a rapid loss of adherence greater than 50% within 3 h. The cells become detached remaining as live refringent free cells for many hours. The first cell dies 21 h after starting the treatment, and 100% are dead after 51 h. Death is due to apoptosis. 2. Peripheral blood monocytes: 98 µg/ml SV produce a partial loss of adherence, damage the plasmatic membrane during the cell retraction, and finally cell death. The first cell dies after a few minutes, more than 50% within 3 h, and 100% within 35 h. The late-dying cells degenerate showing apoptotic signs. Cytoplasmatic lipidic droplets are formed and accumulate in most of the monocytes. 3. Malignant melanoma (ADLL): 98 µg/ml SV produce adherence loss of 50% of the cells within 8 min, and of 100% within 21 min. 50% of the cells die after 11 h. and 100% after 21 h. Death is preceded by a post-mitotic cell fusion (apoptical apoptosis).

**Conclusions:** Under the same experimental conditions, SV shows very different effects in fibroblasts, monocytes and malignant melanoma cells. The differences are observed in adherence loss, survival time, apoptosis induction, and morphodynamic characteristics of cell degeneration.

**WeP45:W23**

**Study of the antiproliferative activity of the 3-HMG-CoA-reductase upon in vitro normal and tumour cells**


**Objective:** To estimate the statins antiproliferative activity, analyses their effect on cell growth kinetics and apoptosis induction.

**Material and Methods:** a) Tissue cultures: Normal human cells (amygdal d-fibroblasts, FA), malignant melanoma cells (ADLL), uterine cervix carcinoma (HeLa), neuroblastoma (SH-SY-5Y) and colon adenocarcinoma (HT-29). Culture media: McCoy R5A as modified by Iwakata and Grace, supplemented with 10% of homologous human serum (defined for cholesterol, triglycerides and lipoproteins concentrations). b) General reagents: SIGMA and Oncogene: Statins: atorvastatin (ATV), cerivastatin (CRV), lovastatin (LV), pravastatin (PRV), and simvastatin (SV) c) Chemoresensitivity predictive tests, d) Videointervalometry

**Results and Conclusions:**
1. **ID50 (µg/ml):**

<table>
<thead>
<tr>
<th>(FA)</th>
<th>(HeLa)</th>
<th>(ADLL)</th>
<th>(SH-SY-5Y)</th>
<th>(HT-29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATV</td>
<td>3.8</td>
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<tr>
<td>CRV</td>
<td>0.96</td>
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<tr>
<td>LV</td>
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<td>9.24</td>
</tr>
<tr>
<td>PRV</td>
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</tr>
<tr>
<td>SV</td>
<td>0.96</td>
<td>15.5</td>
<td>15.4</td>
<td>2.89</td>
</tr>
</tbody>
</table>

2. Videointervalometry confirms that all the studied statins: induce apoptosis, synchronise the cells in the G phase of the cell cycle, apparently they are not mutagenic, and mevalonate inhibits their antiproliferative effects.

**WeP46:W23**

**T cell lines and clones from unstable human atherosclerotic plaques respond to chlamydia pneumo**

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**Objectives:** Recent seroepidemiological studies suggest a relationship be-
ween prior infection with C. pneumoniae and complications of atherosclerosis. However, the potential mechanisms underlying such a relationship remain speculative. Since atherosclerotic lesions are of inflammatory nature, we investigated whether C. pneumoniae is capable of inducing antigen-specific stimulation of intraplaque T cells.

Methods: T cell lines were generated from atherosclerotic plaques (AP) (carotid endarterectomies, n = 8) and tested for their responsiveness against C. pneumoniae elementary bodies; T cell clones generated from the AP derived T cell lines of 2 patients were tested also. Similarly, the responses of peripheral blood T cells from the same patients were analyzed. Proliferation was measured using 3H-thymidine incorporation and expressed as stimulation index (SI).

Results: Four out of eight T cell lines from carotid AP responded to C. pneumoniae. This proliferation could be inhibited with antibodies against HLA-DR. From a total of 57 T cell clones, 17 were C. pneumoniae responsive (3 < SL < 40, mean = 8.0). Of these, all except one were Th1-type (secreting high levels of IFN- and low levels of IL-4 upon stimulation). Interestingly, peripheral blood T lymphocytes from only two patients responded to C. pneumoniae. One of these patients also showed a plaque T cell response, the other not.

Conclusions: Our results clearly show that C. pneumoniae responsive T cells are present in the symptomatic AP of 4 out of 8 patients. These results indicate that C. pneumoniae is capable of triggering the plaque inflammatory response in a subgroup of patients, a phenomenon with potential implications for the stability of plaques.

**WeP47:W23** Induction of CD36 by all-trans retinoic acid

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Objective: To investigate the role of the vitamin A derivative all-trans retinoic acid (aTRA) in the regulation of CD36, an important scavenger receptor for the uptake of oxidized lipoproteins (oxLDL). CD36 is highly expressed in the human atherosclerotic lesions and has previously been shown to be regulated through retinoic x receptor (RXR). Our study provides evidence, that specific retinoic acid receptor (RAR) agonists also induce expression of CD36.

Methods: THP-1 cells were incubated with different RAR or RXR ligands and aTRA with or without specific antagonists. CD36 expression was measured by flow cytometry (FACS) and PCR. Uptake of oxLDL was also analysed by FACS.

Results: Addition of aTRA to THP-1 cells strongly induced CD36 protein and mRNA after 2 days. This was functionally mirrored by an increased uptake of oxLDL. Specific RAR agonists increased CD36 expression. RAR antagonist reduced the response of aTRA, but failed to abolish CD36 induction completely. This indicates that a different pathway is additionally involved besides signaling through RAR. Combination of both RAR and RXR ligands gave a stronger upregulation, than RXR ligation alone.

Conclusions: The study shows that the induction of CD36 by retinoids are mediated through both RAR and RXR. The upregulation of CD36 by aTRA might be important in the pathogenesis of atherosclerosis.

**WeP48:W23** Vitamin E succinate induces apoptosis in hematopoietic and cancer cells: Structural requirements and role of protein kinase

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The vitamin E analogue α-tocopheryl succinate (α-TOS) can induce apoptosis. We pro-apoptotic activity of α-TOS in hematopoietic and cancer cells involves inhibition of protein kinase C (PKC) and lysosomal membrane integrity by apoptosis. Pherbol myristyl acetate prevented multiple features of α-TOS-induced apoptosis, whereas α-TOS-induced PKC activity and increased protein phosphatase 2A (PP2A) activity. Studies with other effectors inferred that PKCα inhibition is due to PKCα activation. Sensitisation by PKCα antisense oligonucleotides and protection by PKCα overexpression confirmed the role of PKCα inhibition in apoptosis. Overexpression of βcI-2 or its gain-of-function mutants indicated that modulation of βcI-2 by PKCα/PP2A is a mechanism mediating α-TOS-induced apoptosis. Structural analogues of α-TOS revealed that, in addition to α-tocopheryl, the succinyl moiety is required for effective apoptosis. Notably, the pro-apoptotic effects of α-TOS were largely restricted to malignant cells, while normal cells were rather resistant. In mice with colon cancer xenografts, α-TOS suppressed tumour growth. Our findings show that the α-tocopheryl moiety of α-TOS is involved in dysregulation of PKC/PP2A signalling, among others affecting the function of bcl-2, and the succinyl moiety in lysosomal/mitochondrial destabilisation. Further, they epitomise elimination of malignant cells by a pharmacologically relevant compound.

**WeP49:W23** The progression of atherosclerotic lesions in hypercholesterolemic rabbits is delayed by chronic treatment with the iNOS inhibitor, L-NIL

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Objective: The presence of an active inducible nitric oxide synthase (iNOS) in advanced atherosclerotic lesions is now well-accepted but its impact on the development of atherosclerosis is not yet known. The aim of this study was to examine the implication of iNOS in the progression of atherosclerosis.

Methods: Rabbits were fed a 0.3% cholesterol diet for 24 weeks. One group of 9 rabbits was then examined (BASELINE). The remaining animals were kept on the diet for another 12 weeks and treated with L-NIL by mini-osmotic pumps delivering the drug intravenously (5 mg/kg/day, L-NIL, n = 8), its vehicle (SALINE, n = 9), or L-arginine (2.25% in drinking water. L-ARG, n = 9).

Results: Most biological parameters (i.e. cholesterol levels, blood pressure) were not affected by the treatments. L-NIL delayed the progression of atherosclerosis on the thoracic aorta (the intima/media ratio increased from 0.98 ± 0.04 (BASELINE) to 1.63 ± 0.17 (p < 0.05) in the SALINE group but not in the L-NIL group (1.32 ± 0.23; NS) and the coronary arteries (the intima/media ratio decreased between the SALINE group and the L-NIL group (0.96 ± 0.19 versus 0.34 ± 0.19, p < 0.05). Lesion development was partially limited in the abdominal aorta by both L-NIL or L-arginine. Inflammatory and smooth muscle cell infiltration were not modified by the treatments but the significant increase in the intimal collagen content of thoracic aortas, from 21.8 ± 4.5% BASELINE to 61.7 ± 7.0%, SALINE and 57.2 ± 7.2%, L-ARG groups was not found in the L-NIL group (40.75 ± 10.6%, NS).

Conclusions: In conclusion, while L-arginine treatment does not appear to have a beneficial role in severe atherosclerotic lesion progression, chronic treatment with the iNOS inhibitor, L-NIL, limits the progression of severe pre-existing atherosclerotic lesions.

**WeP50:W23** The effect of ibuprofen on monocyte function

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Tobacco smoking is a major risk factor of atherosclerosis and a useful model for studying chronic inflammation. We compared monocyte function and lipid profiles in smokers and nonsmokers, before and after oral ibuprofen intake. The adhesion of peripheral blood monocytes (PBM) to native and TNFα-stimulated human umbilical vein endothelial cells (HUVEC), as well as superoxide (O2−) levels and reactive oxygen species (ROS) production in resting and PMA-stimulated PBM were determined. A group of 10 smokers without any other coronary risk factor was compared with an age-matched group of 10 nonsmokers. Tests were performed before and after a two-week course of oral ibuprofen (600 mg/day). In the smokers group before ibuprofen, monocyte adhesion to native and TNFα-stimulated HUVEC was increased (p < 0.001), as were O2− levels in native and PMA-stimulated PBM (p < 0.01). Ibuprofen reduced the adhesion of native and stimulated monocytes to HUVEC (p < 0.001) and O2− generation by resting and PMA-stimulated cells (p < 0.01) in both groups. ROS production by resting and PMA-stimulated monocytes was reduced in smokers (p < 0.001 and ns, respectively) and nonsmokers (p < 0.001 and p < 0.05, respectively). Interestingly, ibuprofen increased HDL-C levels in smokers (p < 0.05) and nonsmokers (p < 0.01).

In conclusion, ibuprofen reduced the adhesion of monocytes to HUVEC, suppressed oxidative stress and increased HDL-C levels in smokers and nonsmokers.
Conclusion: Adhesion molecules in plasma/serum reflect plaque formation in the femoral artery in patients with CAD. Leucocyte counts, however, were related to both plaques and IMT in peripheral arteries.

C-reactive protein (CRP) is an acute phase reactant, and increased levels of CRP levels are associated with the presence of coronary heart disease in man. Previous studies have shown that CRP is present in the arterial wall. Here we studied by RT-PCR whether CRP is expressed by human monocyte-derived macrophages in culture and in coronary artery samples obtained from explanted human hearts. We found expression of CRP in both cultured human macrophages and homogenates of human coronary arteries. Moreover, immunohistochemical staining of frozen sections showed that the CRP protein was absent in normal arterial intima, but was present in atherosclerotic lesions. Most of the CRP was present extracellularly in the superficial proteoglycan and macrophage-rich areas of the arterial intima. Thus, it appears that at least a fraction of the CRP in the arterial intima is synthesized locally and that macrophages are the likely source of the locally synthesized CRP.

Results: CD163 expression was found to be upregulated during phagocytic differentiation and to be suppressed by dexamethasone. Interestingly, downregulation of CD163 was also observed, when monocytes were treated with PPARγ-agonists. The promoter region lacks a canonical TATA-box and contains putative binding sites for the transcription factors C/EBP, Sp1, Ets, and AP-1.

Conclusions: Since CD163 expression is only upregulated in phagocytic cells and since its expression is sensitive to inflammatory stimuli and PPARγ activation, we assume an important function of this protein in inflammatory diseases and atherosclerosis.

Apo A-I regulates the expression of CDC42 and impairs spreading and phagocytic differentiation of human monocytes

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Objectives: Extravasation of monocytes is a critical step in atherogenesis that involves migration and cellular spreading. This process is induced by chemotaxis and regulated by small GTP binding proteins including CDC42. We aimed to elucidate whether the anti-atherogenic High Density Lipoproteins (HDL3) and its major apolipoprotein A-I may influence these processes.

Methods and Results: Human monocytes were isolated and phagocytic differentiation was induced in serum-free macrophage medium supplemented with 50 ng/ml M-CSF for 5 days. The influence of apo A-I on spreading of monocytes was analyzed during differentiation. The morphology of spreaded cells was defined by a flattened appearance together with the presence of filopodia. After day 2 of culture 66 ± 4.9% of monocytes were found spreaded in the absence compared to only 40 ± 0.7% in the presence of apo A-I (n = 3). Baseline expression of CDC42 was determined after cells were allowed to rest for 12 hrs (100%). After day 2 and day 5 in culture CDC42 expression was increased to 255 ± 120% and 340 ± 173%, respectively (n = 3). If HDL3 or apo A-I was added to the culture medium 24 hrs before day 2 and day 5, the expression of CDC42 was markedly downregulated. In addition, the enhanced expression of the β1,- as well as β2-integrins during differentiation, was moderately reduced in the presence of apo A-I.

Conclusion: The presented data show that apo A-I inhibits M-CSF induced spreading of human monocytes in vitro. This probably involves the regulation of CDC42 protein and may define a new and important anti-atherogenic property of apo A-I.

Endothelial function in dyslipidaemic HIV-1 infected patients on protease inhibitors: Significance of immunological status

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Objective: To examine endothelial function of the peripheral circulation in HIV infected patients with dyslipidaemia on protease inhibitors (PIs)

Baseline: We studied 25 HIV infected men on PI treatment with dyslipidaemia (mean age 50 ± 9.4 yrs, BMI 24.2 ± 2.7 kg/m2, CD4+ T-cell count 488 ± 255/mm3, cholesterol 6.8 ± 1.3, triglyceride 5.1 ± 2.9, HDL-cholesterol 0.90 ± 0.16 mmol/L) and 12 age, sex, BMI matched controls with normal plasma lipids (cholesterol 4.9 ± 0.6, triglyceride 0.9 ± 0.4, HDL-cholesterol 1.3 ± 0.2 mmol/L). Endothelial function was studied by measuring post-ischaemic flow-mediated dilatation (PfMD) and glyceryl trinitrate-mediated dilatation (GTMD) of the brachial artery using high resolution ultrasoundography with computer assisted image analysers.

Results:

In the HIV group, multivariate analysis adjusting for baseline arterial diameter, age, smoking and BMI showed that PfMD was inversely with the % CD4+ T-cell count (p = 0.01). Patients with a CD4+ T-cell count > 450/mm3 (n = 11) had significantly lower PfMD of the brachial artery (p = 0.04) than those with a count < 450/mm3 (n = 14).

Conclusions: HIV infected patients with dyslipidaemia on protease inhibitors have preserved endothelial function which may be a consequence of immune suppression, consistent with the role of immunological activity in atherogenesis.

The scavenger receptor CD163 is regulated by pro- and anti-inflammatory mediators and by PPARγ activation

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Objective: CD163, a member of the scavenger receptor cysteine-rich superfamily is expressed on monocytes and most tissue macrophages and might play an important role in the inflammatory response of these cells.

Methods: Regulation of CD163 mRNA and protein expression in human monocytes and macrophages was investigated by Northern blot analysis and flow cytometry, respectively. The putative promoter region of the CD163 gene has been cloned and the transcription initiation sites have been determined by RACE PCR and primer extension analysis.

Results: CD163 expression was found to be upregulated during phagocytic differentiation and to be suppressed by dexamethasone. Interestingly, downregulation of CD163 was also observed, when monocytes were treated with PPARγ-agonists. The promoter region lacks a canonical TATA-box and contains putative binding sites for the transcription factors C/EBP, Sp1, Ets, and AP-1.

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**WeP56:W23** Evaluation and characterization of ELISA measuring autoantibodies against oxidized low density lipoprotein

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**Objective:** Autoantibodies against oxidized LDL (oxLDL) have been measured in many laboratories but comparison of data between laboratories is difficult because of methodological variations and differences in the expression of results.

**Methods:** We have optimized an ELISA which measures autoantibodies against oxLDL and evaluated the effect of different ways of expressing the data on the results.

**Results:** Optimized conditions were as follows: coating concentration 2 μg/ml of LDL on polystyrene plates, 1% human serum albumin as a blocking agent, sample dilution 1:50, conjugate dilution 1:8000 and 0.2% human serum albumin in sample and conjugate diluents. The amount of autoantibodies expressed as a ratio between oxLDL and native LDL (natLDL) as titers against oxLDL or as differences between binding to oxLDL and natLDL showed significant differences between groups of coronary heart disease (CHD) patients, whereas the differences disappeared when the results were expressed as a ratio between antibody titer against oxLDL and a standard serum (oxLDL/standard) or as the oxLDL/natLDL ratio corrected with the standard serum (oxLDL/standard)/natLDL/standard.

**Conclusion:** We have developed an optimized ELISA for measuring of anti-oxLDL antibodies. This test may become useful for analyses of risk to develop atherosclerosis and CHD.

**WeP57:W23** Atorvastatin therapy reduces circulating TH1-lymphocytes in patients with stable angina

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**Objective:** Aberrations of the global immune system with a switch of T-helper (TH) cells toward the interferon gamma (IFN-γ)-producing phenotype (TH1 cells) are suggested to be of functional importance in the development of atherosclerosis. Although several non- lipid lowering effects of the HMG-CoA reductase inhibitor atorvastatin are known, its influence on circulating TH1 lymphocytes has not been investigated.

**Material and Methods:** Peripheral venous blood was collected from 6 male patients with stable angina (SA) and angiographically confirmed coronary artery disease at baseline, after 2 months of atorvastatin therapy (20 mg/d) and from 10 male controls. IFN-γ was measured in lymphocytes by flow cytometry after 4 hour stimulation with phorbol-12-myristate-13-acetate and ionomycin. Endothelial function was assessed by determining flow-mediated (FMD) and nitroglycerine-mediated vasodilation (NMD) using high resolution ultrasound of the brachial artery.

**Results:** There was no difference in controls and SA at baseline concerning IFN-γ-producing TH cells (CD4+/IFN+ cells) (16.03 ± 4.57 vs. 13.52 ± 6.65%; p = NS). After 2 months of atorvastatin therapy CD4+/IFN+ cells were significantly reduced (13.52 ± 6.65 vs. 7.53 ± 2.18%; p < 0.05). Furthermore, the ratio of FMD/NMD increased (0.36 ± 0.10 vs. 0.46 ± 0.06; p < 0.05). Treatment with atorvastatin decreased total cholesterol at an average of 27.1 ± 11.6% (276.3 ± 62.8 vs. 196.8 ± 29.9 mg/dL; p < 0.03).

**Conclusion:** The short term effect of atorvastatin therapy includes improvement of endothelial function and a decrease in circulating IFN-γ-producing TH cells. These 2 mechanisms likely contribute to plaque stabilization and the clinical benefit conferred by this agent.

**WeP58:W23** Effect of fatty acids on expression of endothelial leukocyte adhesion molecules

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**Objective:** Consumption of diet rich in long chain polyunsaturated fatty acids has been linked with a low prevalence of atherosclerosis and inflammation. The aim of the study was to evaluate the influence of linoleic acid in comparison with palmitic acid with respect to cytokine-induced expression of endothelial leukocyte adhesion molecules (ICAM-1, VCAM-1, E-selectin).

**Methods:** Normal human coronary artery endothelial cells (HCAEC) were obtained from clonetics (San Diego, CA, USA). HCAEC were cultured in microvascular endothelial cell growth medium. In the experiments, the cells were preincubated with linoleic acid and palmitic acid, respectively (10 μmol/L, 1 day, 2 days) or in control conditions, after which human IL-1α (10 ng/ml) was added for 1 day. The monoclonal antibodies used were anti-ICAM-1-FITC, anti-VCAM-1-FITC, and anti E-selectin-FITC. Expression was analysed by flow cytometry.

**Results:** The expression of adhesion molecules ICAM-1, VCAM-1 and E-selectin on the surface of the human coronary artery endothelial cells was measured before and after treatment with IL-1α plus fatty acids (linoleic acid, palmitic acid). IL-1α increased ICAM-1, VCAM-1 and E-selectin expression in comparison to controls. Incubation with IL-1α (1d) together with linoleic acid (2d) reduced the expression of ICAM-1, VCAM-1, E-selectin in contrast to palmitic acid.

**Conclusion:** The results indicate that a reduced expression of cell adhesion molecules may be relevant to the antiatherogenic effects of linoleic acid. This is in contrast to the properties of palmitic acid.

**WeP59:W23** Impact of lymphocytes on the differentiation of blood mononuclear phagocytes

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Monocyte-derived macrophages are recruited as the principal inflammatory cells to atherosclerotic lesions where they differentiate into resident macrophages. The factors that direct the differentiation of monocytes into tissue-specific macrophages are largely unknown. Recent studies have suggested that T cells may be capable of modulating the growth and differentiation of macrophages. The development of specific macrophage subsets with either pro- or anti-inflammatory phenotypes cells may crucially influence the fate of atherosclerotic lesions.

Peripheral blood monocytes were isolated from freshly drawn human blood. Monocytes were then either cocultured with autologous lymphocytes or with purified and cultured in the absence of lymphocytes in Teflon bags for two weeks. Macrophages were analyzed for the expression of the scavenger receptor CD36, the low density lipoprotein receptor (LDL-R), the Fc-IgG receptor CD16 and the LPS receptor CD14. Phenotypic analysis of differentiated macrophages was performed by flow cytometry and fluorescence microscopy. In the absence of lymphocytes, macrophages show a strong and persistent staining of CD36 and CD14, whereas only few cells expressed CD16 and the LDL-R. Interestingly, both antigens were now coexpressed on the same cells. Lymphocytes seem to maintain a persistent CD16 expression for at least one week in approximately 30% of all macrophages. In the absence of lymphocytes, CD36 expression was maintained for 8 days on more than 60% of all macrophages. In the presence of lymphocytes the number of CD36 expressing macrophages decreased by almost 10%.

These results provide evidence for the hypothesis that lymphocytes regulate monocyte/macroage maturation and differentiation into distinct macrophage subsets.

**WeP60:W23** The effects of coronary artery disease and clinical stability on endothelial function parameters

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**Objective:** To evaluate the effects of presence, extent and clinical stability of coronary artery disease (CAD) on endothelial function parameters, C reactive protein (CRP) and homocysteine.

**Methods:** Fifty-eight patients with angiographically documented CAD (age 56 ± 10, range 27-78) and 25 patients with normal coronaries (age 53 ± 10, range 35-70) were evaluated for conventional risk factors, plasma levels of homocysteine, CRP and soluble adhesion molecules including sICAM-1, sVCAM-1 and sE-selectin. Patients with CAD were clinically subdivided into stable (SAP/ unstable (USAP) angiina pectoris.

**Results:** s-VCAM-1 and sE-selectin levels were significantly higher in patients with CAD, whereas sICAM-1, CRP and homocysteine levels were similar. In CAD patients who had group with USAP had significantly higher CRP levels (p < 0.001) and leucocyte count (p < 0.05) than the group with SAP. Furthermore CRP and E-selectin levels were correlated with the extent of coronary atherosclerosis as judged by Gensini score (p < 0.01, r = 0.37; p < 0.05, r = 0.28 respectively).
Conclusions: Endothelial markers, namely s-VCAM-1 and s-selectin, are useful for determining the presence of coronary atherosclerosis, whereas CRP is a better predictor of lesion stability.

**WeP61:W23**

**Circulating adhesion molecules ICAM-1, VCAM-1, and E-selectin in patients with coronary artery disease and in subjects with risk factors for atherosclerosis**

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**Objective**: The aim was to assess in both "normolipidemic" and hyperlipidemic patients with coronary artery disease (CAD) and in subjects with risk factors for atherosclerosis including hyperlipidemias, the levels of circulating cell adhesion molecules (sCAMs). Furthermore, the effect of extracorporeal LDL elimination on sCAMs in patients with hypercholesterolemia was studied.

**Methods**: Patients with angiographically defined CAD (n = 155), patients with familial hypercholesterolemia undergoing LDL apheresis (n = 8), and control subjects with and without several risk factors for atherosclerosis (n = 166) were included in the study. Serum lipids and lipoproteins, parameters of lipid peroxidation, and sCAMs were measured.

**Results**: Both hyperlipidemic and "normolipidemic" CAD patients who have average total cholesterol and triglyceride levels show a tendency of elevated sVCAM-1 levels in comparison with control subjects. There is an age-dependent increase of sVCAM-1 in CAD patients. Hypercholesterolemia per se and extracorporeal LDL elimination is without effect on the levels of sCAMs, but elevations of sVCAM-1 and sICAM-1 were seen in hyperlipidemic patients and smokers. sVCAM-1 correlates positively with parameters of lipid peroxidation (conjugated diene and malondialdehyde).

**Conclusion**: Elevated levels of endothelial sCAMs may be a marker of endothelial dysfunction connected with several factors of the metabolic syndrome and with underlying atherosclerosis, but not with hypercholesterolemia itself.

**WeP62:W23**

**Contribution of intercellular adhesion molecule-1 in patients with coronary heart disease**

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**Objective**: The aim of the study was to assess the prevalence of the circulating soluble intercellular adhesion molecule-1 (sICAM-1) and expression of ICAM-1 on leukocytes in patients with coronary heart disease throughout the acute phase of acute coronary syndrome (ACS) compared with stable angina (SA) and healthy controls.

**Methods**: The study was carried out in 35 patients with coronary heart disease – 10 suffering from SA, 25 – with ACS and 10 control subjects. Samples of venous blood were taken at the moment of hospitalization. The expression on leukocytes surface of ICAM-1 was assessed by flow cytometry and serum concentration of sICAM-1 was measured by enzyme-linked immunosorbent assay. Coronary heart disease was confirmed by coronary angiography.

**Results**: Expression of ICAM-1 on monocytes membrane was increased significantly (p < 0.05) in patients with ACS compared with SA patients and control subjects. No difference of that expression on lymphocytes surface was detected in ACS and SA patients, but was increased significantly compared to the control group. Plasma concentration of sICAM-1 showed no difference among ACS and SA patients, but was significantly higher in healthy subjects.

**Conclusion**: Altered sICAM-1 levels and increased expression of ICAM-1 on leukocytes may be indicated as markers of inflammatory activity of coronary heart disease. Measurement of expression of ICAM-1 on monocytes and its increase may be used as prognostic factor of ACS.

**WeP63:W23**

The serum lipids and endothelial injury markers (endothelin-1, von Willebrand Factor) concentrations in children from families with high risk of atherosclerosis

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**Background**: The familial history of premature coronary heart disease in first degree relatives is a highly predictive CHD risk factor. The endothelial activation is the first step in atherogenesis. Many biochemical markers of endothelial injury are known, such as associated with hemostasis and fibrinolysis (thrombomodulin A2, prostacycline, von Willebrand factor – vWF), vasoconstrictor function (endothelin-1, ET-1) and inflammatory response (adhesion molecules). We assessed the serum levels of lipids, vWF and ET-1 in children from families with high risk of cardiovascular diseases (HR).

**Children and Methods**: 48 children were studied – 24 children from HR families, according to NCEP definition: one or two parents had clinical manifestation of cardiovascular disease before the age of 65 years (mother) or 55 years (father). 24 healthy children without familial history of cardiovascular disease were used as the control. Both groups were divided into subgroups in regard to age (6-10 and 11-15 years) and sex. None of the children had any metabolic diseases. The concentration of ET-1 and vWF were assessed using ELISA kits produced by Amersham and Boehringer. Total, LDL, HDL cholesterol and triglycerides levels were measured using enzymatic method. The statistical analysis was done using Student’s t-test and Spearman correlation test, p < 0.05 was considered statistically significant.

**Results**: The serum vWF concentration was significantly elevated in HR group in comparison to control (p < 0.001). The vWF levels were higher in HR boys and girls subgroups and both age groups in comparison to respective control subgroups. There were no differences between the groups in concentrations of ET-1. The analysis of serum lipid parameters has shown no differences in regard to concentration of triglycerides, total, LDL and HDL cholesterol between the HR-group and control. There were no significant correlations between the concentration of vWF, ET-1 and serum lipids.

**Conclusion**: In normolipidemic children from families with high risk of atherosclerosis the serum level of von Willebrand Factor is significantly higher in comparison to control.

**WeP64:W23**

**Monocytes and atherosclerosis. Morphodynamic in vitro characteristics of human peripheral blood monocytes**


**Objective**: To study the morphodynamic characteristics of normal human peripheral blood monocytes long term cultured in vitro.

**Material and Methods**: Isolation of monocytes by means of polymacroscharcharozum sodium diatrizoate. Tissue cultures: McCoy R5a growth medium. Supplements: different sera, and growth factors (EGF, M-CSF, and GM-CSF). The study was carried out by videointervalometry.

**Results**: The observations made 48 h after inoculation were the following: 1) Adhered monocytes: 144-247/mm², average mobility 96.4 μm²/h. 2) Platelets: 27-52/mm³. 3) Lymphocytes: 5-9/mm³. On the following 7 days the cell number decreased until no platelets were seen, and lymphocytes were less than 1/mm³. Monocyte number was retained, but their average mobility diminished to 23.4 μm²/h. The average size increased from 90.5 μm² at 48 h to 303.3 μm² after 7 days, becoming stable starting on the 13 day with 2,770 μm². Mitosis were not observed in the presence or absence of said supplements. Monocytes showed marked membrane undulating movements, endotheciosis and phagocytosis of platelets and dead cells. The loss rate diminished in the presence of GM-CSF, M-CSF, and homologous and heterologous sera. Platelet phagocytosis was modified only by GM-CSF. Adherent monocytes cultured in vitro showed cyclic phenomena of loosening and adherence, in which the free monocytes recovered their original morphology and mobility and then adhered again.

**Conclusions**: The morphodynamic studies of the human normal monocytes are reproducible and permit to define parameters capable of being modified under different experimental conditions having interest for the experimental study of atherosclerosis.
WeP65:W23  Genomic hypercholesterolemia is associated with inflammation

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Hypercholesterolemia is associated with all the pathologic features of the atherosclerotic process. The link between hypercholesterolemia and the inflammatory aspects of atherosclerosis is not yet clear. We wanted to verify whether hypercholesterolemia is associated with higher values of inflammation markers, particularly those molecules with the potential role of reflecting the arterial wall status, in subjects with primary (PH), compared to a group of controls, their relatives with lipid profiles. Therefore we measured C3, C4, C reactive protein, adiponectin molecules in 36 patients with hypercholesterolemia in comparison to a group of 48 normal controls. All patients did not present intercurrent disease or other conditions likely to be associated with an acute phase response, as further confirmed by undetectable values of IL-6.

Results: Hypercholesteroleemics, compared to controls, showed higher mean values of C3 (157.1 ± 43.5 vs 104.1 ± 17.9 mg/dl; p < 0.0001), C4 (31.1 ± 15.7 vs 21.2 ± 6.5 p < 0.0001), median C reactive protein values (0.333, range: 0.300–1.460, vs 0.300, range: 0.300–1.300 mg/dl; p < 0.0001), mean sICAM1 values (258.4 ± 84.9 vs 110.7 ± 30.3 p < 0.0001).

Conclusions: Elevation of circulating inflammation molecules in primary hypercholesterolemia would suggest a direct role of high plasma cholesterol in inducing vascular inflammation.

WeP66:W23  Hyperlipidemia, bone marrow stem cells and atherosclerosis

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The effects of hyperlipidemia (HLP) on vascular wall atherogenesis is likely to be mediated via bone marrow stem cells (colony-forming units, or CFU) for hematopoietic and stromal differentiation lineages. This conclusion is based on analysis of bone marrow biopsy specimens, cultured in human intimal vascular cell and the study of blood mononuclear fraction of patients with HLP in semisolid and liquid test-systems using hystochemical, immunochemoical and electron microscopy techniques.

The following effects of HLP on bone marrow and on CFU were observed:
1. Apoptosis of mononuclear tissue and development of mielofibrosis;
2. Presence of stromal CFU for fibroblasts (CFU-f) in blood and the elevation of stromal/hematopoietic CFU’s ratio depending on the severity of HLP.
3. Appearance of blood in HLP patients of megakaryocytes with high proliferative potential and also CFU for blast colonies and osteoelast colonies; 4. Presence of bone marrow hematopoietic and stromal CFU in loci of lipid intimal infiltration.

The presence of these hematopoietic and stromal CFU in vascular intima and their circulation in peripheral blood suggest the possibility of local hemopoiesis in vascular wall. Penetrating vascular intima, stromal CFU may provide necessary microscouring for local hemopoiesis. Intimal hematopoietic loci may serve as the source of various growth factors and may predetermine future location of clinically manifested plaques.

WeP1:W24  HORMONES AND CARDIOVASCULAR DISEASE

WeP1:W24  High plasma leptin concentrations enhance the development of coronary collateral vessel network in patients with coronary artery stenosis

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Objective: Although myocardial ischemia significantly correlates with the development of coronary collateral vessels (CCVs), there is considerable variation in the number of CCVs in patients with ischemic heart disease. The underlying mechanisms of this variability are not well understood. Leptin is a peptide hormone involved in the regulation of body fat as well as promotion of angiogenesis via activation of endothelial cells. The aim of this study was to investigate the effect of leptin on the formation of CCVs.

Methods and Results: Plasma leptin levels were measured in 64 male patients who underwent coronary angiography between November 1994 and July 1997. These patients were not on insulin treatment and coronary angiography showed severe stenosis (≥75% diameter). The number of CCVs per one diseased vessel was counted in each patient. When these patients were categorized into two groups based on the grade of CCVs development, high plasma leptin concentrations were present in patients with good collateral circulation (1.5 ± 0.77 ng/ml, mean ± SD) but not in those with no or poor collateral circulation (1.12 ± 0.58 ng/ml; P < 0.05).

Conclusions: We conclude that circulating leptin levels might promote the development of CCVs in humans patients with coronary artery stenosis.

WeP2:W24  Simvastatin, transdermal patch and oral estrogen-progestin preparation in hypercholesterolemic postmenopausal women: A randomized, placebo-controlled clinical trial

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Objective: Hormonal Replacement Therapy (HRT) seem to favorably influence the plasma lipid profile. Only few investigations compared the effects of HRT with HMG-CoA inhibitors in post-menopausal women. We performed a randomized, placebo-controlled trial in women with recent-onset spontaneous menopause, aiming at comparing the relative effects of different hypolipidemic strategies.

Methods: Forty-four consecutive healthy women aged ≥45 years, with amereorrhea by 6–60 months, serum FSH > 40 U/L and slight to moderate hypercholesterolemia (LDL-cholesterol, 160–250 mg/dl, HDL-cholesterol < 75 mg/dl and triglycerides < 200 mg/dl) were enrolled and randomized to dietetic advice [placebo, Tapp 1, P], Simvastatin 10 mg [S], 0.625 mg of conjugated equine estrogens [CEE] or 50 µg estrogen transdermal patch [TDE]. In the latter 2 cases the progressive nomegestrol was added to estrogens (days 17–28 of the cicle). Lipoprotein parameters were evaluated after separating VLDLs by ultracentrifugation.

Results: Total and LDL-cholesterol significantly decreased in S, CEE and TDE (but not P) groups compared to baseline (−18.9% ± 17.1 [p < 0.001], −12.6% ± 8.7 [p < 0.01] −10.0% ± 10.9 [p < 0.05]), and −29.2% ± 20.8 [p < 0.001], −18.3% ± 8.8 [p < 0.001], −11.5% ± 14.4 [p < 0.05], respectively) but only Simvastatin showed an effect significantly superior to diet alone (p < 0.01). Apo B was decreased by all drugs (marginally in the TDE group), and Simvastatin was more effective than either P or TDE groups. Triglyceride concentration and VLDL-cholesterol did not vary during treatments. LDL-cholesterol and Apo A-1 increased significantly in the S group (+18.9% ± 19.3 and +11.6%15.5, p < 0.01); lipoprotein(a) was decreased by both HRTs (−31.5% and −21.7% for CCE and TDE respectively, p < 0.05).

Conclusions: HRT, particularly CCE, seems rather effective and well tolerated in post-menopausal hypercholesterolemic women, while low-dosage Simvastatin is superior in LDL-lowering.

WeP3:W24  LCAT catalyzes the esterification of estradiol (E2) in HDL particles

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Objective: To investigate the possibility that lecithin: cholesterol acyltransferase (LCAT) could esterify not only cholesterol but also estrogens. Previous results have provided indirect evidence for this indicating that the LCAT inhibitor, 5,5-dibhydroxy-2-nitrobenzoic acid, reduced the conversion of estrogens to fatty acid esters. Esterified estrogens could according to some studies act as antioxidants in lipoprotein particles in vivo.

Methods and Results: High density lipoprotein (HDL) was isolated from fresh, male plasma and gel filtered removing molecules not attached to HDL. HDL was incubated with labeled estradiol (1H-E2) with and without purified LCAT at 37° for 24 hours. Samples were then subjected to a ultracentrifugation and the radioactivity and protein concentrations were measured from the eluted fractions. Most of the radioactivity obtained coincided with the protein peak in all samples, suggesting that 1H-E2 was attached to HDL. HDL-containing fractions were extracted and subjected to hydrophobic gel chromatography to separate esters from unesterified 1H-E2. The amount of 1H-E2 esters was doubled following the addition of highly purified plasma LCAT. The ester fraction was further analyzed by thin layer chromatography (TLC) demonstrating that the radioactivity conigrated with the E2-17-sterate standard. Saponification
of the ester fraction and a following hydrophobic chromatography on LH20 demonstrated that E2 had not been converted to estrone (E1) or estriol (E3).

Conclusions: The results demonstrated that the formation of E2 monooesters is significantly accelerated by the addition of functional LCAT. The data suggests that plasma LCAT is an important factor not only in the reverse cholersterol transport process but also having a pronounced antioxidant function via generation of estradiol esters.

WeP4:W24 Accumulation of estrone in human plasma lipoproteins
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Objective: To find out if estrone (E1) fatty acid esters could be formed in vitro and incorporated into lipoproteins. Previous studies have suggested that estrogens may exert antioxidant effects in lipoprotein particles.

Methods: Human plasma was incubated with tritiated estrone (1H[3]E1) for 24 hrs at 37°C with and without the LCAT inhibitors 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and iodoacetic acid (IAA). After incubation lipoproteins (VLDL, LDL, HDL) were isolated by ultracentrifugation and purified by gel filtration on Sephadex G-25 to remove unbound estrone and other small-molecular-weight molecules. The lipoprotein-containing fractions were extracted and subjected to hydrophobic gel chromatography on Sephadex LH-20 to separate 1H[3]E1-esters from unesterified 1H[3]E1. After saponification another hydrophobic gel chromatography (LH-20, 96% methanol in toluene) was used to separate E1 from E2 and E3 to exclude conversion of E1 to other estrogens.

Results: The radioactivity present in lipoprotein fractions consisted of both free and esterified 1H[3]E1 and no 1H[3]E1 or 1H[3]E1 esters were detected. More than half of the radioactivity present in lipoproteins was unesterified E1. DTNB and IAA inhibited esterification by 50% or more depending on the lipoprotein fraction.

Conclusions: The 1H[3]E1 was incorporated into lipoproteins during incubation with plasma. Part of the incorporated 1H[3]E1 was in the free form. DTNB and IAA suppressed the E1 esterification by 50% or more.

WeP7:W24 Incorporation of esterified neutral sterols into lipoproteins in human plasma
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Objective: Our aim was to study the formation of neutral steroid esters and their incorporation into lipoproteins in human plasma in vitro.

Methods and Results: Human plasma was incubated with tritiated pregnanolone (1H[3]PREG) for 4 hours at 37°C with and without the lecithin: cholesterol acyltransferase (LCAT) inhibitor 5,5-dithiobis-2-nitrobenzoic acid (DTNB). After incubation LDL and HDL were isolated by sequential ultracentrifugation and purified by gel filtration on Sephadex G-25 to remove molecules not associated with lipoproteins. Most of the radioactivity coincided with the protein peak of LDL or HDL. The lipoprotein containing fractions were extracted and subjected to hydrophobic gel chromatography on Sephadex LH-20 to separate PREG esters from unesterified PREG. All of the lipoprotein bound radioactivity was recovered in the ester fraction. This radioactivity was reduced by 80% in the presence of DTNB suggesting that part of the radioactivity in the ester fraction represented some other lipophilic derivative than fatty acid (FA) ester. A similar experiment was carried out with dehydroepiandrosterone (DHEA). The esterification of DHEA was almost completely inhibited by DTNB.

Conclusions: We confirmed the previously reported finding that neutral steroids are esterified and incorporated into lipoproteins during incubation in plasma. The partial suppression of PREG esterification in the presence of DTNB indicates that this reaction was catalyzed by LCAT. In addition, we observed that some other lipophilic derivative or a FA ester produced by an acyltransferase other than LCAT was formed. The esterification of DHEA was completely dependent on LCAT. It has been reported (Khalil et al., 1998, Atherosclerosis 136, 99-107) that DHEA incorporated in LDL could be able to inhibit the oxidation of LDL and thus protect against atherosclerosis.

WeP6:W24 The effect of menopause on the relation between high triglyceride and low HDL-cholesterol with obesity
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Objective: As the triglyceride (TG) level increased with increasing weight and obesity estimated by body mass index (BMI) and advancing age, this study have been undertaken to investigate the effect of menopausal state on the prevalence of high TG and low high-density lipoprotein cholesterol (HDL-C) and the relationship between this type of lipid abnormality with obesity.

Methods: A sample of 2200 men and women aged 20 years and over was selected randomly from random clusters in Isfahan city. Samples have no history of cardiovascular or any other disease. They were asked to complete standard questionnaires regarding the previous history of major risk factors, then BMI and the ratio of waist to hip (WHR) were calculated, their blood samples were drawn and sent to the central laboratory of the research center to be tested for lipoproteins and TG using enzymatic method. Chi-square test and stepwise logistic regression were used to compare the prevalence of this lipid abnormality among pre and postmenopausal women and the relation between BMI and high TG/low HDL-C among pre and postmenopausal women.

Results: The results showed significant relationship between BMI and high TG/low HDL-C (B = 0.18, P = 0.01) among postmenopausal women while this relationship was not significant among premenopausal women, however the finding among studied men was similar to postmenopausal women (B = 0.26, P = 0.02). On the other hand no significant difference was observed regarding the prevalence of high TG/low HDL-C between pre and postmenopausal women (P > 0.05).

Conclusions: The obtained results showed that decreasing weight among postmenopausal women may be one of the suggested ways for controlling high TG/low HDL-C as one of the important risk factors of cardiovascular disease.

WeP5:W24 Melatonin in patients with coronary artery disease, hypertensive heart disease and dilated cardiomyopathy
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Objective: Recent studies suggest a relationship between melatonin and coronary artery disease (CAD). It was pointed out that this effect could be a consequence of high plasma catecholamine levels by means of desensitization to adrenergic stimuli and thus decrease of melatonin production. This study investigated nocturnal melatonin production in patients with CAD compared to patients with hypertensive heart disease (HHD) and dilated cardiomyopathy (DCM).

Methods: 129 consecutive male patients underwent coronary angiography due to angina pectoris, pathological exercise stress testing, pathological SPECT myocardial perfusion images or suspected dilated cardiomyopathy. Group I: 54 patients with severe CAD (more than 50% stenosis in all coronary arteries) and good left ventricular function (EF > 55%). Group II: 60 patients with HHD (no CAD, good left ventricular function, EF > 55%). Group III: 15 patients with DCM (EF < 40%, dilated left ventricle, no CAD). Urine was collected after coronary angiography between 10 p.m. and 7 a.m. and 6-sulfatoxy-melatonin (aMT6s), the major urinary metabolite of melatonin, was measured radioimmunologically.

Results: Mean nocturnal aMT6s significantly differed between the groups (p = 0.001; ANOVA). Additional beta-blocker treatment did not influence night-time melatonin synthesis. Correlations between melatonin and cardiovascular risk factors were not found.

Conclusions: Our results indicate decreased melatonin production in patients with CAD. Myocardial ischemia without macrovascular CAD, which was present in all patients with HHD, also coincided with low melatonin production, but to a lesser degree. The influence of catecholamines can be ruled out as patients with cardiomyopathy, showing great adrenergic stimuli, have the highest nocturnal melatonin production. In conclusion, melatonin appears to be directly involved in the macro- and microvascular coronary atherosclerosis process.

Effect of oral contraceptives on cGMP and serotonin: comparison of two low-dose ethinylestradiol/levonorgestrel preparations

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Objectives: With hormone replacement in postmenopausal women we have demonstrated effects on biochemical markers surrogating on vasoreactive action (e.g. Mueck et al. 1997; Horm. Metab. Res., 29: 80–83). As little is known with oral contraceptives (OCs), we compared the effect of two OCs containing ethinylestradiol (EE) and levonorgestrel (LNG) on cGMP and serotonin. cGMP can reflect the production of nitric oxide and/or atrial natriuretic peptide; serotonin elicits vasodilation in the presence of intact, but vasocostriction in the presence of damaged endothelium.

Methods: Doubleblind, randomized, parallel-group study; Leios® (gr. A: 0.02 mg EE/0.1 mg LNG, n = 34) vs. Sredil 30® (gr. B: 0.03 mg EE/0.15 mg LNG, n = 33); collection of 8 h night urine at baseline, and during the last week of cycle 3 and 12. The excretion of cGMP and serotonin metabolite, 5-hydroxyindole acetic acid (HIAA), was measured by EIA.

Results: cGMP significantly increased after 12 cycles in both groups: +30% in gr. A, and +22% in gr. B, without statistical difference between the groups. No significant effect on the excretion of HIAA could be observed, neither after 3 cycles nor 12 cycles of treatment.

Conclusions: Both tested OCs can enhance cGMP-excretion after 1 year of treatment surrogating for vasorelaying action. There seems to be no effect on serotonin production. This may be of relevance in women with a higher risk for injuring vascular endothelium, e.g. in smoking women, which would lead to vasocostrictive effects of serotonin.

Estradiol valerate-levonorgestrel therapy decreases 18:1 trans fatty acid content in plasma phospholipids

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Objective: The aim of the work was to study the effect of postmenopausal hormone replacement therapy on plasma lipids and their trans fatty acid content, which possibly adds the risk of developing cardiovascular diseases.

Methods: Two groups of 15 women were studied: one with estradiol valerate therapy alone (group I) and another with estradiol valerate plus levonorgestrel therapy (group II). Plasma cholesterol esters, phospholipids and triacylglycerols and their 18:1 trans fatty acid content were measured from each woman after long term (over 2 years) hormone replacement therapy (measurement 1), four weeks after cessation of the treatment (measurement 2) and three weeks after reintroducing the therapy (measurement 3).

Results: Combined estradiol valerate-levonorgestrel therapy decreased concentrations of plasma cholesterol esters, phospholipids and triacylglycerols. It also decreased plasma 18:1 trans fatty acid concentrations of phospholipids and triacylglycerols. In phospholipid fraction the decrease of 18:1 trans fatty acid remained significant in multivariate analysis after taking concentration of phospholipids as covariate in multivariate analysis of repeated measurements. The pure estradiol valerate therapy decreased the concentration of plasma cholesterol esters but had now effect on other plasma lipids or trans fatty acid content.

Conclusions: Estradiol valerate-levonorgestrel therapy of postmenopausal women decreases concentrations of plasma triacylglycerols and phospholipids and the content of 18:1 trans fatty acids in plasma phospholipids.
we compared the influence of MPA and NET on the endothelial synthesis of the vasoactive substances prostacyclin and endothelin.

**Methods**: Prostacyclin synthesis was examined in endothelial cells of human umbilical vein by measuring its stable metabolite 6-keto-prostaglandin F₁α. Endothelin synthesis was measured directly in the medium of the cultured cells. MPA and NET were tested at the concentrations 0.01, 0.1 and 1 μM, the reaction time was 24 h.

**Results**: MPA had no significant effect on the synthesis of prostacyclin and endothelin at all dosages tested compared to the control value. NET significantly stimulated prostacyclin synthesis at all three concentrations and reduces endothelin synthesis at the highest concentration.

**Conclusions**: These results indicate a significant difference between the progestins MPA and NET. NET can influence the vasotonic balance by a positive effect on prostacyclin synthesis and inhibition of endothelin whereas MPA reacts neutral in this respect. These investigations might contribute to assess the optimal hormone therapy medication for interventional prevention trials like HERS with clinical endpoints.

**WeP13.W24 Low cholesterol synthesis in postmenopausal women with hormone replacement therapy**

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**Objectives**: Cholesterol metabolism and hormone replacement therapy (HRT) are associated with serum cholesterol level, and may have significant implications in the morbidity, and mortality of coronary artery disease. Thus, the relation between cholesterol metabolism and HRT was investigated in postmenopausal women.

**Methods**: Cholesterol metabolism was determined by sterol balance technique in 30 random postmenopausal women without hypolipidemic medication. Ten of the women were on, twenty of them off HRT (estradiol 2 mg and medroxyprogesterone 10 mg). Fecal neutral sterols and bile acids were measured with gas liquid chromatography, cholesterol absorption efficiency (CAE) with oral dual isotope feeding technique and sex hormone binding globulin (SHBG) with immunofluorometric assay.

**Results**: The body mass index, dietary intakes of cholesterol and fat and CAE were comparable in the women on or off HRT, but serum levels of follicle stimulating hormone were lower and SHBG were significantly higher in the women on HRT. The latter women tended to have lower serum cholesterol levels (5.7 ± 0.2 vs 5.9 ± 0.3 mmol/L), but had significantly lower fecal total sterols, mainly due to neutral sterols (9.9 ± 0.8 vs 12.6 ± 0.9 mg/kg/day, P < 0.05), and cholesterol synthesis (10.7 ± 0.6 vs 15.0 ± 1.3 mg/kg/day, P < 0.01) than the women off HRT. Cholesterol synthesis was inversely related to CAE (r = 0.38), and SHBG significantly to HDL cholesterol levels (r = 0.39), CAE (r = 0.36) and cholesterol synthesis (r = −0.42).

**Conclusions**: Inefficient elimination of cholesterol as neutral sterols and low cholesterol synthesis are associated with HRT in postmenopausal women.

**WeP14.W24 Potential atherogenic effects of short-term high dose growth hormone treatment in the rat model**

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Recent experimental and clinical studies have suggested an important role for the growth hormone-insulin-like growth factor-1 (GH-IGF-1) axis in the regulation of cardiac growth and function. However, the changes of coronary vessels after short-term treatment with GH are not completely investigated.

The aim of this study was to evaluate the relationship between growth hormone administration and ultra-structural changes in coronary vessels in rat model.

**Methods**: Recombinant human growth hormone (r-GH) were administered to rats (group 1) at a dose of 0.3 mg/kg for two weeks (n = 10). Control group (group 2) of rats were exposed to daily subcutaneous injections of saline (n = 10). After this period, coronary vessels were dissected and cross-sectional material stained for hematoxylin and electron microscopic examinations. These prepared sections from control and treated groups were stained with Hematoxylin-Eosin for histochemical studies, and with anti-laminin and anti-collagen IV antibodies for immunohistochemical techniques.

**Results**: The slides staining with anti-laminin and anti-collagen IV antibodies were not demonstrated evident differences in vessel basement membrane between the two groups. However, in the GH group, a significant thickening and hypertrophying of endothelial cells and abundant pycnotic vesicles in these cells cytoplasm were detected by electron microscopic observations. Also, the lumen of vessels in GH group was significantly narrowed comparing the control group. In addition, abundant connective tissue was observed surrounding the vessels and an increased smooth muscle cells.

**Conclusion**: 1. Short-term high dose GH treatment influenced on coronary vessels ultra-structural characteristics such as thickening and hypertrophying of endothelial cells. 2. Initiation effects of the GH were observed from second weeks. These finding suggest that short-term high dose GH treatment may triggers endothelial dysfunction leading to premature atherosclerosis process.

**WeP15.W24 Relationship between glucagon in a lipid-glucose load and carotid intima-media thickness**

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**Objective**: Type 2 diabetes is associated with an excessively high risk for atherosclerosis. The relevance of the single factors of the insulin-glucose homeostasis is not completely clarified. Glucagon is produced by the α-cells of the pancreas and plays an important role in lipid and carbohydrate metabolism. So far, there are no data on the relationship between glucagon and intima-media thickness (IMT), an accepted marker of atherosclerosis. The aim of this study was to examine the effect of glucagon secretion after a lipid-glucose load on carotid IMT in various glucose tolerance stages.

**Methods**: Male subjects, aged 35 to 70 years, with body mass index between 22 and 33 kg/m² were examined. 20 of them had type 2 diabetes mellitus, 16 impaired glucose tolerance and 25 had normal glucose tolerance. All subjects were non-smokers and had fasting triglycerides below 4.6 mmol/L. After an overnight fast they were administered a test drink (93 g fat, 82 g glucose). Blood was drawn in the fasting state and 2, 3, 4 and 6 hours after the load. Carotid IMT was determined by B-mode ultrasound.

**Results**: In univariate analysis a positive correlation was found between IMT and age (p < 0.01), postprandial proinsulin (calculated as area under curve; p < 0.05), plasma triglycerides after removing chylomicra (p < 0.05) and a negative correlation between IMT and fasting glucagon level (p < 0.05). Using multile linear regression analysis age, fasting glucagon and triglycerides in plasma rest were found to be independent determinants of carotid IMT.

**Conclusions**: Our data suggest that glucagon could be involved in atherogenesis.

**WeP16.W24 Dehydroepiandrosterol associated to low density lipoproteins reduces their increased susceptibility with ageing to lipid peroxidation**

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The incidence of atherosclerosis and its related diseases increase with aging. The aging process may enhance lipoprotein modification, which leads to the increase in the susceptibility of LDL and HDL to oxidation. DHEA is the most abundant steroid hormone in humans was shown to have antiatherogenic effects and this hormone is decreasing dramatically with aging. In the present study we were interested to determine the presence of DHEA/DHEAS and the evolution of their contents in HDL and LDL lipoproteins with aging. Moreover, we studied the susceptibility of LDL to oxidation with age in the presence or absence of vitamin E or DHEA. We demonstrated that vitamin E is unable to restore the decreased resistance to oxidation of LDL from elderly subjects to the level of LDL obtained from young subjects. Furthermore, our results provide evidence that DHEA is integrative part of LDL and HDL and disappear to almost non-detectable levels with. The DHEA incorporated in the LDL from elderly subjects increased their resistance to oxidation in a concentration dependent manner, by increasing significantly the lag-phase and decreasing the plateau of conjugated diene, hydroperoxide and TBARS formations. The increased resistance provided by DHEA was higher than that with vitamin E. DHEA seems to act either by protecting vitamin E from disappearance from LDL under oxidation or to scavange directly the free radicals produced during the oxidative process. Our results suggest that DHEA exerts an antioxidative effect on LDL, which could have antiatherogenic consequences. Careful clinical trials of DHEA replacement should determine whether this ex vivo effect could be translated into any measurable antiatherogenic (cardio-protective) action.
**P.W25 PSYCHOSOCIAL MECHANISMS IN CVD**

Prevalence of depression in patients with coronary heart disease (CHD) on cholesterol lowering therapy with pravastatin

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Depression worsens prognosis of CHD independent of traditional risk factors and cardiac disease severity.

**Objective:** To determine the prevalence of depression (dep) in a cohort of the LIPID study1 survivors with stable CHD.

**Method:** The LIPID study was a placebo controlled, double blind trial of the efficacy of pravastatin over 6 years in 9014 patients (pts) with CHD and baseline cholesterol of 4–7 mmol/L. A cohort 715 patients were assessed for dep and dep symptoms using the Beck Depression Inventory II (BDI-II) during a further 2 year follow-up.

**Results:** 28.4% of pts demonstrated some depressive symptoms. 20.3% (n = 145) had low dep and 8.1% showed moderate to severe symptoms (5.6% (n = 40) and 2.5% (n = 18) respectively.

**Conclusion:** This stable CHD sample had more depressive symptoms than a healthy population (1–3%) but less than other CHD pts (18%). This accords with their status as survivors.

**References**


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**P.W24 Estriol replacement improves endothelial function for 2 years in octogenarian women and retards progression and regressed atherosclerosis in the rabbit aorta – the relation of NO**


**Objective:** Investigation of the effects of estriol (E3) on endothelial function in elderly and the effect of atherosclerosis.

**Methods:** S1-Human study: 24 elderly women (80 ± 3.5 yrs) were administered CaCl2 with or without E3 (2 mg/day) for 110 wks. Endothelium-dependent flow-mediated (FMD) and -independent dilations of brachial-artery were assessed. S2-Animal study: #1; 3 groups of 24 oophorectomized female rabbits were treated for 10 wks (Gp. I: HCD [0.5% cholesterol diet]. Gp. II: HCD plus E2 (30 µg/kg/day). Gp. III: HCD plus E3 (300 µg/kg/day). #2: 24 rabbits fed HCD for 8 wks were divided into 4 groups. RI; sacrifice, RII; fed a regular diet for 12 wks. additionally: RIII; fed a regular diet plus E2. RIV; fed a regular diet plus E3. S3- In vitro study The effects of E3 on eNOS and iNOS were studied.

**Results:** S1: Plasma E3 and E2 were increased by HRT. The FMD and the plasma NO metabolites were increased by E3 all over the study term. The nitroglycerin response was not changed.

S2: #1 The increased plasma lipid levels by HCD were not affected by E2 or E3. E2 and E3 reduced the atherosclerosis compared to Gp. I. The attenuated ACh or LNMIA (NOS inhibitor) induced responses in Gp. I was restored in RIII and IV. #2: E2 and E3 decreased atherosclerosis and decreased esterified cholesterol in the aorta. eNOS mRNA in vessel increased in R. decreased in RII, however, in RIII, however, did not change in RIII and IV. S3: Pre- or co-incubation with E3 enhanced eNOS mRNA and NO release. ICB28780, an E2 receptor antagonist, inhibited the effect of E3. E3 stabilized eNOS mRNA coacted with TNFα. iNOS induction in J774 cells was inhibited by pre- or co-incubation with E3. ICB28780 inhibited the influence of E3.

**Conclusion:** E3 could be an effective HRT in elderly. E3 may retard progression and cause regression of atherosclerosis partially via NO. E3 upregulated eNOS and inhibited iNOS induction via e2 receptor. These harmonious effects on iNOS and eNOS may have role in the anti-atherosclerotic effects of E3.

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**P.W23 Lipoproteins as carriers of isoflavone phytoestrogens**

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**Objective:** To explore the formation of isoflavone phytoestrogen derivatives and their incorporation into lipoproteins in human plasma in vivo. The isoflavone phytoestrogens daidzein and genistein possess significant antioxidant activity. They are contained in significant amounts in soybean. Intake of soy-derived isoflavone phytoestrogens increase lipoprotein oxidation resistance in humans according to our previous studies. We speculated that lipophilic phytoestrogen derivatives could be formed in plasma and taken up by lipoprotein molecules, a mechanism analogous to that observed for human estrogens.

**Methods and Results:** Human plasma was incubated with tritiated genistein ([3H]Gen) for 24 hours at +37°C with and without the lectin:cholesterol acyltransferase (LCAT) inhibitor 5,5-dithiobis 2-nitrobenzoic acid (DTNB). LDL and HDL were then isolated by sequential ultracentrifugation and purified by gel filtration to remove any non-lipoprotein associated molecules. A peak in the radioactivity coincided with the lipoprotein fraction suggesting the presence of lipoprotein bound [3H]Gen. The lipoprotein fraction was extracted and subjected to hydrophobic gel chromatography on Sephadex LH-20 to separate lipophilic and hydrophilic molecules. On LH-20 the lipoprotein radioactive was partly recovered in a lipophilic fraction in HDL and LDL. DTNB lowered the peak in radioactivity by approximately 20%. Our studies with tritiated daidzein ([3H]Daidzein) show similar results.

**Conclusions:** The incubation of plasma with isoflavone phytoestrogens derivative or derivatives are formed, which accumulate in LDL and HDL in small amounts. It seems that most of the lipophilic product is derived by some other enzyme than LCAT.

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**P.W22 Personal deficiencies and cardiovascular risk profile in middle-aged women: Results from the PSYRECA study**

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**Objectives:** To prove the influence of personal deficiencies on the cardiovascular risk factor profile in middle-aged women.

**Methods:** 239 women of the PSYRECA study were investigated. Fasting blood samples and the anthropometric data were obtained by a physician in the investigation centre. Parameters of lipoprotein and glucose metabolism, as well as inflammation markers were measured using standardised methods. The psychological questionnaires (STAXI – Spielberger 1992, coping styles for stress – Freese 1991) could be completed at home.

**Results:** In middle-aged women the factor anger-expression explains 40.1% of variance and consists of anger-control (~0.81), anger-out (0.085) and impatience (0.76). It is inversely related to total, free and LDL-cholesterol even after adjustment for age, FSH and BMI. Anger-control is positively and anger-out inversely correlated with total, free and LDL- cholesterol. Alpha-lipoproteins, HDL-C and the complement C3 correlate with the degree of passive coping (even after adjustment). Cynical hostility explains 21.6% of variance (components: anger-in 0.83; hostility 0.72) and is positively correlated with ApoE, the complement C3 and the alpha-1-fraction of the protein electrophoresis after adjustment. Free and total HDL- cholesterol are positively associated with the component anger-in, whereas there were no significant correlation of the component hostility, fatalism or wishful thinking with any metabolic parameter after adjustment. The parameters of glucose metabolism are not influenced by personal deficiencies.

**Conclusions:** The personal deficiencies anger-expression, cynical hostility and passive coping may influence negatively the lipoprotein profile and some inflammation marker in middle-aged women.

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**P.W21 Influence of private and work load factors on metabolic profile in middle-aged women**

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**Objectives:** To prove whether private and work load factors influence the change of the pattern within two years.
Methods: 161 middle-aged women of the PSYRECAs study were investigated in 1996 and 1998 (glucose metabolism, lipidoprotein metabolism, anthropometric data). They underwent an oral glucose tolerance test (OGTT). The women completed extensive psychological questionnaires on load factors (daily hassles, critical life events - Rimmann 1993, job demands - Richter, 1994, social stressors at work - Fröse, 1987).

Results: In 1996, all women had a normal OGTT. Within next 2 years 11 women (risk group) developed a metabolic profile which resembled an impaired glucose metabolism (serum glucose > 6.11 mmol/l and/or HbA1c > 6.1% and/or insulin > 0.15 mmol/l accompanied by a triglyceride/HDL-chol ratio > 1.00 and/or BMI > 25 kg/m2), 54% (46%) of the risk group, but only 23% (23%) of the controls were in the upper quartile of daily hassles (critical life events). Analogs, women of the risk group were more frequent in the upper quartile of the work load factors social stressors at work (44% vs. 28%) and job demands (22% vs. 12%).

Conclusion: In middle-aged women with high private and work load factors the risk increases to develop an impaired glucose metabolism.

PSYchoecological RESources and CArdiacvascular risk in middle-aged women

P:W26 REGULATION OF ENDOThelial FUNCTION

WeP1:W26 Intron 4 polymorphism of the endothelial nitric oxide synthase gene is associated with elevated blood pressure in type 2 diabetic patients with coronary heart disease

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Objective: Endothelial nitric oxide synthase (eNOS) gene is responsible for constitutive nitric oxide synthesis and arterial vasodilatation. Recently, intron 4 and exon 7 polymorphisms of the eNOS gene have been associated with coronary heart disease (CHD). Therefore, we studied the association of these polymorphisms of the eNOS gene and CHD in nondiabetic subjects and type 2 diabetic patients with CHD.

Methods: We determined the prevalence of the intron 4 and exon 7 polymorphisms of the eNOS gene by restriction fragment length polymorphism analysis among unrelated 308 nondiabetic subjects with CHD, 251 type 2 diabetic patients with CHD and randomly selected 82 healthy men.

Results: The 4a and Asp298 allele frequencies of the eNOS gene were 0.19 and 0.36 in nondiabetic patients with CHD, 0.21 and 0.27 in type 2 diabetic patients with CHD, and 0.15 and 0.29 in healthy subjects (p = ns). The Asp298 allele in exon 7 of the eNOS gene was not associated with elevated blood pressure in any of the study groups. Among type 2 diabetic patients with CHD, the 4a allele in intron 4 of the eNOS gene was associated with elevated levels of systolic (p = 0.035) and mean arterial blood pressure (p = 0.040) even after adjustment for confounding factors. In nondiabetic subjects these associations were not statistically significant.

Conclusions: We conclude that the 4a allele of the eNOS gene is not associated with CHD or type 2 diabetes, but it is related to elevated blood pressure levels particularly in type 2 diabetic patients with CHD.

WeP2:W26 A novel adipocyte-derived plasma protein, adiponectin, inhibits endothelial adhesion molecule expression through NF-κB signaling pathway

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Objective: Obesity is the common nutritional disorder and one of the major risk factors of atherosclerosis. However, the molecular basis for the link between obesity and atherosclerosis has not been fully elucidated. We found an adipocyte-specific secretory protein, adiponectin, and developed an ELISA system to determine adiponectin concentrations. Plasma adiponectin level negatively correlated with body mass index (BMI) (Arita et. al. BBRC 1999) and was significantly low in patients with coronary artery disease compared with BMI-adjusted control subjects (Ouchi et. al. Circulation 1999). Here we investigated the effects of adiponectin on monocyte adherence and adhesion molecule expression in human aortic endothelial cells (HAEC).

Methods: The adhesion of THP-1 cells to HAEC was determined by adherence assay. The surface expression of vascular cell adhesion molecule-1 (VCAM-1), endothelial-leukocyte adhesion molecule-1 (E-selectin) and intra-cellular adhesion molecule-1 (ICAM-1) was measured by cell ELISA. mRNA levels were determined by northern blotting. NF-κB-DNA binding activity was determined by electrophoretic mobility shift assays. TNF-α-inducible phosphorylation signals were detected by immunoblotting.

Results: Physiological concentrations of adiponectin dose-dependently inhibited TNF-α-induced THP-1 adhesion and expression of VCAM-1, E-selectin and ICAM-1 on HAEC. Adiponectin suppressed TNF-α-induced IkBα phosphorylation and subsequent NF-κB activation without affecting other TNF-α-mediated phosphorylation signals including JNK activation, p38 activation and Akt activation.

Conclusions: Adiponectin modulates inflammatory response of endothelial cells through NF-κB signaling pathways.

WeP3:W26 Hypoxia stimulates the release of the soluble form of Fls ligand and inhibits hypoxia-induced apoptosis in endothelial cells

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Objective: Since endothelial cells (ECs) play important roles in maintaining vascular homeostasis, loss of ECs by apoptosis may participate in the mechanism of atherogenesis. Here, we investigated to the function of sFls in ECs after exposure to hypoxia.

Methods: We used human umbilical vein endothelial cells (HUVECs). After hypoxia stimulation in airtight chamber (1% oxygen), we examined FlsL expression at protein levels by western blot and at mRNA levels by R7-PCR method. To quantified apoptosis, we used FACScan analysis with double-stained propidium iodide and FITC-conjugated antibody to annexin-V.

Results: Exposure of cultured ECs to hypoxia transiently up-regulated the expression of FlsL and increased the levels of sFlsL in the supernatant. The supernatant from hypoxia-exposing ECs inhibited EC apoptosis induced by hypoxia. However, a neutralizing antibody against FlsL abolished this inhibition by the supernatant. In addition, incubation with KB8301, an inhibitor of metalloproteinase, suppressed the release of sFlsL from ECs and enhanced hypoxia-induced EC apoptosis. Furthermore, exogenously added recombinant FlsL inhibited hypoxia-induced apoptosis.

Conclusions: ECs may protect themselves from hypoxia-induced apoptosis through up-regulating sFlsL release. Therefore, the shedding of FlsL could be a therapeutic target in regulating hypoxia-induced EC injury.

WeP4:W26 The impairment in endothelial vasculatory function induced by an acute elevation of free fatty acids was reversed by L-arginine, vitamin C or cox-inhibition

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Objective: An acute elevation of free fatty acid (FFA) levels has previously been found to impair endothelium-dependent vasodilation (EDV). The present study investigated if local administration of vitamin C (n = 9, 18 mg/min), L-arginine (n = 8, 12.5 mg/min) or the COX-inhibitor Diclofenac (n = 9, 0.5 mg/min) could counteract the effects on EDV and endothelium-independent vasodilation (EIDV) seen during i.v. infusion of Intralipid plus heparin alone (n = 10).

Methods: EDV and EIDV were investigated as the vasodilation evoked by local administration of Metacholine (4 μg/min) and Sodium nitroprusside (10 μg/min) in the forearm, measured by venous occlusion plethysmography.

Results: Intralipid plus heparin alone increased FFA levels and induced a reduction in EDV (p < 0.005), that was inhibited by concomitant administration of L-arginine or the COX-inhibitor. Concomitant vitamin C administration actually improved EDV (p < 0.02). Infusion of Intralipid plus heparin increased EIDV (p < 0.004), an effect being counteracted by L-arginine and COX-inhibition, but not by vitamin C. All three interventions counteracted the reduction in the EDV to EIDV ratio (an index of endothelial vasculatory function) induced by Intralipid plus heparin (p < 0.001).

Conclusions: An acute elevation of FFA levels impaired endothelial vasculatory function. This impairment could be counteracted by administration of L-arginine, vitamin C or COX-inhibition.
WeP5:W26
Expression of nitric oxide synthase in endothelial cells is mediated by bilobalide and ginkgolide A
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Objective: Excessive production of nitric oxide (NO) may pose cytotoxic effects to cells in the vascular walls. The extracts of ginkgo biloba leaves (EBG) have been demonstrated to inhibit NO production in endothelial cells. The objective of this study was to investigate the contributions of several active components to the EGB mediated inhibition of NO production in human endothelial cell line (EVC304).

Methods: Cells were incubated with bilobalide or ginkgolide A for 4 hr. The amounts of NO metabolites released by cells were quantified and cellular NO synthase activities were determined. NOS expression was determined by Western immunoblotting analysis. The levels of mRNA were examined by reverse transcription-polymerase chain reaction (RT-PCR) assay.

Results: Bilobalide or ginkgolide A (0.5–1.0 μg/ml) caused a 25–40% reduction of NO metabolites released by endothelial cells. The inducible NO synthase (iNOS) activity was reduced by 20–25% in bilobalide treated cells and by 30–35% in ginkgolide A treated cells with a concomitant reduction in the levels of iNOS protein mass and mRNA.

Conclusions: The results obtained from the present study suggest that bilobalide and ginkgolide A may play an important role in EGB mediated selective inhibition of iNOS in endothelial cells. These two components may have therapeutic implication for the treatment of disorders due to unbalanced production of NO.

WeP6:W26
Improvement of vascular reactivity during pravastatin treatment is affected by circulating nitric oxide inhibitor (ADMA) levels in young adults
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Objective: The aim of the study was to investigate whether the plasma concentrations of asymmetric dimethylarginine (ADMA), an endogenous nitric oxide (NO) synthesis inhibitor, correlate with serum lipids and myocardial blood flow as measured by positron emission tomography (PET) and with the effects of pravastatin treatment.

Methods: This study was randomized, double-blind and placebo-controlled with two treatment groups: placebo (n = 25) and pravastatin (40 mg/day) (n = 26) for 6 months. All measurements were performed at baseline and at the end of the period. Plasma levels of ADMA were measured by HPLC.

Results: There were no significant changes in ADMA during statin intervention. Baseline ADMA correlated significantly with adenine flow change during statin intervention (r = −0.50, p = 0.002) and adenosine flow after statin treatment (r = −0.43, p = 0.049), while similar correlations were not seen after placebo treatment. High baseline ADMA (over median value) concentration predicts the lowering of coronary flow reserve (CFR) after statin intervention (change = −21.7) and low baseline ADMA value (<median) the improvement of CFR after statin treatment (change = +24.4%). ADMA-group by time interaction, p = 0.034 in ANOVA).

Conclusions: NO-related improvement of vascular reactivity during pravastatin treatment is affected by circulating ADMA levels.

WeP7:W26
Paraoxonase gene coding high and low active alleles modulates coronary function in young men
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Objectives: Endothelial dysfunction leading to impairment of coronary flow reserve (CFR) is associated with lipoprotein oxidation, which is affected by antioxidative enzyme paraoxonase (PON). We divided PON genotypes to low (QQ, or MM, ML) and high active (LL, or RR, RR) groups and related them to CFR.

Methods: Study group consisted of 51 healthy men aged 35 ± 4 years. The myocardial blood flow was quantified with [15O]H2O and positron emission tomography at rest and after adenosine infusion. PON genotypes were determined by PCR and restriction enzyme digestion.

Results: The rate-pressure product adjusted CFR was 17% higher (p = 0.001) in high active LL homozygotes compared to 15% higher in high active R-allele carriers (RR, RQ) than in the respective low active groups (ANCOVA; age, BMI, LDL-protein, HDL-cholesterol, triglyceride and hematocrit as covariates).

Conclusions: Our study suggests that PON genotype may be associated with coronary function.

WeP8:W26
Endothelial function of conduit and resistance arteries in nephrotic range proteinuria
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Objective: To test the hypothesis that endothelial dysfunction occurs in nephrotic range proteinuria primarily as a consequence of dyslipidaemia.

Methods: Brachial artery and forearm microcirculatory endothelial function was compared among patients with nephrotic range proteinuria (NRP, n = 14), primary hyperlipidaemia (HL, n = 15) and normal controls (NC, n = 16). Endothelial function was studied by measuring post-ischaemic flow-mediated dilatation (FMD) of the brachial artery using high resolution ultrasonography. Endothelium-independent, glyceryl trinitrate (GTN) mediated brachial artery vasodilatation was also measured. Basal and post-ischaemic blood flow of the forearm microcirculation was measured using venous-occlusion strain gauge plethysmography.

Results: Serum creatinine was similar among groups. The proteinuric group had a mean albumin of 27.6 g/L (standard deviation, SD 1.8) and 24-hour urinary protein excretion of 6.3 g (1.3). Plasma lipids and lipoproteins were not statistically different between the NRP and HL groups. Brachial artery FMD was significantly lower in the NRP and HL groups compared with the controls (NRP 4.7% (1.3%), HL 4.9% (0.7%) and NC 8.3% (0.6%), p < 0.05 vs NC); GTN mediated dilatation and basal and post-ischaemic forearm blood flow were not statistically different among the three groups. Variations in FMD were significantly correlated with LDL-cholesterol (r = 0.37, p < 0.05), but not blood pressure, serum albumin, proteinuria, homocysteine or other plasma lipids and lipoproteins.

Conclusion: Patients with nephrotic range proteinuria have endothelial dysfunction of conduit arteries in the peripheral circulation, similar to that observed in patients with primary hyperlipidaemia. This suggests dyslipoproteinaemia is the principal cause of endothelial dysfunction of conduit arteries in nephrotic range proteinuria. Confirmation of this should be sought with an intervention trial of lipid-regulating therapy.

WeP9:W26
Lipid-lowering improves endothelial function in nephrotic range proteinuria
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Objective: To determine whether lipid-modifying therapy with atorvastatin improves impaired endothelial function in patients with nephrotic range proteinuria (NRP).

Methods: A sequential, open-label study of the effects of atorvastatin on dyslipidaemia and endothelial dysfunction in 9 patients with NRP. Endothelial function was assessed at baseline, after 12 weeks of atorvastatin treatment and after an 8 week wash-out period. Brachial artery endothelial function was studied by measuring post-ischaemic flow-mediated dilatation (FMD) using ultrasonography. Endothelium-independent, glyceryl trinitrate (GTN) mediated vasodilatation was also measured.

Results: At baseline, median serum albumin was 31 g/L (range 20–40) and 24 hour protein excretion was 4.7 g (1.0–16.23). There was no significant change in serum creatinine and 24 hour protein excretion during the study. Total cholesterol (TC) and triglycerides (TG) were significantly lower following treatment with atorvastatin 20 mg (20–40): TC 8.1 mmol/L (5.9–14.9) vs 5.2 (4.0–3.6), TG 2.9 mmol/L (1.3–15.0) vs 1.6 (1.0–3.5), both p < 0.05. Brachial artery FMD improved significantly following atorvastatin treatment: 2.1% (−1.2−5.2%) to 4.7% (0.8–16.3%), p < 0.05. At the end of the 8 week wash-out, FMD had significantly deteriorated to 3.2% (−2.4–8.2), p < 0.05 vs week 12 FMD, and was similar to pre-treatment values. GTN mediated dilatation was unchanged through the study.

Conclusion: Atorvastatin significantly reduced the hyperlipidaemia of...
NRP. This was associated with improved conduit artery endothelial function after 12 weeks of treatment. This is consistent with the hypothesis that dyslipoproteinaemia is the primary cause of endothelial dysfunction in NRP.

WeP10.W26

Fenofibrate improves endothelial function in dyslipidaemic Non-Insulin Dependent Diabetes (NIDDM)

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Objective: To examine the effects of fenofibrate on vascular function in asymptomatic patients with NIDDM and dyslipidaemia.

Methods: 40 patients with dyslipidaemic NIDDM in good glycaemic control (29 males, 11 females; age 54±10 years; HbA1c 6.2±1.4%; Chol 5.3±0.7, TG 2.0±1.0; HDL 1.2±0.4 mM) were randomised to micronised fenofibrate 200 mg/day or placebo for 12 weeks. Forearm microrcalorific function was measured before and after treatment using venous occlusion plethysmography and intrabrachial infusions of acetylcholine (ACH 7.5, 15, 30 µg/min), bradykinin (BK 25, 50, 100 µg/min), sodium nitroprusside (SNP 1.5, 3, 10 µg/min), NG-nitro-l-arginine (L-NMMA 4 µmol/min) and confuision of L-NMMA with ACh and BK. All studies were performed after one week of aspirin 650 mg daily.

Results: Plasma cholesterol, non-HDL cholesterol and triglyceride fell significantly with fenofibrate by 15%, 22% and 42%, respectively, and HDL cholesterol increased by 17% (p < 0.001). Pre-treatment ACh responses were significantly impaired in the diabetes compared with non-diabetics. Compared with placebo, fenofibrate was associated with a significant improvement in the response to ACh (AUC of % increase of forearm blood flow ratio 223 vs 370, p = 0.002), but there were no significant effects on basal blood flow or on responses to BK, SNP, L-NMMA or confuision of L-NMMA with BK or ACh.

Conclusion: Fenofibrate improves endothelial function in NIDDM by enhancing endothelial release of nitric oxide (NO) in response to ACh. Fenofibrate does not, however, influence basal synthesis of NO, release of endothelial derived hyperpolarising factor or smooth muscle cell function.

WeP11.W26

Vitamin C improves endothelial function in healthy postmenopausal women

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Objectives: Oestrogen deficiency is associated with increased cardiovascular risk and endothelial dysfunction. Oestrogen has possible antioxidant effects and improved endothelial function is reported with antioxidants, including vitamin C. We aimed to determine the effect of vitamin C on endothelial function in healthy oestrogen-depleted and oestrogen-replete postmenopausal women.

Methods: Subjects (age 47–57 y) were at least one year post menopause. Ten (serum oestradiol < 50 nmoL/L) were not receiving hormone replacement therapy, while 10 hysterectomised subjects received oestradiol (8 subcutaneous, 2 oral). Forearm blood flow, FBF, (strain-gauge plethysmography) responses to intra-brachial artery infusions of incremental doses of acetylcholine (endothelium-dependent vasodilatation) and sodium nitroprusside (endothelium-independent vasodilatation) were determined at baseline, and following 1.5 gm vitamin C intravenously over 15 minutes.

Results: Groups were similar with respect to age, body mass index, blood pressure, fasting plasma glucose, lipids, and smoking status. FBF responses to acetylcholine were enhanced after vitamin C in the oestrogen-deplete (area under dose-response curve, AUC, 9.9±2.6 vs 15.1±3.2, mean±SEM, p = 0.01) but not in the oestrogen-replete group (16.3±2.3 vs 19.1±2.8, p = 0.1). Vitamin C had no effect on resting FBF (pre vs post vitamin C, oestrogen-deplete, 1.9±0.3 vs 1.8±0.2, oestrogen-replete, 2.5±0.2 vs 2.5±0.3 mL. 100 mL.1. min.1, or response to sodium nitroprusside, (pre vs post vitamin C, AUC, oestrogen-deplete, 7.6±0.7 vs 7.4±0.9, oestrogen-replete, 9.0±0.8 vs 9.2±0.8) in either group.

Conclusions: These results indicate that endothelial function may be improved acutely by antioxidant treatment in oestrogen-deplete postmenopausal women.

WeP12.W26

Endothelial function and cardiovascular risk factors in young healthy adult offspring of parents with type 2 diabetes: Effect of vitamin E in a randomised double-blind placebo-controlled study

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Objective: Antioxidants may have a protective effect against endothelial dysfunction and atherosclerosis. Metabolic abnormalities associated with an increased risk of diabetes and cardiovascular disease have been identified in close relatives of diabetic subjects. We aimed to determine the effect of antioxidant treatment on endothelial function and cardiovascular risk factors in young healthy adult offspring of parents with type 2 diabetes.

Methods: Thirteen offspring (18–38 y, 11 M/2 F, BMI <30 kg/m2) completed a randomised, double-blind, crossover trial (12 weeks vitamin E 800 i.u./day or placebo, 6 week washout). Endothelial function was assessed by forearm blood flow responses (strain-gauge plethysmography) to intra-brachial artery infusions of acetylcholine (endothelium-dependent vasodilation), sodium nitroprusside (endothelium-independent vasodilatation) and NG-nitro-l-arginine, L-NMMA, (nitric oxide synthase inhibition).

Results: There was no difference between groups in BMI, blood pressure, fasting glucose, insulin or lipids. Forearm blood flow responses (area under dose-response curve, AUC) to acetylcholine (active vs placebo, 15.0±4.7 vs 13.4±3.1, p = 0.5), nitroprusside (1.3±2.0 vs 13.4±1.6, p = 0.5), and L-NMMA (−1.0±0.4 vs −1.2±0.2, p = 0.5) did not differ. Vitamin E had no effect on circulating plasminogen activator inhibitor-1 (18.8±2.1 vs 17.9±2.4 U/mL, p = 0.5), von Willebrand factor (100±9 vs 101±7%, p = 0.5), fibrinogen (2.6±0.2 vs 2.6±0.2 g/L, p = 0.5) or urate (0.3±0.2 vs 0.3±0.22%, p = 0.5).

Conclusion: Three months of vitamin E, 800 i.u./day, had no effect on endothelial function or cardiovascular risk markers in healthy adult offspring of parents with type 2 diabetes.

WeP13.W26

Flow-mediated vasodilatation, insulin sensitivity and the metabolic syndrome

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Objective: To evaluate the endothelial dependent vasodilatation in the brachial artery and to study the relationship to insulin sensitivity and the metabolic syndrome in 60-year old clinically healthy men.

Methods: The subjects were recruited from the general population and were all 60 years old (n = 53). Ultrasound images for measurement of lumen diameter of the brachial artery were recorded before and after reactive hyperaemia induced by occlusion of the artery, both with and without ischaemic hand exercise during the occlusion. Glucose infusion rate was determined by euglycaemic hyperinsulinaemic clamp as a measure of insulin sensitivity. The metabolic syndrome was defined according to a definition suggested by a working group associated to the WHO.

Results: Flow-mediated vasodilatation was 3.3% when hyperaemia was induced by occlusion only and 8.7% after occlusion plus ischaemic hand exercise (p = 0.001, n = 51). However, no relationship was observed between any measure of flow-mediated vasodilatation and glucose infusion rate (r = 0.04 and r = 0.08, n = 49, NS). Furthermore, subjects with the metabolic syndrome (n = 13) did not differ in any measure of flow-mediated vasodilatation compared to those with no risk factors (n = 11).

Conclusion: In this study, the ultrasound method to evaluate endothelial function did not show low insulin sensitivity or the metabolic syndrome was associated with impaired flow-mediated vasodilatation in otherwise clinically healthy 60-year old men.

WeP14.W26

Elevation of an endogenous inhibitor of nitric oxide synthase in patients with coronary atherosclerosis

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Objectives: Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide (NO) synthase and has been implicated in systemic atherosclerosis. However, the relationship between NO and ADMA in patients with coronary artery disease (CAD) has not been investigated.

Methods: 40 patients (Group I, 70 ± 7 yrs) with CAD (>70% stenosis of major coronary artery) were compared with 32 patients (Group II, 67 ± 8 yrs) with normal coronary artery. Another 9 healthy subjects were included as a control group (Group III, 64 ± 9 yrs). Fasting plasma samples were used for the determinations of ADMA, L-arginine, and nitrate/nitrite (NOx). Fasting urine was collected for measurement of nitrate excretion, corrected by urinary creatinine (UnCr).

Results: The plasma concentrations of ADMA in Group I patients were 0.49 ± 0.18 μM, which was significantly higher than those in Group II and Group III (p < 0.001, 0.001 respectively). The L-arginine/ADMA ratios in Group I patients were also lower than in the other two groups (Group I vs Group II, p < 0.001; Group I vs Group III, p < 0.001). On the other hand, the plasma NOx and UnCr levels were similar in patients with and without CAD. However, both parameters were significantly higher in the normal control group. There were no significant correlations among plasma levels of ADMA, urinary nitrate excretion and plasma NOx.

Conclusions: The plasma concentration of ADMA in patients with significant coronary stenosis is increased, suggesting that ADMA may be involved in coronary atherogenesis.

WeP15:W26 Antioxidant treatment restores endothelial function in vivo and in vitro in porcine hypercholesterolemia
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Objective: This study aimed to determine whether antioxidant vitamins C and E can restore endothelial function in hypercholesterolemia through changes in eNOS expression, and whether endothelial cell treatment with LDL from experimental animals could reproduce these changes in vitro.

Methods: 36 animals were divided into three groups: a normal-cholesterol (NC) control group (n = 12); a high-cholesterol (HC) group (n = 12); and a high-cholesterol plus vitamins C and E (HCV) group (n = 12), supplemented with 1 g vitamin C and 1000 IU vitamin E/day. Endothelium-dependent vascular responses of the left internal iliac artery (LIIA) to acetylcholine (AC) were determined at the time of sacrifice. Plasma samples were drawn and arteries were obtained and processed for eNOS immunostaining. eNOS expression was studied in porcine coronary endothelial cells (EC) incubated with LDL isolated from porcine plasmas.

Results: HC and HCV had similar cholesterol levels, higher than NC. Vasoconstrictor response to AC in HCV was similar to NC, and both were significantly higher than HC (P < 0.05). Immunohistochemistry staining for eNOS revealed a significant decrease in immunoreactivity in HC, compared with both HCV and NC (P < 0.05). 48 or 96 h treatment of EC with LDL from HC significantly reduces eNOS expression, as compared with LDL from NC and HCV.

Conclusions: Vitamins C and E improve the endothelium-dependent vasoconstrictor capacity in porcine hypercholesterolemia at least by restoring eNOS expression. In vitro results suggest that vitamins can revert or mitigate hypercholesterolemia-derived LDL modification and its harmful effects on endothelial function.

WeP16:W26 Soluble cellular adhesion molecules are increased in patients with low HDL-cholesterol
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Objectives: Cellular adhesion molecules (CAMs) play a critical role in the formation, progression and outcome of vascular lesions, by mediating attachment and transmigration of leukocytes to the vascular endothelial wall. HDL have been shown to inhibit CAMs expression in cultured endothelial cells. Whether enhanced plasma concentrations of soluble CAMs contribute to the high risk for ischemic heart disease in individuals with low plasma HDL-cholesterol (HDL-C) is unknown.

Methods: Plasma levels of soluble vascular cell adhesion molecule-1 (sVCAM-1), intercellular adhesion molecule-1 (sICAM-1), and E-selectin were measured in 65 subjects with HDL-C below the 10th or above the 90th percentile for the Italian population (Low-HDL and High-HDL), 65 hyperlipidemic patients, and 65 healthy controls. 24 Low-HDL patients were given fenofibrate to raise plasma HDL levels.

Results: The plasma levels of sICAM-1 and E-selectin were significantly higher in the Low-HDL individuals (290 and 58 nm/gl) than in the other subjects, who displayed similar scCAMs levels (256 and 47 nm/gl). Plasma sVCAM-1 levels did not differ among the various groups. HDL-C was inversely and significantly associated with sICAM-1 and E-selectin in the Low-HDL subjects, but not in individuals with normal or elevated HDL-C; HDL increase by fenofibrate (21%) lead to a significant reduction of sICAM-1 (~5%) and E-selectin (~6%) but not sVCAM-1.

Conclusions: These data indicate that a "normal" plasma HDL-C level is required to suppress CAMs expression and maintain low plasma scCAMs levels. An increased CAMs expression in the vascular endothelial wall may be a mechanism by which low plasma HDL levels promotes atherogenesis and causes acute atherothrombotic events.

WeP17:W26 Soy protein diet significantly improves endothelial function and lipid parameters
A. Yildirim, L. Tokgozoglu, A. Oto, T. Onducu, I. Haznedaroğlu, D. Akmec, G. Koksal, E. Sade, Ş. Kirazli, S. Kes. Hacettepe University Faculty of Medicine, Ankara, Turkey

Objective: Although the effects of polyunsaturated-monounsaturated and saturated fats on endothelial function have been well documented, the effects of soy protein diet are not well known. Thrombomodulin (TM) is a cell surface glycoprotein located at the luminal surface of vascular endothelium and its increased plasma level reflects endothelial injury. The aim of the study was to evaluate the effects of soy protein diet on plasma lipids and TM levels.

Methods: Fourteen hypercholesterolemic non-smoker male patients (age 51 ± 11, range 39-69) with a normal body mass index were included in the study. After calculating their daily requirements, a diet with 60% of protein from soy was instituted. All anthropometric, lipid parameters and plasma TM levels were assessed at baseline and 6 weeks after diet in the same patients.

Results: There was a significant improvement in plasma cholesterol, low density lipoprotein (LDL) levels and TM levels after diet. High density lipoprotein (HDL) levels were not affected whereas the decrease in triglyceride was borderline (Table).

<table>
<thead>
<tr>
<th></th>
<th>Before diet</th>
<th>After diet</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombomodulin (ng/ml)</td>
<td>49 ± 42</td>
<td>44 ± 17</td>
<td>0.004</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>268 ± 35</td>
<td>223 ± 37</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>244 ± 108</td>
<td>205 ± 61</td>
<td>0.056</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>42 ± 16</td>
<td>41 ± 6</td>
<td>0.950</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>176 ± 33</td>
<td>139 ± 35</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Conclusions: Soy protein diet improves the lipid profile significantly in patients with hypercholesterolemia. Furthermore, the endothelial function as judged by TM levels also improves.

WeP18:W26 N,N'-diacetyl-L-cystine (DINAC) improves endothelial vasodilatory function in atherosclerotic WHHL rabbits
Ulla Brandt-Eliasson, Tommy Abrahamsson, Göran Walldius, Krut Pettersson. Pharmacology CVA, AstraZeneca R & D, SE-431 83 Mölndal, Sweden

DINAC, a novel immunomodulator, stimulates contact sensitivity/delayed type hypersensitivity reactions in mice induced by oxazoline (IPET 1998; 388: 1174-84). The maximal effect was observed at an oral dose of 3 μmol/kg. To study if DINAC can affect dysfunctional endothelial vasodilatation, 40 weeks old WHHL rabbits were given DINAC (3 μmol/kg/day) for 3 weeks (in the drinking water). Untreated WHHL and age matched, non-atherosclerotic (heterozygous) rabbits were controls. At termination, aortic rings were mounted in organ baths and contracted with phenylephrine. Concentration-dose response curves to acetylcholine (Ach) and the calcium ionophore A23187 were used to measure endothelium mediated vascular relaxation (ER). In each aortic ring histological analysis of atherosclerotic lesions was performed. The results (mean ± SEM) are summarised in the table. Maximal ER in response to both Ach and A23187 were improved in DINAC treated animals compared to controls, and were similar to ER in heterozygous rabbits. There was no significant difference in atherosclerosis between vehicle and DINAC rabbits. For both Ach and A23187, the maximal
response was inversely correlated to the amount of atherosclerosis in the aortic rings (p < 0.01).

Att relax (%) A23187 relax (%)

WHRL, standard (n = 9) 15 ± 5 11 ± 7
WHRL, DNAC (n = 9) 40 ± 7 (p = 0.05) 37 ± 2 (p = 0.05)
Hetero, non-ach (n = 9) 40 ± 6 40 ± 4

DNAC thus improved ER in atherosclerotic rabbits, and may represent a new treatment modality for atherosclerosis related diseases.

**WeP19.W26** Plasma asymmetric dimethylarginine (ADMA) increases during oral methionine loading

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**Background:** Homocysteine is a risk factor for atherothrombotic disease, although the underlying pathogenic mechanism is not fully understood. An acute increase of plasma homocysteine during methionine loading is accompanied by a transient impairment of vascular function as measured by diminished flow-mediated vasodilatation. This suggests that homocysteine has an acute effect on the vascular endothelium by inhibition of nitric oxide synthase (NOS).

**Hypothesis:** Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of endothelial NOS. ADMA synthesis from arginine requires incorporation of two methyl groups and thus during synthesis of ADMA two equivalents of methionine are consumed. It is therefore conceivable that methionine loading stimulates ADMA synthesis, leading to inhibition of endothelial NOS.

**Subjects and Methods:** Subjects were ten randomly chosen patients, referred to the laboratory for a methionine loading test. Plasma samples, taken before and 6 hours after oral administration of methionine (0.1 g/kg body weight), were analysed for ADMA and arginine by HPLC.

**Results:** Plasma levels of ADMA increased from 0.48 ± 0.07 μM pre-load to 0.52 ± 0.07 μM 6 h post-load (P = 0.002; paired t-test). Arginine levels decreased from 91 ± 22 μM preload to 87 ± 20 μM 6 h post-load (NS). The Arginine/ADMA ratio dropped from 192 ± 54 pre-load to 170 ± 38 post-load (P = 0.04).

**Conclusions:** In accordance with our hypothesis, ADMA levels increase after methionine loading, resulting in 11% reduction of the arginine/ADMA ratio. We conclude that inhibition of NOS by ADMA may contribute to the acute impairment of vascular function during methionine loading.

**WeP20.W26** The role of nitric oxide in ischemia/reperfusion injury of the atherosclerotic mouse heart

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**Objective:** To investigate the role of nitric oxide (NO) in ischemia/reperfusion injury of the mouse heart with coronary atherosclerosis compared to hearts with normal vessels.

**Methods:** Hearts of the apolipoprotein E/LDL receptor double knockout mice fed an atherogenic diet for 6-8 months were Langendorff-perfused with 40 minutes of global ischemia and 60 minutes reperfusion, and compared with C57BL/6 controls. Heart function was measured by a left ventricular balloon, and coronary flow continuously registered. Other hearts were given the NO inhibitor (NG-nitro-L-arginine methyl ester: L-NAME) or the NO donor (S-nitroso-N-acetylpenicillamine: SNAP). After reperfusion, hearts were stained with Triphenyl Tetrazolium Chloride to measure the infarct size (n = 6-8 in each group).

**Results:** Hearts of animals with atherosclerosis were more susceptible to ischemia/reperfusion injury than hearts of animals with healthy vessels. SNAP protected function and reduced infarct size in atherosclerotic hearts, but the same concentration SNAP was detrimental in normal hearts. A lower concentration of SNAP protected against ischemia/reperfusion dysfunction in normal hearts. L-NAME protected function and reduced infarct size in normal hearts but deteriorated function of atherosclerotic hearts.

**Conclusions:** NO protects against ischemia/reperfusion in hearts of animals with severe atherosclerosis, evident as improved function and reduced infarction by SNAP, but detrimental effects of L-NAME. These findings suggest that impaired NO release contributes to reperfusion injury in coronary atherosclerosis. However, NO-overproduction during reperfusion may be detrimental, perhaps due to peroxynitrite formation.

**WeP21.W26** Spontaneous ischemic events in atherosclerotic mice preclude their hearts

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**Objective:** To investigate if spontaneous noncardiac ischemic events in severely atherosclerotic mice could induce a cardioprotective effect analogous to ischemic preconditioning.

**Methods:** Some apolipoprotein E/LDL receptor double knockout (Apo E/LDLr−/−) mice developed signs of cardiac ischemia after 6-8 months on an atherogenic diet (n = 8). Their hearts were Langendorff-perfused 24-48 hours after onset of symptoms with 40 minutes of global ischemia and 60 minutes reperfusion, and compared with Apo E/LDLr−/− mice without any symptoms (control, n = 7). Hearts were sectioned and stained with TTC to measure infarctions induced by Langendorff. An anti-fibrinogen antibody was employed to evaluate recent infarctions, while masson trichrome staining was used to evaluate the occurrence of old (>1 week) infarctions. Cardiac troponin T (cTnT) was measured in serum and coronary effluent during reperfusion. In vitro reactivity of the thoracic aorta was investigated in organ bath.

**Results:** Animals with suspected spontaneous cardiac events had increased serum cTnT prior to the experiments (0.37 ± 0.09 μg/L vs. control 0.15 ± 0.05 μg/L, p < 0.05). An majority of the hearts of mice with suspected ischemic events had positive staining with anti-fibrinogen Ab and negative staining with masson trichrome, indicating fresh ischemic events. Hearts of infarcted mice had improved left ventricular function (p < 0.05), reduced cTnT release (p < 0.05) and reduced infarct size (p < 0.05) during reperfusion. In vitro contraction to PGF2α was reduced in aortas from these mice.

**Conclusions:** These findings suggest that spontaneous ischemic events occurred in Apo E/LDLr−/− mice, which protected heart function and cell viability analogous to preconditioning. This may be secondary to reduced vessel contraction.

**WeP22.W26** Effects on soluble markers of endothelial dysfunction after 4.5 years treatment with ACE-inhibitor ramipril in patients with coronary heart disease (HOPE substudy)

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**Objective:** To measure possible effects of ramipril on circulating inflammatory and haemostatic markers of endothelial dysfunction in a subgroup of the HOPE (Heart Outcomes Prevention Evaluation) study. Significant reductions in primary clinical endpoints were obtained in the ramipril treated patients.

**Methods:** The substudy was undertaken in 55 patients (>55 yrs, 10 women) randomised in the HOPE study to ramipril (R-group n = 28) or placebo (P-group n = 27). Fasting blood samples were collected at randomisation, and after 6, 12 and 54 months of treatment for determinations of markers of endothelial function: von Willebrand factor (vWF), tissue plasminogen activator antigen (tPAag), thrombomodulin (TM), vascular cell adhesion molecule-1 (VCAM-1) and the soluble forms of E-selectin. Results are expressed as differences in relative changes between the groups.

**Results:** Numerically, a smaller increase in tPAag, E-select and VCAM-1 were noted in the R-group as compared with the P-group during the study period. However, these differences did not reach statistical significance, except for VCAM-1 after 6 months where a 9% decrease was noted in the R-group vs. a 1% increase in the P-group (p = 0.03).

**Conclusion:** In this HOPE substudy there were no significant effects on the measured endothelial cell markers, although some observed changes were in favor of the ramipril treated group. It should be emphasised that the number of patients is small, thus lack of statistical power might be present. Whether positive clinical effects of ramipril may be related to measurable changes on the endothelium has to be evaluated in larger subsamples.

**WeP23.W26** Antioxidant protection with probucol ameliorates endothelial function and klotho gene expression in Otsuka Long-Evans Tokushima Fatty (OLETF) rats

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**Objectives and Methods:** Klotho-deficient mouse presents multiple pheno-
types of human aging including arteriosclerosis. We have reported that nitric oxide (NO) production was reduced in klotho mice. In present study, we examined if oral treatment with the antioxidant, probucol (500 mg/kg/day), increases in klotho mRNA expression or restores cardionic function in Otsuka Long-Evans Tokushima Fatty (OLETF) rats developing hypertension, diabetes mellitus, obesity, and hyperlipidemia (n=6).

**Results:** Systolic blood pressure (152 ± 5 vs. 137 ± 4 mmHg), body weight (688 ± 20 vs. 518 ± 12 g), plasma glucose (392 ± 12 vs. 163 ± 7 mg/dL) and triglyceride (307 ± 44 vs. 50 ± 4 mg/dL) were significantly increased in OLETF rats as compared with control (LETO; Long-Evans Tokushima Osuka) rats (p < 0.05). Endothelium-dependent relaxation of aorta in response to acetylcholine (10−5 M) was significantly attenuated in OLETF rats (60 ± 6%) as compared with LETO rats (98 ± 2%). The expression of klotho mRNA was also significantly decreased in OLETF rats (p < 0.05). Oral treatment with probucol for 10 weeks improved these parameter (130±3 vs. 27±9 mg/dL) and increased in klotho mRNA expression as well as endothelium-dependent relaxation to 87 ± 4% (p < 0.05).

**Conclusions:** We demonstrated that endothelial dysfunction and a decrease in klotho gene expression in OLETF rats are ameliorated by treatment with probucol, suggesting that the antioxidant protection results in an increased klotho gene expression and an improvement in endothelial function against multiple atherogenic risk factors.

**WeP24:W26** Chylomicron remnant induces apoptosis in human umbilical venous endothelial cells

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**Objective:** Since chylomicron remnant (CR) has been regarded as a proatherogenic lipoprotein in postprandial hyperlipidemia, we investigated the effect of CR on apoptosis in vascular endothelial cells.

**Methods:** CR was isolated from plasma of the functionally hepatopatized male rats injected with chylomicron. To test the viability of human umbilical venous endothelial cells (HUVECs), we used WST-1 assay. Apoptotic HUVECs were detected by Hoechst 33342, TUNEL staining, and FITC-conjugated Annexin V. The involvement of the caspase CPP32 (caspase-3) was examined by western blot analysis. The lipid of CR and chylomicron (CM) was analysed by TLC.

**Results:** WST-1 assay revealed that incubation of HUVECs with CR (10 μg protein/ml) significantly decreased viable cells in a time- and dose-dependent manner. In contrast, CM did not affect cell viability at the concentration that contained equal triglyceride to 10μg protein/ml of CR. In the presence of CR, HUVECs displayed cell shrinkage and condenced and/or fragmented nuclei, and TUNEL-positive HUVECs increased dose-dependently. FACS can analysis revealed that CR increased Annexin-V binding to HUVECs. In addition, western blot analysis showed that HUVECs treated with CR (10 μg/ml) induced the proteolytic cleavage of CPP32 into its active subunit p17. The amount of lysophosphatidylcholine (lysoPC) in CR was increased by 7-fold compared to CM. Furthermore, we observed lysoPC also induced apoptosis and activation of CPP32 in HUVECs.

**Conclusion:** Our findings demonstrate that CR induces apoptosis in HUVECs by an activation of CPP32. This proapoptotic effect of CR may contribute its atherogenesis.

**WeP25:W26** Proline7 substitution in the prepro neuropeptide Y (NPY) is associated with enhanced brachial artery flow-mediated dilatation in middle-aged men


**Objective:** Leucine7 to Proline7 polymorphism in the signal peptide region of the preproNPY gene is associated with vascular endothelial function of middle-aged subjects. This study was undertaken to elucidate the function of the vascular endothelial cells in subjects having Proline7 substitution in the preproNPY.

**Methods:** The subjects were participants (n = 152, mean age 54 years) of an epidemiological twin study aiming to detect risk markers of subclinical coronary heart disease in middle-aged men. Flow-mediated dilatation (FMD) of the brachial artery was used to measure endothelial function with high resolution ultrasound. Low FMD is known to be an early marker of subclinical atherosclerosis. Classic CHD risk factors were measured using standard methods.

**Results:** FMD of the subjects with the Proline7 substitution (n = 20) was 48% higher (7.3± 4.9%) compared with men having the wild type (n = 132) signal peptide sequence (-1.97 ± 4.05%), p = 0.018. Adjusting for age, serum lipids, blood pressure, BMI, basal vascular diameter, medication, diabetes or IGT, smoking and history of CHD or hypertension did not modify this result. The vasodilatory response to exogenous nitroglycerin tended to be slightly higher in men with Proline7 mutation (15.2 ± 6.76% vs 12.9 ± 6.60%, p = 0.14).

**Conclusions:** Our results suggest that these men having Proline7 substitution in the NPY signal peptide sequence have enhanced flow-mediated vasodilatation. This may reflect decreased vasoconstrictive effect of the arterial smooth muscle in subjects with this mutation.

**WeP25:W26** A role of protein kinase C in the regulation of cytosolic phospholipase A2 in bradykinin-induced PG2 synthesis by human vascular endothelial cells


**Objective:** To elucidate the mechanism by which bradykinin (BK) enhances PG2 production in human umbilical vein endothelial cells (HUVECs).

**Methods:** PG2 and IP3 was measured by RIA. Cytosolic free Ca2+ concentration ([Ca2+]i) was measured by fura2/AM. Ca2+ uptake, release and Ca2+ release from the Ca2+ stores were measured by fura2/AM. Cytosolic phospholipase A2 (cPLA2) activity was measured by a modified Dole extraction procedure. Protein kinase C (PKC) activity was measured with PKC enzyme assay system. The quantitative analysis of mRNA was measured by competitive PCR.

**Conclusions:** BK-induced PG2 synthesis was observed in a dose- and time-dependent manner. Pretreatment with PKC inhibitor (GF109203X) or EGTA significantly reduced BK-induced increase of PG2. Addition of BK resulted in an increase in [Ca2+]i, Ca2+ uptake, and Ca2+ release from the Ca2+ stores. cPLA2 activity was increased by the addition of BK. Pretreatment with PKC inhibitor reduced BK-induced increase in [Ca2+]i, and cPLA2 activity. PKC activity was increased by the addition of BK. The BK-induced increase in PKC activity was significantly reduced by pretreatment with PKC inhibitor. Phorbol 12-myristate 13-acetate (PMA) increased cPLA2 activity and PG2 synthesis but had no effect on Ca2+ kinetics. The expression of cPLA2 mRNA was increased by the addition of BK or PMA. BK-induced cPLA2 mRNA was blocked by pretreatment with PKC inhibitor and was superinduced by pretreatment with cycloheximide.

**WeP27:W26** Effect of high density lipoprotein on the apoptosis in human vascular endothelial cells


**Objective:** The apoptosis of endothelial cells has been reported to be involved in atherogenesis and high density lipoprotein (HDL) has anti-atherogenic effect. In this study, the effect of HDL on the apoptosis in human vascular endothelial cell (HUVEC) was investigated in association with the calcium ion (Ca2+) kinetics and nitric oxide (NOx).

**Methods:** The apoptotic cells were detected by morphological changes, staining with propidium iodide and ladder patterns of DNA fragmentation upon electrophoresis. The number of the morphologically altered apoptotic cells were counted with phase-contrast microscope. IP3 was measured by RIA kit. The intracellular calcium ion ([Ca2+]i), Ca2+ uptake from an extracellular luminal space and Ca2+ release from the calcium storage sites were measured by fura2/AM. 4Ca uptake from the space and 4Ca release in saponified HUVEC, respectively. NOx was measured by Griess method.

**Results:** The number of the apoptotic cells in HUVEC was few in the basal condition, but increased time-dependently in the serum-free condition. On the other hand, HDL potently decreased the increased induced apoptotic cells, this HDL-induced inhibition of the apoptosis was reduced by the addition...
of NO inhibitor L-NAME and L-arginine (LNMMA) and reappeared by the combined treatment with LNMMA and L-arginine (L-Arg). HDL increased IF-γ (Ca2+)-Ca2+ uptake and Ca2+ release from the calcium storage sites and also augmented NOX, but this enhancement of NOX was inhibited by LNMMA, and recovered by the combined treatment with LNMMA and L-Arg.

Conclusion: These results suggest that HDL inhibits the apoptosis induced by the serum-free condition, and its inhibitory effect is potent. The HDL-induced inhibition of the apoptosis is mediated by the increased NOX via the increase in [Ca2+]i in HUVEC.

Conclusions: Both cerivastatin and fenofibrate improve endothelial dysfunction in mixed hyperlipidemia although their lipid-modifying effects are different. However, the improvement of FMD is much more pronounced after cerivastatin treatment.

WeP30:W26 Atorvastatin improves microvesSEL permeability in subjects with familial hypercholesterolemia (FH)

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Objective: The endothelium plays a pivotal role in atherosclerosis as a target for risk factors that act to remodel vascular wall, impair vasorelaxation and disrupt permeability. Statins prevent endothelial dysfunction and improve endothelium dependent vasorelaxation. We aimed to evaluate the putative role of atorvastatin in improving vessel wall permeability in subjects with FH. More, to throw light on the hypothesis that a dysfunction of the peripheral endothelium (arterioles and capillaries) is closely related to insulin resistance, HOMA IR was employed to evaluate insulin sensitivity.

Methods: Twenty-two subjects discontinued lipid-lowering therapy for at least 6 weeks. All (49 ± 8 years old) were non-diabetic, non-obese (BMI: 25.2 ± 3.2 kg/m²) normotensive (129/81 ± 11/7 mmHg). Atorvastatin was administered at 20 mg/day for 1 month and then at 40 mg up to 1 year. Lipids, lipoproteins, the transcapillary albumin escape rate (TERaCh, %/h, i.e. 125I-albumin) and HOMA IR were performed at baseline, 1, 6, 12 months (ANOVA repeated measures).

Results: Total-ch (baseline 379 ± 43 mg/dl), LDL-ch (301 ± 43) and Apo-B (222 ± 56) reduced by 37, 47 and 43%, respectively, at 1 year, with most lowering at a month (33, 39 and 30%); (p < 0.0001). Triglycerides (baseline 133 ± 57 mg/dl) reduced by 20% at 1 month and steaded over the year (p = 0.0002). HDL-ch (baseline 51 ± 12 mg/dl) and Apo-A1 (145 ± 20) both increased of 12% at 1 year (p = 0.001). TERaCh (baseline 8.9 ± 2.0%h) was stable at 1 month (9.4 ± 2.8%h) but reduced at 6 months (7.7 ± 2.0%h); this result was confirmed at 1 year (7.6 ± 2.1%h) (p = 0.02). HOMA IR (baseline 2.07 ± 0.92 µU/ml × mmol/l) did not change during the study.

Conclusions: 1-yr atorvastatin improves widespread microvascular endothelial leakiness to albumin in subjects with FH. Improvement of the dysfunction of the peripheral endothelium does not associate in these patients with an enhancement of insulin sensitivity.

WeP31:W26 PPAR-alpha activator (fenofibrate) restores endothelium responses in spontaneous hypertensive rat

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Objective: Spontaneous Hypertensive Rat (SHR) is a model of essential hypertension associated with a metabolic syndrome. Moreover, SHR have abnormal endothelium-dependent vasorelaxation since high doses of acetylcholine induce endothelium-dependent contraction of SHR aorta while these doses induce endothelium-dependent relaxation in normal rats. These abnormal endothelial function could contribute to spontaneous occurrence of myocardial or cerebral ischemia. Because fibrates are commonly used in patients with metabolic syndrome we tested the effect of fenofibrate on endothelial function of SHR aorta.

Methods: SHR and normal (Wistar Kyoto, WKY) were treated for 2 weeks with fenofibrate (0.2% in food). Then, endothelial-dependent relaxation to acetylcholine was tested in phenylephrine-constricted aorta.

Results: Fenofibrate suppressed the acetylcholine induced aorta contraction in SHR (table), but did not change endothelial function in WKY rat.

Percentage of relaxation to acetylcholine in phenylephrine-constricted aorta

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>WKY + fenofibrate</th>
<th>SHR</th>
<th>SHR + fenofibrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ach 3.10⁻⁷ M</td>
<td>65 ± 5</td>
<td>57 ± 5</td>
<td>60 ± 5</td>
<td>63 ± 6</td>
</tr>
<tr>
<td>Ach 3.10⁻⁸ M</td>
<td>93 ± 2</td>
<td>96 ± 5</td>
<td>43 ± 6*</td>
<td>82 ± 5*</td>
</tr>
</tbody>
</table>

*p < 0.05 vs WKY; *p < 0.01 vs SHR

Conclusions: This data suggests that PPAR-alpha activators could improve endothelial function in hypertension associated with a metabolic syndrome.
**WeP32: W26**  
Vasorelaxation and tPA release induced by insulin are impaired in women with polycystic ovary syndrome and hyperinsulinism  
E. De Negri, R. Forni, L. Ferrini, A. De Giorgi, C. Guarnaletti, G. Dell’Orso, R. Pedrinelli, F. Fruzzetti, F. Carmassi. Dpt. of Internal Medicine, Dpt. of Gynecology, Univ. of Pisa, Italy  
**Objective:** Women with polycystic ovary syndrome (PCOS) have often insulin-resistance (IR) and increased risk of atherosclerosis. Hyperinsulinemia associated with PCOS can stimulate Plasminogen activator inhibitor-1 (PAI-1) release and trigger hormonal changes. As vasodilatory response to insulin is blunted in IR states, impaired vasodilation and fibrolysis may be correlated to the increased risk of atherosclerosis.  
**Methods:** PAI-1 and tissue plasminogen activator (tPA) levels were evaluated basally and during insulin infusion in the forearm vascular bed of 8 healthy young subjects and 4 young women with PCOS and hyperinsulinemia. Insulin was infused for 120 min in the brachial artery at a rate calculated to raise local venous concentrations of 100 μU/mL. Blood samples were obtained from brachial artery and vein.  
**Results:** Elevated basal PAI-1 levels were found in PCOS women (p<0.01 vs. controls), correlating with BMI and androgen levels. In PCOS women, vasodilatory response to insulin was blunted. PAI-1 balance increased both in normal subjects (p<0.01) and PCOS patients (p<0.05), tPA balance increased (p<0.01) in normals, but not in PCOS women.  
**Conclusions:** In PCOS women with IR, blunted vasorelaxation and tPA release was observed during insulin infusion of physiologic doses of insulin, as well as elevated basal PAI-1 level. An impairment of vasorelaxation and fibrolysis could be involved in the development of atherosclerosis in IR states.

**WeP33: W26**  
Involvement of GATA proteins in the VCAM-1 induction in human endothelial cells  
M. Uemura, C. Mataki, T. Hamakubo, T. Kodama. Research Center for Advanced Science and Technology, University of Tokyo, Tokyo, Japan  
We established a bioassay system to detect the intensity of monocyte-endothelial cell adhesion and then screened newly synthesized compounds to discover cell adhesion inhibitors. One of these inhibitors, K-7174, suppressed the expression of VCAM-1 both in cell surface expression and in mRNA level, without affecting the induction of ICAM-1 or E-selectin in cytokine-stimulated endothelial cells. K-7174 had no effect on the stability of VCAM-1 mRNA, and gel shift assay revealed that its inhibitory effect on VCAM-1 induction was mediated by a direct effect on the binding activity of GATA protein family to the VCAM-1 gene promoter. K-7174 did not influence the binding to any other binding motif including NFκB. Studies using specific antibodies and antisense oligonucleotides to GATA proteins revealed that multiple GATA proteins and their complex formation were involved in the induction of VCAM-1 by TNFα. These results provide evidence for the importance of GATA proteins in the cytokine induction of VCAM-1, and also indicate that GATA proteins are hopeful as targets for development of anti-inflammatory agent for clinical use.

**WeP34: W26**  
α-tocopherol succinate inhibits adhesion of monocytes to endothelial cells under flow by inducing caspase-dependent p65  
J. Neuville, P. von Hundelshausen, N. Gellert, C. Weber. Institute for Prevention of Cardiovascular Diseases, Ludwig Maximilians University, Munich, Germany  
Activation of endothelial cells by an inflammatory stimulus renders their adhesiveness for monocytes. Pre-treatment of human umbilical vein endothelial cells (HUVEC) with α-tocopherol succinate (α-TOS), but not with α-tocopherol (α-TOH) or α-tocopherol acetate (α-TOA), inhibited adhesion of monocytes to TNFα- or IL-1β-stimulated HUVECs, and the inhibitory effect was suppressed by co-treatment of the cells with a caspase inhibitor. Moreover, transfection of HUVEC with a gain-of-function bel-2 gene prevented the anti-adhesive effect of α-TOS, α-TOS, but not that α-TOH or α-TOA, treatment of HUVECs exhibited features of early apoptosis, caspase activation and specific cleavage of p65, a subunit of NFκB. α-TOS pre-treated cells showed neither B degradation nor nuclear translocation of p65 following TNFα stimulation. Expression of VCAM-1 was lower in TNFα-stimulated, α-TOS pre-treated cells. Transfection of endothelial cells with a VCAM-1 gene suppressed the inhibitory action of α-TOS. The role of a caspase in p65 sission was confirmed by experiments in which HUVECs were transfected with a caspase non-cleavable p65 gene. These data explain why the succinyl analogue of vitamin E inhibits adhesion of monocytes to activated endothelial cells, and suggest a relation between induction of apoptosis and NFκB activation in endothelial cells.

**WeP35: W26**  
Myocardial vascular responsiveness improves after cholesterol reduction by selective LDL-apheresis  
T. Sampietro, F. Bigazzi, B. Dal Pino, S. Fusaro, G. Sassi, C. Marchetti, G. Sambuceti, M. Tuoni, O. Parodi, A. Bionda. CNR Institute of Clinical Physiology, Pisa; Department of Internal Medicine, University of Pisa, Italy  
Our previous work showed a regulatory role of plasma cholesterol (CH) on vascular function regards endothelial adhesiveness and cutaneous microcirculatory blood flow. To answer the question whether massive CH removal by selective LDL apheresis (dextran sulfate columns, treated plasma = 2.5–3 times the patient plasma volume) could actually affect coronary circulation reactivity, we studied myocardial perfusion in 7 consenting, heterozygous patients (6 M and 1 F, mean age 47.4 ± 6.3 years) with familial hypercholesterolemia (FH); all but one had coronary artery disease undergoing apheresis therapy. Just before and soon after apheresis, myocardial blood flow at rest (MBF/rest) and after adenosine stimulation (MBF/rest) was assessed with 13N-ammonia and positron emission tomography. After apheresis, the mean percent reductions of CH, 1.00 CH. ApoB, HDL-Ch, TG, and Lp(a) were 77%, 95%, 87%, 6%, 64% and 89%, respectively. Adhesion molecule values (sELAM-1, sICAM-1) were significantly reduced, p<0.003 and p<0.0001, respectively. MBF/rest values showed no significant change after apheresis (baseline values were 0.71 ± 0.06 mI/min/g and after apheresis were 0.86 ± 0.25 mI/min/g). Following apheresis, MBF/rest increased significantly (from 1.31 ± 0.2 to 2.05 ± 0.8 mI/min/g, p<0.05), while blood pressure rate was unmodified. These results demonstrate a direct role for CH in regulating coronary vascular reactivity, at least in FH patients, and they suggest that ‘aggressive’ LDL apheresis may be a useful tool to acutely reverse coronary vascular dysfunction.

**WeP36: W26**  
Significance of up and down regulation of vascular endothelial growth factor receptor in angiogenesis  
S. Murata, M. Onodera, J. Wang, I. Morita. Tokyo Medical and Dental University, Tokyo, Japan  
**Objective:** Vascular endothelial growth factor (VEGF) is an endothelial cell specific growth factor. The growth signal of VEGF is transferred through its specific tyrosine kinase receptor, VEGF-R2. VEGF production is known to be regulated by various substances and conditions in several tissues and cells. By contrast, the regulation of VEGF-R2 expression remains unknown.  
**Methods:** Endothelial cells were examined for the tube forming activity and the VEGF-R2 expression. The former was assayed in vitro by the Type 1 collagen gel method and the latter was quantified by the fluorescence image analysis.  
**Results:** 1) Endothelial cells exposed to high glucose concentration (33 mM) for 30 days increased the tube formation induced by VEGF, but not by serum and bFGF. Immunohistochemical study showed that VEGF-R2 expression was up regulated by the high dose glucose treatment. 2) Delection assay of the VEGF-R2 promoter in endothelial cells treated with long term high glucose and VEGF showed that the 4th SP1 site located between positions −116 and −95 in the VEGF-R2 promoter plays a very important role in VEGF-R2 gene expression. 3) Collagen and osteoblast conditioned medium caused up regulation of VEGF-R2 expression in endothelial cells and to enhance the tube formation. 4) Eicosapentaenoic acid (EPA) pretreatment caused down regulation of VEGF-R2 expression in endothelial cells and to inhibit the tube formation.  
**Conclusions:** Up and down regulation of VEGF receptor in endothelial cells is as important as the change in VEGF production in tissues near by, and both changes can affect the angiogenesis very much.

**WeP37: W26**  
A Mediterranean diet, high in monounsaturated-fat and a low fat diet improve endothelial function in hypercholesterolemic patients  
José López-Miranda, Francisco Fuentes, Francisco Sánchez, Purificación Gómez, Elior Pae, Pablo Pérez Martínez, Carmen Marin, Pedro Castro, José M. Ordoñez, José Jiménez Pérez, Francisco Pérez Jiménez. Hosp. Reina Sofia, Córdoba, Hosp. Alto Guadalquivir Andújar, Spain; USDA HNRCA Tufts University, Boston MA, USA  
Endothelial dysfunction is an early event in atherogenesis and it is present
in adults with risk factors for atherosclerosis, such as hypercholesterolemia. We examine the influence of diet, with different fat content, on endothelial function in 22 hyperlipidemic males.

**Methods:** The study design included an initial 28-day period, during which all subjects consumed a saturated fat-rich diet (SAT diet: 38% as fat, 20% saturated fat), and after this all participants were randomized in a crossover design and subjected to two diet periods: a National Cholesterol Education Program (NCEP-I) type diet (28% as fat, <10% saturated fat) and a high-cholesterol diet (MUFA diet, with the typical composition of a Mediterranean diet enriched in olive oil (MUFA diet: 38% as fat, 22% MUFA). At the end of each dietary period, an endothelial function study was performed, using high-resolution ultrasound; we measured the diameter of the right brachial artery at rest, during reactive hyperaemia (with increased flow causing endothelium-dependent dilatation), and after sublingual glyceryl trinitrate (GTN; causing endothelium-independent dilatation). We measured the flow velocity in the systolic peak and at the end in diastole, calculating the resistance index (RI).

**Results:** Flow-mediated dilatation was greater with MUFA-diet than with SAT-diet (0.054 cm vs. 0.041 cm, p < 0.05). RI during reactive hyperaemia and during GTN-induced dilatation were lower with MUFA-diet and with NCEP-diet than with SAT-diet (both p < 0.05). Changes in LDL cholesterol (LDL-C) plasma levels, induced by diet intervention, were correlated with percent diameter changes (r = 0.23, p < 0.036) and RI after reactive hyperaemia (r = 0.24, p < 0.032).

**Conclusions:** A Mediterranean diet, high in MUFA-fat, and a high-carbohydrate low-fat diet improve endothelial function in hyperlipidemic males. The endothelial changes observed could be related with the modification in LDL-C with the dietary intervention.

**WeP38:W26**

**Role of protein tyrosine kinase in thrombin-induced endothelial cell migration**

H.J. Kruse,1, I. Wieczorek,2, G. Bauriedel,3, S. Schellong1.1 Departments of Angiology; 1Medical University of Dresden; 2Medical School of Hanover, Germany

**Objective:** After endothelial desquamation due to angioplasty endothelial migration is the initial response aimed at regaining an intact monolayer. We have studied cellular mechanisms that regulate the migratory activity of human endothelial cells (HUAEC).

**Methods:** Random motility of single HUAEC was evaluated using a standard videotape tracking system. Moreover, after wounding an intact endothelial monolayer with a 200 μm pipette tip migration was assessed by calculating the number of migrated cells into the wound area during 24 h. In addition, the release of endothelin-1 (ET-1) in response to wounding was measured by RIA.

**Results:** Stimulation of HUAEC with thrombin and with the thrombin receptor-activating peptide (TRAP-6) significantly increased migratory velocity by 89% and 67% (thrombin and TRAP-6, resp. vs. control). Concomitantly, after wounding 330 ± 35 and 279 ± 51 vs. 156 ± 22 cells had migrated (thrombin and TRAP-6, resp. vs. control). Migration was significantly inhibited by the tyrosine kinase inhibitor herbimycin-A (~64% and ~52%, thrombin and TRAP-6 vs. control). In contrast, migratory activity was not altered by the calcium channel blocker nifedipine. Whereas mechanical injury did not affect the release of ET-1 in unstimulated cells, thrombin-activated HUAEC produced more ET-1 (44%) than non-activated cells.

**Discussion:** Thrombin stimulates migratory activity of HUAEC. Generation of thrombin in the vicinity of an injured endothelium may thus accelerate wound repair. Protein tyrosine kinase(s) reduce migratory velocity of HUAEC. In vitro wounding of an endothelial monolayer does not lead to an increased release of ET-1.

**WeP39:W26**

**Oxidized LDL-induced endothelial cell apoptosis: Role of mediator phospholipids and bFGF**

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**Objective:** Oxidized low-density lipoproteins (oxLDL) promote endothelial cell (EC) apoptosis. Methods: Bone marrow cells were incubated with 50 μg/mL Cu-oxLDL or native LDL. Membrane integrity and nuclear morphology were assessed by epi-fluorescence microscopy after staining the cells with a mixture of calcein-AM, propidium iodide, and Hoechst 33342. The role of mediator phospholipids in oxLDL was studied using agents that selectively degrade or antagonize the platelet-activating factor (PAF)-like effects. The role of basic fibroblast growth factor (bFGF) was assessed by evaluating its expression and effects of exogenous bFGF.

**Results:** OxLDL, compared with native LDL, increased at 24 hours the percent of cells with apoptotic nucleus (42 ± 5% vs. 7 ± 3%; n = 3, P < 0.01) without evidence of membrane disruption. OxLDL reduced bFGF mRNA levels by 30–40% (n = 3), bFGF protein concentrations by 40% (310 ± 22 vs. 178 ± 38 pg/mg protein cell lysate; n = 6, P < 0.05), and DNA by 40% (98

**WeP40:W26**

**Impact of calcium on endothelial contraction and on prostacyclin and endothelin-1 formation**

H.J. Kruse,1, I. Wieczorek,2, S. Schellong1.1 Departments of Angiology; 1Medical University of Dresden; 2Medical School of Hanover, Germany

**Objective:** Whereas cytosolic calcium is closely related to prostacyclin (PGI2) formation, its contribution to both endothelin-1 (ET-1) release and regulation of endothelial permeability remains unclear. We have determined the effect of thrombin on endothelial release of prostacyclin and ET-1, and on endothelial contractility.

**Methods:** Changes in cytosolic Ca2+ concentration were monitored in thrombin-stimulated endothelial cells (HUAEC) by fluorescence using fura-2. PGI2 and ET-1 release were determined by RIA. Endothelial contractility was assessed by computer-assisted quantification of intercellular gap formation.

**Results:** Activation of HUAEC resulted in typical biphasic Ca2+ signals. Whereas PGI2 synthesis mainly depended on receptor-operated Ca2+ influx, thrombin-induced ET-1 release was almost abolished after depletion of intracellular Ca2+ stores with thapsigargin. Pharmacological modulation of intracellular Ca2+ stores did not significantly affect endogenous gap formation. Pre-activation of protein kinase C by phorbol ester augmented thrombin-stimulated endothelial contraction whereas pre-treatment with the tyrosine kinase inhibitor herbimycin-A almost completely inhibited contraction.

**Discussion:** Whereas thrombin-induced PGI2 formation in HUAEC depends on calcium influx, ET-1 release is mainly regulated by intracellular calcium. In contrast, ET-1 does not play a crucial role in the regulation of endothelial contractility. Therefore, additional postreceptor events are required to induce endothelial contraction including protein kinase C and protein tyrosine kinase(s).

**WeP41:W26**

**Impact of calcium on endothelial contraction and on prostacyclin and endothelin-1 formation**

H.J. Kruse,1, I. Wieczorek,2, S. Schellong1.1 Departments of Angiology; 1Medical University of Dresden; 2Medical School of Hanover, Germany

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± 12 vs. 60 ± 14 cm² x 10⁶/3-cm dish; n = 6, P < 0.05). After pretreatment of oxLDL with 200 µg/mL recombinant PAF acetylhydrolase for 1 hour, apoptosis, bFGF down-regulation, and inhibition of DNA synthesis were negligible. The PAF receptor antagonist WEB 2086 (10 µM) significantly suppressed oxLDL effects. However, lyophilized PAF (up to 1 µM) was only weakly active compared to oxLDL. Effects of oxLDL were almost entirely prevented by supplemented of exogenous bFGF (10 ng/mL).

Conclusions: In endothelial cells, oxLDL promotes apoptosis, inhibits DNA synthesis, and down-regulates bFGF without membrane disruption. These effects are mediated by phospholipids that act through PAF receptors as indicated by their susceptibility to xanthine oxidase and inhibition by the PAF antagonist. Effects of bFGF suggest bFGF's pivotal role in endothelial response to oxLDL, a key process of atherogenesis.

**WeP42-W26**

Mast cell-mediated downregulation of Bcl-2 expression triggers endothelial cell apoptosis in vitro

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**Objectives:** Mast cells are present in coronary atherosomas where they localize to the shoulder region, the site of erosion or rupture in myocardial infarction. These mast cells are known to express TNFα, a proinflammatory cytokine capable of inducing endothelial cell dysfunction and apoptosis. Since the presence of an intact, functional endothelium is critical for the stability of the atherosclerotic plaque, mast cells might participate in its destabilization and rupture by affecting the integrity of the endothelium.

**Methods and Results:** Here we show, by means of RT-PCR, western blotting, immunocytochemistry, propidium iodide-staining and DNA-laddering, that in isolated and cultured rat myocardial endothelial cells, stimulation of isolated rat atherosclerotic plaque cells with subsequent degranulation, downregulated the expression of the anti-apoptotic protein Bcl-2, with subsequent induction of apoptosis. The mast cell-mediated apoptotic effect, which resides in the granule remnant-fraction, was comparable to the effect obtained with purified TNFα. No effect was seen with another known inducer of apoptosis, i.e. chymase, also present in the mast cell granule remnants. The Bcl-2 mRNA expression level was downregulated rapidly (30 min), and was followed by a major decrease in Bcl-2 protein. In contrast, there was no significant change in the expression level of either Bax, a pro-apoptotic protein, or p53. As a consequence, cytochrome c was found to be increased and rapidly released from mitochondria into cytoplasm.

**Conclusions:** Mast cell stimulation and degranulation results in a rapid downregulation of Bcl-2 mRNA- and protein-levels followed by the induction of endothelial cell apoptosis. Thus, mast cells may participate in the erosion of atherosclerotic plaques by inducing endothelial cell apoptosis.

**P:H5 GENETIC SCREENING**

**WeP1:H5**

Effects of polymorphism of angiotensin-converting enzyme, apolipoprotein E and endothelial nitric oxide synthase (eNOS) genes on progression of arterial wall thickening in Japanese population


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**Objective:** To investigate the effects of the polymorphism of angiotensin-converting enzyme (ACE), apolipoprotein E (apo E) and endothelial nitric oxide synthase (eNOS) genes on the progression of arterial wall thickening in Japanese population.

**Methods:** A high-resolution B-mode ultrasonic examinations of the common carotid and femoral arterial intima-media thickness (IMT) were repeated after a follow-up of 36 months for 39 men and 59 women who were recruited from participants of a local health check program. The IMT increased by 0.05 ± 0.004 mm in the carotid artery and by 0.07 ± 0.006 mm in the femoral artery. The increase in carotid IMT was significantly higher in the subjects with D allele of the ACE gene than those without (p < 0.0005). Multiple regression analysis showed that cigarette-years and presence of the D allele of the ACE gene were the strongest predictors of the progression of carotid IMT (R² = 0.247, p < 0.001). The polymorphism of the apo E or eNOS gene did not affect the progression of arterial wall thickening during 3 years follow-up study periods.

**Results:** ACE gene polymorphism is associated with the progression of the carotid arterial wall thickening.

**Conclusions:** The D allele of the ACE gene may be a genetic determinant of the carotid arterial wall thickening in Japanese general population.

**WeP2:H5**

Polymorphisms in apo E gene promoter. Frequency in Spanish newborns, elderly and coronary patients. Relationship with coronary stenosis levels

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Apolipoprotein E (apo E) macrophage expression in vascular tissue is related with atherosclerosis and the atherosclerotic lesions development. It has been demonstrated that two polymorphisms in the apo E gene promoter, -491 A/T and -219 G/T modify the expression in hepatocytes and astrocytes.

**Objective:** To determine the frequency of -491 A/T and -219 G/T polymorphisms in Spanish newborns, elderly and coronary patients and its possible association with the coronary lesion extent.

**Methods:** Both polymorphisms and apo E genotype were determined by PCR and restriction analysis in 103 newborns (NB), 115 corona population > 70 years old (EL) and 147 corona population < 60 years old with coronary lesions quantified by angiographic study (CP).

**Results:** The frequencies of -491 A and -219 T were 0.82 and 0.45 in NB, 0.81 and 0.43 in EL and 0.82 and 0.41 in CP respectively. In the CP group, -219 T/T patients have higher coronary extent scores than -219 G/G patients, although these values did not reach statistical significance.

**Conclusions:** Our data indicate that there are no significant differences in allelic frequencies between NB, EL, CP groups but a relationship could exist between -219 T/G polymorphism and the extent of coronary lesions.

**WeP3:H5**

Spectrum of mutations in LDL receptor gene in Czech hypercholesterolemic patients

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**Objective:** The purpose of our study was to identify molecular determinants for the development of hyperlipidemia and/or atherosclerosis in Czech population. Especially for classical FH, when an impairment of the LDL receptor function due to mutations is one of the most common metabolic disorder. In our country there is no evidence of mutation spectrum in LDL receptor gene still.

**Methods:** Patients with high total and LDL cholesterol, normal serum triglyceride levels and with family history of premature CHD were selected for the study. DNA sequence variations were detected using heteroduplex, SSCP, DGGE, PCR/RFLP analysis and in detail characterized by DNA sequencing.

**Results:** Molecular searching for mutations in the coding and flanking intronic sequences of the low density lipoprotein receptor gene, resulted in identification of 18 sequence variations, 13 of which are new and were not described previously.

**Conclusions:** Knowledge of mutations causing classical FH is an aid for unambiguous diagnosis, facilitates genetic consulting at early age together with preventing disease manifestation.

This study was supported by grant IGA MH CZ. NE/554-3.

**WeP4:H5**

High throughput genotyping of apoE using molecular beacons and real-time monitoring of fluorescence PCR


The Apolipoprotein E gene (APOE), a key player of the lipid transport, exist as three main alleles in the general population: E2, E3 and E4. These polymorphisms correspond to mutations in the coding sequence of the APOE gene resulting in amino acid substitution (Arg and Cys) at position 112 and 158 of the protein: E3 (Cys 112, Arg 158), E4 (Arg 112, Arg 158) and E2 (Cys112, Cys 158). This allelic variation contributes to susceptibility to atherosclerotic cardiovascular disease and Alzheimer disease.

**XIIIth International Symposium on Atherosclerosis, Stockholm, Sweden, June 25-29, 2000**
To date, genotyping for ApoE is mainly performed with RFLP analysis, and separation of the DNA fragments on acrylamide gels. This method is very consuming and gives rise to unequivocal results even after optimization of PCR and restriction reactions. The aim of this study was to establish and apply a novel methodology, based on Molecular Beacons, for high-throughput sequence variant analysis of the APOE gene. Molecular Beacons are hairpin-shaped oligonucleotide probes that report the presence of specific nucleic acids in homogenous solutions with excellent allele discrimination. When they bind to their targets they undergo a conformational change that restores the fluorescence of an internally quenched fluorophore. We designed Molecular Beacons to detect DNA variations at the positions 112 and 158 of the APOE genes. This method correctly identified the three major isoforms of the APOE: E2, E3 and E4. The assay was carried out entirely in sealed PCR tubes and was simple to perform and interpret. Molecular Beacons PCR enabled the high throughput, rapid (96 samples in 2 hours), easy to perform and reliable genotyping of APOE alleles.

**Conclusions:** The 373P and the 451Q mutations in CETP are overrepresented in Caucasians with extreme low levels of HDL cholesterol, while the 405V mutation was only slightly overrepresented in subjects with extremely high levels of HDL cholesterol.

**WeP6.H5 Identification of differentially expressed genes in macrophages from rabbits with high (HAR) and low (LAR) atherosclerotic response**

R. Burkhardt, D. Teppeier, J. Thiery, Institute of Clinical Chemistry, University Hospital LMU, Munich, Germany

**Objective:** We have previously described two strains of New Zealand White rabbits with high (HAR) and low (LAR) atherosclerotic response to diet induced hypercholesterolemia. The aim of the present study was to identify new candidate genes of atherosclerosis susceptibility and resistance in macrophages from both strains of rabbits.

**Methods:** mRNA was isolated from peritoneal macrophages of 6 HAR and 6 LAR rabbits fed a 0.5% cholesterol diet for 21 days. Using a RT-PCR based suppression subtractive hybridization assay we either enriched for genes with higher expression in LAR rabbits or with higher expression in HAR rabbits. The enriched cDNAs were inserted into a T/A-cloning vector. We then rescreened for clones representing mRNAs that were truly differentially expressed and determined their sequences. The sequences were compared with a nucleotide sequence database (GenBank).

**Results:** Several of the sequences identified in macrophages from both strains of rabbits showed no homology with genes in the database. However, at present we have identified two cDNAs with higher expression in macrophages from HAR rabbits, which show homology with human NUMB protein mRNA (89%) and the human mRNA for the KIAA 0196 gene (93%). Among the known genes with higher expression in macrophages from LAR rabbits we found a high homology with human arginase (91%), rabbit fibrinectin mRNA (99%) and rabbit ferritin H-chain mRNA (98%).

**Conclusion:** Suppression subtractive hybridization is suitable to identify differentially expressed genes in hypercholesterolemic rabbits with high and low atherosclerotic response. Some of the genes identified so far, are involved in macrophage differentiation and uptake mechanisms. Ongoing studies will provide evidence about the role of the identified genes in atherogenesis or atherosclerosis prevention.
T:W17 GENETIC ANIMAL MODELS OF LIPOPROTEIN METABOLISM

Effects of amiodipine and atorvastatin on the development of atherosclerosis in ApoE*3-Leiden transgenic mice

D.J.M. Delsing1, J.W. Jukema2, H. van der Boom3, E.H. Offerman4, A. van der Laarse1, I.M. Havekes1, H.M.G. Princen1, J.TNO-PG, Gauhuis Laboratory, Leiden; 2Department of Cardiology, Leiden University Medical Centre, Leiden, The Netherlands

Objective: The anti-atherosclerotic effects of calcium-antagonists (CAS) are under debate. Results of the REGRESS-study suggested that CAS have a synergistic effect with statins. The present study in mice was designed to test the hypothesis that CAS are able to oppose the atherosclerotic effects of cholesterol only when they are administered together with a cholesterol-lowering drug, such as a statin.

Methods and Results: Four groups of 15 female ApoE*3-Leiden transgenic mice each received one of the following diets: 1) high cholesterol diet (HC, control group), 2) HC + CA (amiodipine, amlo), 0.002% (w/w), 3) HC + statin (atorvastatin, atorva), 0.01% (w/w), 4) HC + atorva + amlo. The HC diet resulted in total plasma cholesterol concentrations of 21.8 ± 3.4 mM. Amlo did not have an effect on cholesterol levels, whereas atorva caused a 53% decrease of plasma total cholesterol, mainly confined to the VLDL, IDL and LDL fractions. After 28 weeks of treatment, atherosclerosis in the aortic root was determined. The control group showed a lesion area of 0.33 ± 0.15 mm². Amlo alone had no effect on the lesion area (0.26 ± 0.13 mm²). Atorva reduced the lesion area by 77% to 0.08 ± 0.08 mm² (p < 0.001). The combination of atorva and amlo reduced the lesion area by 91% as compared to the control group (0.03 ± 0.02 mm², p < 0.001) and by 61% as compared to the atorva only group. The difference between these latter groups, however, was not statistically significant. Almost all atherosclerotic lesions found were severe, as characterized by the presence of cholesterol clefts, mineralization, large lipid pools, loss of smooth muscle cells and necrosis.

Conclusions: These results indicate that atorva potently reduces atherosclerosis in ApoE*3-Leiden mice, and suggest that amlo may indeed have a synergistic effect. The observed lack of significance can in part be explained by the large reduction in plasma lipids accomplished by atorva treatment and the advanced stage of the atherosclerotic process.

T:W17 EFFLUX OF CHOLESTEROL ON HUMAN ATHEROSCLEROTIC PLAQUES DOES NOT OCCUR AS READILY AS THAT ON NORMAL CELL MEMBRANES

Byung-Hong Chung, J.P. Segrest, Karen Hart. University of Alabama Medical School, Birmingham, AL, USA

The regression of atherosclerotic (A) plaques in vivo evaluates commonly in vitro in cultured cells by measuring the release of cellular cholesterol (CH) into culture medium containing acceptors of CH. Whether CH on A-plaques is as readily releasable as that on normal cell membranes is not clear. We have examined the potencies of fresh human plasma, HDL, apoproteins of HDL (apo HDL) or complexes of phosphatidylcholine (PC) and apo HDL (R-HDL) to promote the release of CH from isolated human A-plaques and red blood cell (RBC) membranes. Fresh plasma containing active lecithin cholesterol acyltransferase and cholesteryl ester transfer proteins was very effective in releasing CH from RBC; upon incubation of fresh plasma with 3x excess amount of RBC, plasma CH mass can be increased by 19% owing to an exclusive increase in the cholesteryl ester level. Little or no increase of plasma CH mass occurred when plasma was incubated with isolated human A-plaques containing an unesterified CH level similar to that on RBC. R-HDL was able to release CH from A-plaques in a concentration-dependent manner while HDL or apo HDL was not effective. Supplementation of fresh plasma with R-HDL markedly increased the potencies of plasma to release CH from RBC but to a much less extent from A-plaques. The incubation of A-plaques with R-HDL resulted in the significant enrichment of A-plaques with PC, causing a marked increase of the PC to sphingomyelin ratio of A-plaques. Upon enrichment of A-plaques with PC, plasma or apo HDL became effective in releasing CH from A-plaques. Our data suggest that regression of A-lesions in vivo may require the modification of A-plaque composition.

T:W19 ASSOCIATION BETWEEN CAROTID INTIMA MEDIA THICKNESS AND ACUTE CORONARY ARTERY DISEASE

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Objective: We inquired the important relationship between intimal media thickness of the carotid artery, atherosclerotic plaque, inflammatory indexes and acute coronary artery disease.

Methods: In this study we enrolled 57 patients (35% women, mean age 61.7 years) hospitalized in our Operative Unit for acute coronary disease. Non-invasive measurements were made with high-resolution B-mode ultrasonography (model ACUSON Sequoia 256C ver 4.0). The study is based on the use of CCB index, mean intima-media thickness measured on 4 regions on both carotid bifurcations.

Results: CCB index is associated with severity of atherosclerotic artery disease (mean bilateral carotid artery wall = 1.65 vs 1.044 p = 0.0022).

Important association was observed between intimal carotid plaque and higher inflammatory indexes (CRP in mg/dl and WBC in 1000/mm³, myocardial lesion extension (CK in U/L) and lower Left Ventricular Ejection Function (%):

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<th>CRP on entry</th>
<th>CRP max</th>
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</table>

Conclusion: The presence of significant atherosclerotic plaque (CCB

**WeT2W19** Lipoprotein content in circulating immune complexes as a marker of coronary- and extra-coronary atherosclerosis

A.A. Orekhova, V.V. Tertov, I.A. Sobenin, A.N. Orekhov. *Institute for Atherosclerosis Research, Russian Academy of Natural Sciences, Moscow, Russia*

**Objective:** In the blood of patients with coronary atherosclerosis we have found circulating immune complexes containing modified low-density lipoprotein (LDL) and auto-antibodies against modified LDL. In this study we estimated correlation between LDL content in circulating immune complexes (LDL-CIC) and severity of coronary- and extra-coronary atherosclerosis and found diagnostic and prognostic value of LDL-CIC.

**Methods:** To determine LDL-CIC we measured total cholesterol in polyethylene glycol precipitates. Coronary atherosclerosis was evaluated by angiography and extra coronary atherosclerosis by ultrasonography.

**Results:** LDL-CIC and apoB/ApoA-I but not total cholesterol, triglycerides, LDL cholesterol, high-density lipoprotein cholesterol, lipoprotein[a] correlated significantly with the severity of coronary atherosclerosis. The accuracy of the diagnosis of coronary atherosclerosis by LDL-CIC was 78%. In case of carotid and femoral atherosclerosis diagnostic value of LDL-CIC was even higher. In 1-year follow-up study, LDL-CIC levels were increased with an increase of cardiovascular risk. On the other hand, in the group of patients treated with long-acting garlic powder tablets cardiovascular risks were decreased, while LDL-CIC was unchanged.

**Conclusion:** LDL-CIC may be employed as a marker in the diagnosis of advanced atherosclerosis.

**WeT3W19** Bacteria in atheromatous plaque in acute ischaemic stroke

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1B. Internal Medicine Clinic; 2Pathology laboratory of General hospital of Trikala; 3Neurosurgery clinic, Peripheral hospital of Larissa; 4Pathology laboratory of "Alexandra" hospital, Greece

**Objective:** To study whether there is any relation between infection and atherosclerosis in the acute ischemic stroke.

**Material-Methods:** Material of the study were the histology findings of 20 patients – of them 18 aged 30 ± 15 had road accidents and 2 aged 30 ± 5 who were admitted with acute ischemic stroke. There was histology correlation with hematogenous causes. Special immunohistological pigmentation was used for bacteria tracing on the surface of the plaque.

**Results:** Of the 20 preparations, 18 were negative for bacteria while 2 of the patients with acute ischemic stroke were positive for bacteria on the surface of the plaque, with inflammatory infiltration.

**Conclusion:** Tracing bacteria in the atheromatous plaque shows the existing relation between microbial infection, atheromatous plaque and acute ischemic stroke.

**WeT4W19** Age-related peculiarities of atherosclerotic lesions of the major head arteries in post-ischemic stroke patients

V.A. Rogozhin, V.V. Kuznetsov1, I.I. Glazovsky1. *Clinic-Diagnostic Center; 1Institute of Gerontology, Kiev, Ukraine*

**Objective:** Study on age peculiarities of the major head arteries (MHA) lesions structure in the patients with residual ischemic stroke phenomena in the carotid area.

**Subjects and Methods:** 116 patients (60 men and 56 women) aged 30–79 years with residual ischemic stroke phenomena in the carotid area were examined by means of transcranial ultrasound dopplerography of the neck and head major vessels (Logidop 5, Kranshuhler, Germany).

**Results:** Within the general structure of MHA lesions ischemic stroke patients (ISP), pathology of extracranial (EC) parts of the carotid area prevailed (88%) over that of intracranial ones (12%). Deformations of the carotid area parts presented the main reason of acute cerebral blood circulation disturbances at age groups 30–39 years (66%) and 40–49 years (30%). With age, there increased a frequency and the degree of stenotic damages of MHA, being conditioned by an atherosclerotic process. Thus, stenosis of one of the carotid arteries was found in 22% of ISP aged 30–39, at 40–49 – in 50%, at 50–59 – in 56%, at 60–69 – in 73%, and at 70–79 – in 49%. The two-sided stenosis of carotid arteries was seen in ISP only after 50 years, making 20% at 50–59 and 60–69. At 70–79, the frequency of stenosis of both carotids increased to 33%.

**Summary:** The percentage of extracranial stenoses of the carotid area, bilaterally, in ISP increases with age. The MHA deformations (curvings, carotid artery loops) have lead to acute blood circulatory disturbances at younger ages.

**T:W20 TRIGLYCERIDES AND CVD**

**WeT1W20** The prevalence of high serum triglyceride and low HDL cholesterol among an Iranian sample

N. Sarraf-Zadeh, N. Mohammadi-Fard. *Isfahan, Cardiovascular Research Center, Isfahan, Iran*

**Objective:** To study the prevalence of high triglyceride (TG) and low high-density lipoprotein cholesterol (HDL-C) among Iranian men and women.

**Methods:** This study include 2200 men and women aged 20 years and over who were randomly (free from clinically overt disease at entry) were randomly selected from 40 random clusters in Isfahan city. Participants were asked to complete standardized questionnaires regarding the major risk factors for coronary heart disease (CHD), then blood samples were drawn from participants after being fasting for about 14 hours. The sera were analyzed for total-C, HDL-C and TG using precipitation techniques by the Enzymatic Method. Results are expressed as the prevalence (%) correlation coefficient was used to find the relation between TG and HDL-C, TG and LDL/HDL cholesterol etc. P value < 0.05 was considered to be significant.

**Results:** The total prevalence of high TG (≥2.2 mmol/l) and low HDL-C (<0.9 mmol/l) was 19.7% or high TG and high LDL/HDL cholesterol was 12%. These data based on sex and different age groups are presented in the following graphs.

**Conclusion:** These results expressed high frequency of high TG and low HDL-C or high LDL/HDL cholesterol among Iranian people, with higher frequency among women specially in greater age groups.

**WeT2W20** A case of familial hypercholesterinaemia II/A associated with valvular aortic stenosis

Á. Németh, T. Szamossi, E. Horváth, J. Szabolcs, K. Lozsádi. *Semmelweis University of Medicine II, Department of Pediatrics, Budapest; National Institute of Cardiology, Budapest, Hungary*

**Objective:** Authors present a case of a 15 years old boy with hypercholesterinaemia II/A type combined with aortic stenosis.

**Methods:** The patient's valvular aortic stenosis was discovered at the age of 2. In 1989 he was examined because of xanthomatosus skin alterations, and familial hypercholesterinaemia was found at the Department of Pediatrics, Pécs. 1993 an atherosclerotic plaque was removed from the orifilit of the left coronary artery in EC (Department of Pediatrics II, SOTE) In 1994 an extended examination of the lipid metabolism revealed normal level of receptor, but decreased function.

Because of the aggravation of his aortic stenosis, he was operated at Munich preceded by repeated LDL pheresis.

**Results:** The LDL pheresis was continued in Hungary as well, and the patient lipid profile nearly normal today, but unfortunately because of his growth and development he is waiting for the change of his artificial aortic valve.

**Conclusions:** Authors consider worthwhile to present the case because of the young age of the patient, and the unusual presentation of the disease. (Only one similar case was published in the literature.)
**T:W21 CELLULAR STRESS AND GENE REGULATION**

**WeT1:W21** Cloning of a novel gene related to LDL stimulation in human umbilical vein endothelial cell line ECV304

W.C. Zhang, B.S. Chen, G. Wu, H. Xu, W.W. Zeng, Institute of Basic Medical Sciences, CAMS & PUMC, Beijing, China

**Objective:** Cloning and characterization of novel genes related to atherosclerosis to investigate the molecular basis of an elevated blood low-density lipoprotein (LDL) level on promoting vascular diseases

**Method:** ECV304 cells were maintained in medium 199 (M199). LDL was added to the culture medium on subconfluent cell monolayers at the concentration of 400 μg/mL. The total RNA of the LDL-treated and untreated ECV304 cells was extracted by use of Trizol agent (Gibico) according to the manufacturer’s instruction. For differential display analysis, the total RNA of the LDL-treated and untreated ECV304 cells was used as the template for synthesizing the first-strand cDNA using MMLV reverse transcriptase (Gibco). Performing differential display PCR using first-strand cDNA as template, the cycling parameters are as follows: 94°C 1 min; 60°C 1 min; 68°C 3 min totally 20 cycles. 33 differentially expressed bands on gel was cut out with razors, each fragment was reamplified and cloned into plasmid vector pGEM-T and sequenced; comparison of the DNA homology with the Genbank database using BLAST. 6/33 fragments without corresponding the full-length cDNA in the Genbank were analyzed by Northern blot. 2/6 were confirmed being differentially expressed, 2/6 fail to detect any signals and the remaining 2/6 were false positives. We have tried to identify a full-length cDNA corresponding to the fragment 42 - 1 and succeeded in isolating three independent clones from the main arterial tissue cDNA library. The pBlue-script phagemids (42-1-3; 42-1-5; 42-1-6) were excised by in vivo excision protocol using ExAssist helper phage and E. coli XLORL strain (Strategene). The insert DNA was analyzed by Xhol/EcorI digestion. Their length were all about 1.7 kb the same as the transcript of Northern blot.

**Results:** The cDNA full-length is 1726 bp, the 3’-terminal region contained the sequence of the fragment 42 - 1 obtained by DDDDPR - PCR. Comparing with the homology with the sequence data in Genbank shows that it’s a novel gene.

**Conclusions:** We have successfully cloned a novel gene responsive to LDL stimulation from human umbilical vein endothelial cell line ECV304, it’s structural characteristics reveals that it may be a new member of the zinc finger genes related to atherosclerosis.

**T:W22 REVERSE CHOLESTEROL TRANSPORT**

**WeT1:W22** Plasma PLTP concentration measured by sandwich ELISA

T. Oka1, M. Itou1, T. Egashira1, N.E. Miller2, H. Hattori1, 1 R & D Center, BML Inc., Saitama, Japan; 2 Department of Cardiovascular & Biochemistry, St Bartholomew’s & The Royal London School of Medicine & Dentistry, London, UK

Plasma phospholipid transfer protein (PLTP) is a factor that plays an important role in LDL metabolism and reverse cholesterol transport. To date, plasma PLTP levels have been measured by phosphatidylcholine (PC) transfer activity. To measure plasma PLTP of PLTP, we have newly developed a sandwich enzyme-linked immunosorbent assay (ELISA). An ELISA for PLTP was established using two specific monoclonal antibodies raised against recombinant human PLTP (hPLTP). PLTP concentration in this ELISA could be measured in the range from 1.2 to 40 μg/mL. The intra- and inter-assay coefficient variation were less than 5%.

The concentration of PLTP in plasma from 132 nonodipidemic Japanese subjects was 12.0 ± 3.0 μg/mL (mean ± SD). PLTP concentration was positively correlated with LDL cholesterol (r = 0.72, p < 0.001), apo A-1 (r = 0.62, p < 0.001) and HDL2 cholesterol (r = 0.72, p < 0.001), and was negatively correlated with TG (r = -0.45, p < 0.001) and apo B (r = -0.40, p < 0.001). There was a weak positive correlation between PLTP concentration and PC transfer activity in plasma (r = 0.29, p < 0.05).

We have newly developed an ELISA to determine plasma levels of PLTP. This ELISA could be a useful tool for the determination of PLTP concentrations in plasma.

**WeT2:W2** Marked decrease in plasma apolipoprotein A-1 and HDL-C in a case with Werner syndrome

J. Kobayashi1, S. Murano2, K. Yokote1, S. Morii1, A. Matsunaga2, J. Sasaki2, K. Takahashi1, H. Bujo1, Y. Saito1, 1 Chiba University, Chiba; 2 Fukushima University, Fukushima, Japan

**Objective:** To study the potential factors responsible for the extremely decreased levels of plasma apo A1 and HDL-C in a case of Werner syndrome.
**Case:** The patient was a 39-year-old Japanese male who was diagnosed as Werner syndrome of homozygote for mutation 4, with body height of 160 cm and weight of 48 kg (body mass index 18.8 kg/m²). His plasma total cholesterol (TC), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C) and apo A-I levels were 7.2, 2.1, 1 mmol/L and 128 mg/dL, respectively. His plasma levels of HDL-C and apo A-I declined spontaneously to as low as 0.2 mmol/L and 10 mg/dL, respectively, with concurrent reciprocal increase in plasma TG levels. Plasma HDL-C, apo A-I and TG levels gradually returned to original values but subsequently the same decrease in HDL-C and apo A-I levels were observed repeatedly. The activity of plasma cholesterol ester transfer was normal. The plasma TG had a negative correlation with plasma HDL-C and apo A-I levels in both the proband (r = -0.703 p < 0.0001) and other hyperlipidemic patients (n = 71; age 41 ± 15 years, M/F = 48/23, TC 5.9 ± 1.5, TG ≥ 1.3, HDL-C 1.2 ± 0.37 mmol/L) (r = 0.401 p = 0.0005). However, plasma apo A-I and HDL-C levels were much lower than in the proband than in other hyperlipidemic subjects at the same plasma TG levels. The result of SSCP and direct sequence of the exon 3 and 4, and the promoter region of the apo A-I gene of the patient revealed no single nucleotide changes.

**Conclusions:** In the present patient, impaired hydrolysis of TG in TG-rich lipoproteins may have reduced the formation of nascent HDL more drastically than in other hyperlipidemic patients, resulting in unusually low plasma levels of HDL-C and apo A-I.

**Objective:** To determine the impact of mutations in apo A-I, LCAT, and glucocerebrosidase (GC) genes and in the enhancer of apo A-I at S' apo C-III gene on the genetic basis of hyperalphalipoproteinemia (HALP).

**Methods:** We screened the coding sequence of apo A-I and LCAT genes and the S' apo C-III region by single strand conformation polymorphism (SSCP), heteroduplex analysis and DNA sequencing, and analyzed the N370S and L444P variants in GC gene by restriction fragment analysis, in 67 unrelated subjects with low HDL-cholesterol (HDL-C) levels (<100 mg/dL).  

**Results:** We detected 3 mutations in apo A-I gene (L144R, W108R, g.1833C > T) and 3 mutations in LCAT gene (S108T, I117T, I532C > A), in 6 HALP subjects. Seven subjects carried a novel silent mutation in LCAT gene (g.4886C > T), which was assayed in 92 HALP and 100 control samples and classified as a polymorphism. Allelic frequencies of polymorphisms g (−641) C > A, g (−630) G > A, g (−625) T > del, g (−452) C > T and g (−455) T > C, located at S' apo C-III gene, were in normal range and no other mutation was found in this region. Three HALP subjects were found to carry the N370S mutation in the GC gene.  

**Conclusions:** Nine HALP subjects (13.5%) were found to carry a mutation in apoA-I, LCAT or GC genes. A novel polymorphism in the LCAT gene not linked to HALP has been identified.
and after a fat-containing test meal (M); plasma lipids; PLTPa: radioactively labeled phospholipid liposomes incubated with HDL from C donor’s pool and the subjects’ plasma as the PLTP source; CETPa: incubation of C plasma donors’ pool VLDL and HDL (labeled with radioactive cholesterolsterol) together with the subjects’ plasma as the CETP source; CETP concentration measurement by RIA.

**Results:** PLTPa and CETPb (median) and CETP concentration (mean ± SD) values in the F and M periods

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<th>D (M)</th>
<th>C (F)</th>
<th>C (M)</th>
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<td>CETP µmol/L</td>
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**Conclusion:** Plasma PLTPa, CETPb and concentrations are not altered in Type 1 D when properly matched with C subjects for gender, age, body weight and fasting plasma lipid levels.

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**WET8.W22**

**Lecithin: cholesterol acyltransferase activity in type 1 diabetes**

J. Valabhis, J. Donovan, A.J. McColl, M. Schachter, W. Richmond, R.S. Elkasles. Unit of Metabolic Medicine, Imperial College School of Medicine, St Mary’s Hospital, London, UK

**Introduction:** The incidence of coronary heart disease (CHD) is high in type 1 diabetes, despite normal or increased HDL cholesterol. The enzyme lecithin: cholesterol acyltransferase (LCAT) facilitates HDL-mediated reverse cholesterol transport.

**Objectives:** We aimed to demonstrate altered LCAT activity in type 1 diabetes.

**Method:** LCAT activity was measured as the rate of decrease of unesterified cholesterol during in vitro incubation of plasma at 37°C (assay coefficient of variation 6.14%). Ten type 1 diabetic subjects (duration of diabetes 17 [7–21] years (median [IQR]); HbA1c 7.25 [6.65–9.03]%) were matched with 10 control subjects for age, sex and BMI.

**Results:** Type 1 diabetic subjects had higher HDL unesterified cholesterol concentrations [0.47 [0.36–0.53] vs. 0.38 [0.24–0.42] mmol L–1; Mann-Whitney p = 0.05]; higher HDL total cholesterol concentrations of borderline significance (1.85 [1.55–2.07] vs. 1.62 [1.06–1.80] mmol L–1; p = 0.055) and higher apolipoprotein A1 concentrations (1.68 [1.57–1.89] vs. 1.49 [1.28–1.71] g L–1; p < 0.05). Fasting LCAT activity was similar in type 1 diabetic and control subjects (62 [41–88] vs. 53 [45–61] mmol mL–1 h–1; p = 0.3). If both groups were analysed together, significant independent predictors of LCAT activity were fasting plasma phospholipid concentrations (r = 0.74; p < 0.001) and fasting plasma triglyceride concentrations (r = 0.67; p < 0.01). Regression of LCAT activity against triglyceride concentration produced a steeper slope in type 1 diabetic compared to control subjects (analysis of covariance p = 0.01).

**Conclusion:** The different relationship between LCAT activity and triglyceride concentration may contribute to the increased CHD risk in type 1 diabetes.

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**T.W23 IMMUNE AND INFLAMMATORY MECHANISMS IN ATHEROSCLEROSIS**

**Wet1.W23**

**Increased levels of ICAM-1 in smokers**

A. Schumacher,1 H. Arnesen,2 I. Seljedal,2 L. Sommervoll,1 1Westfold Central Hospital, Tønsberg; 2Ullevål University Hospital, Oslo, Norway

**Objective:** To investigate the effects of smoking on the levels of soluble cellular adhesion molecules (sCAMs) in two different populations: One with established atherosclerotic heart disease, and one with apparently healthy controls.

**Methods:** Fasting venous blood samples were obtained from 197 patients with documented coronary heart disease (CHD), sampled at least 10 days after acute events, and from their age- and sex-matched controls without signs of atherosclerotic disease (mean age 55 years, 16% women). Soluble VCAM-1, ICAM-1, E-selectin and P-selectin levels were measured using commercial ELISA methods (R & D Systems Europe, UK), and the results are expressed as mean ± SD.

**Results:** Among the CHD patients, 22.3% were smokers at the time of sampling, compared to 26.4% of the healthy controls. Soluble ICAM-1 levels were significantly higher among smokers in both populations studied. Smoking CHD patients had 367.0 ± 159.9 ng/mL compared to 311.5 ± 122.5 ng/mL in non-smoking patients (p < 0.05). Smoking healthy controls had 291.0 ± 105.5 ng/mL, compared to 236.1 ± 50.6 ng/mL in non-smokers (p < 0.01). No significant differences were observed between smokers and non-smokers in the other sCAMs measured. Among demographic and biochemical variables tested in multivariate analysis, sICAM-1 levels were significantly correlated to the number of "cigarette years" (1 cigarette year = 20 cigarettes/d for 1 year) (p < 0.001).

**Conclusion:** Soluble ICAM-1 seems to be a sensitive marker of endothelial dysfunction in smokers, both in patients with atherosclerotic heart disease and among healthy individuals without signs of atherosclerosis.

**Wet2.W23**

**Development of a peptide-based ELISA for the detection of oxidized low density lipoprotein (oxLDL) with various degrees of oxidative modifications**

Paulo Boschov1, Luiz Juliano1, Maria Aparecida Juliano1, Hiro Goto2, Magnus Gidlund3,1, INFAR UNIFESP; 2IC-HC, FMUSP; 3IMTSP, São Paulo, Brazil

**Objective:** To develop a peptide-based ELISA that will allow the analysis of auto-antibodies strictly directed against oxLDL with various degrees of oxidative modifications.

**Methods:** Monoclonal antibodies (mAb) were developed against oxLDL and screened for a reactivity against highly oxLDL (10 µM CuSO₄, 18 Hr) or minimally oxLDL (10 µM CuSO₄, 10 minutes) and b peptides (25-mers) derived from the apoB.

**Result:** We were able to define two mAb, MH-2 and MH-3, that recognizes either highly oxLDL or Low oxLDL. Epitope screening with peptides revealed that the respective epitope consisted of both different and common amino acid sequences.

**Conclusion:** One of the major obstacle in the analysis of the role of oxLDL in atherosclerosis is both the great heterogeneity of the particles as well as methods for direct measurement in serum. As these mAb also react with serum oxLDL, they can be a useful tool to analyze the role of subpopulations of oxLDL in several diseases, including atherosclerosis.

(Supported by FAPESP, LIM18-HC-FMUSP)

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**Wet3.W23**

**In ofissnings of patients with premature coronary heart disease (PCHD) and B35 and B35 are associated with higher levels of some metabolic risk factors**

L.M. Doborendzidze, S.N. Maximov1, R.P. Manishchka1, A.S. Netchev, N.A. Gratsiansky, Center for Atherosclerosis, IPCM; 2Institute of Gerontology, Moscow, Russia

**Background:** We have previously shown that premature coronary heart disease (PCHD) is associated with HLA-A10, DR2, DR5 and B35.

**Objective:** To study frequencies of these antigens in ofissnings of patients with PCHD and their relation to levels of coronary risk factors, some parameters of carbohydrate metabolism and fibrinolysis.

**Material and Methods:** Ofissnings (n = 44, age 5–27 years, mean age 16.6 years, 23 males) of 32 patients with documented PCHD. HLA were identified by microlymphocytotoxic test. Data from blood donors were used as reference. Anthropometric parameters, levels of cholesterol (CH), high density lipoprotein (HDL) CH, triglycerides, apoprotein B, lipoprotein a, fibrinogen, characteristics of fibrinolysis, plasma glucose and insulin were determined by conventional methods.

**Results:** Ofissnings of patients compared with blood donors had higher frequencies of HLA-A10, DR2 and DR5 (34.1 and 14.5%, p = 0.002; 56.8 and 31.9%, p = 0.003; 52.3 and 29.9%, p = 0.007, respectively) but similar frequency of HLA-B35 (p = 0.163). Compared with ofissnings without these antigens subjects with HLA-A10 had higher basal insulin (6.2 ± 1.3 and 9.3 ± 1.9 µU/mL, respectively, p = 0.042), subjects with DR2 – lower HDL CH (47.7 ± 1.56 and 41.3 ± 1.67 mg/dL, respectively, p = 0.01) and higher serum glucose (4.4 ± 1.14 and 4.9 ± 1.16 mmol/L, respectively, p = 0.032), while subjects with B35 – higher fibrinogen (2.5 ± 0.13 and 3.3 ± 0.28 g/L, respectively, p = 0.026) and plasminogen activator activity (8.1 ± 1.89 and 17.6 ± 4.68 IE/ml, respectively, p = 0.004).

**Conclusion:** Compared with blood donors ofissnings of patients with PCHD had higher frequencies of HLA-A10, DR2, DR5 and some of antigens were associated with higher levels of metabolic risk factors.
WeT4:W23 Regulation of CTLA-4 on human T-cells

Objective: To analyse the regulation of CTLA-4 in human peripheral lymphocytes.

Methods: RT-PCR was used for mRNA determination and FACs analysis for the intracellular and surface constitutive and stimulated expression of CTLA-4 and for expression of surface markers.

Result: CTLA-4 is constitutively expressed intracellularly in CD3+ and CD4+ lymphocytes. The effect of IL-2 was more marked on the intracellular expression than on the surface expression and was dose-dependent. Also IFN-γ increases the CTLA-4 expression. RT-PCR analysis shows that nonactivated human peripheral blood T lymphocytes express an alternatively spliced form of CTLA-4 mRNA. The short mRNA (550bp) is down-regulated by activation.

Conclusion: CTLA-4 is constitutively expressed intracellularly in resting T cells. Cytokines such as IL-2 and IFN-γ can regulate CTLA-4 expression.

WeT5:W23 PAF-Acetylhydrolase activity in plasma and lipoprotein subfractions of normolipidaemic children and adults
S.-P. Karabina1, S. Bessi2, M. Elsay3, A. Siamopoulou1, M.J. Chapman4, A.D. Tselepis1
1Department of Chemistry; 2Department of Pediatrics; 3Department of Internal Medicine, Medical School, University of Ioannina, Greece; 4INSERM U321 Hôpital de la Pitié, 75015, Paris Cedex 13, France

Objective: Human plasma platelet activating factor acetylhydrolase (PAF-AH) is an antithrombolytic enzyme mainly distributed on low density and high density lipoproteins (LDL and HDL). We studied the PAF-AH activity in plasma and lipoprotein subfractions of children and sex matched adults.

Methods: Thirty normolipidemic children aged 7–14 years and 10 sex-matched normolipidemic adults aged 40–60 years were studied. Plasma lipoproteins were fractionated by density gradient ultracentrifugation into 9 subfractions (VLDL + IDL, LDL1–5, HDL2, HDL3, VHDL). Their protein and lipid components as well as lipoprotein mass were determined. PAF-AH activity in plasma and in each lipoprotein subfraction was measured by the TCA precipitation method. After precipitation of all apoB-containing lipoproteins with magnesium-ethanol sulfate, PAF-AH activity was also determined in the supernatant containing the total HDL and plasma proteins (HDLPr).

Results: There were no significant differences in the plasma lipid profile between the two groups. Similarly, no significant differences were observed in the chemical composition and in mass of each subfraction between children and adults. In both groups the predominant LDL subfraction was the intermediate. Plasma PAF-AH activity was significantly lower in children compared to adults (46.7 ± 8.2 nmol/min vs 62.0 ± 14.9 nmol/min, P < 0.01). The same phenomenon occurred for the enzyme activity in HDLPr (1.96 ± 0.46 nmol/min vs 5.91 ± 1.84 nmol/min, P < 0.006). No difference was observed in PAF-AH activity in each lipoprotein subfraction between the two groups.

Discussion: The plasma PAF-AH activity is influenced by the age in a lipoprotein-dependent manner which needs further investigation.

WeT6:W23 Immune inflammation of artery wall during atherosclerosis
V.A. Nagorev, V.S. Rabonovich, S.V. Maltseva, O.J. Jakovleva. Institute of Experimental Medicine, Russian Academy of Medical Sciences, Lab. of Atherosclerosis, St. Petersburg, Russia

Objective: Investigation of cellular and molecular aspects of atherosclerosis

Methods: The material of 32 urgent autopsies (within 1.5–3 h) after death from acute cardiovascular insufficiency, together with HD of patients aged 45–65, was mainly used in the study. Scanning and transmission electron microscopy was applied. We used monoclonal antibodies against CD4, CD8, CD68, CD40, CD40L, IL-1β, TNF-α.

Results: Immunoregulatory signaling molecules (CD40-CD40L) are shown to play important, and probably key role in the initiation of atherosclerotic lesions of arteries. Adhesion and migration of monocytes and T-lymphocytes (Th) occur on endothelium, producing CD40L and IL-1β. Monocytes, not transformed into foam cells, T-lymphocytes (CD4+) and smooth muscle cells express CD40, CD40L and produce TNF-α. It is likely that a pathological response similar to the delayed-type hypersensitivity reactions is brought forthvia self-regulation mechanisms. Macrophages and T-lymphocytesproducing pro-inflammatory cytokines and free radicals provoke atherosclerotic modification of apo B-containing lipoproteins.

Conclusions: Focal development of immune inflammation is considered as the important condition in initiation and progress of atherosclerotic damage of arteries.

WeT7:W23 Comparative study of cell composition in human arteries with clinically significant atherosclerotic sequelae
E.R. Andreieva, I.V. Andrianova, A.N. Orekhov. Institute of Experimental Cardiology, Cardiology Research Center, Institute for Atherosclerosis Research, Moscow, Russia

Objective: Cells of the vessel wall intima comprise two subpopulations: resident cells (RC) – the constitutive component of the intima, and inflammatory cells (IC), most of them emigrating from the peripheral blood. We carried on the comparative study of intimal cell composition in human arteries with clinically significant atherosclerotic sequelae: carotid and coronary arteries and aorta.

Methods: The total cell number as well as IC and RC number in uninvolved was estimated in the intima (I) and in initial lesion (L), fatty streak (I), fibrinoid plaque (Va) and fibrous plaque (Ve), according to AHA classification. We investigated the effects of IC and RC on plasma factor (K) levels against CDLC+CD14+ monocytes. RC were recognized as cells, nonstained with inflammatory cell cocktail.

Results: Total cell number in human aortic intima was approximately 2-fold higher than in coronary and carotid intima in normal as well as in atherosclerotic areas. In all examined arteries the total as well as RC/IC cell number increased in the range: 0–I–II–Va–Ve with the maximum in lipid-rich lesions (types II and Va). The share of IC was significantly higher in lipid-rich lesions (type II and Va) of coronary and carotid arteries, reaching 45% in Va lesion in carotid artery and 34% in coronary arteries compared to 20% in aorta.

Conclusions: Thus, the bell-shaped increase of intimal cellularity occurs within the 0–I–II–Va–Ve range. The higher share of IC in atherosclerotic lesions of coronary and carotid arteries may imply to a formation of atherosclerotic lesions with more pronounced clinically significant sequelae. This study was made possible in part by the Grant # 1B-12 from Russian Federal Program of Civil Research.

WeT8:W23 Different level of leukocyte activation and its cause during atherosclerosis (AS) progression
E. Koval. State medical academy, Dniepropetrovsk, Ukraine

Objective: To study composition and functional state changes of lymphocytes (LC), granulocytes (Np) and monocytes (Mc) in peripheral blood and its cause as a possible marker of AS progression and CAD destabilisation.

Methods: 572 men immunograms (IG) with AS risk factors (65), stable (362) and unstable angina, myocardial infarction (145) were examined (indirect immunoperoxidase of CD receptors, IEA method of leukocyte (LT) B2 determination, superoxide anion (SA) production in NST tests, fluorescent expression-method of the cellular lipids determination were used).

Results: it was found changes of Le cell subpopulations: CD5+ from 52.1 ± 1.0% to 65.2 ± 1.2% (p < 0.01), CD22+ from 21.7 ± 1.1% to 34.2 ± 1.5% (p < 0.01), CD8+ from 27.4 ± 0.9% to 37.6 ± 1.5% (p < 0.05) CD45R0+ from 26.3 ± 1.1% to 32.0 ± 1.0% with increasing of CD25+ from 28.6 ± 1.1% to 40.7 ± 1.0% (p < 0.05), CD38+ from 29.6 ± 1.1% to 40.0 ± 1.5% (p < 0.05). The levels of NST-tests were increased both by Np– from 17.8 ± 1.3% to 29.8 ± 1.3% (p < 0.05) and by Mc– from 19.9 ± 1.0% to 25.2 ± 1.2%, lowering of the synthesis of LTb4 by Np from 38.3 ± 1.1 pg/106 cells (c) to 29.0 ± 0.9 pg/106 c with increasing for Mc from 20.8 ± 0.7 pg/106 c to 37.0 ± 0.7 pg/106 c and parallel increasing the C level in CIC from 22.4 ± 1.9 mg/dl to 33.5 ± 1.1 mg/dl (p < 0.05). The close correlations and regression lines were established between the grade of lipid accumulation (LA) in Le with both CD25, CD8, CD4 expression and between phagocyte’s lipid levels and LTb4 and SA production. These connections were more closely determined by Ig type than dislipidemia. In stable course LA moderately influenced on cellular activation (r = 0.53), SA production (r = 0.54). In unstable cases these influences were considerably more strong (r = 0.72).

Conclusions: LA in main leukocyte populations is not a mechanical process but considerably amplifies their activation which may cause, in turn, oxidised LDL modification and to support in this way AS and CAD progression. The data confirm the existence of higher level of leukocyte activation during unstable disease course, one may utilise as a marker of destabilisation.
Development of a peptide-based ELISA for the detection of antibodies against oxidized low density lipoprotein (oxLDL)

Paulo Boschov1, Luiz Juliano1, Maria Aparecida Juliano1, Magnus Giilhoud1,2, INFAR-UNIFESP, 2LC-CH and IMTSF, FMUSP, São Paulo, Brazil

Objective: To develop an ELISA that will allow the analysis of auto-antibodies directed against oxLDL.

Method: Twenty-five 20-25-mers were designed and synthesized from the apoB protein and tested with sera from patients with high or low antibody-reactivity against highly oxidized LDL (10 μM CuSO4, 18 Hrs).

Results: We could define four regions of the apoB molecule that were recognized by patients with a high anti-oxLDL antibody titer.

Conclusions: The role of anti-oxLDL in the pathogenesis remains unclear and both beneficial detrimental effects have been reported. This new ELISA presents a good tool to dissect the auto-antibody profile directed against oxLDL to evaluate their role in several disease conditions including atherosclerosis.

(Supported by FAPESP, LIM38-HC-FMUSP)

Higher estradiol concentrations are associated with elevated lipids in men with coronary artery disease

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Elevated serum testosterone is known to be associated with increased risk for coronary artery disease (CAD) in women. The role of sex steroids in the pathogenesis of CAD in men remains unknown. The aim of the study was to determine levels of testosterone and estradiol and to relate them to the blood lipid profile in 68 men with angiographically proven CAD.

Methods and Results: In 68 stable CAD pts (untreated with lipid-lowering drugs), the blood levels of estradiol, total cholesterol (TCh), LDL-Ch, HDL-Ch and triglycerides (TG) were measured. The mean levels of lipids in the pathogenesis of CAD in men remains unknown. The aim of the study was to determine levels of testosterone and estradiol and to relate them to the blood lipid profile in 68 men with angiographically proven CAD.

Methods and Results: In 68 stable CAD pts (untreated with lipid-lowering drugs), the blood levels of estradiol, total cholesterol (TCh), LDL-Ch, HDL-Ch and triglycerides (TG) were measured. The mean levels of lipids in the pathogenesis of CAD in men remains unknown. The aim of the study was to determine levels of testosterone and estradiol and to relate them to the blood lipid profile in 68 men with angiographically proven CAD.

Conclusions: Increased levels of estradiol are associated with elevated levels of atherogenic lipids in male CAD patients whereas total testosterone does not seem to influence lipid levels. This indicates that estrogens may contribute to atherosclerotic process in males.

Thyroid function and lipid abnormalities in older cremen women

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Objective: Dyslipidemia and thyroid abnormalities are both common disorders in older women. The effect of subclinical hypothyroidism, an asymptomatic state associated with abnormally elevated serum thyroid-stimulating hormone (TSH) and a normal serum free thyroxine (T4) concentration, on serum lipoproteins remains controversial. Moreover the relationship between high density lipoprotein cholesterol (HDL-C) and thyroid function in older women is unclear.

The aim of this study was to evaluate the frequency and to determine the lipid disorders of subclinical hypothyroidism in older women who were referred to our primary prevention outpatient lipid clinic for metabolic and cardiovascular assessment.

Results: Full fasting serum lipid profile and thyroid function tests, were performed in 326 apparently healthy women aged 75 (±15) by standard biochemical and immunochromatographic methods.

Results: TSH was high (≥3.20 μL/U) in 24 women (7.4%). Although by pooled analysis in these clinically hypothyroid women mean values of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), HDL-C, triglycerides (TG), lipoprotein (a) Lp(a) were higher compared with those of women with normal TSH (by 17 mg/dl or 6.2%, 14 mg/dl or 7.4%, 2 mg/dl or 3.5%, 17 mg/dl or 9.6% and 2.2 mg/dl or 8.8% respectively), none of these differences reached statistical significance. Significant elevations were observed only in the levels of TCH and Lp(a) – both p < 0.025 – in two groups of subclinically hypothyroid women: those aged 70–79 and 60–69.

Conclusions: In older women attending our primary prevention outpatient lipid clinic, subclinical hypothyroidism is comparatively common, affecting however only modestly modifiable risk factors for cardiovascular disease.

Postmenopausal hypercholesterolemia in women sent to a lipid clinic

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Objective: To study clinical and analytical findings of hypercholesterolemia (HC) postmenopausal women (POST) sent to a Lipid Clinic, focusing in coronary Endogenic HC (PRE) in POST and postmenopausal women (PRE).

Methods: Observational study. Review of medical records from patients sent to the Lipid Clinic. Setting: 400-bed community hospital attending 250,000 inhabitants. Data were compared with other hypercholesterolemic cohorts sent to the same clinic. LDL-C were calculated by Friedwald formula. Only were evaluated data obtained from patients who weren’t on hypolipidemic drug therapy.

Results: Data from 807 patients were analyzed: 340 women, mean age 52.6 ± 15.7 years. 221 POST, and 467 men, 50.5 ± 13.3 y. PHC was the more frequent primary dyslipidemia in POST, getting significative difference with premenopausal women (PRE). Sex distribution of patients sent to the clinic changes in the cohort aged more than 50, increasing percent of women (48.65% versus 34.44% in younger, p < 0.001). Serum Lp(a) level was higher in POST (48.8 ± 43.1 vs 26.2 ± 26.9 mg/dl, p < 0.05) and considering Lp(a) > 25 like cardiovascular risk threshold significative difference was found too. Fibrinogen level was higher in POST (418.7 ± 105.5 vs 282.0 ± 14.1 mg/dl, p < 0.001). In comparison with that, fibrinogen level was similar in men at every age. HDL-C in PHC was lower in POST (52.1 ± 13.7 vs 61.2 ± 15.0 mg/dl, p < 0.01) and within the POST the lowest level was present in the cohort of women aged >60 years (48.4 ± 12.5 vs 57.4 ± 13.8, p < 0.01). Ratio LDL-C/(apoB) was higher in PHC women than PHC men, at every age. However POST had a non significative tendency to decrease this ratio comparing with PRE (1.23 ± 0.13 vs 1.31 ± 0.80, p = 0.055).

Conclusions: Postmenopausal hypercholesterolemia is frequent cause to sent patients to study in the Lipid Clinic. POST with PHC, comparing with PHC in PRE, has higher Lp(a) and fibrinogen levels, lower HDL-C and a tendency to decrease the LDL-C/(apoB) ratio. All these findings may contribute to the increase of coronary heart disease rates in this women age group and configure a worse coronary risk profile comparing with PHC in PRE, and not only opposite PRE non-dyslipidemic women.

Menopausal effect on the relation between haemostatic factors and lipoprotein levels

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Objectives: Beside higher cholesterol or blood pressure levels among postmenopausal women, clotting factors and their relationship with serum lipoproteins may be another possible mechanism or risk factors for the higher incidence of IHD among post menopausal women. This hypothesis was tested in this study.

Methods: The study includes 220 women aged 18–65 years without cardiovascular or any other disease. Neither premenopausal women were using oral contraceptives nor postmenopausal women were using estrogen replacement therapy. Women who missed three consequent menstruation periods were classified as postmenopausal women. Participants were asked to be in a fasting state for 14 hours, then their blood samples were sent to the central laboratory of the research center. Plasma lipoproteins were measured by

enzymatic method, Lp(a) by Eliza method, fibrinogen and factor VII activity by fibrinometric method.

Results: The mean plasma levels of fibrinogen, factor VII activity and total cholesterol were significantly higher and HDL-cholesterol lower, among postmenopausal women P < 0.05, however, no significant difference regarding serum triglyceride (TG) or Lp(a) levels were observed.

The biochemical-haemostatic interrelationship among premenopausal and postmenopausal women is shown in the following table:

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Total-C</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>TG</th>
<th>Lp(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>0.1787</td>
<td>0.25</td>
<td>0.177</td>
<td>0.28</td>
<td>0.0519</td>
</tr>
<tr>
<td>Factor VII</td>
<td>0.2075</td>
<td>0.03</td>
<td>0.129</td>
<td>0.03</td>
<td>0.0714</td>
</tr>
<tr>
<td>Postmenopausal Women</td>
<td>0.155</td>
<td>0.05</td>
<td>0.2035</td>
<td>0.30</td>
<td>0.0628</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.20</td>
<td>0.04</td>
<td>-0.0691</td>
<td>0.79</td>
<td>0.2939</td>
</tr>
</tbody>
</table>

Conclusions: In conclusion, the observed differences in fibrinogen and factor VII levels and its relationship to plasma lipids may explain part of the higher prevalence of cardiovascular diseases among postmenopausal compared to premenopausal women.

WeT5:W24 Hormone replacement therapy and risk factors of cardiovascular disease in postmenopausal women with non-insulin-dependent diabetes mellitus

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Objective: To estimate the influence of hormone replacement therapy (HRT) on biochemistry parameters of lipid and glucose metabolism and haemostatic factors in postmenopausal women with non-insulin-dependent diabetes mellitus (NIDDM).

Methods: 25 postmenopausal NIDDM women aged 56 ± 7 years were treated in 28-day cycles with 2 mg estradiol valerate (EV) daily for 21 days (4 women after hysterectomy) or EV 2 mg for 21 days plus cyproterone acetate 1 mg for the last 10 days (21 women with intact uterus). 20 controls were postmenopausal NIDDM women aged 58 ± 4 years without HRT.

Results: After 3 months of treatment low-density cholesterol lipoprotein (LDL-C) decreased 8% (NS), high-density cholesterol lipoprotein (HDL-C) increased 15% (p < 0.05), triglycerides (TG) increased 3% (NS), plasma fibrinogen (F) decreased 15% (p < 0.05), spontaneous platelet aggregation (Agg.) decreased 14% (p < 0.05), glycylated haemoglobin (Hba1c) decreased 4% (p < 0.05).

Conclusions: 1) HRT in postmenopausal women with NIDDM was associated with favorable changes in biochemistry parameters of lipid and glucose metabolism and haemostatic factors. 2) HRT was well tolerated in postmenopausal women with NIDDM.

WeT6:W24 Dehydroepiandrosterone sulphate, lipid profile and thrombogenic factors in young and middle age men

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Objective: Dehydroepiandrosterone sulphate (DHEA-S) is a steroid hormone synthesized mainly by the adrenal cortex. Decrease of its concentration may probably influence atherogenesis.

Methods: Study included 60 randomly selected men aged 20-50. Serum DHEA-S was measured by ELISA, cholesterol (TCH), HDL-CH, triglycerides (TG) by enzymatic colorimetric assay. Protein C and AT III were determined by colorimetric assay and fibrinogen using modification of Clauss method.

Results: Mean TCH was found to be increased whereas markers of anticoagulant activity (Protein C and ATIII) as well as fibrinogen values were in normal range. Mean DHEA-S value was also in the normal range.

WeT1:W25 Blood fibrinogen levels and associations with other risk factors in turkish adults

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Objective: To measure fibrinogen values in Turkish adults and assess associations of fibrinogen levels with several coronary risk factors investigated.

Methods: In the 1599 men and women of the 2575 adults visited in the third follow up of the TEKHFAR Cohort in 1997/8, plasma fibrinogen and blood lipids were measured by Behring turbidimetry and Bayer Reflotron respectively and validation of the results were done in a reference laboratory.

Results: Mean fibrinogen levels were 2.68 and 2.88 g/l in men and women respectively. Median age was 46 for men and 48 for women. Fibrinogen levels were independent of age in women but increased with age in men (0.1 g/l for every 5 years; r = 0.29 p < 0.001). Multivariate analysis indicated smoking as an independent significant marker for fibrinogen levels in both sexes. Waist circumference, triglycerides, and HDL-cholesterol (HDL-C) in women and waist/hip ratio in men were significant determinants of fibrinogen levels. HDL-C was borderline significant in men. In the univariate analysis, physical activity showed a weak indirect significant association with fibrinogen in both sexes. Body mass index, systolic and diastolic blood pressures in women and LDL-C in men showed weak but significant associations with fibrinogen levels. LDL-C/HDL-C showed an indirect association with fibrinogen in women. Direct association between HDL-C and fibrinogen was found but was difficult to explain.

Conclusions: Slightly higher fibrinogen levels in Turkish adults compared to Caucasians are thought to contribute to the coronary heart disease risk in our population.

WeT2:W25 Psychological features of patients with ischaemic heart disease and coronary behavior type “A”

A.S. Melentyev, V.P. Zaicev, I.A. Melentyev, E.A. Kolesnikova. Russian State Medical University, Moscow, Russia

Objective: To study behavioral type “A” at patients with different forms ischemic heart disease (IHD).

Methods: The computer psychometrical investigation included 83 patients with angina pectoris (AP) and 120 patients with acute myocardial infarction.
T.W26 REGULATION OF ENDOThelial FUNCTION

**WeT1.W26** Bezbrazafate reduces heart rate and blood pressure in patients with hypertriglyceridemia


**Objective:** To determine whether bezafibrate affects hemodynamics in hypertriglyceridemic (HTG) patients and to assess whether insulin resistance, elevated non-esterified fatty acids (FFA) and cGMP (endothelial function) are involved in this regulation.

**Methods:** The effects of bezafibrate (six weeks 400 mg once daily) on heart rate (HR) and BP in relation to plasma lipids, insulin, FFA, aldosterone, catecholamines, 24 h urinary sodium excretion and urinary catecholamines were investigated in 17 endogenous HTG patients in a double-blind placebo-controlled cross-over fashion. cGMP was measured as a marker for NO production. At the end of both treatment periods, fasting blood samples were drawn and BP and HR were measured automatically for 30 min at 2min intervals.

**Results:** Bezbrazafate significantly decreased plasma total TG (−61%) and total cholesterol (−25%). Hemodynamic parameters decreased upon bezafibrate therapy: HR from 69.4 ± 2.5 to 66.6 ± 2.6 per min (p = 0.009), SBP from 137.4 ± 5.4 to 132.2 ± 5.3 mmHg (p = 0.001), DBP from 81.3 ± 2.7 to 79.0 ± 2.6 mmHg (p = 0.07) and MBP from 101.6 ± 3.7 to 98.7 ± 3.9 mmHg (p = 0.06). Both FFA and insulin decreased significantly upon bezafibrate therapy (−55% and −57%, respectively). cGMP significantly increased upon bezafibrate therapy (+24%, p = 0.005), whereas the other parameters were not affected.

**Conclusion:** Bezbrazafate reduces HR, BP, insulin and FFA in HTG patients. These improved hemodynamics might be caused by improved endothelial function.

**WeT2.W26** Reduced levels of NO in patients with uncomplicated type II diabetes mellitus

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**Objective:** It has been reported that poor glycemic control is associated with endothelial dysfunction in type II diabetes mellitus. The enhanced oxidative stress showed in diabetes could contribute to endothelial dysfunction. The present study was designed to evaluate the effect of metabolic control on circulating nitric oxide (NO) levels and on systemic oxidative stress.

**Methods:** Serum nitrite and nitrate (NO₂⁻ + NO₃⁻) concentration, an indirect index of circulating NO, LDL susceptibility to oxidation, vitamin C and E plasma content, LDL vitamin E levels, were assessed in 50 uncomplicated type II diabetic subjects, 25 in poor metabolic control (HbA₁C ≥ 8.0%) and 25 in longstanding good metabolic control (HbA₁C ≤ 7.5%) as compared to 25 well matched healthy subjects. Following basal measurements, 13 diabetic subjects in good metabolic control were randomly assigned to receive vitamin E (300 mg b.i.d.) for 4 weeks whereas 12 subjects of the same group received placebo.

**Results:** Circulating NO levels were significantly reduced and oxidative stress significantly increased in both diabetic groups as compared to healthy subjects. However serum NO₂⁻ + NO₃⁻ content was significantly lower and oxidative stress significantly enhanced in poorly controlled than in well controlled diabetic subjects. Interestingly, serum NO₂⁻ + NO₃⁻ levels were significantly and inversely related to HbA₁C in poorly controlled NIDDM whereas in well-controlled diabetic patients they were significantly and inversely related to native-LDL lipid peroxide content. Vitamin E supplementation normalizes serum NO₂⁻ + NO₃⁻ levels.

**Conclusions:** Although good metabolic control can significantly increase serum NO₂⁻ + NO₃⁻ content an antioxidant treatment with vitamin E is necessary to normalize NO levels in type II diabetes mellitus.

**WeT3.W26** Relation between endothelial dysfunction and laboratory parameters in patients with combined hyperlipidemia

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**Objective:** Impaired endothelial function (EF) has been described in hypercholesterolemic and hypertriglyceridemic subjects. The purpose of this study was to determine any relation between EF and several metabolic parameters in men with combined hyperlipidemia.

**Population:** Twenty-nine non-smoking otherwise healthy men with untreated combined hyperlipidemia, aged 47.4 ± 7.8 years (mean ± SD), with body mass index 27.7 ± 2.69, systolic blood pressure (dSBP) 125.5 ± 11.4 and diastolic blood pressure (dDBP) 83 ± 7.6 mmHg, participating in the Fenofibrate versus Atorvastatin Trial (FAT).

**Methods:** Flow-mediated dilation (FMD) and laboratory parameters mentioned below were measured. FMD was assessed in brachial artery using a linear 7.5 MHz ultrasound-probe. Laboratory parameters included serum levels of total cholesterol (C), HDL-C, LDL-C, LDL particle size, triglycerides (TG), fibrinogen (FBG), ferritin (FERR), glycemia (G), C-reactive protein (CRP), apolipoprotein AI (Apo-AI), apolipoprotein B (Apo-B), lipoprotein(a) (Lp(a)), homocyst(e)ine (Hc), uric acid (UA), and oxidative stress markers – malondialdehyde (MDA) and superoxideliasmutase (SOD). A statistical correlation (Pearson) between FMD and each of the laboratory values was calculated, p < 0.05 was considered as significant.

**Results:** as expressed mean ± SD. The FMD was 2.17 ± 0.20%, total C 7.53 ± 1.22 mmol/L, HDL-C 1.25 ± 0.32 mmol/L, LDL-C 4.39 ± 0.89 mmol/L, LDL particle size 24.7 ± 0.7 nm, TG 5.40 ± 4.66 mmol/L, FBG 2.73 ± 0.50 g/L, FER 192 ± 185 μg/L, G 5.27 ± 0.54 mmol/L, CRP 1.21 ± 0.71 mg/L, Apo A-I 1.33 ± 0.24 g/L, Apo B 3.35 ± 0.25 g/L, Lp(a) 2.0 ± 2.6 g/L, Hc 21.1 ± 2.6 μmol/L, UA 415 ± 202 μmol/L, MDA 4.07 ± 1.94 μmol/L, SOD 1.11 ± 0.22 U. The only statistically significant correlation between FMD and any of the other variables (including age, BP and BMI) was found for CRP (r = 0.42, p = 0.039).

**Conclusion:** Endothelial dysfunction in subjects with combined hyperlipidemia is caused by interaction of many factors and can not be easily predicted by any of the above mentioned individual laboratory parameters. A correlation was found between FMD and CRP level. It could be explained, at least in part, by a role of the vessel wall inflammation in the process of atherosclerosis.

**WeT4.W26** Plasma markers of endothelial dysfunction in dyslipidemic patients: Outcome after a 3-months treatment

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A negative impact of dyslipidaemias on vascular endothelium has been previously suggested and the positive effect of statins might in part come from an improvement of vascular endothelium function. To assess this hypothesis, we measured biological markers of endothelial dysfunction before and upon treatment of dyslipidemias. Soluble markers of endothelial dysfunction were performed in 2 populations of patients: 10 hypercholesterolaemic (HC) (TC > 6.5 mmol/L and TG < 3 mmol/L), and 13 hypertriglyceridaemic (HTG) (TG > 1.9 mmol/L). All were free
from detectable atherosclerosis assessed by treadmill electrocardiography and duplex ultrasonography. We measured lipids, soluble thrombomodulin (STM), von Willebrand factor (vWF), E-selectin, P-selectin, VCAM-1 and ICAM-1. HC were given fluvastatin 20 or 40 mg/day and HTG fenofibrate 200 mg/day. At baseline the only marker to differ between both groups was STM that was higher in HC than in HTG (34 ng/ml (31–39) vs 29 ng/ml (22–34). After a 3 months therapy, STM significantly decreased in HC patients treated with fluvastatin (32 ng/ml, 29–37 vs 34 ng/ml, 31–39; p = 0.039) while none of the markers significantly varied upon fenofibrate. Fluvastatin is able to improve a marker of endothelial damage after 3 months.

**WeT5:W26**

**Is cutaneous microcirculation endothelium-dependent vasomotion impaired in patients with coronary artery disease?**

University Hospital of Brest, Brest, France

**Objective:** To compare cutaneous microcirculation endothelium-dependent vasomotion measured by Laser-Doppler techniques in patients (pts) with coronary artery disease (CAD) and healthy controls (CTRL).

**Methods:** Laser-Doppler (Periflux 4001–2, Perimed, Jarllilla, Sweden) was used to measure forearm cutaneous microcirculatory flow in 2 populations: 26 pts with documented CAD (20 M, mean age 58 ± 10 years) and 17 CTRL (14 M, mean age 48 ± 6 years). We measured flow at baseline (F), and peak (P) and duration (D) of reactive hyperemia induced by brachial arterial occlusion (phygromonometric cuff) for 5 min. Risk factors and complete lipid profile were collected systematically. Diabetic pts and pts with heart failure were excluded.

**Results:** Mean values are summarized in the table.

<table>
<thead>
<tr>
<th></th>
<th>F (PU)</th>
<th>P (PU)</th>
<th>F-P (PU)</th>
<th>PSF</th>
<th>D (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CAD pts</strong></td>
<td>5.8 ± 2.2</td>
<td>30 ± 13</td>
<td>24.4 ± 12</td>
<td>5.5 ± 2</td>
<td>133 ± 40</td>
</tr>
<tr>
<td><strong>CTRL</strong></td>
<td>5.2 ± 1.6</td>
<td>43 ± 19</td>
<td>37.5 ± 19</td>
<td>8.6 ± 4</td>
<td>189 ± 92</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>NS</td>
<td>0.019</td>
<td>0.001</td>
<td>0.004</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Only HDL was significantly lower in CAD vs. CTRL (1.3 ± 0.3 vs. 1.6 ± 0.4 mmol/L).

**Conclusion:** Endothelium-dependent vasomotion in the forearm cutaneous microcirculation measured by Laser-Doppler seems to be impaired in pts with CAD. Studies with larger populations are needed to evaluate the usefulness of this technique in the follow-up of atherosclerotic pts.

**WeT6:W26**

**Impaired endothelium-dependent vasodilation in hypertriglyceridemia can be improved by eicosapentaenoic acid**

T. Okumura, F. Fujikawa, S. Tsuibo, S. Morimoto, M. Masai, A. Miyoshi, T. Iwasaki. Hyogo College of Medicine, Nishinomiya, Japan

**Objective:** Hypercholesterolemia impairs the endothelium-dependent vasodilating response (EDR) and statins could improve it. On the other hand, the effects of lowering serum triglyceride level on EDR is not well established. To test this, we investigated EDR of hypertriglyceridemia and the effect of eicosapentaenoic acid (EPA) supplementation.

**Methods and Results:** We studied the responses of forearm blood flow (%FBF) to intra-arterial infusion of acetylcarnitine (ACH) and sodium nitroprusside (SNP) by strain-gauge plethysmography. Studies were performed in patients of eight hypertriglyceridemic and five healthy control subjects. All subjects had no atherosclerotic disease. There were no difference in age, body mass index, mean blood pressure, smoking habits and glutathione peroxidase between two groups. Mean levels of serum lipids were 211 and 186 mg/dl of total cholesterol (NS), 293 and 76 mg/dl of triglyceride (P < 0.05), 50 and 56 mg/dl of HDL cholesterol (NS), respectively. %FBF evoked by ACH was attenuated in hypertriglyceridemia patients (P < 0.05). EDR evoked by SNP was preserved in those compared to normal subjects. After 3 months of administration of EPA (1800 mg/day), serum triglyceride level decreased to 137 mg/dl and attenuated EDR evoked by ACH was improved.

**Conclusions:** EDR in hypertriglyceridemia is attenuated, and EPA supplementation for 3 months can improve it.

**WeT7:W26**

**Intercellular adhesion molecule-1 (ICAM-1) expression by endothelial cells and soluble ICAM-1 levels do not correlate during cardiopulmonary bypass**

M. Vallel1,2, P. Bannon2, C. Hughes2, R. Dean1, L. Kritzhalde1,3, 1The Heart Research Institute; 2Royal Prince Alfred Hospital Cardiothoracic Surgical Unit; 3Department of Cardiology, Concord Hospital, Sydney, Australia

**Objectives:** Cardiopulmonary bypass (CPB) causes systemic inflammation which is associated with increased levels of soluble adhesion molecules, such as ICAM-1. The relationship between endothelial cell (EC) expression of ICAM-1 and soluble ICAM-1 is unknown.

**Methods:** Plasma was taken from 10 adult patients undergoing first time, elective coronary artery bypass grafting. Samples were taken for each patient before CPB, during CPB (before and after release of aortic cross clamp), 3 and 24 hours after operation (post-op). Soluble ICAM-1 levels were measured by ELISA. The same plasma samples were incubated with passage 2 human umbilical vein EC (HUVEC) monolayers and cell surface expression of ICAM-1 measured by ELISA.

**Results:** All plasma samples were analysed and corrected for haemodilution. Soluble ICAM-1 levels were increased in plasma taken 24 hours post-op (117% of pre-CBP; p = 0.019), but all other samples contained identical ICAM-1 to the pre-CBP control. HUVEC expression of ICAM-1 was decreased only by plasma sampled 3 hours post-op (85% of control; p = 0.008), but no upregulation of ICAM-1 was seen.

**Conclusions:** Soluble ICAM-1 levels increase late post-operatively but HUVEC expression of ICAM-1 is decreased by exposure to early post-op blood. EC expression and soluble forms of ICAM-1 need not correlate during cardiopulmonary bypass.

**WeT8:W26**

**The influence of daily exercise to the physical strength in patients with myocardial infarction at chronic phase**

Y. Yasunobu1, Y. Katou1, S. Nishimura1, I. Inoue2, 1Myoshii Medical Associated Hospital; 2Myoshii General Hospital, Myoshii, Japan

**Objective:** To examine whether daily exercise produces a good effect on exercise tolerance in patients with myocardial infarction at chronic phase.

**Methods:** Patients with a history of myocardial infarction were measured cardiologic rehabilitation under anaerobic threshold (AT) point at acute phase were studied (14 subjects, 11 males; age, 46 to 84 years). AT, peak oxygen output (peak VO2) obtained from symptom-limited incremental exercise by breath-by-breath respiratory gases, and blood flow of lower extremity by strain gauge plethysmography were measured after the second week form the previous admission and after several months from getting out of the hospital. We got some information about daily exercise after leaving the hospital and their occupation from them. We investigated the correlation between the changes of kinetics of pulmonary gas exchange in the two measuring, doing daily exercise, and having an occupation.

**Results:** 1. The increasing rate of peak VO2 was significantly higher in patients who were exercising daily than in those who were not (p = 0.0032).

2. The positive correlation between the increasing rate of peak VO2 and max reactive hyperemia was observed (r = 0.593, p = 0.0308). The increasing rate of basal flow and max reactive hyperaemia were significantly higher in patients who had an occupation than those who did not (p = 0.0324).

**Conclusion:** The influence of a continuously getting exercise to basal flow and endothelial function of lower extremity may be more extensive than that of short-time exercise. Daily exercise may produce a good effect on keeping and increasing the physical strength in patients with myocardial infarction at chronic phase through the improving endothelial function.

**WeT9:W26**

**Effect of niludipin and verapamil on the peripheral vascular resistance**

M. Brodmann, U. Lischnig, I. Friedl, A. Lueger, D. Scherr, E. Pilger. G. Stark. Division of Angiology, Department of Internal Medicine, Karl Franzens University Graz, Austria

**Objective:** Arterial hypertension is a relevant concomitant disease in patients suffering from peripheral vascular disease (PAD). In the choice of the antihypertensive treatment a thought should be given concerning the vasodilative effect of the chosen substance. The aim of the following study was to evaluate the effect of the calcium channel blockers niludipine and verapamil on the vascular resistance of isolated perfused guinea pig hind limbs.
Methods: For this reason a catheter was inserted in the distal abdominal aorta. After equilibration with tyrode’s solution and preconcentration with noradrenaline 3 μM, nifedipine and verapamil were administered at dosages of 0.1, 1 and 10 μM.

Results: At the lowest dosage of 0.1 μM nifedipine a significant reduction of the peripheral vascular resistance (8 ± 1% x ± SEM, n = 5) could be reached already, whereas this could not be seen with verapamil. A further increase of the dosage showed for both substances a reduction of the peripheral vascular resistance, whereas at a concentration of 1 μM nifedipine also for verapamil a significant reduction could be reached (11 ± 5%). At the highest concentration of 10 μM the peripheral vascular resistance was more significant (p > 0.01) reduced by nifedipine than by verapamil (26 ± 7% vs. 18 ± 2%).

Conclusions: The different reduction of the peripheral vascular resistance by different calcium channel blockers is caused on the one hand by the distribution of the calcium channels and on the other hand by the preferred binding of different calcium antagonists at the special channel types. According to the presented results nifedipine reduces in lower dosages the peripheral vascular resistance much more significantly than verapamil, whereas this difference is diminished in higher dosages of both substances. This may be a sign for the reduction of the selectivity of the specific blocking (mostly for verapamil) of the L-type and T-type calcium channel in peripheral vessels.

WeT11C2W26 Effect of fluvastatin on endothelial function in patients with peripheral atherosclerosis

Objective: Endothelium-dependent vasodilation is impaired in coronary and peripheral arteries of patients with hypercholesterolaemia. The objective of our study was to assess whether the lipid-lowering fluvastatin therapy improves peripheral endothelial function in patients with established atherosclerosis.

Methods: In 11 pts (9 male and 2 female) aged 45–65 years with established atherosclerosis the brachial artery diameter was measured with high-resolution ultrasound and ACUSON128XP10 system at rest, during reactive hyperemia and after 500 μg of nitroglycerin.

Results: Flow-mediated dilatation, total cholesterol and LDL-cholesterol were significantly lower after 2 month fluvastatin treatment (40 mg daily).

Parameter | Before | After
---|---|---
Total cholesterol, mmol/l | 6.7 ± 1.2 | 5.1 ± 1.3*
LDL-cholesterol, mmol/l | 4.8 ± 0.9 | 3.0 ± 1.0
Diameter BA, mm | 4.6 ± 0.6 | 4.6 ± 0.6
Baseline flow, m/s | 0.64 ± 0.18 | 0.70 ± 0.20
PMD, % | 4.7 ± 3.9 | 7.0 ± 3.1**
NDD, % | 101.± 6.7 | 107. ± 4.6
Mean ± SE; * = p < 0.004, ** = p < 0.007.

Conclusion: The ultrasound methods have improved our diagnostic capabilities in estimation of endothelial function under the lipid-lowering therapy in patients with peripheral atherosclerosis.

WeT11C2W26 Endothelial dysfunction – influence of atorvastatin

Background: Great Studies (4S, WOSCOPS) show that the reduction of mortality and cardiovascular events is higher than the one which is explained by the regulation of lipid profile. This studies suggest antioxidant effects of statins.

Methodology and Results: The study observes secondary prevention in 36 patients with coronary heart disease. We prescribed them Sortis 10–40 mg, to attain optimal lipid profile. Before the introduction of the therapy, we followed the parameters of vascular mechanic a. brachialis in basic condition, during endothelium-dependent vasodilation EDV (effect of reactive hyperemia) and during endothelium-independent vasodilatation EMDV (3 min. after sublingual application 0.4 mg nitroglycerin). The control measurements were performed 1, 3 and 6 months after starting of the therapy with Atorvastatin. The study following the vascular remodeling a. carotis int. with analysis of intima-media complex thickness IMT, and left ventricular mass index LVM, determined by Deveroux method, as the most valid indicator of vascular remodeling.

Conclusion: Atorvastatin decreases the level of arterial endothelial dysfunctions with increasing response to endothelial-dependent vasodilatation, and with decreasing to endothelial-independent vasodilatation. During the long-term therapy with Atorvastatin we notice a tendency of regression vascular and ventricular remodeling in hyperlipidemic patients with CHD.

Well12C2W26 Comparison of the effect of prostaglandin E1, prostacycline and adenosine on peripheral vascular resistance
M. Brodmann, U. Lischning, I. Friedl, A. Laeger, E. Pilger, G. Stark. Division of Angiopathy, Department of Internal Medicine, Karl Franzens University Graz, Austria

Objective: Prostacycline and prostaglandin E1 and adenosine are highly effective vasodilators. These drugs are widely used in the treatment of peripheral arterial occlusive disease. The aim of the study was to compare the vasodilatory potency of these three substances in the isolated perfused guinea pig hind limb.

Methods: After equilibration with Tyrode’s solution and preconcentration with noradrenaline 3 μM, prostaglandine E1 and adenosine were administered at dosages of 0.1, 0.3 and 1 μM, whereas prostacycline was administered at a dosage of 0.01, 0.03 and 0.1 μM.

Results: 0.01 μM prostacycline and 0.1 μM prostaglandine E1 and 0.1 μM adenosine were the lowest dosages at which a significant vasodilatation could be reached for each substance. The reduction of the peripheral vascular resistance at comparable dosages of 0.1 μM was 11 ± 2% (x ± SEM, n = 5) at for adenosine, 12 ± 1% (n = 5) for prostaglandine E1 and significantly higher in the presence of prostacycline. Even at a dosage of 0.01 μM prostacycline a considerable reduction in peripheral vascular resistance (16 ± 2% versus 12 ± 1%) could be reached compared to a ten fold higher dosage of prostaglandine E1 and adenosine. At the highest concentration of 1 μM the vasodilatory effect of adenosine was significantly less expressed compared to that of prostaglandine E1 (18 ± 3% versus 33 ± 4%).

Conclusions: In summary prostacycline, at a ten fold lower concentration, showed comparable vasodilatory effects as adenosine and prostaglandine E1. The rank order at the vasodilatory potency between these three substances is prostacycline > prostaglandine E1 > adenosine.

WeT11C2W26 Biochemical parameters of endothelial dysfunction, serum lipids and antioxidant status in men with arterial hypertension
R. Grabys, M. Cholewa, A. Marszałek. 1. Dept. of Internal Diseases, Military Hospital, Olszyn; 2. Clinic of Internal Diseases and Cardiology, Central Military Clinical Hospital, Warsaw, Poland

The aim of the study was to evaluate vascular endothelium function in men with untreated arterial hypertension (AH) on the ground of endothelin 1 (ET 1) concentrations measurements. We also studied the function of antioxidative system and concentrations of lipids radicals in this group. In 52 men (mean age 50.1; range 36–65 yrs) with new diagnosis of AH we evaluated following biochemical measurement: ET 1 (ELISA), total cholesterol (TC), HDL-cholesterol, triglycerides (TG) (enzymatic assay). To determine the function of antioxidative system we measured total antioxidative status (activity of superoxide dismutase and glutathione peroxidase) (TAS; colorimetric assay).

The results are compiled in the table:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>AVG</th>
<th>SD</th>
<th>Abnormal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET1 (fmol/ml)</td>
<td>18</td>
<td>3.7</td>
<td>2.83</td>
<td>83.00</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>32</td>
<td>236</td>
<td>39.70</td>
<td>78.10</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>32</td>
<td>46.3</td>
<td>11.30</td>
<td>12.50</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>32</td>
<td>187.0</td>
<td>120.95</td>
<td>37.50</td>
</tr>
<tr>
<td>TAS (nmol/l)</td>
<td>30</td>
<td>13.6</td>
<td>2.53</td>
<td>35.50</td>
</tr>
</tbody>
</table>

We didn’t find statistically significant correlation between concentrations of ET1 and age, values of blood pressure, TC, HDL-cholesterol. We observed statistically significant correlation (p < 0.05) between concentrations of ET1 and TG (r = 0.6). We didn’t find significant abnormalities in TAS in studied group. It seems that used parameter (TAS) doesn’t reflect real changes in antioxidant status in studied group.
**T:H5 GENETIC SCREENING**

**WeT14:W26** Reactive oxygen species in peripheral blood of patients suffering due to coronary heart disease (CHD)


**Department of Physiology; Department of Internal Disease**: Military Medical Academy; **Department of Cardiovascular Surgery**: Medical Academy Lödz, Poland

The role of reactive oxygen species (ROS) and other substances released by morphotic elements and vascular endothelium in regulation of hemodynamics processes in physiological conditions and atherosclerosis has been described in the last few years.

The aim of this study was to estimate ROS: superoxide anion (O2-), nitric oxide (NO) generation in peripheral blood of patient with CHD.

Our examination were carried out on 19 patients with CHD (age 40-50 yrs). The comparative group consisted of 15 healthy men (age 40-50 yrs). The Local Ethical Board permits on this investigations. The blood was taken from cubital vein to heparinized tubes. The generation of O2- by granulocytes (PMNL) at rest and after stimulation by opsonized zymosan was determined acc. to Bellavire et al method. NO generation by PMNL was measured using selective electrode conjugated with ISO NO apparatus – World Pression Instruments (USA).

We indicated that in peripheral blood of patients with CHD the O2- and NO were significantly increased in comparison to control (healthy men). This observation can suggest that simultaneous increase of O2- and NO generation may be inactivated by the vasoconstriction effect of nitric oxide.

Supported by Grant N 4 4050 CO 414.1.

**Conclusion**: This study surveys 75 hypertensive patients in the fifth and sixth decade, divided in three equal groups. In the first group we prescribed Tricat 2.5 mg, in the second Telmisartan 40 mg and in the third we prescribed both of them. Before the introduction of the therapy, we followed the parameters of vascular structure (Carotid Intima-Media Complex Thickness-IMK-mm) and ventricular geometry (Left Ventricular Mass Index-LVMI-g/m2). The control measurements were performed 1, 3 and 6 months after starting of the therapy. Following the vascular remodeling with analysis of IMT, after 6 months we get decreasing from 12.5% in group I, 13.58% in group II and 18.07% in group III. LVMI determined by Devereux method, as the most valid indicator of ventricular remodeling, after 6 months shows the decreasing from 11.93% in group I, 10.94% in group II and 15.04% in group III.

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMT (mm)</td>
<td>LVMI</td>
<td>IMT (mm)</td>
<td>LVMI</td>
</tr>
<tr>
<td>Basal</td>
<td>0.81 ± 0.11</td>
<td>129 ± 12</td>
<td>0.81 ± 0.12</td>
</tr>
<tr>
<td>After 1 m.</td>
<td>0.80 ± 0.12</td>
<td>127 ± 12</td>
<td>0.80 ± 0.12</td>
</tr>
<tr>
<td>After 3 m.</td>
<td>0.77 ± 0.11</td>
<td>123 ± 12</td>
<td>0.76 ± 0.11</td>
</tr>
<tr>
<td>After 6 m.</td>
<td>0.71 ± 0.09*</td>
<td>116 ± 11*</td>
<td>0.70 ± 0.10*</td>
</tr>
</tbody>
</table>

*p < 0.01 vs baseline.

**Conclusion**: During the longterm therapy with Ramipril and Telmisartan, we notice a tendency of regression ventricular and vascular remodeling in hypertensive patients.

**WeT15:W26** Common carotid wall shear stress is reduced in patients with heart failure


**Unit of Internal Medicine, Mornanno General Hospital, Mornanno (CS); University of Magna Graecia**, Catanzaro, Italy

**Objective**: In patients suffering from heart failure cardiac output is reduced and endothelial dysfunction appears. Wall shear stress (WSS), that is the frictional force exerted on the endothelium by the flowing blood, depends on arterial geometry and cardiac output. This hemodynamic force is an important factor activating the endothelial production of nitric oxide. Aim of the present study is to determine WSS levels in conditions of low cardiac output.

**Methods**: We have calculated common carotid mean WSS in 14 patients with heart failure (NYHA class I–IV) and in 14 healthy controls matched for sex, age, and body mass index, using the following formula: WSS = blood velocity * blood viscosity/arterial diameter.

**Results**: In the present group, WSS resulted inversely related to NYHA class (r = 0.68, p = 0.008), and directly proportional to the ejection fraction (r = 0.71, p = 0.004). Furthermore, WSS was lower in patients than in controls (5.2 ± 2.5 vs 7.6 ± 3.4, p = 0.03).

**Conclusions**: The results of the present study demonstrate that WSS is low in patients suffering from heart failure and is related to the severity of the disease. This finding might contribute to explain the presence of endothelial dysfunction in these patients.

**WeT16:W26** Ventricular remodeling - effects inhibitors ACE and AII receptor antagonists in hypertensive patients

I. Petrovic, Z. Petrasinovic, D. Petrovic, V. Kanjulj, M. Ostojić


**Background**: Ramipril administration reduces thrombus formation and surface expression of platelet GPIIb/IIIa in ischaemic stage, and it reduces ventricular and vascular remodeling. Angiotensin induces inflammatory activation of vascular SMC, and Telmisartan is All receptor antagonist with beneficial effects on cardiac and vascular structure and function.

**Methods and Results**: The study observe 75 hypertensive patients in the fifth and sixth decade, divided in three equal group. In the first group we prescribed Tricat 2.5 mg, in the second Telmisartan 40 mg and in the third we prescribed both of them. Before the introduction of the therapy, we followed the parameters of vascular structure (Carotid Intima-Media Complex Thickness-IMK-mm) and ventricular geometry (Left Ventricular Mass Index-LVMI-g/m2) The control measurements were performed 1, 3 and 6 months after starting of the therapy. Following the vascular remodeling with analysis of IMT, after 6 months we get decreasing from 12.5% in group I, 13.58% in group II and 18.07% in group III. LVMI determined by Devereux method, as the most valid indicator of ventricular remodeling, after 6 months shows the decreasing from 11.93% in group I, 10.94% in group II and 15.04% in group III.

**Conclusion**: The present study we evaluated the results of a selective screening combining cut-offs levels and family study to identify genetic forms of HC in children.

**Methods**: Forty-two HC children (age 5–12 years) were identified on the basis of total cholesterol (TC) > 90th age-sex specific percentile. Lipid profile was obtained in their family members (n = 217). HC were classified as follow: 1) probable Familial Hypercholesterolemia according to the presence of a first degree relative with HC (TC > 90th age-sex specific percentile); 2) Familial Combined Hyperlipidemia according to the presence of a first degree relative with high TG (>90th age-sex specific percentile). Children with non familial relatives were classified as having non familial HC.

**Results**: Sixteen children (38%) were identified as probable FH, 15 (36%) as FCHL and 11 (26%) did not show a familial lipid disorder. Table shows clinical characteristics and lipid levels (mg/dl) in probands and affected relatives in FH and FCHL families.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FH</th>
<th>FCHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>9/7</td>
<td>6/5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 ± 3</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>64 ± 13</td>
<td>64 ± 13</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>204 ± 17</td>
<td>181 ± 17</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>106 ± 14</td>
<td>106 ± 14</td>
</tr>
</tbody>
</table>

*p < 0.05; †p < 0.01; ‡p < 0.001 for comparison FH vs FCHL.

**Conclusions**: The ascertainment through HC and family study allowed to identify about 1/4 of HC children which do not have evidence of familial lipid disorder and therefore do not require further action. In addition, 1/3 of HC belongs to families with evidence of FCHL or probable FH.
Thursday June 29, 2000: Workshop Abstracts

**W:27 LIPOPROTEIN(a)**

**ThW1:27** Assembly of lipoprotein(a)
S.P.A. McCormick, Department of Biochemistry, University of Otago, Dunedin, New Zealand

The assembly of lipoprotein(a) [Lp(a)] is a two-step process. The first step is thought to be a noncovalent interaction between apolipoprotein (apo) B and apolipoprotein(a), while the second step comprises a disulfide bridge between apoB48 and apo(a)Cys4326. Evidence for the initial noncovalent interaction comes from studies of two forms of apoB lacking apoB48 and apo(a)Cys4326. This association can then reassociate with apo(a) to reconstitute the plasma. The protein interactions involved in the initial binding between apoB and apo(a) are not fully understood. The aim of our current research is to identify the apoB sequences that are important for this initial binding to apo(a).

Recent work targeting the Cys4326 residue in the mouse apoB sequence in a human/mouse hybrid apoB has shown that variation in the carboxyl-terminal sequences of apoB affects the efficiency of Lp(a) formation. In addition, studies of Lp(a) assembly with carboxyl-terminally truncated human apoB proteins have indicated that apoB amino acids 4330–4397 are important for the initial interaction with apo(a). With the 4330–4397 region, we have identified a stretch of amino acids (residues 4372–4392) that are highly conserved between human and mouse apoB. A synthetic apoB peptide spanning the 4372–4392 sequence has proved to be an effective inhibitor of Lp(a) assembly. Computer analysis of this region predicts an amphiphilic alpha-helix containing two sets of paired lysine residues on opposite sides of the helix. Circular dichroism studies of the synthetic peptide have confirmed its alpha-helical nature. Our results indicate that apoB amino acids 4372–4392 play an important role in Lp(a) assembly. Additional studies are currently being performed to further characterise the structural features of this new putative apo(a) binding site.

**ThW2:27** Lipoprotein (a) as a risk factor for cardiovascular disease
Lars Berglund, Columbia University, New York, NY, USA

Lipoprotein(a), [Lp(a)], has been identified as a risk factor for cardiovascular disease (CAD) in numerous but not all prospective studies. Potentially, several different features of the Lp(a) particle, such as apo(a) protein properties and the apoB/ApoB ratio, could contribute to its atherogenicity, separately or in conjunction. Curiously, mean Lp(a) levels are twice as high in Blacks compared to Whites, but studies to date have failed to establish a significant association between elevated Lp(a) levels and CAD among Blacks. The lack of understanding of this racial difference has made it difficult to conclude with full confidence that Lp(a) is a risk factor for CAD. Further, presence of small apo(a) isoforms has been established in CAD in Whites but not in Blacks. The majority of Whites with high Lp(a) levels also possess at least one small apo(a) isoform, but for Blacks, high mean Lp(a) levels are present over a wider range of apo(a) isoforms. The high correlation between elevated levels of Lp(a) and small apo(a) isoforms in Whites makes it difficult to ascertain whether one is a confounder for the other with regards to CAD. In Blacks, the more common combination of high Lp(a) levels with larger apo(a) sizes provides opportunities to test whether Lp(a) level or apo(a) size is more predictive of CAD. We compared Lp(a) levels, apo(a) sizes and the level of Lp(a) particles carrying small apo(a) sizes in Blacks and Whites, and found that elevated levels of Lp(a) particles carrying a small apo(a) size were significantly associated with CAD in both groups. The results provide one basis for explaining the nature of the association between Lp(a) and CAD, as well as for the previously observed difference in association between Lp(a) and CAD among Blacks and Whites. The plasma level of Lp(a) carried by small-size apo(a) may be the atherogenic subpopulation of particles that determines the degree of risk for CAD contributed by Lp(a).

**ThW3:27** LP(a) metabolism and in vivo proteolytic fragmentation
G.M. Kostner, S. Frank. Institute of Medical Biochemistry, University of Graz, Austria

The knowledge of specific features of the Lp(a) metabolism is still fragmentary. Early turnover studies in man revealed that individuals with high plasma Lp(a) levels exhibit a faster rate of synthesis whereas the FCR appeared to be uniform. There are currently two possibilities discussed on Lp(a) assembly: i) Apo(a) is secreted from liver and associates with LDL outside the liver cell mainly in circulating blood, and ii) Lp(a) is intracellularly assembled. These latter findings were delineated from in vivo kinetics using stable isotopes.

Concerning the catabolism of Lp(a) it appears that the liver is the major organ yet LDL-receptors may play an inferior role. There is still intensive work going on aimed at elucidating specific binding mechanisms of to the liver cell.

Another organ, which might be important in the removal of Lp(a) from circulation, is the kidney. Not only that an arterio-venous concentration difference in Lp(a) in the order of >5% was found, Lp(a) might also bind to megalin, a member of the LDL-receptor family present in kidney cells. Another point underlining the role of kidney in Lp(a) catabolism is the presence of large apo(a) fragments in the urine. Although the total apo(a) immune reactivity found in urine accounts only for 1% catabolism/day, there is the possibility that small fragments not recognized by antibodies might be also secreted.

One major question is which enzyme might be responsible for Lp(a) fragmentation and also what physiological significance might such a proteolytic cleavage have? There is little doubt that the proteases attacking Lp(a) belong to the family of metallo-proteases of the elastase and collagenase type. Results of our ongoing studies will be presented aimed at elucidating the role of various enzymes in Lp(a) catabolism.

**ThW4:27** Sequence changes in putative enhancer regions upstream of the apolipoprotein(a) gene
L. Packer, B. Knight. MRC Clinical Sciences Centre, London, UK

Objective: Plasma concentrations of the atherogenic lipoprotein(a) [Lp(a)] are almost entirely determined by sequences at or closely linked to the apolipoprotein(a) [apo(a)] gene locus. Two regions upstream of the apo(a) gene have been identified which enhance the activity of the apo(a) promoter in reporter-gene constructs in vitro, DHIII, situated about 28kb, and DHIII, about 20kb upstream of the apo(a) gene. The aim of this study was to investigate whether sequence changes in these regions could influence Lp(a) concentrations.

Methods: The enhancers were amplified and sequenced from subjects chosen to cover the whole range of Lp(a) concentrations. The sequence changes were introduced into reporter-gene constructs, the resulting change in apo(a) promoter activity measured and those that had an effect were tracked through families to discover any association between the different alleles and Lp(a) levels.

Results: No base changes were found in the DHIII region. In the DHIII region, 3 common base substitutions were found, an A to G change at position −1230, A to C change at −1617 and a G to T substitution at −1712. The frequency of these sequence changes were 0.54 (A), 0.84 (C) and 0.89 (G) respectively in a Caucasian population. Changing the A to a G in reporter-gene constructs increased the activity of the downstream apo(a) promoter approximately 4-fold, while changing the C to a A and the G to T decreased activity by 50% and 30% respectively. Family studies have shown that the G at −1230 is associated with significantly higher Lp(a) concentrations and the T at −1712 with significantly lower levels.

Conclusions: These sequence changes could provide a significant contribution to the variation of plasma Lp(a) concentrations, but are not solely responsible for determining the large range of concentrations seen in a Caucasian population.

**ThW5:27** Immunohistological localisation of Lp(a) in the human kidney
H. Diepingeler 1, I. Leiter 1, E. Torekwalder 1, W. Salvenmoser 2, W. Horninger 1, K. Lhotz 3, P. König 1, F. Kronenberg 3, 1 Institute of Medical Biology and Human Genetics; 2 Institute of Zoology; 3 Department of Urology; 1 Department of Clinical Nephrology; University of Innsbruck, Austria

Objective: Lipoprotein(a) [Lp(a)] is a genetically determined risk factor for...
Hypertension is frequently present in patients with parenchymal renal disease even before the development of impaired excretory function, and is nearly universal in patients developing end-stage renal failure. Many patients with chronic renal failure die prematurely from cardiovascular disease—end-stage renal failure carries a risk of death comparable to many malignancies—but much of this excess mortality appears to be due to hypertensive heart failure rather than atherosclerotic myocardial infarction.

Mechanisms of hypertension in renal disease include sodium chloride retention, increased renin secretion and increased sympathetic tone, the afferent signal arising in diseased kidneys. Hypertension is poorly controlled in up to 60% of haemodialysis patients, despite aggressive treatment, but can be perfectly controlled, at least in selected patients, by dietary salt restriction combined with rigorous control of extracellular volume by long haemodialysis; this results, apparently paradoxically, in reduced peripheral vascular resistance. Exceptionally good survival figures have been published from one centre employing this strategy.

Observations over up to 4 years in large numbers of dialysis patients have shown that blood pressure is inversely, not directly, associated with risk of death. Very few studies have demonstrated that hypertension is a significant risk factor for death in chronic renal failure, although this can be demonstrated in the centre employing long haemodialysis with dietary salt restriction. The most tenable explanation is that the majority of dialysis patients already have significant hypertensive or ischaemic heart failure, the clinical signs of which are altered as a result of renal disease and its treatment. Treatment to alter the high cardiovascular mortality of renal patients must therefore start early in the course of renal disease.

Amelioration of lipid induced glomerulopathy by lovastatin

A.K. Walli1, P. Fraunberger1, E.F. Greene2, H.J. Gräfe3, D. Seidel4
1Department of Clinical Chemistry, Ludwig-Maximilians-University Munich; 2Institute of Pathology, Deutsches Krebsforschungszentrum Heidelberg, Germany

Objective: Dyslipoproteinemia plays an important role in progression of renal disease. In contrast to rats which transport cholesterol mainly in the HDL fraction, guinea pigs like humans carry cholesterol in LDL. Rates of hepatic cholesterol synthesis and response of lipoprotein metabolism to diet and drug therapy are similar in guinea pigs to those observed in humans. The aim of the study was 1. to characterize alterations in the lipoprotein profile, lipoprotein receptor status and HMG-CoA reductase activity in hepatic and renal tissue in the presence and absence of hypercholesterolemia and lovastatin administration; 2. to attempt to delineate hypolipidemic and direct effects of the drug on reduction of glomerular injury; 3. to correlate biochemical data with morphological changes in the kidney of guinea pigs treated with lovastatin.

Methods: Male Dunkin-Heartley guinea pigs were unilaterally nephrectomized and fed either a regular or 0.3% cholesterol enriched diet in the presence or absence of lovastatin for eight months. Lipoprotein status, LDL receptor activity and the activity of HMG-CoA reductase were determined in liver and kidney. Quantitative analysis of glomerular and tubulointerstitial morphological changes were performed.

Results: Cholesterol feeding increased VLDL and LDL cholesterol by several folds. Administration of a high dose of lovastatin lowered plasma cholesterol by about 50% in contrast to a low dose of lovastatin. Glomerular fat content, cell number, monocyte/macrophage number, matrix increase were significantly reduced by lovastatin at both dosages.

Conclusion: Our data show that HMG-CoA reductase inhibitorLovastatin ameliorates the lipid-induced glomerulopathy in guinea pigs possibly due to its antiproliferative and antiinflammatory effects independent of cholesterol lowering.

Sclerotic change of aorta and survival of patients with end-stage renal disease. A prospective study

T. Shoji1, M. Emoto1, R. Kikuya2, K. Shinozuka1, E. Kimoto1, A. Yamada1, T. Tabata1, Y. Nishizawa1
1Second Dept. of Internal Medicine, Osaka City University; 2Division of Internal Medicine, Innou Hospital, Osaka, Japan

Objective: Atherosclerosis is advanced and mortality rate is high in end-stage renal disease (ESRD). The purpose of this study was to examine the impact of aortic atherosclerosis on the survival of patients with ESRD.

Methods: ESRD patients on maintenance haemodialysis (N = 245) were enrolled in the study. Aortic pulse wave velocity (PWV) was measured as an index of aortic stiffness, and the survival was followed.

Results: During the follow-up period (mean 56 months), 76 deaths (31%) were observed. Kaplan-Meier analysis indicated that diabetic patients had a higher mortality rate than nondiabetics. Also, when the subjects were divided into two groups according their initial PWV values, the high PWV group had a significantly poorer prognosis in both diabetics and nondiabetics. Cox proportional hazard model indicated that PWV was a significant factor associated with mortality in this population independent of age, gender, diabetes and serum creatinine level.

Conclusions: Aortic stiffness as measured by PWV is an independent factor predicting the prognosis of patients with ESRD on haemodialysis.

Correlation between the carotid intimal-medial thickness and histological arteriosclerosis of radial artery in patients with chronic renal failure

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Objective: To assess the relationship between the intimal-medial thickness (IMT) of the carotid artery and the histopathological degree of arteriosclerosis of the radial artery, and to explore the factors affecting arteriosclerosis of the radial artery.

Subjects and Methods: Twenty-five patients with pre-dialysis chronic renal failure (CRF) (serum creatinine >4.0 mg/dL: 40 to 75 years old; 12 men and 13 women) were recruited from Yodogawa Christian Hospital. The tissues from the radial artery, which were resected during the arterio-venous shunt operation, were histologically examined after H.E. staining. The IMT of the carotid artery were measured with high-resolution B-mode ultrasonography.

Results: Out of 25 patients, arteriosclerosis was histopathologically evident in 18 patients (group 1), but not in 7 patients (group 2). In group 1, three patients had intimal plaque and 6 patients had calcification in the media. The IMT of the carotid artery of the group 1 were significantly greater than those of the group 2 (2.05 ± 1.10 vs. 0.92 ± 0.20 mm, p < 0.05). Multiple regression analysis revealed that the histopathological degree of arteriosclerosis of the radial artery were significantly, independently affected by age and hypertension, similar to the factors affecting IMT of the carotid artery.

Conclusion: These results demonstrated that significant relationship between the carotid artery IMT and the histopathological degree of arteriosclerosis of the radial artery.

Lipoprotein-X stimulates MCP-1 expression in mesangial cells: a possible role in monocyte infiltration in familial LCAT deficiency

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Objective: Familial LCAT deficiency is a rare genetic disorder whose clinical symptoms include corneal opacities, hemolytic anemia, proteinuria and subsequent renal failure. Lipoprotein-X (Lp-X) is present in the plasma of LCAT deficient patients. Many of these patients develop glomerulosclerosis. A key event in the pathogenesis of glomerulosclerosis is the infiltration of circulating monocytes in glomeruli. Mesangial cells can express MCP-1, an important chemoattractant for monocytes. The objective of this study was to examine the effect of Lp-X on the induction of mesangial cell MCP-1 expression.

Methods: Lp-X was isolated from the plasma of a LCAT deficient patient. Mesangial cells were isolated from the kidneys of male Sprague-Dawley rats.

Results: Treatment of cells with Lp-X (50–100 μmol/mL) stimulated mesangial cell MCP-1 mRNA expression (137–220%) and MCP-1 protein secretion (235–375%). Lp-X-like liposomes (50–100 μmol/mL) also stimulated mesangial cell MCP-1 mRNA expression (124–162%) and MCP-1 protein secretion (214–317%). Lp-X-induced mesangial cell MCP-1 expression resulted in enhanced monocyte chemotaxis.

Conclusions: Lp-X may participate in the pathogenesis of glomerulosclerosis and subsequent renal failure in familial LCAT deficient patients by stimulating monocyte infiltration via a mechanism involving MCP-1 (Supported by the RGC and the ICSM).
**W:29 OXIDATION AND AHEROGENESIS**

**ThW1:29** Isoprostanes: Indices of oxidant stress in atherosclerosis


Isoprostanes are free radical catalyzed isomers of arachidonic acid. They are formed initially in situ in cell membranes from which they are cleaved, circulated and are excreted in urine. Specific assays based on mass spectrometry have been developed for a range of isoprostanes. Their urinary excretion reflects oxidant stress associated with inflammation, reperfusion after tissue ischemia, and oxidant stress in response to xenobiotics. Isoprostanes may be immunolocalized in vascular smooth muscle cells and monocyte macrophages in human atherosclerotic plaque hand are elevated in atherosclerotic vessels, circulating LDL and atherogenic lipoprotein particles with high density lipoprotein. Similar biochemical abnormalities characterize a variety of mouse models of atherosclerosis. Isoprostanes also act as incidental ligands at membrane and nuclear receptors for prostanooids.

**ThW2:29** Oxidized phospholipids as ligands for macrophage scavenger receptors

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It has generally been assumed that oxidized LDL (OxLDL) is recognized by scavenger receptors by virtue of changes in apoB, either changes in its primary structure (e.g., acetylation or other masking of lysine amino groups) or changes in configuration. However, studies in this laboratory have shown that the ligand moiety of OxLDL (but not that of native LDL), when reconstituted into a microemulsion, binds in a saturable fashion to mouse peritoneal macrophages and competes for the binding of intact OxLDL. Further studies showed that liposomes containing oxidized phospholipids also competed. Unexpectedly, the reconstituted lipid and the isolated lipid-free apoB from OxLDL competed with each other. Moreover, a monoclonal antibody against oxidized phospholipids was shown to bind to both the lipid moiety and the apoprotein moiety. These findings are now shown to result from the presence of oxidized phospholipids covalently bound to apoB. Direct examination of apoB isolated from OxLDL after exhaustive extraction with chloroform/methanol showed that more than 70% of several phosphorus remained attached to the protein whereas apoB from native LDL showed almost no retained phosphorus.

Monoclonal antibodies that recognize oxidized phospholipids and oxidized LDL also react with apoptotic cell membranes (but not normal cell membrane) implying a commonality of ligand between OxLDL and apoptotic cells, probably oxidized phospholipids. These results by no means rule but the presence of additional ligands that bind to macrophage scavenger receptors. Moreover the relative importance of oxidized phospholipids as ligands may vary from one scavenger receptor to another. We have now examined the ligand-binding specificity of CD6 expressed in transfected cells. The results closely parallel those obtained with intact mouse peritoneal macrophages.

**ThW3:29** Role of LOX-1 in atherosclerosis

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One of the critical events in the pathogenesis of the early stage atherosclerotic lesions is the focal accumulation of lipid-laden foam cells derived from macrophages. In various cholesterol-fed animal models of atherosclerosis, it was found that localized attachment of circulating monocytes to arterial endothelial cells appears to precede the formation of foam cells. Recently the molecular mechanism of the formation of atherosclerosis has been elucidated. It has been suggested that monocyte recruitment into early lesions might involve, changing the endothelial adhesiveness for monocytes and lymphocytes. In vivo and in vitro studies have identified molecules, such as ICAM-1, VCAM-1 and ELAM-1, that can support the adhesion of leukocytes, monocytes and lymphocytes. Moreover, oxidized LDL and lysophosphatidyl choline, (which is derived from oxidized LDL), induce the expression of these adhesion molecules. In addition, it has been demonstrated that monocyte-macrophages can ingest chemically modified LDL, such as oxidized LDL, and thereby become foam cells. Recently we identified and characterized the novel receptor for oxidized LDL, named LOX-1. The expression of LOX-1 is found on the surface of endothelial cells smooth muscle cells and macrophages. I want focus my talk on the formation of foam cells, especially monocyte recruitment and significance of oxidized LDL and its receptor LOX-1, in terms of atherosclerosis.

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**ThW4:29** Evidence for dissociation of lipoprotein lipid oxidation and atherosclerosis in different animals

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Oxidation of low-density lipoprotein (LDL) is now commonly thought to play a central role in atherosclerosis. Indeed, oxidized lipoproteins are present in atherosclerotic lesions and in vitro oxidized LDL has many potential pro-atherogenic activities. However, direct evidence that LDL oxidation causes (rather than being a consequence of) atherosclerosis has not been forthcoming. Vitamin E (α-tocopherol, TOH) is the major antioxidant associated with LDL and commonly thought to be anti-atherogenic. Surprisingly however, in human lesions relative normal concentrations of TOH coexist with small amounts of tocopherol-derived oxidation products and substantial amounts of oxidized lipids, formed largely in the presence of the vitamin. Also, overall human and animal intervention studies with vitamin E on atherosclerosis have yielded disappointing results. We discovered that the role of TOH in lipoprotein lipid oxidation is complex. As formulated by the tocopherol-mediated peroxidation (TMP) model, the vitamin requires compounds capable of eliminating the TOH-derived radical for effective protection of lipoprotein lipids. We have identified compounds that inhibit TMP in vitro. Where tested these compounds also inhibit the accumulation of the primary products of lipoprotein lipid oxidation in the vessel wall of atherosclerosis prone rabbits and mice. However, such inhibition is not always associated with a decrease in the extent of atherosclerosis. Also, probed (an antioxidant unable to inhibit TMP) substantially attenuates atherosclerosis in the aorta of apolipoprotein E−/− mice and cholesterol-fed, balloonated rabbits without concomitant inhibition of aortic lipid oxidation. Conversely, disease can be promoted in rabbits by hypoxia without an increase in lipid oxidation. Together, our findings question whether lipoprotein lipid oxidation is a general cause of atherosclerosis.

**ThW5:29** Expression of Macrophages (Mf) Scavenger receptor, CD36, in cultured human aortic smooth muscle cells, in association with the presence of peroxisome proliferator activated receptor-gamma – gain of Mf-like phenotype in vitro and its implication in atherogenesis


CD36 is one of the major receptors for oxidized low density lipoproteins belonging to macrophages (Mf) scavenger receptor (SR) class B, and thought to play an important role in the foam cell formation from monocyte-Mf in the atherosclerotic lesions. Although it has been hypothesized that smooth muscle cells (SMCs) may be the other origin of foam cells in vivo, supporting data available are still very limited. In the current study, we have tested the expression of a variety of SRs including CD36 in eight lots of primary human aortic SMCs (HASMCs) explanted from eight different donors. Functional CD36 was expressed in the cultured HASMCs and the levels of expression were widely ranged between the lots. SR class A was expressed abundantly in CD36-negative (CD36 (−)) lots. Other Mf markers such as CD32 and CD68 were expressed in all lots tested. These data suggest that the cultured HASMCs gained Mf-like phenotype. To know the mechanism for the above phenotypic change, we have tested the expression of a nuclear receptor, peroxisome proliferator activated receptor-g (PPARg) in these cells. This nuclear receptor was abundantly expressed in CD36-positive (CD36 (+)) lots, whereas c-fms was expressed abundantly in CD36 (−)/SR-A (+) lots. The synthetic ligand of PPARg, troglitazone, up-regulated the expression of CD36 only in CD36 (+) lots. These observations demonstrate that cultured HASMCs can gain Mf-like phenotype, and that expression of CD36 may be associated with that of PPARg in these cell types. The present study may support the possibility that HASMCs is one of the origins of foam cells in vivo.
**MONOCYTE CHEMOATTRACTANT PROTEIN-1 PROMOTES MACROPHAGE OXIDATION OF LOW DENSITY LIPOPROTEIN**

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**Objective:** To investigate the atherogenic role of MCP-1/CCR2 interaction beyond monocyte recruitment and to test the hypothesis that MCP-1 promotes oxidation of LDL and generation of oxidative stress by macrophages.

**Methods:** CCR2 expression in normal and atherosclerotic aortas of apolipoprotein E-deficient (apoE−/−) mice and its modulation by native LDL in mouse peritoneal macrophages was evaluated by RT-PCR. Macrophages were incubated with LDL in the presence of increasing concentrations of MCP-1 (0–100 ng/mL). The effect of MCP-1 on the oxidation of LDL was assessed by two independent measures: analysis of thiobarbituric acid reactive substances (TBARS) and gas chromatography-mass spectrometry (GC-MS) determination of isoprostane F2α-VI, if2α-VI, a sensitive marker of lipid peroxidation and oxidative stress. In vivo studies to examine the effect of MCP-1 inhibition on aortic oxidative stress were performed in 6-month old apoE−/− mice divided into 3 groups: one group sacrificed at baseline and the second and third were injected intravenously with a neutralizing anti-MCP-1 monoclonal antibody and a control isotype-matched IgG, respectively. After 2 weekly injections with respective antibodies, the mice were sacrificed at day-14 and the aortas prepared for analysis for if2α-VI levels by GC-MS.

**Results:** CCR2 mRNA was abundantly expressed in established aortic atherosclerotic lesions but absent from arteries lacking grossly-visible atherosclerotic lesions in apoE−/− mice. Both resident and adherent peritoneal macrophages expressed CCR2 and the expression was markedly augmented by native LDL. MCP-1 induced a dose-dependent increase in macrophage oxidation of LDL assessed by analysis of TBARS and if2α-VI generation. Furthermore, intravenous injection of neutralizing anti-MCP-1 monoclonal antibody significantly reduced oxidative stress in the artery wall by 40% compared with baseline and control IgG injection in apoE−/− mice. The aortic if2α-VI levels of baseline and control antibody-injected mice were not significantly different.

**Conclusions:** These results provide first evidence that MCP-1 promotes oxidation of LDL by macrophages and generation of oxidative stress. Thus, MCP-1/CCR2 interactions could play atherogenic role(s) beyond monocyte recruitment and potentially contribute to the inflammatory nature and progression of atherosclerotic lesions.

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**GENETICS OF LIPOPROTEIN METABOLISM**

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No gene has been established that has invariant context independent effects on the risk of cardiovascular disease (CVD). Most geneticists agree that the causation of phenotypic variability is a consequence of non-linear, dynamic interactions between genes, proteins, cellular organelles, cells, tissues and organ systems as well as the interactions between each of these classes of agents. Furthermore, it is widely acknowledged that these interactions are influenced by interactions with exposures to environments external to the individual that are indexed by time and ecological space. So why does medical science continue to seek to identify and characterize independent invariant Cartesian effects of candidate agents using study designs and analytical models that assume no interactions? Disciplinary hubris, lack of mathematical skills, narrowness of intellectual interest, intellectual property considerations and inadequate training in biology have been suggested as reasons for the inconsistency. We review here genetic studies that test the assumptions of biological and statistical independence of predictors of CVD risk. These studies document that the influence of genotypic variation in candidate genes on risk of CVD are not independent of background genotype, age, body size, gender, alcohol consumption, smoking and other measures of context. Such studies clearly establish that the full utilization of genomic information can only be expected if the forgotten role of context is reinstated in the search for the genetic predictors, and an etiological understanding, of CVD. The cost to medicine and public health of ignoring context in the search through the immense resource of genetic data now available for meaningful information will be great. Awareness of the complexity of the problem in the academic and industrial communities is a necessary first step to avoiding this cost. (Supported by NIH grants HL 39107, HL 51021 and HL 54457).

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**SITES OF ACTION OF PROTEIN PINASE C (PKC) AND PHOSPHATIDILINOSITOL 3-KINASE (PI3K) ARE DISTINCTLY OXIDIZED LOW-DENSITY LIPOPROTEIN-INDUCED MACROPHAGE PROLIFERATION**

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**Background and Objective:** Oxidized low density lipoprotein (Ox-LDL) can induce macrophage proliferation in vitro. To explore the mechanisms involved in this process, we reported that activation of protein kinase C (PKC) is involved in its signaling pathway (ATVB. 17:3013, '97) and that expression of granulocyte/macrophage colony-stimulating factor (GM-CSF) and its subsequent release in the culture medium are important (JBC. 273:28305, '98). However, a recent study from other laboratory showed the involvement of phosphatidylinositol 3-kinase (PI3K) in this process (JBC. 273:4915, '98). In the present study, we compared the contribution of PKC and PI3K to Ox-LDL-induced macrophage proliferation.

**Results:** Ox-LDL-induced macrophage proliferation was inhibited by 90% by a PKC inhibitor, calphostin C, and 50% by a PI3K inhibitor, wortmannin. Ox-LDL-induced expression of GM-CSF and its subsequent release were inhibited by calphostin C but not by wortmannin, whereas recombinant GM-CSF-induced macrophage proliferation was inhibited by wortmannin by 50% but not by calphostin C. Ox-LDL activated PI3K at two time points (10 minutes and 4 hours), and the activation at the second but not first point was significantly inhibited by calphostin C and anti-GM-CSF antibody.

**Conclusions:** The present results suggest that PKC plays a role upstream in the signaling pathway to GM-CSF induction, whereas PI3K is involved, at least in part, downstream in the signaling pathway after GM-CSF induction.

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**ABCA7, A NOVEL ABC TRANSPORTER POTENTIALLY INVOLVED IN MACROPHAGE LIPID METABOLISM**

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**Objective:** Identification of macrophage cholesterol-responsive genes

**Methods:** Human lipid-laden macrophages were screened for the expression of ATP binding cassette (ABC) transporters. ABC transporters translocate a variety of substances, ranging from ions to small peptides, across cell membranes.

**Results:** We recently demonstrated that the ABC transporter ABCA1 is a key regulator of HDL metabolism (1–3), which is mutated in Tangier disease, a syndrome associated with premature atherosclerosis. Here we report the cloning of a novel member of the A subfamily of full-size ABC transporters, tentatively termed ABCA7, from human macrophages. The ABCA7 cDNA predicts a polypeptide, consisting of 12 transmembrane domains and two ATP binding cassettes, that exhibits significant homology with ABCA1. The identification of alternative mRNA splice variants suggests the existence of potential ABCA7 isomers. Unlike ABCA1, which is expressed in a multitude of tissues, ABCA7 mRNA expression was detected predominantly in myeloid-lymphoid tissues.

**Conclusions:** Our finding that ABCA7 is regulated by cholesterol import and export in human macrophages suggests that this ABC transporter may be involved in macrophage lipid metabolism.

**References**

W:31 Prevention of CVD

ThW3:30 A Cholesterol-lowering gene maps to chromosome 13Q
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Objective: To map a putative Cholesterol-lowering gene in a large Arab family

Methods: We studied an Arab family with familial hypercholesterolemia (FH) in which certain relatives with a mutated LDL receptor gene have normal or minimally elevated LDL levels. Certain homozygous FH individuals have LDL levels similar to heterozygous FH persons. Furthermore, part of the non-FH family members display lower than normal LDL levels. We performed a genome wide search and used parametric as well as non-parametric linkage analysis.

Results: We identified a locus on the long arm of chromosome 13 defined by markers D13S156 and D13S158 linked to the LDL-cholesterol lowering phenotype by means of an affected sibpair analysis. We extended our study and performed a multipoint quantitative-trait (QTL) linkage analysis and verified this locus as a QTL for LDL levels within this family. To test the relevance of this QTL in an independent normal population we then performed an IBID linkage analysis on healthy young DZ twins from the German population with markers at the 13q locus. We found strong evidence for linkage at this locus with LDL (p < 0.0002), HDL (p < 0.004), total cholesterol (p < 0.0002), and body mass index (p < 0.0001).

Conclusions: These data provide further support for the existence of a new gene influencing lipid concentrations in man.

ThW4:30 A Genome-wide scan for low HDL-Cholesterol in genetically isolated finnish families with premature coronary heart disease
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Background: Low HDL-C is one of the most important risk factors for CHD. At least 50% of the variation in the HDL levels is seen to be genetically determined. Identification of major genes affecting this trait would provide basis for the characterization of the molecular aetiology and metabolic mechanisms of this disease.

Objective: To use a genome-wide scan to identify major loci for low HDL-C using families with multiple affecteds collected from the genetically isolated population of Finland.

Methods: 25 well documented Finnish pedigrees with premature CHD and isolated low HDL-C. Inclusion criteria for the probands were: Age of 30-60 years, angiographically or clinically verified CHD and HDL-C level below the age and sex specific 10th percentile. The genome scan was performed using 388 informative multiallelic markers with average marker to marker interval of 7.5 cM (modified Weber set 9.0). A total of 176 individuals, including 83 affected family members, were genotyped and both parametric and non-parametric linkage and affected sib pair methods were adapted for statistical analyses.

Results and Conclusions: A total of seven chromosomal regions were identified to reveal a pairwise LOD score >1.0, one of them being the region of the ATP-binding cassette transporter 1 gene on chromosome 9. We are currently performing fine mapping of these chromosomal regions with markers providing an average intermarker interval of 2 cM to obtain conclusive evidence for the involvement of some of these loci in the genetic predisposition to low HDL-C.

W:31 PREVENTION OF CVD

ThW1:31 Global mandate in CVD prevention: From molecules to markets
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A major component of the process of globalisation sweeping across the planet is the consolidation of the pharmaceutical industry with widening ‘knowledge gap’ between them rich and poor countries. This is accompanied by a resurgence of small biotechnology companies whose explosive growth parallels that of startups in the telecommunications and information technology sectors.

To ameliorate (and even exploit) this commercial trend of globalisation, research agencies, donors and industry itself are forming global alliances to ensure that, not only are drugs and vaccines developed for the diseases that affect developing countries, but that these agents when they reach market are affordable for these countries.

Particular challenges for these initiatives lie in the realm of public health law, particularly international trade law as pertains to the World Trade Organisation and the TRIPS agreements.

Global alliances for cardiovascular prevention provide leadership and coordination of efforts in for example health advocacy (such as the Tobacco Free Initiative); and research and development (such as the this Cardiovascular Health Initiative in Developing Countries initiative). There is little doubt that the world will become an increasingly unpredictable and exciting place to live in new millennium; with unimaginable possibilities for mankind to transform his body, his ymnd, and his environment.

ThW2:31 Conventional risk factors: Evolving concepts of risk and expanding strategies for prevention
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The importance of the conventional risk factors for cardiovascular disease is widely underestimated. The principal reasons for this are twofold. First, random and systematic errors in the measurement of risk factors such as cholesterol, blood pressure and smoking result in substantial underestimates of the strengths of the associations. And second, the arbitrary division of risk factor levels into normal and abnormal levels (when many associations are actually continuous) fails to take account of the effects of risk factors in the large proportion of most populations. The conventional risk factors are therefore likely to have stronger effects on risk in a much larger proportion of the population than is typically recognised. As a consequence of this, many at-risk individuals may well have been denied potentially lifesaving prevention interventions. Preventive strategies that recognise the true strengths of the associations between risk factors and disease and interventions that are based upon an individual’s absolute disease risk (rather than a single risk factor level) may prove to be more effective and efficient. Identification of the most cost-effective means for disease prevention is particularly important for low-income countries since they suffer most of the burden of cardiovascular disease. In such countries, resources for the prevention of cardiovascular disease are scarce and the selective implementation of the most efficient strategies will be essential. New studies to determine the best mechanisms...
for the implementation of established low-cost interventions, within the constraints imposed by the limited resources available in low-income countries, are required.

**ThW3:31**

**High apolipoprotein (apo) B and low apoA-I improve the prediction of fatal myocardial infarction at all levels of cholesterol and triglycerides – mortality data from the amors (apolipoprotein-related mortality risk) study**

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**Objective:** To evaluate the importance of apoB (reflecting atherogenic particles LDL, VLDL and IDL) and apoA-I (anti-atherogenic particles) in predicting fatal acute myocardial infarction (AMI).

**Methods:** In 98722 males and 76 831 females (<20 to >80 yrs) examined 1985–96 at health controls total cholesterol (TC), total triglycerides (TG), apoB and apoA-I (automated imm. turb. method, fresh serum, acce. to WHO-IFCC) were measured. After a mean follow up of 67 months 864 males and 359 females died in AMI.

**Results:** A high apoB and a low apoA-I were stronger risk factors than TC in both males and females below and over 70 yrs (age standardized, univariate analysis). Similarly TG was a strong risk factor both in males and even stronger in females. When adjusting AMI risk for age, TC and TG there still remained a highly significant and graded increase in risk ratios of 3–9 times for those with highest apoB and lowest apoA-I levels as compared to those with lowest apoB and highest apoA-I values. The ratio apoB/apoA-I summarizes the additional risk over and above that of TC and TG at all lipid levels. Similar findings were obtained for both males and females irrespective of age.

**Conclusions:** By measuring both apoB and apoA-I, which are easily standardized, automated and sensitive to dietary variation, the predictivity of AMI is considerably increased. These measurements may help to select out those at highest/lowest risk at all lipid levels and may therefore be useful in clinical practice when deciding upon lipid lowering therapy.

**ThW4:31**

**Relations of lipoprotein subclass levels and LDL size to reduction in coronary atherosclerosis in the PLAC I trial**

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**Objective:** To evaluate the influence of pravastatin treatment on coronary atherosclerosis in subjects who have predominantly large LDL (pattern A, LDL size > 20.6 nm) or small LDL (pattern B, LDL size <20.5 nm).

**Methods:** Frozen plasma samples were analyzed from a subset of participants in the PLAC I trial at baseline and after 6 months treatment with pravastatin (n = 119) or placebo (n = 104). Lipids, lipoprotein subclasses, and mean LDL and HDL particle size were measured by NMR spectroscopy. The primary angiographic endpoint was change in minimum lumen diameter (MLD).

**Results:** In the placebo group, subjects classified as pattern B at baseline had MLD that decreased more than pattern A (0.09 vs 0.04 mm/m², p = 0.05). As compared to placebo, pravastatin limited atherosclerosis progression in the entire treatment group (0.02 vs 0.06 mm/m², p = 0.003), and this response was greater for pattern B (0.02 vs 0.09 mm/m², p = 0.008) as compared to pattern A subjects (0.01 vs 0.04 mm/m², p = 0.11). For the whole group, progression was correlated positively with on-trial levels of intermediate-size VLDL (p = 0.01), total cholesterol (p = 0.009), LDL cholesterol (p = 0.003), LDL particles (p = 0.0004), small LDL (p = 0.003) and small HDL (p = 0.01). Negative associations were observed for HDL size (p = 0.03).

**Conclusions:** (1) LDL pattern B subjects have more coronary atherosclerosis progression than pattern A subjects; (2) pravastatin reduces progression rates in both pattern A and B subjects, but more in pattern B; (3) LDL and HDL particle size and subclass levels are important predictors of coronary atherosclerosis progression.

**ThW5:31**

**Moderate physical exercise prevents carbohydrate-induced hypertriglyceridaemia and accumulation of postprandial chylomicron remnants**

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**Background:** Low-fat, high-carbohydrate (CHO) diets are associated with elevation of fasting and postprandial plasma triglyceride (TG) concentrations as well as accumulation of atherogenic small dense LDL.

**Objectives:** To investigate (i) whether moderate physical exercise can prevent the high CHO diet-induced increase in postprandial lipaemia and (ii) the underlying mechanisms by studying the effect on postprandial apo B-48 and B-100 concentrations in the triglyceride-rich lipoproteins (TRL).

**Methods:** Eight healthy, normolipidaemic, postmenopausal women aged 60 ± 4 years underwent oral fat-tolerance tests in a random order 1) after 3 days on a diet with 35%, 50% and 15% of energy (E) derived from CHO, fat and protein, resp., 2) after 3 days on a high-CHO diet (70 E%, 15 E% and 15 E%, resp.) and 3) after 3 days on the same high-CHO diet and daily 60 minute sessions of brisk walking. ApoB-48 and B-100 concentrations were measured in the fasting and postprandial TRL fraction by quantitative SDS-PAGE.

**Results:** The increase in plasma TG concentration (fasting +59% and postprandial +36%, both p < 0.01) induced by the high-CHO diet compared with the high fat diet was significantly attenuated by exercise. In the fasting state there was a marked accumulation of TRLs containing apo B-100 (+296%, p < 0.01) after consuming the high-CHO diet without exercise compared with the high fat diet. This effect was normalised by exercise. The high-CHO diet induced a significant accumulation of postprandial TRLs containing apo B-48 compared with the high fat diet (+111%, p < 0.05), which was completely normalised by the exercise.

**Conclusions:** Elevation of postprandial lipaemia due to high-CHO diets may be explained by expansion of the fasting VLDL pool. This in turn could increase the concentration of these particles with chylomicon postprandiality. The CHO-induced increase in chylomicrons remnants was entirely prevented by moderate exercise.

**ThW6:31**

**Comparison of a mediterranean (med) diet to low fat (lf) diet on lipids in patients (pts) with coronary heart disease (chd)**

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**Objective:** To compare LF to Med diet effects on lipids and major lipoproteins in pts with CHD on statin therapy.

**Method:** 68 pts with CHD documented by coronary angiography were randomised to LF (20–25% energy (E) from fat and 8–10% saturated) or Med (35–40% E from fat and >50% of fat being monounsaturated). Lipids were measured prior to lipid lowering drug therapy, at randomisation (×2) and 3 mths (×2).

**Results:** At randomisation and 3 mths 86% pts LF and 80% pts Med were on statins. Similarly, 80% LF and 85% Med pts were taking aspirin.

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<td>LDL-C</td>
<td>3.92 2.64 2.51 2.58 2.46</td>
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**Conclusion:** A Med diet is as efficacious for lipid management as a LF diet in pts with CHD on statin therapy. Current dietary recommendations for CHD could consider a Med diet as a treatment and preventative dietary option. This would extend choice for pts and possibly enhance adherence.
signaling proteins play an important role in the growth and patterning of multicellular embryos, from insects to man. The loss or attenuation of Hh signaling can lead to developmental defects, such as cyclopia, limb deformities, and absence of the axial skeleton. Inappropriate activation of Hh signaling pathways in contrast is associated with neoplastic growth, as in basal cell carcinoma, one of the most common forms of human cancer.

The role of cholesterol in the Hedgehog signaling pathway first became apparent several years ago upon the discovery that in the presence of cholesterol, the C-terminal domain of Hedgehog executes an autoproteolytic cleavage reaction. This reaction results in attachment of cholesterol to the N-terminal fragment, the fragment active in signaling. This covalent cholesterol-peptide product adheres to the signaling cell so as to localize the signal and produce spatially patterned responses in tissues such as the spinal cord, where differentiation into distinct neuronal types is exquisitely sensitive to the level of Hh pathway activation.

In addition to its role in Hedgehog biogenesis, pharmacologic and genetic studies suggest that cholesterol has a distinct role in cellular response to the Hedgehog signal. Treatment of laboratory animals with synthetic compounds that interfere with cholesterol biosynthesis and/or transport causes cyclopia and other birth defects that are reminiscent of severe holoprosencephaly in humans. In addition, cyclopamine and related steroidal alkaloids from plants have long been known to cause cyclopia. Finally, several genetic perturbations of cholesterol transport and synthesis are associated with various birth defects including an abnormally high incidence of holoprosencephaly. In our studies of these perturbations of cholesterol homeostasis, we have demonstrated that both natural and synthetic steroidal compounds produce birth defects by inhibiting Hedgehog protein signaling, acting at the level of cells’ ability to respond to the Hedgehog signal. Although the precise mechanism by which these compounds block cellular response to the Hedgehog signal is not resolved, we are utilizing cultured cell studies to identify the cellular targets of these compounds and to understand how Hedgehog signaling is affected.

Apolipoprotein A-I stimulates transport of intracellular cholesterol to cell surface caveolae

Dmitri Sviridov, Noel Fidge, Christopher Fielding. Baker Medical Research Institute, Melbourne, Australia and Cardiovascular Research Institute, University of California, San Francisco, USA

The effect of lipid-free human plasma apolipoprotein A-I (apo-A-I) on the transport of newly synthesized cholesterol to cell-surface caveolae in human skin fibroblasts was studied. Changes in transport of newly synthesized cholesterol and other birth defects that are reminiscent of severe holoprosencephaly in humans. In addition, cyclopamine and related steroidal alkaloids from plants have long been known to cause cyclopia. Finally, several genetic perturbations of cholesterol transport and synthesis are associated with various birth defects including an abnormally high incidence of holoprosencephaly. In our studies of these perturbations of cholesterol homeostasis, we have demonstrated that both natural and synthetic steroidal compounds produce birth defects by inhibiting Hedgehog protein signaling, acting at the level of cells’ ability to respond to the Hedgehog signal. Although the precise mechanism by which these compounds block cellular response to the Hedgehog signal is not resolved, we are utilizing cultured cell studies to identify the cellular targets of these compounds and to understand how Hedgehog signaling is affected.

Apolipoprotein B (apoB) lipoproteins carry the bulk of triglyceride (TG) and cholesterol esters (CE) in plasma, and have atherogenic potential. ApoB is essential for the assembly and secretion of apoB lipoproteins, and the biosynthesis of these lipoproteins in hepatocytes has been extensively examined during the past 20 years. It is clear from these studies that apoB is a very unusual secretory protein in that it has very hydrophobic sequences that seem to interact with the translocation (T) channel (C) of the endoplasmic reticulum (ER) so that T is very slow or even stop, leaving nascent apoB in a binopic orientation in the TC and exposing domains of apoB to the cytosol. Cytosolic apoB interacts with heat shock protein-70, facilitating ubiquitination and proteosomal degradation of the protein. The proteosomal degradation occurs co- or post-translationally while apoB is still in the TC and associated with Seel1. In the presence of adequate TG or CE, the T of nascent apoB is more efficient and less proteosomal degradation occurs. The effects of core lipids on T depends on the presence of microsomal triglyceride transfer protein (MTP). The resulting partially lipidated apoB lipoprotein can later accumulate additional lipid by MTP independent pathways. This may also occur by fusion of the partially lipidated apoB lipoprotein with a lipid droplet already in the ER.

Insulin, PI 3-Kinase and apolipoprotein B biogenesis

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Insulin action inhibits the secretion of very low density lipoprotein (VLDL) by liver through a mechanism that favors the degradation of freshly synthesized apo B. A direct action of post-receptor signaling molecules on an “apo B-specific” process is implicated because of the selectivity of the insulin effect for apo B.

Our recent findings indicate that the insulin effect on apo B requires the activation of phosphatidylinositol 3-kinase (PI 3-K). A role for PI 3-K in effects on apo B is further strengthened by the following new findings. 1) p85β, the regulatory subunit of PI 3-K, cross-links to apo B in situ using a cell-permeant, cross-linking agent, indicating that apo B is within 12 A˚ of PI 3-K. 2) Incubation of hepatocytes with IL-4, but not EGF, mimics insulin effects supporting that an insulin receptor substrate is a likely intermediate in signal transduction. 3) The S6-kinase inhibitor, rapamycin is unable to block insulin-mediated effects on apo B secretion suggesting effects are not related to transcriptional events. 4) Label incorporation studies support that apo B degradation is accompanied by inhibition of B100 synthesis. The inhibition of B100 translation occurs even when co-translational degradation is inhibited by pre-incubation of hepatocytes with lactacystin, a specific proteasomal inhibitor.

The significance of insulin-mediated suppression of hepatic apo B secretion is that the process most likely reduces hepatic VLDL secretion during the time interval that intestinal triglyceride-rich lipoproteins are being secreted. The inhibition thereby minimizes competition of VLDL and chylomicrons for catalytic pathways modulating post-prandial hypertriglyceridemia.


W:32 Intracellular Lipid Metabolism

ThW:432 Intracellular lipidation of apoB


ApoB acquires lipids in at least two steps during the formation of VLDL. The first step occurs co-translationally and is terminated shortly after the completion of the molecule. The product is a partially lipidated VLDL-precursor. This VLDL precursor acquires the major amount of lipids in the second step, forming VLDL. The assembled VLDL is first observed in a non-Rough ER compartment suggesting that the second step occurs outside Rough ER. VLDL particles isolated from the microsomal lumen contains a network of chaperons (BiP, PDI, GRP 94, Calreticulin and CaBP2), indicating an ongoing protein folding process also when the VLDL-precursor is converted to VLDL. Investigation of the relation between the length of apoB and the efficiency of the second step demonstrated that this efficiency increased dramatically when apoB reached the length of apoB 48, indicating that the last portion of apoB 48 contains sequences of importance for the second step. The second step can be inhibited by Brefeldin A. A similar inhibition was also observed when a GDP restrictive mutant of ARF1 (T31N) was transiently overexpressed in MDV-RH 7777 cells, indicating that ARF1 is involved in the regulation of the second step. A cell free system carrying out the second step was developed and used to follow the signal from ARF1 to the VLDL assembly. Brefeldin A inhibited the VLDL assembly also in the cell free system. Moreover such an inhibition was also observed when a synthetic polypeptide corresponding to the N-terminal 17 aa of ARF was included in the incubation. Together these results strengthen the conclusion that ARF1 regulated the VLDL assembly. Using the cell free system, we could demonstrate that an ARF1 dependent activation of phospholipase D and the subsequent formation of phosphatidic acid were essential for the VLDL assembly.

ThW:332 Biosynthesis of lipoproteins

H.N. Grinstead1, J.-S. Liang1, M. Pan1, D. Mitchell2, M. Zhou1, R. Parthasarathy1, E.A. Fisher4. 1Columbia University, New York; 2Mt. Sinai School of Medicine, New York, NY, US

Apolipoprotein B (apoB) lipoproteins carry the bulk of triglyceride (TG) and cholesterol esters (CE) in plasma, and have atherogenic potential. ApoB is essential for the assembly and secretion of apoB lipoproteins, and the biosynthesis of these lipoproteins in hepatocytes has been extensively examined during the past 20 years. It is clear from these studies that apoB is a very unusual secretory protein in that it has very hydrophobic sequences that seem to interact with the translocation (T) channel (C) of the endoplasmic reticulum (ER) so that T is very slow or even stop, leaving nascent apoB in a binopic orientation in the TC and exposing domains of apoB to the cytosol. Cytosolic apoB interacts with heat shock protein-70, facilitating ubiquitination and proteosomal degradation of the protein. The proteosomal degradation occurs co- or post-translationally while apoB is still in the TC and associated with Seel1. In the presence of adequate TG or CE, the T of nascent apoB is more efficient and less proteosomal degradation occurs. The effects of core lipids on T depends on the presence of microsomal triglyceride transfer protein (MTP). The resulting partially lipidated apoB lipoprotein can later accumulate additional lipid by MTP independent pathways. This may also occur by fusion of the partially lipidated apoB lipoprotein with a lipid droplet already in the ER.
W:33 PROLIFERATION AND DIFFERENTIATION OF SMOOTH MUSCLE CELLS

ThW3:33 Coronary smooth muscle differentiation from proepicardial cells
M. Majesky, T. Landerholm, J. Lu, X.-R. Dong, P.-T. Ku. Baylor College of Medicine, Houston, Texas, USA

Lineage maps in vertebrate embryos indicate that the vascular system is a mosaic structure made up of different types of smooth muscle cells (SMCs) that arise from independent embryonic origins. Guided by these lineage maps, we isolated committed progenitors for coronary SMCs from the proepicardial organ (PEO) of HH17 quail embryos and studied their differentiation in vitro. We found that the default pathway for PEO differentiation was cytokinergic-positive epicardial cells. By contrast, exposure to endothelial-derived factors (PDGF-BB, TGF-β1, desert hedgehog) produced epicardial to mesenchymal transformation (EMT) and stepwise differentiation to coronary SMCs. SMC differentiation was strictly dependent on intact rhoA/GAP160 rho kinase (p160ROCK) signaling and required transcriptionally active serum response factor (SRF). To test the role of rhoA-p160ROCK signaling in coronary wall formation in vivo, we prepared chick/quail chimeric embryos by grafting gene-modified quail PEOs into the pericardial coelom of HH17 host chick embryos. Hearts and coronary vessels were examined 10d later. Chick hearts that received vehicle-injected quail PEOs were easily distinguishable from native hearts. By contrast, chick hearts that received quail PEOs pretreated with inhibitors of rhoA-p160ROCK signaling had few, if any, coronary SMCs and exhibited a hypertrophic myocardial wall. QCPN-positive quail nuclei were largely absent from the subepicardial and myocardial layers of chick hearts that received rhoA-p160ROCK-inhibited quail PEOs, indicating a failure of epicardial differentiation to complete EMT at the surface of the heart. These finding suggest that formation of coronary SMCs from proepicardial cells in vivo requires signaling via rhoA-p160ROCK, and that epithelial-derived cells are required in multiple ways for normal heart development.

ThW4:33 Fibronectin activates the p42/44 map kinase cascade and facilitates cell cycle entry of smooth muscle cells in primary culture
J. Roy, M. Kazi, J. Thyrberg, U. Hedin. Division of Vascular Surgery, Karolinska Hospital, SE-171 76, Stockholm, Sweden

Objectives: Rat arterial injury is accompanied by an accumulation of fibronectin (FN) around smooth muscle cells (SMCs). In order to elucidate the role of FN in SMC activation, we studied the effect of FN on the expression of Cyclin D1, Cyclin A, p27kip1, and Rb protein and on the activation of the p42/44 mitogen-activated protein kinase (MAPK) cascade.

Methods: SMCs were enzymatically isolated from rat aortas, seeded on FN and cultured under serum-free conditions for up to 6 days. Some of the cultures were stimulated with serum for 24 h. After regular time intervals, samples were prepared for immunoblotting and immunocytochemistry. There was a gradual increase in Cyclin D1 and p27kip1 expression during the 6 day period of culture. However, the expression of Cyclin A and hyperphosphorylation of the Rb protein required serum stimulation. We also detected phosphorylated ERK1 and ERK2 throughout the culture period.

Conclusions: FN facilitates the cell cycle entry of quiescent SMCs into the G1 phase of the cell cycle, but further progression into the S-phase is mitogen-dependent. Furthermore, there was a distinct pattern of sustained MAPK phosphorylation that differs significantly from growth factor induced...
MAPK activation. Our results suggest that FN plays a permissive role for the action of growth factors on SMC proliferation after arterial injury.

**ThW5.33** Rho-associated kinase regulates migration and proliferation of vascular smooth muscle cells both in vitro and in vivo


*1Dept. of Medicine and Clinical Science and Dept. of Cardiovascular Surgery, Kyoto University Grad. Sch. of Med., Kyoto, Japan*

**Objective:** Rho-associated kinase (ROCK), a putative effector of small GTPase Rho, has been shown to mediate the calcium sensitization of vascular smooth muscle cells (VSMCs) and play a key role in the pathogenesis of hypertension. However its role in vascular remodelling has remained unknown.

**Methods:** We examined the effect of a newly-developed specific ROCK inhibitor, Y-27632 (0–10 μM), on migration and proliferation of cultured human aortic SMCs in vitro and on neointimal formation of balloon-injured rat carotid arteries in vivo (Wistar rats, 14–16 weeks).

**Results:** [1] Y-27632 dose-dependently suppressed PDGF-BB- and LPA-induced VSMC migration. Both Y-27632 treatment and overexpression of dominant negative ROCK inhibited PDGF-BB, LPA and serum-induced DNA synthesis of VSMCs. In Y-27632-treated VSMCs, mitogen-induced tyrosine phosphorylation of FAK and paxillin was blocked. Serum-induced deprivation of CDK1/cip1 and kip1 was attenuated by Y-27632. [2] Systemic administration of Y-27632 (35–70 mg/kg/day) via osmotic pumps started three days prior to injury and decreased systolic blood pressure by 20 mmHg. Fourteen days post-injury, in Y-27632-administered rats, neointimal formation was dramatically reduced to 22% of the control rats (Intima/media ratio; 0.20 ± 0.08, Y-27632-treated rats vs. 0.90 ± 0.16, vehicle). ROCK activation in injured vessels was detected by the elevated phosphorylation of myosin phosphatase and myosin light chain.

**Conclusion:** Activation of ROCK underlies the pathogenesis of both hypertension and proliferative vascular disorders. Rho/ROCK blockade can be a common therapeutic strategy for hypertension and atherosclerosis.

**ThW6.33** Cell density determines apoptosis and cell cycle arrest in vascular smooth muscle cells in association with p21WAFl/CIP1

J. Ako, M. Yoshizumi, M. Akishita, S. Kim, M. Hashimoto, K. Iijima, N. Sudoh, Y.Q. Liang, T. Watanabe, Y. Ohike, K. Toki, Y. Ouchi. Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

There is a controversy about the fate of vascular smooth muscle cells (VSMC) treated with antioxidants, cell cycle arrest or apoptosis. We hypothesized that cell density affects the effect of antioxidants on VSMC.

**Methods:** Rat aortic smooth muscle cells (RASM) were cultured in DMEM with 10% FBS. RASM were plated at two different concentrations, high cell density (HCD; 50,000/cm²), and low cell density (LCD; 10,000/cm²). Pyrrolidinedithiocarbamate (PDTC) was added to exponentially growing RASM. Apoptotic change was examined using Hoechst 33258 under fluorescent microscopy. DNA synthesis was measured with [3H] thymidine incorporation.

**Results:** Incubation with 0.1 mM PDTC for 24 hr induced apoptosis in RASM in LCD confirmed by fluorescent staining with Hoechst 33258. In contrast, RASM in HCD did not undergo apoptosis. The DNA synthesis decreased in a dose-dependent manner in both groups after 24-hr incubation with PDTC (2.4% of control in LCD, 14.6% in HCD: 0.01 mM PDTC: 0.6% in LCD, 2.8% in HCD: 0.1 mM). In HCD, the down-regulation of DNA synthesis was reversible after the removal of PDTC. However, DNA synthesis did not resume in LCD due to induction of apoptosis. The expression of cyclin D1 and cyclin A did not change significantly in either LCD or HCD. A potent cdk inhibitor, p21WAFl/CIP1, was expressed as early as 8 hours after the treatment of PDTC only in HCD. p21WAFl/CIP1 was not expressed in LCD after PDTC treatment.

**Conclusion:** Cell density may be a key regulator of PDTC-induced apoptosis in VSMC, and the expression of p21WAFl/CIP1 is associated with PDTC-induced cell cycle arrest.

**ThW7.33** The heparan sulfate chains on perlecain affects smooth muscle cell adhesion and spreading when co-coated with fibronectin

K. Lundmark1, P.K. Tran1, U. Hedlin1, A.W. Clowes2, T.N. Wight2. 1Division of Vascular Surgery, Karolinska Hospital, SE-171 76 Stockholm, Sweden; 2University of Washington School of Medicine, Seattle, USA

**Objective:** We work on the notion that composition of the basement membrane may play a key role in regulating smooth muscle cell (SMC) functions. In this study we have focused on how the heparan sulfate proteoglycan perlecain modifies the effect of fibronectin on cell-adhesion and morphology.

**Methods:** Matrices were prepared by co-coating fibronectin (FN) or laminin (LN) with either heparin (Hep), perlecain (PCN), chondroitin sulfate (CS) or hyaluronic (HA). SMC were allowed to adhere to the substrate for 3 h before fixation, staining and quantification in an ELISA reader. SMC were also stained for actin and examined under light and interference reflection microscope.

**Results:** SMC adhesion to FN (10 μg/ml) was reduced in a dose-dependent manner by both FN and Hep. FN (2 μg/ml) and Hep (1 μg/ml) reduced adhesion by 70–90%. The presence of either FN or Hep also inhibited cell-spreading, stress fiber formation and development of focal contacts. Hep-aminase treatment of FN abolished these effects. FN and Hep did not inhibit SMC adhesion to LN (10 μg/ml). Other glycosaminoglycans such as CS and HA had no effects on adhesion and morphology of SMC plated on a FN- or LN-containing matrix.

**Conclusion:** Both the heparan sulfate chains of perlecain and heparin reduce cell-adhesion and spreading when co-coated with fibronectin.

**W:34 LIPOPROTEIN RECEPTORS**

**ThW1.34** Complex genetic functions of the LDL receptor gene family

Joachim Herz. Department of Molecular Genetics, UT Southwestern, Dallas, Texas, USA

The low density lipoprotein (LDL) gene family represents a class of multifunctional cell surface receptors that is involved in the cellular uptake of biologically diverse ligands. Five members of this gene family are currently known, that control distinct physiological processes. For instance, the LDL receptor and the LDL receptor-related protein (LRP) work in concert to mediate the removal of cholesterol and triglyceride-containing lipoproteins by the liver and LRP also participates in the homeostasis of proteases and protease inhibitors. All members of the family are abundantly expressed in the brain where they bind ApoE, an integral component of several lipoproteins. Genetic association studies indicate that ApoE has important roles in neurodegenerative processes but the underlying mechanisms are presently not understood.

We have recently described a molecular pathway by which neuronal ApoE receptors transmit signals across the plasma membrane, leading to the activation of intracellular signaling cascades. Signaling through this pathway may be altered in neurodegenerative disorders like Alzheimer disease. Similar signaling events may take place in other tissues, for instance the vascular wall, where the expression of lipoprotein receptors is highly regulated during atherogenesis.

**ThW2.34** Structure and function of the class B type I scavenger receptor, SR-BI, an HDL and LDL receptor which mediates selective lipid uptake

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The class B type I scavenger receptor, SR-BI, is a physiologically relevant, high affinity cell surface HDL receptor which mediates selective lipid uptake. It also binds LDL with high affinity. The mechanism of selective lipid uptake is fundamentally different from that of classic receptor-mediated uptake via coated pits and vesicles (e.g., the LDL receptor pathway) in that it involves efficient transfer of the lipids, but not the outer shell proteins, from HDL to cells. In mice, SR-BI (mSR-BI) plays a key role in determining the levels of plasma HDL cholesterol and in mediating the selective delivery of HDL-cholesterol to steroidogenic tissues and the liver. The structure, ligand binding properties and physiologic functions of SR-BI will be discussed.
Roles of ApoE receptors and their family

Tokuo Yamamoto, Tohoku University Gene Research Center, Sendai, Japan

ApoE plays a key role in the transportation and metabolism of plasma cholesterol and triacylglycerol. It is recognized by several receptors including the LDL receptor, LDL receptor related proteins, VLDL receptor (VLDLR), and apoE receptor 2 (apoER2). VLDLR and apoER2 consist of five common functional domains that resemble LDLR. Although they are structurally closely related, their expressions are completely different: VLDLR mRNA is abundant in heart and skeletal muscle, and apoER2 mRNA predominates in brain, but they are almost absent in the liver. In chicken, VLDLR is expressed almost exclusively in oocytes and mediates the uptake of yolk precursors, VLDL and vitellogenin. Chicken mutants lacking VLDLR are sterile and exhibit severe hyperlipidemia, demonstrating that VLDLR is crucial in non-mammalian vertebrate oogenesis. In contrast to nonmammalian vertebrates, mice lacking VLDLR exhibit modest decreases in body weight, body mass index and adipose tissue mass, while their plasma cholesterol levels, triacylglycerol levels, and lipoprotein profiles are not altered. Furthermore, knockout mice lacking both VLDLR and LDLR exhibit a modest hypercholesterolemia, whereas apoE knockout mice exhibit a profound hypercholesterolemia. These data suggest the presence of several apoE receptors. To identify and characterize several cDNAs containing the ligand-binding region. Our recent characterization on apoE receptors and their family proteins will be presented.

Intracellular trafficking – Consequence of receptor mediated uptake of lipoproteins

U. Beisiegel, J. Heeren. Medical Clinic, UKE, Hamburg, Germany

Objective: To study the intracellular pathway of chylomicron remnant (CR) constituents after endocytosis by lipoprotein receptors, most probably by the LDL receptor-related protein (LRP).

Methods: Human chylomicrons were isolated from Apo C-II deficient patients and hydrolyzed in vitro by lipoprotein lipase (LpL) to obtain CR. The CR were labeled with iodine and used for biochemical studies (binding, uptake, degradation and recycling) in human hepatoma cells and human fibroblasts with and without LDL receptor (LDLR). In addition native CR and CR labeled with Dil (fluorescent phospholipid analogue) were incubated on the same cells for immunofluorescence analysis.

Results: The biochemical studies showed that apo E and apo C were not degraded, in contrast to LDL-apo B. The labeled apoproteins remained in the cell and apo E was detected within peripheral endosomal compartments. In the presence of HDL, as an extracellular acceptor, the radioactivity was re-secreted into the medium and labeled apo E was associated with HDL. The Dil-label, in contrast, was found in the lysosomal compartment. This intracellular pathway which leads to lipoprotein disassembly occurred in both LDLR positive and negative cells, suggesting that LRP is a candidate receptor for CR recycling.

Conclusions: The uptake of human CR, possibly via LRP, leads to the intracellular disassembly of the particles. The surface constituents, mainly apo E, are recycled when HDL is present as an extracellular acceptor.

TGF-β1 increases the expression of lacteal-like oxidized LDL receptor-1 (LOX-1)

M. Minami, N. Kume, H. Kataoka, M. Morimoto, T. Kita. Department of Geriatric Medicine, Kyoto University, Kyoto, Japan

Objective: LOX-1 is a novel membrane receptor for atherogenic oxidized LDL, which can be expressed by vascular endothelial cells and macrophages. We have examined whether TGF-β1, which inhibits the expression of class A scavenger receptors in macrophages, plays crucial roles in vascular diseases including atherosclerosis, can induce expression of LOX-1.

Methods: I. Cultured bovine aortic endothelial (BAEC) or smooth muscle (BASMC) cells were treated with recombinant human TGF-β1 (0.1–10 ng/ml), then immunoblot analyses and Northern blot analyses were performed. 2. After pretreatment with or without actinomycin D (2.5–5 ng/ml) for 30 minutes, BAEC were stimulated for 48 hours with 1 ng/ml of TGF-β1, then Northern blot analyses were performed. 3. Murine peritoneal macrophages were cultured overnight and treated with TGF-β1 (0.1–10 ng/ml), and then Northern blot analyses were performed.

Results: 1. Immunoblot and Northern blot analyses showed that treatment with TGF-β1 increased the expression of LOX-1 protein and mRNA in both BAEC and BASMC. Treatment with 1 ng/ml of TGF-β1 for 8 hours resulted in a 4.2-fold and 2.8-fold increases in the amounts of LOX-1 protein in BAEC and BASMC, respectively. 2. Pretreatment with actinomycin D completely abolished LOX-1 mRNA induction elicited by TGF-β1, suggesting that TGF-β1 may stimulate transcription of the LOX-1 gene. 3. TGF-β1 also induced LOX-1 expression in murine peritoneal macrophages in a dose- and time-dependent fashion; treatment with 10 ng/ml of TGF-β1 for 4 hours resulted in a 4.7-fold increase in the amount of LOX-1 mRNA.

Conclusions: TGF-β1 can highly induce LOX-1 expression in vascular endothelial cells, smooth muscle cells and macrophages. TGF-β1 appears to be one of the key regulators that can modulate the expression of scavenger receptors.

Very-low-density lipoprotein receptor deficient mice are protected against diet-induced obesity and insulin resistance

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Objective: To investigate whether very-low-density lipoprotein receptor deficient (VLDLR /−/) mice develop a clear phenotype when fed a high-fat diet.

Methods: Homozygous knockout male mice that lack the VLDLR receptor and wild-type littermates were fed a high-fat diet (HF; 45% of the calories are provided by corn oil) for a period of 17 weeks. Their body weight, food intake, plasma insulin, lipid, and ketone levels, glucose tolerance, intestinal fat absorption and the fatty acid composition of liver, heart, muscle and fats were measured.

Results: After 17 weeks on the HF diet, VLDLR /−/ mice showed a slight increase in their body weight (25.3 ± 2.3 g to 29.7 ± 4.8 g), whereas wild-type littermates became obese (from 28.7 ± 1.6 g to 44.1 ± 5.7 g). No differences were observed with respect to food intake. Furthermore after 17 weeks of HF feeding, VLDLR /−/ mice exhibited significantly lower plasma insulin levels as compared to wild-type mice (1.3 ± 0.8 versus 4.6 ± 2.5 ng/ml) and were able to clear an intraperitoneal injected glucose bolus more rapidly than wild-type mice. These results clearly indicate that VLDLR /−/ mice are protected against HF diet-induced insulin resistance. There were no significant differences found between VLDLR /−/ mice and their controls with respect to plasma lipids, fatty acid oxidation (ketone bodies), intestinal fat absorption or fatty acid tissue composition.

Conclusion: In contrast to their wild-type littermates, VLDLR /−/ mice do not become obese, nor insulin resistant after 17 weeks of high-fat feeding. These findings are indicative for a fundamental role of the VLDLR receptor in the entry of fat-derived calories into tissues.

Evidence for the role of megalin in renal resorption of transthyretin

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Megalin is expressed on the luminal surface of various epithelia including the renal proximal tubules. In the kidney it plays an important role in tubular uptake of macromolecules filtered through the glomerulus. By two-dimensional gel electrophoresis followed by mass spectrometry analysis we identified transthyretin (TTR) as an abundant protein in the urine of patients with renal tubule failure. TTR is a plasma protein that functions as a transporter of thyroxine and retinol. It was recently shown that by binding RBP, megalin has an essential role in transsplanchnic transport of retinol. It is possible that in vivo, megalin might also be responsible for the tubular resorption of TTR. To investigate this hypothesis we performed different TTR binding assays by surface plasmon resonance (SPR) analysis and by using immobilized rat yolk sac cells with high expression levels of megalin. Binding of purified TTR, free as well as in complex with thyroxine or retinol, to immobilized megalin was shown by the SPR analysis. Radiolabeled TTR was rapidly taken up by cultured cells and the uptake was partially inhibited by a polyclonal megalin antibody and by the receptor-associated protein (RAP), a chaperone-like protein inhibiting ligand binding to megalin and other LDLR family receptors. The present data indicate that TTR represents a novel megalin ligand of potential importance in the transsplanchnic transport of retinol and thyroxine.


THURSDAY
**W35 MONOGENIC HYPERLIPIDEMIA**

**ThW1:35**

**Familial hypercholesterolemia: Factors affecting phenotypic expression.**

Joep C. De Fruyst, Permeke tu Sauvage Noiting, John J.P. Kastelein.

**Department of Vascular Medicine, Academic Medical Centre, Amsterdam, The Netherlands**

**Aim:** To assess the contribution of genetic variants in several genes to the phenotypic expression of Familial Hypercholesterolemia (FH).

**Method:** Genetic analysis by conventional PCR in a well-defined and well-documented cohort of patients with FH.

**Results:** Specific mutations in the low-density lipoprotein receptor (LDL-R) gene were associated with mildly elevated LDL-cholesterol levels (below 250 mg/dl), while other mutations predominantly led to severely elevated levels. Mutations in the lipoprotein lipase (LPL) gene further deteriorated lipoprotein levels and significantly increased the risk for cardiovascular disease (CVD) in FH patients. The D-variant in the gene for the angiotensine converting enzyme (ACE) was more frequent in FH patients with CAD, while the ID-variant was associated with higher blood pressure. The T1-genotype of the microsomal triglyceride transfer protein (MTP) gene was associated with significantly lower LDL-cholesterol levels. Genetic variation in the genes coding for the cholesterylster transfer protein (CETP) and apolipoprotein E (apoE) was associated with variation in LDL levels, with the Tg1B2- and apoE2-variants resulting in higher LDL-cholesterol levels. In patients with the apoE2-variant the frequency of cardiovascular disease was higher. A functional mutation (C7T7) in the methylene-tetrahydrofolate reductase (MTHFR) gene was not associated with elevated homocysteine levels nor with CVD risk.

**Conclusion:** The phenotype of FH in terms of levels of LDL-cholesterol and CVD risk is determined by LDL-R mutation type and genetic variation in the genes coding for LPL, ACE, MTP, CETP and apoE.

**ThW2:35**

**The underlying gene in Tangier disease**

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**Objective:** One of the major features in homozygous Tangier disease is an almost complete deficiency of normal high density lipoprotein (HDL). Heterozygous relatives show half normal HDL values. As low HDL is a strong predictor of ischaemic heart disease and myocardial infarction, we aimed on identification and analysis of the gene underlying Tangier disease.

**Methods:** For chromosomal localization of Tangier disease a modified linkage analysis procedure was developed, the so-called traffic light scheme. Candidate genes were mapped to contigs of the resulting linkage region and sequenced in Tangier patients. The established contigs were furthermore exploited for analysis of the genomic structure of the final candidate gene.

**Results:** We identified the ABCA1 (also called ABC1) gene on chromosome 9q31 to be the Tangier disease gene. Sequencing revealed numerous mutations in ABCA1 in Tangier patients, some of them obviously destroying the function of the gene. Complete mRNA encoded by 50 exons and the promoter region were characterized.

**Conclusions:** The ABCA1 has an essential influence on HDL in Tangier patients and their heterozygous relatives and the colomniant fashion of inheritance regarding this trait indicates a clear gene dosage effect. Thus, ABCA1 is a very promising target for an intervention that aims at improving so-called reverse cholesterol transport by increasing HDL. Our data provide the basis for developing such a strategy that is aimed on upregulation of ABCA1.

**ThW3:35**

**Phenotypic expression and pathophysiology of genetic cholesteryl ester transfer protein deficiency**

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Genetic deficiency of cholesteryl ester transfer protein (CETP), which was found in 1985 in Japan, is characterized by the marked elevation of HDL cholesterol. We found various different mutations in the CETP gene, responsible for the majority of CETP-deficient patients. Their lipoprotein profiles were characterized by the presence of small polydisperse LDL as well as markedly large and cholesterol-rich HDL. These abnormal lipoproteins had such atherogenic biological properties that the patient had developed atheroma for LDL receptor and that the latter did not have ability to mediate cholesterol efflux. We found a unique area (Omagari, Japan) where a CETP gene mutation accumulates. In this area, prevalence of marked HALP was 10 times higher than in other areas of Japan and there was a U-shaped relationship between HDL-cholesterol levels and ischemic ECG changes. The quantification of carotid and aortic atherosclerosis determined by ultrasound showed the significantly increased atherosclerotic area in the CETP-deficient patients (Osogami, Japan) compared with nonosogami patients. Combined reduction of CETP and hepatic triglyceride lipase worsened the susceptibility for atherosclerosis in the patients. Recently, we have found the expression of CETP in both cultured human macrophages (Mf) and foam cells in the atherosclerotic lesions. Cholesterol efflux from Mf obtained from CETP deficiency was markedly reduced, whereas overexpression of CETP increased HDL-mediated cholesterol efflux from the cells. These findings indicate that CETP facilitates cholesterol efflux from the cells as well as transfer of CE between lipoproteins, suggesting that this molecule plays crucial roles in multiple steps of the reverse cholesterol transport (RCT). In conclusion, the genetic deficiency of CETP is expressed as an atherogenic phenotype resulted from the above impairments of RCT, even though those patients have very high plasma HDL-cholesterol levels.

**ThW4:35**

**Non apoB related familial hypobetalipoproteinemia: Genetic and metabolic studies**

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Virtually all of the well-characterized cases of FHBL are due to defects in the apoB gene on chromosome 2, specifying unnatural truncations or null alleles. In heterozygotes, both the normal apoB100 and the truncated apoB35 circulate in plasma in greatly reduced concentrations. Metabolic studies in heterozygous humans indicate that low rates of production VLDLapoB are responsible. In some cases high fractional rates of catabolism of VLDLapoB also contribute. The limited low production of apoB100 is accompanied by reduced production rates of triglycerides. The latter may account for the reported cases of fatty liver in FHBL. Animal and cell culture studies confirm the human results, and further demonstrate that while the subjects remain most of circulating apoB100, renal proximal tubular cells remove most of the apoB truncations, mediated by the megalin receptor. However, the genetics of the vast majority of cases of FHBL remain unexplained. We report a FHBL kindred in which linkage to apoB, apoE, and MTP have been ruled out. Rather, we have identified a heretofore undetected candidate locus at 3p21.1-2. We used genome scanning, followed by two-point and multipoint linkage calculations, using dichotomous or quantitative traits and a variety of parametric and non-parametric programs (e.g. GENEHUNTER, SOLAR, LOKI). In the affected members of this family, VLDLapoB100 production is reduced and LDLapoB100 (not VLDLapoB) fractional catabolic rates are increased. Thus, the genetic and metabolic bases of FHBL in this family differ from those in the apoB-linked families, demonstrating the heterogeneity of FHBL.

**ThW5:35**

**Investigation of a novel defect in patients with familial hypercholesterolemia (FH): Identification of a protein that binds to the cytoplasmic tail of the low density lipoprotein (LDL) receptor.**

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**Objective:** To identify proteins involved in the internalisation of the LDL-receptor via clathrin-coated pits that might be defective in a novel form of familial hypercholesterolemia in which LDL-receptor internalisation has been shown to be defective (Norman et al, 1999 J Clin Invest 104: 616-628).

**Methods:** The inducible Lex A-based yeast two hybrid system (Clontech) was used to screen a human liver cDNA library fused to a prey protein with a bait comprising the cytoplasmic tail of the human LDL receptor. Positive clones were re-tested, and the inserts amplified by PCR and identified by sequencing and database searching.

**Results:** Out of 35 positives, 16 different clones were probable false positives, 6 were identical to human pigment epithelium derived factor, and 13 were identical to a known cDNA for a hypothetical protein (Nomura et al, 1994 DNA Res 1: 223-229), which is predicted to have domains that suggest a possible role in receptor organisation. Mammalian cells transfected with this cDNA in pcDNA4/HSMAX (Invitrogen) expressed a soluble His-tagged protein of expected size. Mutations in the LDL receptor cytoplasmic tail known to influence internalisation reduced binding of the novel protein in the yeast two hybrid system. Northern analysis and cDNA sequencing show that the mRNA is normal in the patient's cells; this was confirmed by linkage analysis in the family.

**Conclusion:** A novel protein of previously unknown function has been identified that binds to the cytoplasmic tail of the LDL receptor. Preliminary
evidence suggests that it may play some part in internalisation, but that it is not the defective gene in our patients with unusual phenotypic FH.

### W36 MANAGEMENT OF CARDIOVASCULAR RISK IN WOMEN

#### ThW1:36

**Coronary risk factors for atherosclerosis in women (COR A) a case-control study**

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**Design**: Population based case-control study. 200 women aged 30–80 years with incidental CHD and at least 200 population based controls free of CHD.

**Objective**: To define environmental (nutrition, lifestyle, anthropometry, hormones) and genetic risk factors predisposing particular women for CHD.

**Results**: Preliminary results of the first 100 cases indicate that a waist-to-hip ratio of >0.8 is a strong predictor for CHD risk at any age, while BMI is less powerful. This is in line with the findings that diabetes, elevated fasting C-peptide and low HDL are several fold more frequent in CHD patients. Also, hypertension is more frequent in cases, while elevated LDL is less predictive. Cases do smoke more often, but especially at older age of >60 years.

**Conclusions**: These preliminary data point to the metabolic syndrome as the principle risk factor for CHD in women at any age. Interestingly smoking is more characteristic of older women with CHD. The results underline the importance of developing effective interventions and implementing general measures like hormone replacement therapy.

#### ThW2:36

**The effects of hormone replacement therapy on plasma homocysteine and C-Reactive Protein levels**

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**Objective**: To evaluate the effects of hormone replacement therapy (HRT) on plasma C reactive protein (CRP) and homocysteine levels.

**Methods**: Forty-six postmenopausal women (age 48 ± 5, range 40–60) were prospectively evaluated for the effects 6 months of HRT on lipid parameters, CRP and homocysteine levels. HRT regimen was continuous 0.625 mg/day conjugated equine estrogen (CEE) + 2.5 mg/day medroxyprogesterone acetate (MPA) or 0.625 mg/day CEE alone depending on the hysterectomy status.

**Results**: Estrogen alone significantly increased CRP levels, but the combination therapy did not have significant effect on CRP. However, the decrease in homocysteine levels was maintained with both regimens. The HDL increasing effect of estrogen was blunted by the addition of progesterone which also increased the triacylglycerides (Table).

### ThW7:35

**Overproduction of apoE in patients with type III hyperlipoproteinemia and in patients with other forms of hypertriglyceridemia**


**Objective**: Recent studies in transgenic mice have shown that overexpression of human apoE results in hypertriglyceridemia (HTG) and increased secretion of VLDL-TG by the liver. The aim of the present study was to investigate the role of apoE overproduction in the etiology of human HTG.

**Methods**: The plasma kinetics of total, VLDL and HDL apoE were investigated in normalistic (NL) subjects (n = 5), HTG patients (pts., TG > 2.3 mmol/L, n = 5) and in pts. (n = 2) with type III (TypeIII) hyperlipoproteinemia. Each subject, after an overnight fast, underwent a primed (12 h) infusion of stable-isotope labelled [D3]-leucine, and apoE kinetics were determined by multicompartamental analysis of apo. enrichments curves.

**Results**: HTG and TypeIII pts. had reduced rates of VLDL apoE catabolism and no evidence of VLDL apoE overproduction. Elevated total plasma and VLDL apoE concentrations. TypeIII pts. (10- 40-fold, resp.) were associated with reduced apoE catabolism, but also sig. elevated rates of apoE production. Similarly, HTG pts. (with 2- 6-fold higher total and VLDL apoE levels) had sig. elevated apoE production rates – plasma apoE transport rates (TRs, mean ± SEM): (NL) 2.9 ± 0.78, (HTG) 5.0 ± 0.59 (P < 0.05), and (TypeIII) 11.80 mg kg-1 d-1; VLDL apoE TRs: (NL) 1.9 ± 0.18, (HTG) 4.52 ± 0.61 (P < 0.01), and (TypeIII) 11.95 mg kg-1 d-1. In fasted NL subjects, one-half of newly-synthesized apoE was found in VLDL and one-half was in HDL. In TypeIII and HTG pts., more than 80% of newly-synthesized apoE was found in VLDL.

**Conclusion**: These results demonstrate that patients with TypeIII HLP or HTG, having reduced rates of VLDL apoE-100 catabolism, are characterized by overproduction of plasma and VLDL apoE.

of vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1). E-selectin and P-selectin were determined before and after 3 and 6 month of therapy.

Results: Fifty-two women completed the study. According to the increases in estradiol levels we identified 22 women who responded adequately to HRT (R). The control group (C) consisted of 27 women with no change in estradiol. We found a significant decrease in ICAM-1 in R group compared to C group (278 vs 289 before, 239 vs 295 after 3 month and 226 vs 301 ng/ml after 6 month of HRT; p < 0.01 for both). There was also a significant decrease in E-selectin (49.9 vs 49.7 at the beginning, 37.1 vs 50.8 after 3 month and 34.7 vs 51.7 after 6 month for R and C group, respectively, p < 0.01 for both). The changes in VCAM-1 and P-selectin were smaller and non significant.

Conclusions: Combined HRT of estradiol and NETA reduced the levels of some soluble CAM after 3 and 6 month in healthy postmenopausal women. These findings could indicate a less activated state of the endothelium with consequent reduced risk for atherosclerotic disease.
ThH:6  EXPANDING POSSIBILITIES IN RISK MANAGEMENT

How to manage risk of macrovascular disease in patients with type 2 diabetes

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Macrovascular disease is the major cause of morbidity and mortality in type II diabetes. Outcome of coronary events and coronary interventions is poor and primary prevention is a major challenge. Risk factors include not only diabetes-specific factors such as hyperglycaemia but also cigarette smoking, hypertension and dyslipidaemia as in the general population.

Results of the UKPDS trial have demonstrated that for a given reduction in glycaemia microvascular complications benefit to a greater extent than macrovascular complications. It is clear that a greater impact on the prevention of macrovascular disease will result from modification of the major coronary heart disease risk factors.

There is now an increasing evidence base for glycemic control and antihypertensive therapy, β-blockade, aspirin, angiotensin-converting enzyme inhibitors and lipid-lowering agents (particularly statins) for the prevention of macrovascular disease in type II diabetic patients.

ThH:2:6  How to manage the risk of CHD in elderly hypercholesterolemic patients

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Age is a major risk factor for the development of atherosclerosis and its clinical consequences. Indeed, elderly persons above the age of 70 are at high risk for coronary heart disease, stroke and peripheral vascular disease. A large proportion of these patients display hypercholesterolemia, hypertension or diabetes. Large trials conducted in middle-aged patients demonstrated that treatment of hypertension, diabetes and hypercholesterolemia was associated with a significant reduction of cardiovascular events. Extrapolation of the benefit to elderly patients should be cautious since they differ from middle-aged subjects in several respects: risk factors such as serum levels of cholesterol are weaker predictor of CHD, percentage of stroke is higher, side effects of drugs (orthostatic hypotension, hypoglycaemia) are more likely to occur and may have severe consequences. Furthermore, the incidence of risk factors may lead to a wide and expensive use of drugs. Despite these limitations recent data render a non-treatment behavior unacceptable both scientifically and ethically. A metaanalysis of studies with statin strongly suggests that the subgroup of elderly patients benefited the treatment as their younger counterparts. Three placebo-controlled outcome trials on antihypertensive drug treatment of isolated systolic hypertension have established that lowering blood pressure reduce the incidence of fatal and non-fatal cardiovascular events and may also lower cognitive impairment. However, decision to treat should be adapted to each single situation. Those patients 70 to 80 with a reasonable life expectancy and no comorbidity should be treated as their younger counterparts when they have hypertension or hypercholesterolemia.

ThH:3:6  How to manage patients with familial hypercholesterolemia

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Optimal management of patients with familial hypercholesterolemia (FH) includes making the diagnosis, risk stratification and implementation of lifestyle changes, starting and especially follow-up of drug therapy, testing of relatives and noninvasive assessments. Several scoring systems aid in the clinical diagnosis. As DNA diagnosis, considered the gold standard, has become available, cases of isolated hypercholesterolemia not thought to be due to FH have been shown to be caused by LDL receptor defects. Risk stratification includes assessment of age, sex, family history and other genotypes, lipid-related factors and lifestyle. The drugs of choice are statins, often in combination with other lipid-lowering drugs. Reaching recommended target LDL cholesterol levels (<2.6 mmol/l if established CHD, <3.4 mmol/l if 2 or more risk factors, <4.1 mmol/l if 1 or 0 risk factors) may be facilitated by new statins and abeprphen. Barriers and methods related to testing of relatives and issues related to long-term adherence and lifestyle will be discussed. Unique issues related to treatment of women include when to start therapy, contraception, menoopause and counsel in regards to progeny. Treatment of children presents ethical and practical issues that require careful consideration. MED PED (Med Early Diagnoses – Prevent Early Deaths) is an organization founded by R. R. Williams dedicated to diagnosis, education, treatment, research and advocacy. Two reports recently published by the World Health Organization aid in achieving government and research goals.

ThH:4:6  How to manage the future risk of patients with acute coronary syndromes

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The recent large-scale trials of HMG CoA reductase inhibitors have conclusively established their value for secondary prevention in CHD patients. Benefits include improved survival, a decrease in CHD events including myocardial infarction and need for revascularisation, and prevention of stroke. These benefits occur after either myocardial infarction or unstable angina, with cholesterol levels which are elevated or in the "average" range of usual patients, and are independent of other therapies for which benefits are also proven, including aspirin and beta-blockers. Ongoing trials are addressing remaining questions including the role of these agents earlier after acute coronary syndromes and the most appropriate dosing and target cholesterol with treatment.

Among other therapies, recent findings will expand the role of ACE inhibitors. Results of trials with vitamin E and hormone replacement therapy have been disappointing. Other studies will further elucidate the potential value of folic acid and other interventions that might impact on other biomedical and behavioural risk factors.
Thursday June 29, 2000: Poster Abstracts

P:W27 LIPOPROTEIN(a)

**ThP1:W27**

**Correlation between systemic transvascular transport of albumin and low density lipoprotein in patients with coronary atherosclerosis**

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**Objective:** Elevated systemic transvascular transport of albumin (TERab) is associated with coronary atherosclerosis. By use of a non-invasive in vivo isotope method we investigated the relationship between TERab and the systemic transvascular transport of low density lipoprotein (TERLDL) in patients with coronary atherosclerosis.

**Methods:** Nineteen patients (17 men and 2 women; age range 43-65 years), who had angiographically verified coronary atherosclerosis, were studied. Commercially available 125I-albumin and autologous LDL isolated by sequential ultracentrifugation and iodinated with 131I-iodide were used. The tracers were injected intravenously and blood samples were drawn every 10th minute during the subsequent hour. TERab and TERLDL were calculated as the slopes of the two plasma decay curves.

**Results:** TERab was higher than TERLDL, mean (95% confidence interval) 5.4 (4.7-6.1) vs 3.6 (2.7-4.5) %/hour; P < 0.001, in accordance with the smaller diameter of albumin than LDL. There was a tight correlation between TERab and TERLDL, R = 0.88, P < 0.0001, which was independent of age, sex, and blood pressure.

**Conclusion:** Since TERab and TERLDL are tightly correlated, the relationship between elevated TERab and coronary atherosclerosis may reflect a systemic transvascular leakiness of macromolecules including LDL.

**ThP2:W27**

**Estrogen use, lipoprotein (a) and future risk of coronary heart disease (CHD) in a cohort of women in golmsted county**

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We have previously shown that high lipoprotein (a) (Lp(a)) level, semiquantitatively measured as sinking pre-B lipoprotein, is an independent risk factor for future CHD in a cohort of 4,968 women (Circulation 96: 1390, 1997).

**Objective:** To determine the effect of estrogen use on CHD risk in postmenopausal women with high Lp(a) level in the same cohort.

**Methods:** 2,853 postmenopausal women free of cardiovascular disease at baseline were identified and followed for incidence of CHD, defined as myocardial infarction, cardiac sudden death and angina corroborated by objective evidence of ischemia. Risk factors assessed were age, triglycerides, total cholesterol, body mass index, diabetes, hypertension, smoking status, lipoprotein (a) and estrogen use at baseline. Univariate and joint relationships of the risk factors to CHD were assessed using the Cox proportional hazards model.

**Results:** For all postmenopausal women, high Lp(a) level was associated with CHD even after adjusting for other risk factors (P = 0.0116). 619 postmenopausal women had high Lp(a) levels at baseline and 113 (18%) of those were on estrogen treatment (mean duration of use = 3.3 years). During an average follow-up of 14.6 years, there were 124 CHD events. On multivariate analysis, age (RR 1.04, P < 0.0001), Diabetes (RR 2.366, P < 0.0001), Triglycerides (RR 1.73, p = 0.01) were joint risk factors for future CHD, while estrogen use (RR 0.374, p = 0.002) reduced the risk of future CHD.

**Conclusions:** During 9,048 person-years follow-up of 619 postmenopausal women with high Lp(a) levels, postmenopausal estrogen use at baseline reduces significantly the future risk of CHD even after adjusting for other risk factors for CHD.

**ThP3:W27**

**Complexity of association between apolipoprotein (a) gene size and Lp(a) levels in Caucasians & African Americans – does size matter?**

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Apolipoprotein (a) gene size, which varies with the number of kringle 4 repeats (K4), is a major determinant of plasma Lp(a) concentrations; Lp(a) levels are inversely associated with apolipoprotein (a) gene size. The purpose of our study was to determine if, in any individual, Lp(a) associated with the smaller allele is always predominant over Lp(a) associated with a larger allele, and to study interaction between the alleles. We determined apolipoprotein (a) gene size (pulsed-field gel electrophoresis), apolipoprotein (a) protein size (Western blotting), and plasma Lp(a) levels (ELISA) in 358 individuals (219 Caucasians (C) and 139 African Americans (AA)). The median Lp(a) level in C was 26.1 nM and 116.5 nM in AA. Of the 350 subjects with 2 apolipoprotein (a) alleles (8 homozygotes), the smaller allele dominated (contributed >70% to plasma Lp(a) levels) 56% in C and 45% in AA. The larger allele dominated 29% in C and 23% in AA; codominance occurred 15% in C and 33% (p < 0.01) in AA. In AA, codominance increased with Lp(a) levels (p = 0.08), while there was no trend in C. In C, the smaller allele dominated in 44% of the subjects with Lp(a) ≤ 30 nM, increasing to 84% in Lp(a) > 100nM (p < 0.001). This pattern was not seen in AA. Although we detected 2 alleles in 350 subjects, two plasma isoforms were not always detected. In C, only 11% of alleles with ≥ 22 K4 had no detectable apolipoprotein (a) isoforms, whereas 36% of alleles with ≥ 22 K4 lacked a detectable isoform (p < 0.01). In AA, the situation was reversed-22% of alleles with ≥ 22 K4 and 11% of alleles with ≥ 22 K4 lacked a detectable apolipoprotein (a) isoform (p = 0.13). In C, the proportion of individuals with a single apolipoprotein (a) phenotype decreased from 62% to 44% as Lp(a) levels increased (p = 0.07). The difference between allele sizes did not influence the Lp(a) level proportion to each allele in C or in AA. However, we did find, in AA, when the smaller of two alleles had > 27 K4 or the larger allele had > 34 K4, the smaller allele almost always dominated. We did not find a rule for allele dominance in C. Also, there was no significant correlation between the Lp(a) levels proportion to each allele in C or in AA, after controlling for apolipoprotein (a) allele size. Lp(a):apolipoprotein (a) association is complex and controlled by many factors including, but not limited to, apolipoprotein gene size.

**ThP4:W27**

**Interactions between apo(a)/Lp(a) and apo e in late-onset Alzheimer disease**

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**Objective:** To determine whether apo(a), the distinctive glycoprotein of Lp(a) which shares a series of common features with apoE, is also implicated in Alzheimer disease.

**Methods:** Plasma Lp(a) levels, the size of the apo(a) isoforms and the pentanucleotide repeat polymorphism within the 5’ flanking region of the apo(a) gene were examined in 285 patients with Alzheimer disease and 296 non-demented controls genotyped for apo E.

**Results:** Among apoE e4 carriers, lipoprotein(a) was associated with a progressive, age-dependent increased risk of late-onset Alzheimer’s disease (odds ratio for subjects aged >80 years: 6.0 (95% CI 1.2-30.8, p < 0.01)). In contrast, among non-apolE e4 carriers aged >80 years, lipoprotein(a) was associated with a reduced risk of Alzheimer’s disease (odds ratio: 0.4, 95% CI 0.2-0.9, p < 0.05).

**Conclusion:** In this particular convenience sample, lipoprotein(a) constitutes an additional susceptibility factor for late-onset Alzheimer disease among apoE e4 carriers. In contrast, lipoprotein(a) may protect against late-onset Alzheimer disease in non-apolE e4 carriers.
**ThP5.W27**

Association of serum lipoprotein(a) levels, apo(a) size and sequence polymorphisms in the apo(a) gene with coronary heart disease

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**Objective:** We have evaluated the potential association between Lp(a) levels, apo(a) size and apo(a) gene TTTTA polymorphisms in 357 patients with significant coronary stenosis proven by angiography [mean age: 57.7 ± 8.6 yr (35–78)], and in 195 healthy subjects [60 ± 7.7 yr (38–74)].

**Methods:** Lp(a) levels were measured by ELISA, apo(a) isoform size [number of Kringle IV (KIV) repeats] was determined by SDS-agarse gel electrophoresis, and analysis of the TTnTTTAn polymorphism was carried out by PCR and detected on 10% acrylamide gels. For statistical analyses, both groups were divided into low (at least one isoform <22 KIV) and high (>22 KIV) apo(a) isoform sizes, and into low (<10 on both alleles) and high number (>10) of TTnTTTAn repeats.

**Results:** Lp(a) levels were higher (31.1 ± 34.7 mg/dl vs 18.3 ± 27.2 mg/dl, P < 0.0001), apo(a) isoform sizes <22 KIV were more frequent (P = 0.03), and TTnTTTAn repeats <10 were less frequent (P = 0.003) in cases than in controls. Lp(a) levels were correlated with apo(a) isoforms (P < 0.0001) but not with the TTnTTTAn sequences in both groups. In the CHD group, apo(a) isoforms are inversely correlated with the number of TTnTTTAn repeats. Small apo(a) isoforms were associated with a higher incidence of more coronary branch disease (P = 0.01) and with the presence of myocardial infarction in case-history (P = 0.04).

**Conclusion:** High Lp(a) levels, small apo(a) isoform size, and <10 TTnTTTAn repeats are associated with CHD; moreover, in the case of small apo(a) isoforms, the disease is clinically more severe. The number of TTnTTTAn repeats appears to influence the development of CHD via Lp(a) levels.

**ThP6.W27**

Less frequency of low-molecular weight apolipoprotein(a) phenotypes in aged men

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**Objective & Methods:** In order to determine whether the elevated lipoprotein(a) (Lp(a)) levels may affect the difference of human life expectancy, we compared the frequency of genetically determined apolipoprotein(a) (apo(a)) phenotypes and serum Lp(a) levels in 40 Japanese male and 34 female above 80 years old (aged subjects) and 517 the other younger adult subjects (control subjects; 221 male and 296 female subjects).

**Results:** Low-molecular weight apo(a) phenotypes associated with high serum Lp(a) levels (F, B, S1) were significant less frequent in the aged male than in the control and control female subjects (2.5 vs 16.1, 18.9%; p < 0.05, 0.02, respectively). F, B and S1 allele frequencies were also decreased in the aged male compared to the control, control female and aged female subjects (1.3 vs 9.7, 11.4, 10.3%; p < 0.025, 0.01, 0.05, respectively). Significant lower plasma Lp(a) levels in the aged male than in the control, control female and aged female subjects was observed (mean ± SD: 130.2 ± 9.2 vs 17.1 ± 18.0, 18.0 ± 19.8, 18.6 ± 13.4; p < 0.05, 0.05, 0.025, respectively).

**Conclusions:** The decline of the frequency of low-molecular weight phenotypes in the aged male has been suggested the possibility that Japanese male having these phenotypes might be dead before 80 years old, and this may be reconfirmed the role of Lp(a) as a primary genetic risk for atherosclerotic diseases. Lower Lp(a) levels in the aged male may be in part explained by this difference in apo(a) allele frequencies.

**ThP7.W27**

A high lipoprotein(a) level may be a riskfactor for valvular aortic stenosis in the elderly

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**Objective:** Aortic valve calcifications have structural similarities to atherosclerotic plaques. The aim of the present study was to investigate whether high plasma lipoprotein(a) (Lp(a)) levels and other atherosclerotic risk factors are associated with the presence of valvular aortic stenosis.

**Methods:** The study included 101 patients (41 women and 60 men) with valvular aortic stenosis, mean age 71 ± 8 years and 101 age and sex matched control subjects. All patients underwent aortic valve replacement at the University Hospital in Umeå, Northern Sweden between June 1997 and October 1998. The control subjects were examined echocardiographically. Lp(a), apolipoprotein A-1, apolipoprotein B, total cholesterol, t-PA and clinical data were registered.

**Results:** The proportion of individuals with Lp(a) ≥ 480 mg/dl differed significantly between cases (21%) and controls (5%) (p = 0.002). The results from the conditional logistic regression analyses showed an association between the risk of valvular aortic stenosis and Lp(a) ≥ 480 mg/dl (Odds Ratio = 5, p = 0.003). This result remained after adjustment for smoking, systolic blood pressure, body mass index, apolipoprotein A-1, apolipoprotein B, t-PA and total cholesterol.

**Conclusions:** A high Lp(a) level may be a predisposing factor for the development of calcific aortic valve disease.

**ThP8.W27**

The lipoprotein (a) levels in patients with neoplasia

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The Lipoprotein (a) (Lp(a)) increased risk for atherosclerosis and is an independent risk factor for cardiovascular disease.

**Objective:** To investigation the Lp(a) levels in patients with diversity of cancer. (Ca).

**Material and Methods:** We examined the Lp(a) levels in 272 patients (87 cases Ca of uterine system, 48 cases Ca of gastrointestinal system, 45 cases Ca of lung, 43 cases Ca of liver-biliary-pancreas, 34 cases Ca of breast and 15 cases Ca of different systems (larynx, skin, brain), 147 males and 125 females (Group A), with a mean ± SD age of 70 ± 13 years (17–98 years). The Lp(a) levels of Group A compared with those 104 randomly selected controls (Group B). All results were analyzed in the same laboratory after a 12 to 14 hour overnight fast. Differences between groups were tested by Student's t-test. Data was analyzed using the Statistical Package SPSS.

**Results:**

<table>
<thead>
<tr>
<th>Group</th>
<th>Lp(a) (mg/dl)</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (n = 272)</td>
<td>18.2 ± 26.7 (1.5–153)</td>
<td>39.2 ± 36 (28–176)</td>
<td>&lt;0.0007</td>
</tr>
</tbody>
</table>

**Conclusions:** The aim of this study was that the Lp(a) levels of patients with neoplasia in Greek population were decreased statistically significant.

**ThP9.W27**

Gender difference in serum levels of Lp(a) in coronary artery disease patients

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**Objective:** Previous studies of the role of Lp(a) in the development of coronary artery disease (CAD) in men have shown contradictory results, while only limited data are available in women. We compared Lp(a) levels in men and women with and without angiographic evidence of CAD to determine whether there was a gender difference associated with disease.

**Methods:** From a cohort of 1075 consecutive patients referred for angiography at Vancouver teaching hospitals, Lp(a), measured using a solid phase two-site immunoradiometric assay (Mercordia Apo(a) RIA), was available for 181 women and 339 men. One hundred and thirty-six women (mean age 64) and 296 men (mean age 61) had angiographic evidence of CAD while 45 women (mean age 62) and 43 men (mean age 57) had no evidence of CAD.

**Results:** There was no significant difference in Lp(a) levels between men (median 138 U/L) and women (median 141 U/L) without CAD. However, median Lp(a) levels were significantly higher in women with CAD compared to CAD positive men: 447 U/L vs. 274 U/L (p < 0.001, Mann-Whitney U test). In addition, women with CAD had significantly higher levels of HDL-cholesterol, LDL-cholesterol, apolipoprotein B and total cholesterol than men with CAD. There was no significant difference in triglycerides between men and women with positive angiographic findings.

**Conclusions:** While there was no difference in Lp(a) with respect to gender in disease-free individuals, women with CAD had almost two-fold higher levels of Lp(a) than men with CAD. This contradicts previous studies which have shown no gender differences in Lp(a) values.
ThP10:W27  
SR-67029/SB-248424 Reduces synthesis of apolipoprotein(a) and mRNA for apoa1 in baboon primary hepatocytes  
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Objectives: Aminophosphonates such as SR-67029/SB-248424 (SR) significantly reduce plasma Lp(a) concentrations in orally treated cynomolgus monkeys. It was therefore decided to investigate the mechanism of action of SR on the synthesis of apoa1 protein and on the mRNA for apoa1 in baboon primary hepatocytes.

Methods: Primary cultures of baboon hepatocytes that synthesise apoa1 were selected as the test system since partially processed immature and secreted mature apoa1 can be detected. Initial studies showed that SR reduced the synthesis of mature and immature apoa1 over a concentration range of 20–80 μM. Pulse-chase experiments performed with [35S]methionine suggested that this effect occurs at a very early stage. Hepatocytes were therefore incubated with SR at 80 μM for time periods of 24, 48 and 72 hours. Lp(a) in the medium was determined by ELISA and at the same time points in parallel incubations mRNA was prepared for TaqMan quantitative PCR determination using monkey probe/primer sets. The mRNA levels were normalised to beta-actin.

Results: Lp(a) concentrations in the medium were reduced by 30%, 60% and 85% at 24, 48, and 72 respectively. The level of mRNA for apoa1 at 48 hours was reduced by 54% and at 72 hours by 47%. No effect was found for apoA-1.

Conclusions: Taken together with the observed reductions in apoa1 mRNA in a cynomolgus monkey study, these results suggest that aminophosphonates such as SR cause their reduction in Lp(a) in vivo by an effect on the steady state concentration of apoa1 mRNA.

ThP11:W27  
Impact of family history on cardiovascular risk in hypercholesterolemic patients  
Philippe Giral, Eric Bruckert, Christophe Rioux, Hafida Chikri, M. John Chapman, Alain Mallet, Gérard Turpin. IFR Cœur Muscle Vasculaire, Groupe hospitalier Pitié – Salpêtrière – Université Pierre et Marie Curie, Paris, France

Background: A family history of cardiovascular disease is a risk factor for subsequent development of a clinical event. We assessed the impact of family history on cardiovascular disease in a large cohort of hypercholesterolemic patients.

Methods and Results: This case-control study involved two groups of 821 subjects. The first group was constituted of patients referred for secondary prevention and was age (± 1 year) and sex-matched with a primary prevention patient group and in our Prevention Unit during the same quarter. A family history of cardiovascular disease obtained from each patient included construction of the family tree over two generations and the determination of the occurrence of coronary artery disease, stroke, peripheral arterial disease, and the age of these events. The frequency of a positive CVD family history was higher in patients than in controls (59% versus 51%; p < 0.001). Multiple logistic analysis showed that the OR for a positive versus negative family history of cardiovascular disease adjusted to the other cardiovascular risk factors remained significantly associated (OR: 1.4; 95% CI = [1.2–1.6]) with the occurrence of disease. Correspondence analysis revealed a strong graphical relationship between the distribution of cardiovascular diseases in the patient and in his/her father. There was a close relationship between the age at which the cardiovascular event occurred in the patients and that in siblings (r = 0.47; p < 10^-4).

Conclusion: Cardiovascular disease in the father predisposes to similar disease in offspring; furthermore such vascular disease tends to occur in the same decade among siblings.

ThP12:W27  
Generation of apolipoprotein b transgenic mice with mutations in a putative apolipoprotein(a) binding region  
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The assembly of lipoprotein(a) [Lp(a)] is a two-step process involving an initial non-covalent interaction between apolipoprotein B and apoa1, followed by a disulfide bond between apoB Cys4326 and apoa1 Cys4057. The apoB region involved in the initial binding to apoa1 has yet to be characterised. Lysine residues have been implicated, as lysine analogs inhibit Lp(a) assembly and deletions of lysine-binding domains in apoa1 disrupt Lp(a) formation. Studies using synthetic apoB peptides have identified a putative apoa1 binding region (apoB amino acids 4372–4392) which contains multiple lysine residues. The aim of this project was to identify the apoB amino acids that are important for the initial binding step to apoa1. In particular, we tested the role of lysine residues in the first step of Lp(a) formation.

All four lysine residues in the putative apoa1 binding region (Lys4372, 4379, 4385 and 4392) were mutated to serine residues in a yeast integrating vector. Homologous recombination was used to target the mutations into a yeast artificial chromosome (YAC) spanning the entire human apoB gene. Successful mutagenesis was confirmed by pulse field gel and PCR analysis. Purified YAC DNA was microinjected to generate transgenic mice expressing the mutated human apoB gene. Western blots identified two founder mice which expressed the full-length mutant apoB.

The mutant apoB was tested for its ability to form Lp(a). Time course assays of Lp(a) formation revealed that the apoB mutant did form Lp(a), but at a slower rate than wild-type apoB. This is most likely due to an increased affinity for apoa1 during the non-covalent binding step, confirming the importance of the lysine residues in this putative apoa1 binding region. However, the mutations did not seriously disrupt the interaction, suggesting the overall structure of the region may play a bigger role than individual residues.

ThP13:W27  
Polygenic hypercholesterolemia is associated with elevated plasma Lp(a) and apolipoprotein (a) fragments levels in plasma and urine  
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Objective: Hypercholesterolemia (HC) is generally associated with mildly elevated Lp(a) levels. Apoa1 proteolytic fragments, found in plasma, urine and in human atherosclerotic plaques, may be implicated in Lp(a) catabolism. Therefore, the impact of HC on apoa1 fragmentation was evaluated.

Methods: We measured plasma Lp(a), plasma and urinary apoa1 fragments levels by ELISA in 83 patients with polygenic type IIa hypercholesterolemia (LDL-C ≥ 4.13 mmol/l, TG < 2.24 mmol/l) and in 75 normolipidemic subjects.

Results: Lp(a) levels were significantly elevated in patients as compared to controls (0.37 ± 0.41 and 0.26 ± 0.32 mg/ml respectively; median: 0.15 and 0.08 mg/ml; p = 0.039). Although the apoa1 isoforms distribution did not differ, patients had significantly higher plasma and urinary apoa1 fragments levels than controls (5.3 ± 5.4 vs 2.2 ± 2.7 μg/ml in plasma; p < 0.0001; 74.8 ± 7.9 vs 41.3 ± 6.7 ng/ml per mg of creatinine in urine; p < 0.0001). The ratio of plasma apoa1 fragments/Lp(a) levels was also significantly higher in patients than in controls (2.1 ± 1.5 vs 1.8 ± 1.26%, p < 0.0001). In both populations, apoa1 fragments in plasma and urine correlated strongly and positively with plasma Lp(a) (p < 0.0001).

Conclusions: We demonstrate for the first time that apoa1 fragments in plasma and urine are higher in type IIa patients than in controls. Higher apoa1 fragments levels in plasma cannot be attributed to a decreased urinary excretion, since urinary apoa1 levels are also elevated. We suggest that high LDL levels may modulate Lp(a) metabolism through an increased apoa1 fragmentation.

ThP14:W27  
Sudden hearing loss is associated with low levels of lipoprotein (a)  
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Objective: To study whether the plasma levels of Lipoprotein (a) [Lp(a)] are altered in patients with sudden hearing loss (SHL) and possibly contribute to increased blood viscosity and insufficient perfusion of the inner ear, which are assumed to play a role in the etiology of SHL.

Methods: In a case-control study 68 patients with sudden hearing loss were compared to 68 controls with regard to Lp(a) plasma levels as well as other parameters in lipoprotein metabolism which have been discussed to play a role in the pathogenesis of SHL. Blood was drawn from patients in the acute symptomatic phase and total plasma levels of Lp(a), triglycerides (TG), cholesterol, LDL and HDL were determined.

Results: SHL in the acute phase proved to be associated with decreased levels of Lp(a); mean (and median) values in mg/dl were 11.7 (5.0) for patients and 21.0 (8.0) for controls (p = 0.008 for mean values). Changes in total TG and cholesterol were statistically significant: average values of total cholesterol (p = 0.039) were mildly elevated in the patient group (216 vs 200 mg/dl), mainly due to elevated LDL (229 vs 221 mg/dl) with no change in HDL values (56 mg/dl). Total TG were also increased with 150 vs 117 mg/dl (p = 0.024).

Conclusions: Plasma levels of Lp(a) are decreased in the acute phase of sudden hearing loss. Lp(a) plasma levels seem not to contribute to increased blood viscosity as a potential etiologic factor for SHL. The potential (patho) physiological role of the low levels of Lp(a) in SHL will have to be determined in further studies.

Conclusion: Lp(a) apheresis is the innovative and unique treatment of severe CHD patients with elevated Lp(a).

ThP17: W27 Antifibrinolytic effect of isolated human Lp(a) and apo(a) in an in vitro clot lysis assay

Conflicting results concerning the exact role of Lp(a) or apo(a) in thrombosis and fibrinolysis have been published, probably due to differences in the methodology used and to the lack of stability of Lp(a). The objective of the study was to evaluate the antifibrinolytic activity of Lp(a) and apo(a) isolated from human plasma on the lysis of a clot generated from rabbit euglobulin which does not contain apo(a).

Lp(a) was rapidly isolated by ultracentrifugation at a density <1.12. Apo(a) fractions were obtained from Lp(a) by a mild DTT reduction. Apo(a) concentrations in the Lp(a) and apo(a) fractions were determined by ELISA. Purity of fractions was verified by immunoblot using anti-apo(a) or anti-apoB antibodies.

Clots of rabbit euglobulin formed in vitro using 0.150 ml of rabbit euglobulin to which 1.5 I.U. bovine thrombin was added. Clots lysis kinetics of control and samples supplemented with Lp(a) and apo(a) was optically monitored (OD at 340 nm) every 15 minutes during 20 hours at 37°C. Addition of increasing amounts of Lp(a) (0.1 to 0.4 μg/clot) inhibited clot lysis in a dose-dependent way. After mild reduction of Lp(a), this inhibition was markedly enhanced (ca. 3-fold). Furthermore, apo(a) from different individuals produced different lysis inhibition profiles.

In conclusion, an in vitro assay has been developed that may be useful for a better understanding of the role of Lp(a) in the fibrinolysis-thrombosis area and for individual characterization of antifibrinolytic activity of Lp(a)/apo(a) in patients.

ThP18: W27 Time course and dose dependence of activity of the aminophosphonate SR-74829/SB253149 on plasma Lp(a) in cynomolgus monkeys

Substituted phenyl aminophosphonates such as SR-74829/SB253149 decrease apo(a) secretion by cultured hepatocytes and plasma Lp(a) in Cynomolgus monkeys. We studied in vivo the time and dose dependence of the activity of SR-74829/SB253149 after oral administration to groups of 4-5 Cynomolgus monkeys.

In a first study animals were treated for 4 weeks with SR-74829/SB253149 at doses of 12.5, 25 and 50 mg/kg with a 2-week washout period. Lp(a), apo B and cholesterol (cholesterol) were measured weekly. The activity of SR-74829/SB253149 was dose dependent and measurable after one week of treatment. At the dose of 50 mg/kg, plasma Lp(a) and apo B levels were decreased by ~25% and ~31% respectively while total chole was decreased by ~19%. Similar conclusions were made from a study where doses of 25 and 75 mg/kg were given for 2 weeks. In a third study plasma Lp(a) and chole were determined daily in animals dosed with 50 mg/kg/day for 10 days followed by 2 days off drug. Lp(a) and chole decreased significantly after the second dose and reached a plateau at day 8 (~36% for Lp(a) and ~32% for chole). These changes were independent of the predrug plasma levels of Lp(a) and returned to pretest values within 2 days without treatment. No decreases in Lp(a), chole and apo B were measured in the control animals.

In conclusion, when given orally to Cynomolgus monkeys, SR-74829/SB253149 decreases plasma Lp(a) rapidly and reversibly in a time and dose dependent fashion.

ThP19: W27 Lipoprotein(a) and potency of the grafts after coronary bypass surgery
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Lipoprotein(a) [Lp(a)] seems to be an undefined risk factor for the development of coronary atherosclerosis. In one study we have checked the association at Lp Ln level, total cholesterol (TC), triglycerides (TG), apolipoprotein AI (apo AI) and apolipoprotein B (apo B) concentrations with patency of the
grafts during the first year after coronary artery by pass surgery (CABG). In 132 male patients (mean age 52 ± 9 years) the grafts were visualized by non-invasive three-dimensional electron beam contrast tomography (n = 31) in 5.4 ± 3.0 months after operation. We have examined the patency of 244 vein and 92 autografts. Comparison of patients (I group, and in the II group - with ○1 occluded vein graft) indicated that there was difference only in Lp(a) level, which was 24 ± 29 mg/dl in the I group, and in the II group - 42 ± 41 mg/dl (p = 0.62). All patients were divided in 2 groups according to Lp(a) level: group A (Lp(a) > 30 mg/dl), group B (Lp(a) < 30 mg/dl). In group A there were significantly more patients with occluded vein grafts than in group B: 82% and 54% respectively (p = 0.008). There was no correlation between Lp(a) level and number of occluded vein grafts. The potency of autografts was not related to the parameters of lipid profile and traditional risk factors of atherosclerosis like age, smoking, hypertension, family history of CHD.

**Conclusion:** Lp(a) concentration can be used as a biochemical marker for the estimation of the risk of vein grafts occlusion during the first year after coronary artery bypass surgery. Patients with elevated Lp(a) should received more intensive lipid lowering therapy.

**ThP20-W27** Differential gene expression in monocytes from proband with elevated Lp(a)

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**Objective:** Numerous case-control studies demonstrate a strong correlation between increased plasma Lp(a) levels and premature cardiovascular diseases. The apo(a) gene is highly homologous to plasminogen and it was suggested that Lp(a) shares binding sites with plasminogen or may compete with plasminogen on cell surfaces and in the subendothelial matrix. This may contribute to the pathogenic risk associated with elevated levels of Lp(a).

**Methods:** To investigate differential gene expression in monocytes mediated by elevated Lp(a) two different strategies were followed: 1. We compared mRNA expression of monocytes from patients with Lp(a) hyperlipidaemia as the dominating risk factor and control monocytes. 2. We compared the gene expression induced by purified Lp(a) in control monocytes Differential gene expression was analyzed using an Incyte/Syntenti microarray with 8,000 cDNA's bound to a glass surface.

**Results:** The expression of several candidate genes identified by microchip hybridization was already investigated by Northern Blot or Western Blot experiments in 6 probands with elevated Lp(a) and 14 healthy controls. Whereas most of the differentially expressed genes were simply due to variations of the mRNA levels in different donors, a high expression of PAI-2 mRNA and protein was detected in probands with elevated Lp(a). Purified Lp(a) also induced PAI-2 mRNA in control monocytes.

**Conclusions:** Our results indicate that Lp(a) induces the expression of PAI-2 in monocytes in-vitro and in-vivo. The interference of Lp(a) with the plasminogen system may explain in part the atherogenic properties of this unusual lipoprotein.

**ThP21-W27** Relationship between Lp(a) values in acute phase and follow-up of coronary artery disease

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**Purpose:** To determine changes in Lp(a) levels between the acute phase and the follow-up of coronary artery disease.

**Methods:** We choose 96 patients (mean age 42 ± 5 years) from a cohort of 230 patients (pts) admitted to our hospital for an acute coronary event and who were not under hypolipidemic treatment nor at the admission neither during the follow-up. The diagnosis of acute myocardial infarction in 60 pts (62.5%) and unstable angina in 36 pts (37.5%). After 12 hours of fastening, determinations of Lp(a) were carried out by the same laboratory in the seventh day of hospitalization and after a follow-up of 31 ± 12 months. Data are presented in mg/dl as the median and P5-P95. To analyze differences the Wilcoxon test was used. We also determined correlation coefficient (r)

<table>
<thead>
<tr>
<th>mg/dl</th>
<th>Acute phase</th>
<th>Follow-up</th>
<th>Variation (%)</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a)</td>
<td>25 (2-91)</td>
<td>22 (2-89)</td>
<td>-24%</td>
<td>0.893</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Results** are presented in the table.

**Conclusions:** Lp(a) concentrations show significative higher levels in the acute than in the chronic phase with a high correlation coefficient between both phases.

**ThP22-W27** Small triglyceride-rich lipoprotein cholesterol exceeds low-density lipoprotein cholesterol in patients with hypertriglyceridemia

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**Objective:** We evaluated TG-rich lipoprotein (TGRLP) as a source of CE in plagues, including the relation between hypertriglyceridemia (HTG) and cholesterol content in TGRLP.

**Methods:** In 103 patients whose fasting serum TG level exceeded 300 mg/dL, cholesterol level in each lipoprotein fraction was measured by high-performance liquid chromatography. Two cases were followed for 6 months.

**Results:** Four peaks were found in each sample: large TGRLP, small TGRLP, cholesterol (S-TGRLP), low-density lipoprotein cholesterol (LDLc), and high-density lipoprotein cholesterol (HDLc). Total cholesterol (TC) increased with increases in TG. The increment of S-TGRLP was nearly equal to the increment of TC, while LDLc and HDLc did not increase despite increased TG. The S-TGRLP/LDLc ratio increased with increasing TG, and S-TGRLP exceeded LDLc when TG was >500 mg/dL.

**Conclusions:** S-TGRLP exceeds LDLc in patients with HTG, suggesting that S-TGRLP may be a source of CE in arteriosclerotic plaques, and increased S-TGRLP may be a basis for HTG atherogenicity. Changes in S-TGRLP are reversible.

**ThP23-W27** Prevalence of high molecular weight apoprotein(a) isoforms in subjects with very low plasma levels of Lp(a)

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**Objective:** to analyze the expression of apoprotein(a) [apo(a)] isoforms and the single-isoform contribution to Lipoprotein(a) [Lp(a)] plasma values in 631 unrelated probands of age > 18 yrs.

**Methods:** apo(a) isoform identification and single isoform contribution to total plasma Lp(a) levels of these subjects were assessed by 1.5% agarose gel followed by western blot and densitometric scan of chemiluminescent bands.

**Results:** In our sample 34.7% subjects were heterozygous for apo(a) isoforms. Females showed significantly higher Lp(a) levels than males (median 5.6 vs. 3.8 mg/dL). Lp(a) levels after log transform and age adjustment significantly correlated to apo(a) isoform size (Pearson R = -0.41). In the heterozygous subjects also the rate of expression of the dominant (smaller) apo(a) isoform was correlated (R = 0.49). A multivariate analysis in the same group showed that the correlation of Lp(a) with apo(a) isoform (Multiple R = -0.289) increased its predictive power when the rate of expression was included (multiple R = -0.319). The 40.4% of the heterozygous subjects under-expressed the smaller dominant apo(a) isoform. In the group with non detectable Lp(a) values (<0.5 mg/dL) the analysis of subjects with detectable apo(a) isoform (167 of 232), showed that 16.7% had low (<21 krigle IV repeats) molecular weight dominant apo(a) isoforms, 34.1% had intermediate (21–26 k IV repeats) isoforms and only 49.1% had high (> 26 k IV repeats) isoforms.

**Conclusion:** the analysis of single-isoform contribution to Lp(a) levels highlights that the presence of a low molecular weight apo(a) isoform has less predictive power on Lp(a) levels than in studies with simple apo(a) isoform genotyping.

**ThP24-W27** Apoprotein(a) phenotype in patients with coronary artery disease

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**Objective:** Relationship between serum Lp(a) levels and apoprotein(a) phenotype was investigated in patients with coronary artery disease (CAD).

**Methods:** Consecutive 261 patients who were evaluated by coronary angiography were enrolled. CAD was defined as 75% or greater stenosis.
Results: CAD group consisted of 119 men and 31 women (age 62 ± 10 yr [SD]). Non-CAD group was 62 men and 50 women (age 65 ± 11 yr [SD]). Serum Lp(a) levels were 28 ± 23 mg/dL in CAD group, and 19 ± 19 in non-CAD group (P = 0.01). FOA (mg/dL) was not varied between CAD and non-CAD groups. However, Lp(a) levels in the most common phenotype S3 and S4 were 37 ± 28 and 19 ± 14 in CAD group, which were higher than those in non-CAD group (21 ± 19 and 11 ± 12; P < 0.01). In phenotype S3, CAD group was older than non-CAD group (68 ± 19 vs. 58 ± 12; P < 0.01); prevalence of diabetes mellitus was higher in CAD group than non-CAD group (38% vs. 10%; P < 0.01). In phenotype S4, there were more men found in CAD group than in non-CAD group (75% vs. 55%; P < 0.05).

Conclusions: Higher Lp(a) levels in CAD could not be explained by apo(a) phenotype, but acquired factors such as diabetes.

**ThP25:W27** Smoking is strongly associated with white blood cell count elevation and subclinical atherosclerosis

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Background: The aim of this study was to investigate the potential relationship between WBC count, fibrinogen and smoking in hypercholesterolemic men with subclinical carotid and femoral atherosclerosis.

Methods: A cross-sectional study in a University Hospital. Carotid and femoral arterial atherosclerosis was evaluated by high resolution B-mode ultrasound and the results were classified into two categories: absence of any atherosclerotic plaque, or presence of one or more arterial plaques at each arterial site.

Results: WBC count, plasma fibrinogen levels and the daily consumption of cigarettes were increased in hypercholesterolemic patients with carotid or femoral plaques as compared to patients in which plaques were not detectable. In comparison to patients who had never smoked, both fibrinogen levels and WBC count were positively correlated with tobacco consumption, but when adjusted for WBC count, fibrinogen was no longer related to cigarette smoking (r=0.06; ns). Multivariable analysis revealed that WBC count remained significantly associated with atherosclerosis when fibrinogen levels (OR for carotid artery = 1.64, CI: 1.00 to 2.70; OR for femoral artery = 1.79, CI: 1.18 to 2.72) were taken into account. However, WBC count and fibrinogen were not predictive of the presence of carotid or femoral plaque when smoking was introduced into the logistic model.

Conclusions: White blood cell count and fibrinogen are additionally related to subclinical atherosclerosis in hypercholesterolemic hypercholesterolemic men. However, this association is dependent of smoking.

**ThP26:W27** Triglycerides and Lipoprotein(a) predict the presence and severity of coronary artery disease in postmenopausal women


Objective: Evaluate traditional risk factors as predictors of coronary artery disease (CAD) among postmenopausal women.

Methods: Subjects with non-obstructive coronary angiography were considered to the control group (n = 62) and patients with CAD involving 1 or more than 2 coronary vessels were divided into one-vessel (n = 61) and multi-vessel (n = 59) groups, respectively.

Results are presented in the table.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>One-Vessel</th>
<th>Multi-Vessel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>64 ± 11</td>
<td>64 ± 8</td>
<td>63 ± 8</td>
</tr>
<tr>
<td>Menopause (yr)</td>
<td>47 ± 6</td>
<td>48 ± 5</td>
<td>48 ± 4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29 ± 2</td>
<td>28 ± 2</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>245 ± 48</td>
<td>240 ± 41</td>
<td>254 ± 45</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>128 ± 82</td>
<td>172 ± 90</td>
<td>192 ± 106</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>26 ± 15*</td>
<td>34 ± 18*</td>
<td>38 ± 20*</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>168 ± 40</td>
<td>160 ± 40</td>
<td>170 ± 40</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>51 ± 12</td>
<td>50 ± 10</td>
<td>48 ± 14</td>
</tr>
<tr>
<td>apo A1</td>
<td>1.95 ± 0.8</td>
<td>2.0 ± 0.9</td>
<td>1.9 ± 0.8</td>
</tr>
<tr>
<td>apo B</td>
<td>1.5 ± 0.4</td>
<td>1.4 ± 0.3</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Lipoprotein(a)</td>
<td>12 ± 11*</td>
<td>13 ± 28*</td>
<td>47 ± 29*</td>
</tr>
</tbody>
</table>

*p < 0.01 for differences between all groups.

**ThP28:W27** Serum glycated lipoprotein(A) levels in type 2 diabetic patients

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Lipoprotein(a)[Lp(a)] is an independent risk factor for atherosclerosis. Glycation of lipoproteins is implicated in the development of the macro- and microvascular complications of diabetes mellitus.

Objective: To evaluate the alteration of serum glycated Lp(a) levels with glycemic control in type 2 diabetic patients.

Methods: The glycation of Lp(a) was measured by enzyme-linked immunosorbent assay after m-aminophenyl boronate affinity gel chromatography.

Results: The percentage of glycated Lp(a) in diabetic patients (4.2 ± 1.5%) was significantly higher (P < 0.001) than that in control subjects (2.2 ± 0.4%) and significantly decreased with glycemic control (2.4 ± 0.5%; P < 0.001). Improved glycemic control had no effect on serum Lp(a) levels. The level of glycated Lp(a) was also positively correlated with that of HbA1c (r = 0.72, P < 0.001).

Conclusions: Glycated Lp(a) may be a potentially interesting factor contributing to the diabetic complications.

**ThP29:W27** Effect of etofibrate therapy in patients with high levels of lipoprotein(a)

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Objective: To evaluate the effect of etofibrate on lipoprotein(a) in plasma of patients with levels higher than 30 mg/dL.
**Methods:** We selected 20 consecutive dyslipidemic patients (10 males and 10 females) of a total of 112 that received medical attention at the dyslipidemias outpatient department. Levels of Lp(a) in plasma higher than 30 mg/dL were found after 3 months on a hypolipidemic diet. We excluded patients on acute cardiac event 3 months before the assay, diabetes, hypothyroidism, nephropathy, hepatopathy, body mass index equal or over 30 and those taking hypolipidemic drugs. We started on etofibrato 500 mg daily after dinner. We determined Lp(a), cholesterol, triglycerides, HDLc and LDLc before etofibrato (baseline), in weeks 4th, 12th and 24th.

**Results:** The median plasma Lp(a) shows a sustained decrease from 83.57 to 61.73 (week 4th), 59.43 (week 12th) and 63.32 mg/dL (week 24th), all with p < 0.01. In 4 patients with the lowest baseline mean levels of Lp(a) of the whole group, Lp(a) increased from 55.90 to 63.63 mg/dL on week 24th (not significant).

**Conclusions:** Etofibrato reduces Lp(a) levels more than 32% after 4 weeks of its administration and maintains this trend during the 24 weeks of observation in patients with the highest levels, while Lp(a) increases slightly among those with the lowest levels. These results of etofibrato on Lp(a), have not been reported before.

**P:W28 HYPERTENSION, KIDNEY DISEASE, AND ATHEROSCLEROSIS**

**ThP1:W28 Association of angiotensin converting enzyme gene polymorphism with essential hypertension among Korean men and women: age- and gender-related differences**
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**Background:** A number of investigations in various populations have shown discordant evidence on the association of the insertion-deletion (I/D) polymorphism of angiotensin converting enzyme (ACE) gene with hypertension. The aim of this study is to examine whether the ACE I/D polymorphism is associated with essential hypertension in relation to blood pressure-related variables in a large sample of healthy subjects.

**Methods:** A cross-sectional study was conducted on a total of 13,914 healthy subjects (8,261 men, 5,653 women) aged between 20 to 79 years. ACE genotypes were determined using polymerase chain reaction. Subjects with vascular event, abnormal renal, thyroid dysfunction, or electrolyte levels were excluded.

**Results:** Odds ratio of ACE DD genotype for hypertension differed between gender, men having 15% increased risk (OR = 1.15, 95% confidence interval (CI), 1.07–1.23) (p = 0.03) but women no increased risk. However, increased risk in men was not significant (OR = 1.13, 95% CI, 1.05–1.21) (p = 0.08) after adjustment for age, obesity, cholesterol, alcohol consumption, smoking, and diabetes mellitus. With regard to age groups, only a subgroup of men bearing DD genotype, aged 50–54 years, showed 61% (p = 0.001) increased risk for hypertension compared to those bearing ID and II.

**Conclusions:** Among Koreans studied, ACE DD genotype was at increased risk of hypertension only in men aged 50–54 years, born in World War II period. Finding suggests that ACE polymorphism may be one of the factors involved in the predisposition of hypertension, in conjunction with environmental event occurring early in life, with sex-related difference.

**ThP2:W28 Serum and LDL concentrations of vitamin E and lipid peroxide in renal disease**
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**Objective:** We determined the levels of vitamin E and lipid peroxide concentrations in serum and low density lipoprotein (LDL) to establish the roles of lipids in the development of renal injury.

**Methods:** We examined 67 patients with renal disease and 12 normal controls. Fasting blood samples were drawn from the patients and controls, and serum LDL was isolated by density gradient ultracentrifugation. Lipid levels, vitamin E and lipid peroxide concentrations were measured in LDL and serum. Vitamin E concentration was measured by high performance liquid chromatography (HPLC) and lipid peroxide was measured as concentration of malondialdehyde (MDA), an end product of lipid peroxidation.

**Results:** There was a strong correlation between serum and LDL concentrations of vitamin E, and serum and LDL concentrations of cholesterol and triglycerides, respectively. There was also a significant correlation between serum and LDL concentrations of vitamin E, and protein excretion quantity in urine. Serum and LDL concentrations of vitamin E did not differ significantly among the chronic nephritis group, chronic renal failure group and control group. Similarly, there were no differences in serum and LDL concentrations of vitamin E among the chronic nephritis group, diabetic nephropathy group and control group. Similar to vitamin E-concentration, MDA concentration did not differ significantly among the renal disease groups and the control group.

**Conclusions:** Serum and LDL concentrations of vitamin E correlate strongly with serum and LDL lipid levels, respectively. However, these concentrations did not differ significantly among the renal disease groups and the control group.

**ThP3:W28 Effect of diabetes on intermediate density lipoprotein level in end-stage renal disease**
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**Objective:** Intermediate density lipoprotein (IDL) is an independent risk factor for atherosclerosis, and it is elevated in end-stage renal disease (ESRD). In this study, we examined the effect of diabetes, another atherogenic risk factor, on IDL levels in subjects with ESRD.

**Methods:** The subjects were 287 ESRD patients with (DM/ESRD, N = 80) and without diabetes (NonDM/ESRD, N = 207) and 330 healthy control subjects. Age and gender were comparable among the three groups. Lipoprotein fractions were measured by ultracentrifugation.

**Results:** As compared with the healthy controls, IDL-cholesterol was elevated in the hemodialysis groups, and DM/ESRD patients had significantly higher IDL levels than NonDM/ESRD patients. IDL-cholesterol correlated positively with plasma triglycerides (TG). When the subjects were stratified according to plasma TG, IDL-cholesterol was still higher in the two ESRD groups than the healthy control level, whereas no difference was found between the DM and NonDM groups. Multiple regression analysis indicated that factors affecting IDL-cholesterol included gender, ESRD and plasma TG. Also, plasma TG was affected by BMI, ESRD, and diabetes or blood glucose level.

**Conclusions:** IDL-cholesterol level was raised in ESRD patients with diabetes. The results suggested that this effect of diabetes were mediated partly by hypertriglyceridemia associated with diabetes or hyperglycemia.

**ThP4:W28 (Familial) dyslipidemic hypertension syndrome: delineation in FCHI**
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**Background:** Familial Combined Hyperlipidemia (FCHL), the most frequent genetic lipid abnormality in man has been proposed as the leading cause of dyslipidemia in Familial Dyslipidemic Hypertension (FDH). It was the objective of this study to quantify and analyse the simultaneous occurrence of hypertension and hyperlipidemia in FCHL families.

**Methods:** We assessed blood pressure and hyperlipidemia in 27 families with FCHL, consisting of 235 relatives and 140 spouses, aged 30–60 y. Hypertension was defined as a blood pressure above 140/90, or the use of antihypertensive medication. Multiple backward linear regression analysis was used to derive a biological formula describing blood pressure in FCHL families.

**Results:** One-third of 27 FCHL families were diagnosed with FDH. Sixty-four of 235 (27.2%) relatives had dyslipidemic hypertension, compared to twenty of 140 (14.3%) spouses (p = 0.005); odds ratio = 2.25 (95% CI 1.29–3.91). Multiple linear regression analysis showed that age, FCHL status and waist circumference significantly contributed to systolic blood pressure in female FCHL relatives.

**Conclusion:** In conclusion, apart from age, affected FCHL status was identified as a significant contributing factor to the expression of systolic and diastolic blood pressure in FCHL women. A common mechanism for dyslipidemia and elevated blood pressure in FCHL plausibly relates to increased waist circumference. Our novel findings have implications for the delineation of (Familial) Dyslipidaemic Hypertension as a hypertensive syndrome within FCHL.
**Effects of the angiotensin-II-antagonist valsartan on blood lipids and glucose in hypertensive patients**

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With respect to cardiovascular risk factors often associated with hypertension such as blood lipids and glucose, antihypertensive agents should not impair lipid and glucose metabolism. These potential effects have to be proven for each antihypertensive therapy in controlled studies.

Thus, we investigated the influence of the antihypertensive agent valsartan which belongs to the new substance class of angiotensin-II-antagonists. After a 3-week placebo run-in period, 123 patients with moderate hypertension have been randomised in a double-blind, 12-weeks study to receive 80 mg valsartan or placebo. Main inclusion criteria were: DBP > 90 mmHg and ≤105 mmHg, LDL-cholesterol (LDL-C) < 160 mg/dl, total cholesterol < 250 mg/dl, triglycerides (TG) < 250 mg/dl, HbA1c < 7.5%, BMI < 35 kg/m², no lipid-lowering and antidiabetic drugs at least 12 weeks prior randomisation, no type 1 diabetes. Primary parameter was LDL-C, secondary parameters were total cholesterol, triglycerides, HDL-C, apolipoprotein B, VLDL-C, VLDL-TG, fasting plasma glucose, and HbA1c.

After 12 weeks therapy, 112 patients completed the study, 52 pts. in the placebo group and 60 pts. in the active treatment group. In the valsartan-group LDL-C statistically significant decreased by 6.9 mg/dl compared with an increase by 4.9 mg/dl in the placebo group (p < 0.05 vs placebo). Moreover, total cholesterol significantly decreased by 7.6 mg/dl compared with valsartan compared with placebo with an increase by 5.8 mg/dl. The other parameters did not show any changes versus placebo. Valsartan was safe and well tolerated as well as effective in lowering DBP by 9.0 mmHg and SBP by 14.1 mmHg.

The present study documents that antihypertensive therapy with valsartan is neutral on blood glucose and has beneficial effects on lipids.

**Rapid detection of angiotensinogen M/T235 polymorphism by fluorescence probe melting curves**

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**Objectives:** Angiotensinogen (AGT) gene M/T235 polymorphism has been shown to be associated with hypertension, diabetic nephropathy and coronary heart disease. The aim of this study was to present a rapid, single step method for AGT genotyping that uses LightCycler technology.

**Methods:** Six DNA samples, two from each of the AGT genotype groups MM, MT and TT, were selected for the mutation detection analysis by LightCycler. To confirm the LightCycler results, PCR products were also analyzed by solid-phase minisequencing and sequencing. The M/T235 genotyping by LightCycler combined both rapid-cycle PCR with real-time monitoring of the amplification process and generation of allele-specific fluorescent probe melting profiles.

**Results:** The genotypes of six specimens obtained from LightCycler instrument matched with the genotypes obtained from sequencing and minisequencing.

**Conclusions:** Previous methods for AGT M/T235 genotyping have been laborious and time consuming. We have here described a method based on the LightCycler technology that can be used to genotype 32 samples in less than 45 min by a single sample processing step.

**Impact of blood pressure on early carotid structural changes in a female population study**

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**Objective:** To evaluate the impact of blood pressure on early carotid structural changes in a female population based study (Progetto Atena).

**Methods:** 310 healthy women (30–69 years, 74.5% postmenopausal) underwent high resolution B-mode ultrasound to assess intima-media thickness (IMT) of common carotid artery (CCA) and carotid bifurcation (BIF) and CCA external and internal lumen diameter. A qualitative evaluation of plaque occurrence (IMT > 1.2 mm), and quantitative computer assisted measurements of the single maximum IMT (TMAX) and of the mean of maximum IMT (MMAX) were performed in each subject. Standard procedures for blood pressure measurements were used.

**Results:** Women with CCA plaques had significantly higher age-adjusted values of systolic (< 0.05) and pulse pressure (p < 0.01), compared with participants without plaques at this site, whereas no differences were found between the two groups in the BIF. The prevalence of CCA atherosclerotic lesions increased gradually from the lowest to the highest quartile of systolic pressure. Systolic and pulse pressure in the upper quartile were associated with a greater extent (MMAX) rather than severity (TMAX) of CCA wall abnormalities. Systolic blood pressure was a predictor of CCA plaques, independently of age, body mass index and HDL cholesterol (OR 1.34, 95% CI 1.01–1.78). Furthermore, systolic pressure significantly contributed to increased CCA diameters, beyond its effect on IMT (adaptive arterial remodeling).

**Conclusions:** The present findings suggest a greater and independent association of blood pressure, mainly the systolic, with early structural changes in the CCA of clinically healthy women.

**Homocysteine metabolism in patients with end-stage renal disease under maintenance dialysis**

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**Objective:** To understand mechanism(s) leading to hyperhomocysteinemia in ESRD hemodialysed patients we measured fasting plasma concentrations of homocysteine (cysteine (cys) and its related amino acids, methionine (met), cyst(e)ine (cyst), and cystathionine (cystathionine). All patients received daily oral folate (160 µg-320) and vitamin B6 (10–20 mg) supplement.

**Methods:** Blood samples from 47 women (mean age 57 yrs.) and 84 men (mean age 55 yrs.) were taken before dialysis. Plasma concentrations of the amino acids were determined by an isotope dilution method using gas-chromatography/mass spectrometry. Vitamin B6 was measured by HPLC, vitamin B12 and folate acid by an IMX-assay.

**Results:** Plasma concentrations of cys were increased in 95% of the patients (28.6 ± 11.9 µM). Normal levels of vitamin B12 and B6 were found. There was significantly positive correlation between cys and cys (r = 0.434, p < 0.05), cys and cys correlated negatively with folate acid and vitamin B12 (r = -0.281 and r = -0.229; p < 0.01, respectively). No correlation could be found between the ratio cys/cys to vitamin B6.

**Conclusions:** Despite vitamin substitution dialysed patients have extremely high homocysteine plasma concentrations than healthy subjects. Most of their homocysteine seems to be converted to cyst(e)ine while vitamin B6 substitution seems to preserve this pathway. Supported by a grant of BONFOR (107/48 A).

**A study to evaluate cardiovascular risk markers in patients with end stage renal failure – evidence of increased activation of factor XII**

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**Background:** Patients with end stage renal failure (ESRF) are known to suffer significantly elevated mortality rates from cardiovascular disease. The aim of this study is to evaluate the role of established and new risk factors with the incidence of cardiovascular events.

**Study Design:** 65 chronic hospital haemodialysis patients both with and without a history of cardiac events were recruited together with 70 apparently healthy controls. Blood samples were collected at the commencement of the study and during the first year. Blood sample collection will continue for two more years. The relationship of established (lipid profile) and newer (activated factor XII, homocysteine, fibrinogen and von Willebrand factor) risk markers to cardiovascular events will be further investigated.

**Results:** During the first year of the study a total of 10 (15%) patients have died, 6 (6.2%) patients have suffered a cerebrovascular accident, 1 of which died and 10 (15%) a myocardial event, of which 6 died. Preliminary data indicates a significant (p < 0.0001) elevation of FXIIa in the patient group (8.27 ng/ml ± 2.82 ng/ml) compared to a control group (1.53 ng/ml ± 0.73 ng/ml). During the first 12 months of the study insufficient events have occurred to reach a conclusion on the ability of FXIIa to predict outcome in this population of ESRF patients.
Conclusion: As expected there is a high incidence of mortality and morbidity in this population of ESRF patients. In addition we have established that FXIIa is significantly elevated. Factor XII is activated to FXIIa on the surface of triglyceride-rich lipoproteins and it has been reported that FXIIa is associated with cardiovascular risk. Further study will examine the relationship between the activated factor XII and lipid metabolism. It is anticipated that the relationship of events with FXIIa and other markers will be established during course of the study.

**ThP10:W28** The diagnostic value of homocysteine; methylenalonic acid and cystathionine in renal patients

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**Background:** Hyperhomocysteinemia is an independent risk factor for atherosclerotic vascular diseases. The metabolites methylenalonic acid (MMA) and cystathionine (CYS) provide more information about the specificity or shortage of B-vitamin deficiencies. MMA is a sensitive indicator for vitamin B12 deficiency and CYS is a metabolite of the vitamin B6 dependent catabolic pathway.

**Objective:** The aim was to evaluate the prevalence of increased serum metabolite concentrations in renal patients, and whether the measurement of metabolite concentrations is more useful diagnosing B-vitamin deficiency than mere homocysteine (HCY).

**Design:** We investigated 66 transplanted patients and 38 patients undergoing hemodialysis. The metabolites were investigated by gas chromatography/mass spectrometry. Vitamin B6 was determined by HPLC. Folate and vitamin B12 were measured with an Abbott IMX Analyser.

**Results:** The metabolites were increased in 97% of transplanted patients, and in 100% of patients with hemodialysis. Vitamin deficiency was found in 32% res. 55%, CYS, the most sensitive indicator, was increased in 94% res. 95%. HCY was elevated (>15 μmol/L) in 73% res. 92% at which the median level in transplanted patients was (21 μmol/L) moderately but in dialysis patients stronger increased (36 μmol/L).

**Discussion:** In renal patients the remethylation of HCY to methionine is strongly disturbed. Normally about 75% of HCY are remethylated to methionine by the kidney. In dialysis patients we found a marked elevation of CYS but normal vitamin B6. This indicates a strong formation of HCY which is not remethylated to methionine and therefore catabolised to CYS. Additionally, renal patients have low folate which also contributes to an inhibited remethylation.

**Conclusion:** The determination of metabolites allows a more specific diagnosis of the disturbance in homocysteine/methionine metabolism.

**ThP11:W28** The effect of the 677-MTHFR mutation on homocysteine level in elderly subjects and renal patients

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**Background:** Hyperhomocysteinemia is an independent risk factor for atherosclerotic vascular diseases. The very common 677C → T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene is deemed to be responsible for moderate hyperhomocysteinemia.

**Objective:** The aim was to evaluate the effect of the 677-MTHFR mutation on the homocysteine (HCY) level in elderly subjects and in renal patients who are at high risk for CAD.

**Design:** We investigated 92 senior subjects (aged 65–75 y.), 90 high aged subjects (85–102 y.), 63 transplanted patients and 38 patients undergoing hemodialysis. HCY was investigated by HPLC. Folate was measured with an Abbott IMX Analyser (Illinois, USA). For genetic analysis, DNA was isolated from whole blood with the QIAamp Blood kit. The 677-миссenses mutation in the MTHFR gene was analysed after PCR and enzyme digestion.

**Results:** Compared to wild type or heterozygous elderly subjects, elderly individuals homozygous for the 677-MTHFR mutation showed a significantly higher HCY level when the folate level was below <6 μg/L. The prevalence of the 677-MTHFR mutation was in elderly subjects not different compared to younger persons. In our renal patients with a markedly increased HCY level, the 677-MTHFR gene mutation affected a further increase of the HCY concentration in serum when the folate level was low. In renal patients with sufficient folate supply, the 677-MTHFR mutation had no effect on HCY concentration.

**Conclusion:** The 677-MTHFR mutation is the only confirmed common risk factor for moderate hyperhomocysteinemia. The homozygous mutant genotype is associated with hyperhomocysteinemia when the folate level is low <6 μg/L.

**ThP12:W28** Endothelial nitric oxide synthase gene GLU298 --> ASP variant – association with arterial hypertension, but not with coronary artery disease

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Endothelial-derived nitric oxide (NO) is synthesised by L-arginine by endothelial nitric oxide synthase (eNOS). Within the eNOS gene a common polymorphism has been described, which converts Glu to Asp at position 298 (894G --> T). The Asp variant is associated with lower NO levels and was found to be associated with myocardial infarction in Japanese but not in whites.

LURIC is a prospective case-control study on environmental and genetic risk factors for CAD and arterial hypertension. A standardised patient history was obtained from 1918 people (1339 male, 579 female). Blood pressure was measured by oscillometric device after a rest of at least 15 minutes (average between three measurements). In all participants CAD was diagnosed or excluded by coronary angiography. eNOS Glu298 --> Asp was determined by amplification of a part of exon 7 with subsequently DpnII digest, which cuts the T but not the G allele.

The frequencies of the eNOS GG, GT and TT genotypes were 43.4%, 45.2% and 11.4% in men and 45.9%, 43.8% and 10.3% in women with CAD, and were not significantly different from those without CAD (44.2%, 47.5% and 8.3%, P = 0.40 in men; 45.5%, 40.1% and 14.4%, P = 0.33 in women). Furthermore, the mutation was not associated with MI (P = 0.56 in men, P = 0.69 in women). However, we found in males a significant association with stroke (P = 0.024), hypertension (P = 0.035), and the use of ACE inhibitors (P = 0.006). In summary, we could confirm data from other studies that Glu298 --> Asp is a genetic variant associated with hypertension but in contrary we don’t see the proposed role as a major risk factor for CAD in the Caucasian population.

**ThP13:W28** Immunological factors in early human atherosclerosis

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**Objectives:** To identify inflammatory mediators in atherosclerosis.

**Methods:** Antibodies and other factors tested were determined by ELISA. Seventy-five men with borderline hypertension (BHT) and 75 normotensive controls (diastolic pressure, 85–94 and <80 mm Hg respectively) were from a family screening program. Atherosclerosis was determined by carotid ultrasound.

**Results:** Anti-Hsp65 antibody and Hsp60 levels were raised in BHT and associated with early atherosclerosis (p < 0.01). OxLDL induced HSP and proinflammatory cytokines mediated by platelet activating factor (PAF)-like lipids, one of which is lysophosphatidylcholine (LPC). Anti-oxLDL and anti-LPC antibodies were decreased in BHT (p < 0.01). Anti-EC antibodies were raised in BHT (p < 0.01). Antibodies to β2-glycoprotein-1 (β2GPI), a co-factor for anti-phospholipid antibodies and shown here to be a major antigen on endothelial cells, were associated with BHT, atherosclerosis and metabolic factors, as were anti-PAF antibodies. IL-6 was raised in BHT and associated with early atherosclerosis (p < 0.01). C-reactive protein was only significantly associated with smoking.

**Conclusions:** Immunological changes are present in early stages of cardiovascular disease. Activation of EC by anti-Hsp65, Hsp60 and antibodies to EC-related antigens such as β2GPI and PAF may induce inflammation and influence atherogenesis, whereas anti-ox-LDL and anti-LPC antibody may protect against early disease.
**P:W29 Oxidation and Atherogenesis**

**P:W29 Oxidation and Atherogenesis**

**ThP14:W28** Young men with high-normal blood pressure have lower insulin sensitivity and smaller LDL size than those with optimal blood pressure.

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**Objective:** Those with borderline hypertension are known to be insulin resistant than those with normal blood pressure (BP), which has been classified into 3 categories: optimal, normal, and high-normal BP according to the guideline by WHO/IHSH in 1999. To study whether those with high-normal BP are insulin resistant than those with optimal BP.

**Method:** Insulin resistance (IR) index was calculated using the homeostasis model assessment and LDL size was measured by polycrylamide gel electrophoresis in comparison to Krauss and Burke in 188 young healthy men (29% were 18 years old and BMI averaged 21.6 ± 3.6(SD) kg/m²) with systolic and diastolic BP < 140/90 mmHg. Results were adjusted for BMI using analysis of covariance.

**Results:** Optimal, normal and high-normal BP were found in 90 (48%), 52 (28%), and 46 men (24%), respectively. As BP rose, IR index increased (1.66 ± 0.68, 1.89 ± 0.66, 1.94 ± 0.69, p = 0.03). In addition, LDL particle size decreased (271 ± 5, 269 ± 5, 269 ± 5 Å, p = 0.008). There was no difference in serum triglycerides and HDL cholesterol.

**Conclusions:** Young men with high-normal BP are insulin resistant and have smaller LDL size than those with optimal BP.

**ThP15:W28** An association of three polymorphisms in the gene coding for endothelin-1 (ET-1) in essential hypertension.

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**Objective:** As a potent vasoconstrictor, endothelin-1 is a candidate gene for cardiovascular diseases. The aim of the study is to prove an association of three polymorphisms in the ET-1 gene (6bp21–23) with essential hypertension.

**Methods:** Normotensive and hypertensive subjects of the age of 45–55 years were included in 3 ET-1 gene polymorphisms (CA/C'T, 11/12 repeat polymorphism, I/D polymorphism in the promoter and TaqI polymorphism in the intron 4) by PCR methods. Data were evaluated by Kruskal-Wallis Anova, Mann-Whitney U test and Fisher-exact test.

**Results:** The case-control difference was found in hypertensive women (not in men) when I/D promoter alleles with TaqI polymorphism were evaluated together (p = 0.008). The allelic length of CA/C'T repeat polymorphism was significantly higher in hypertensive subjects (p = 0.03). Linkage concurrence between CA/C'T and TaqI polymorphisms in hypertensive subjects (p = 0.0018) as well as in normotensive group (p = 0.02) was found. In hypertensives, I/D promoter polymorphism was correlated with homozygosity in CA/C'T repeat polymorphism (p = 0.02). The conclusion: Complex interactions among alleles inside the gene coding for ET-1 gene could participate in pathogenesis of essential hypertension.

**ThP16:W28** Evaluation of endothelial function and plaque activity in essential hypertension.

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**Objective:** To evaluate endothelial function (EF) and plaque activity in essential hypertension (EH).

**Methods:** The study included 35 moderate to severe EH patients (mean age 49.5 ± 6.9 years, 19 men and 16 women) without clinical manifestations of CAD and 22 healthy normotensive individuals of age and sex matched. EF was evaluated by means of two tests: flow-mediated vasorelaxation (FMVR) of brachial artery (high-resolution ultrasound) and activity of von Willebrand factor (VF) in plasma (ristocetin method). Spontaneous (SA), ADP- and collagen-induced platelet aggregations (PA) were evaluated ex vivo on laser aggregometry. Endothelial dysfunction assessed by FMVR and ultrasonic evaluation of carotid arteries were performed in all patients. According to risk factors count a high risk group (n = 8) was selected among hypertensive patient.

**Results:** Data on EF evaluation and PA are summarized in the table. Hypertensives exhibited disturbed EF which manifests as depressed FMVR and elevated activity of PA in plasma. Elevated PA closely correlated with FMVR markers. Endothelial dysfunction and plaque activation were found in about half of hypertensive patients. High correlation of EF markers with CAD risk factors but not with hemodynamic parameters (r = 0.62, p < 0.01 and r = 0.63, p < 0.01 for FMVR and WF, respectively) was supported by more severe changes of EF and PA in high risk group even compared to all EH patients.

**Conclusions:** Patients with essential hypertension have endothelial dysfunction but this is heterogeneous phenomenon depending mainly on cumulative effect of CAD risk factors. Endothelial dysfunction is partly responsible for plaque activation. The results of our study may help to explain the link between essential hypertension, atherosclerosis and atherothrombosis.

**ThP17:W28** Prevalence of hypertension in CHD patients in the Asia-Pacific region: the apsc study.


**Objective:** To determine the prevalence and treatment rates of hypertension among patients presenting with acute coronary syndromes in randomly selected hospitals throughout the Asia-Pacific region.

**Methods:** Data formation was collected from all in-patient and out-patient medical records of 4,112 consecutive patients. Hypertension was defined as a documented past history in the notes, a SBP ≥ 140 mmHg and or DBP ≥ 90 mmHg recorded at least 24 hours after admission or the prescription of anti-hypertensive medication during the survey period.

**Results:** Blood pressure was recorded in over 96% of patients in all countries. Hypertension prevalence ranged from 58% (Indonesia and Thailand) to 82% (Singapore) of all patients, with an average 8% higher prevalence among women. Overall, 89% of patients diagnosed as having hypertension received drug treatment. Calcium channel antagonists were the most commonly prescribed drugs, given in 45% of patients, beta-blockers in 39%, ACE inhibitors in 37% and diuretics in 24%, though drug preferences varied widely between countries. Despite high rates of therapy, in more than half (58%) of all hypertensive patients blood pressure failed to fall below 140/90 mmHg.

**Conclusions:** Hypertension is a more frequent risk factor among CHD patients in Asia than in Western Europe. The majority of patients receive drug therapy over 6 months follow-up. However more than half fail to achieve target levels for adequate control. Population measures, closer monitoring of treatment and more effective drugs are urgently required.

**P:W29 Oxidation and Atherogenesis**

**ThP1:W29** Lysoosomal accumulation of oxidized PC-apoB complex derived from oxidized LDL in macrophages.


**Objectives:** Oxidized phosphatidylcholine (OxPC), one of the products formed in oxidized low density lipoprotein (OxLDL), has been found in foam cells in vivo. OxPC is able to bind with apolipoprotein B (apoB) in OxLDL to form complex. Here, the intracellular fate of OxPC-apoB complex after internalization of OxLDL by macrophages was investigated.

**Methods and Results:** Murine macrophage cell line J774.1 was incubated with either OxLDL or acetylated LDL for 24 h, then the cells were further incubated for up to 24 h in new media without lipoprotein. Modified apoB in the cells was quantitated by sandwich ELISA using anti-OxPC mAb (DH3) and anti-apoB pAb. Intracellular OxLDL decreased rapidly for the first 4 h to approximately 20% of that before medium change, then the apparent metabolism of OxPC-apoB complex ceased. As the period of OxLDL loading to macrophages prolongs, OxPC-apoB complex remained in the cells after 24 h chase increased. Acetylated LDL in the cells decreased quickly and disappeared after 4 h of chase. Subcellular fractionation using sucrose density
gradient ultracentrifugation of macrophages, which had already accumulated OxPC-apoB complex by 24 h incubation with OxLDL and further 24 h chase, showed that the complex was co-localized with endosome and lysosome markers. Immunohistochemical double staining study demonstrated that OxPC and apoB co-localize in foam cells in early atherosclerotic lesions from human coronary artery.

**Conclusion:** These results suggest that OxPC-apoB complex originated from OxLDL accumulates in foam cells in human atherosclerotic lesions as well as in macrophages in vitro.

**ThP2/W29**

_**Selective distribution of oxysterols in human plasma, plasma lipoproteins and atherosclerotic lesions**_

_**Jacob Vaya**_1, Saeed Mahmood1, Tony Hayek2, Ehud Grenadiz2, Simcha Milo2, Aaron Hoffman1, Michael Aviram1,2,3,4

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**Introduction:** The presence of oxidized sterols (oxysterols) in human serum and lesions has been linked to the initiation and progression of atherosclerosis. Data concerning the origin, identity and quantity of oxysterols in biological samples are controversial and inconsistent. This inconsistency may arise from different analytical methods or handling conditions used by different investigators.

**Objective:** In the present study oxysterol levels and distribution were analyzed by a single method, in native and in vitro oxidized human low and high density lipoproteins, in plasma from patients after myocardial infarction and in human atherosclerotic coronary and carotid endothelial lesions.

**Methods:** Oxysterol distribution patterns were analyzed by GC-MS, as well as by GC.

**Results:** Oxysterol levels were calculated with a limit of detection of 0.064 ng. Plasma derived from atherosclerotic patients demonstrated the presence of elevated levels of 7b-OH, 27-OH, and 7a-OH cholesterol. In human coronary and carotid lesions, obtained from endarterectomy samples, 27-OH was the major oxysterol, with about 85% as sterols esterified to fatty acids. Unlike in human aortic lesions, in atherosclerotic polyploiprotein E deficient mice, 27-OH was entirely absent and the main oxysterols were 7-keto and 7a-OH cholesterol (about 30% each), where the latter was minor (5%) in human lesions. We conclude that the major oxysterol present in oxidized lipoproteins is 7-keto, in plasma 7b-OH and 27-OH, and in human lesions 27-OH cholesterol.

**Conclusion:** These results may support intervention to attenuate atherosclerosis by altering oxysterol formation through appropriate agents that would affect the selective formation of the above oxysterols.

**ThP3/W29**

_**Oxidized HDL stimulates cyclin D1 gene expression in cultured human arterial smooth muscle cells**_

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The Proliferation of arterial smooth muscle cells (SMC) plays important roles in the process of atherogenesis. The aim of this study was to observe the effect of oxidized high density lipoprotein (Ox-HDL) on cyclin D1 gene and c-fos oncogene expression in cultured SMC. Human plasma HDL (native HDL, N-HDL) was isolated by density gradient ultracentrifugation. Ox-HDL was prepared by copper ion method. The total RNA was extracted from cultured SMC after 2, 12 and 24 hour exposure to N-HDL and Ox-HDL respectively. mRNA was measured using digoxigenin labeled c-fos and cyclin D1 genes fragments as the probes by dot blot and Northern hybridization. Meanwhile, the cell number of SMC proliferation was measured by MTT method. The results showed that N-HDL has no effect on proliferation of SMC, but Ox-HDL stimulated significantly the proliferation of SMC. N-HDL has no effect on c-fos and cyclin D1 gene expression, but Ox-HDL significantly increased c-fos and cyclin D1 gene expression (P < 0.01). Ox-HDL increased the content of fos gene mRNA of SMC at 2 hour (P < 0.01), reached peak value at 12 hour (P < 0.01) and recovered to the normal level at 24 hour. Ox-HDL increased the content of cyclin D1 gene mRNA of SMC at 2 hour (P < 0.01) and reached the peak value at 24 hour (P < 0.01). These results suggest that the atherogenic role of Ox-HDL are probably related to its stimulating effects on the transcriptional expression of fos and cyclin D1 gene of SMC.
**ThP6:W29**

Native high-density lipoprotein reverses activating effects of oxidized low-density lipoprotein on polymorphonuclear leukocytes (PMNL): evidence for a paroxonase-independent mechanism

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**Objective:** The present investigation examines whether native or modified (oxidized or tryptophanized) high-density lipoprotein (HDL) and apoAI are able to prevent oxidized LDL-induced activation of the PMNL respiratory burst.

**Methods:** PMNL were isolated from heparinized blood by density gradient centrifugation. Human LDL and HDL were obtained from pooled plasma by very fast ultracentrifugation. APOAI was isolated by delipidation of freshly prepared human HDL as described by Scano and Edelstein. Oxidation of native LDL and HDL (0.2 g/l protein) with 1 mM sodium hypochlorite was carried out at 37°C for 40 min. Furthermore, native HDL and apoAI were treated with tryptophan/EDTA (278 µg trypsin per mg protein) for 1 h at 37°C. ROS generation was measured by chemiluminescence with an Autolumat LB 953 (Berthold Co., Wildbad, FRG). Paraoxonase (PON) activity was determined in an acrylamide assay using phenylacetate as substrate. HDL exerted its inhibitory effects independently of whether it was in previous contact with the cells or with oxidized LDL suggesting a fast-acting protective mechanism. Tryptophanized HDL, but not tryptophanized apoAI, both lacking PON activity, showed similar protective effects.

**Conclusion:** The data support an important role of the lipid moiety in the protective action of the HDL particle against deleterious effects of oxidized LDL on polymorphonuclear leukocytes.

**ThP7:W29**

OX-LDL Responsive genes in vascular endothelial cells identified by differential display analysis

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**Objective:** Identification of genes expressed responsible for oxidatively modified low-density lipoprotein (Ox-LDL) stimulation on vascular endothelial cells is important for understanding the molecular basis of atherosclerosis.

**Method:** Primary cultured human umbilical vein endothelial cells (HUVEC) were treated with 50 µg/ml Ox-LDL or vehicle, and 32 hours later the HUVEC RNA was extracted. Total RNA was used in differential display to screen for Ox-LDL responsive genes. Products of reverse transcriptase-PCR, made with pairs of arbitrary and anchored oligo (dT) primers, were separated on denaturing polyacrylamide gels; candidate bands were excised and reamplified for cloning and sequencing. The homology of the cDNA sequences was retrieved in the GenBank. The Ox-LDL responsive cDNA fragments is confirmed by northern blot analysis and the novel cDNA fragments were used to screen human aorta cDNA library. Positive clones were sequenced and used as probes to re-confirm the transcripts in HUVEC by northern blot analysis.

**Results:** We identified three up-regulated and one down-regulated genes. Two up-regulated genes were thymosin β4, a major actin-sequestering protein associated with endothelial cells differentiation; and ICAM-1, endothelial cells adhesion molecule involved in the early stage of atherosclerosis. Another up-regulated novel gene is a 1000 bp in length, encodes a protein of 210 amino acids with deduced molecular weight of 24 KD. Search in several databases confirmed the unique identity of this sequence. One down-regulated novel gene with the length of 2177 bp, contains a 1401 bp open reading frame which encodes a protein of 467 amino acids. By database analysis, the predicted NH2-terminal signal sequence is composed of 21 amino acids. The mature protein contains lipocalin and thiol-protease-his motifs.

**Conclusions:** These data suggest that Ox-LDL can alter the expression of multiple genes, including the known proteins and novel genes in cultured vascular endothelial cells. These responses may contribute to atherogenesis.

**ThP8:W29**

Allcin reduces atherosclerosis in mice and inhibits LDL Degradation in isolated mouse macrophages

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Many studies have shown that Garlic (Allium sativum) has beneficial effects on cardiovascular risk factors. These anti-atherosclerosis related properties of garlic are attributed particularly to allicin (diallylthiosulinate), produced during crushing of the cloves.

**Objective:** The aim of the current work was to study the mode of action of garlic.

**Methods:** Aortic atherosclerosis in C57BL/6 mice model of atherosclerosis, in spite of any significant effect on the plasma lipid profile. We further studied possible anti-atherogenesis mechanisms of garlic and found that: I. Allicin inhibits copper-induced LDL oxidation but on contrast, does not inhibit oxidatively-resistant, AAPH, II. Allicin reacts with homocysteine. Since homocysteine is a known risk factor for atherosclerosis its reaction with allicin in the plasma may reduce the risk for the disease. III. Allicin inhibits degradation of LDL and oxidized-LDL by isolated mouse-macrophages. Thus, it may inhibit foam-cells formation in early atherosclerosis. Further studies are required to elucidate the exact effect of allicin on atherogenesis in vivo.

**ThP9:W29**

Passive smoking in children is associated with increased isoprostane 8-EP-F2α

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**Objective:** The deleterious influence of passive smoking (PS) on atherogenesis is under debate. The effect of PS on in-vivo oxidation injury, in particular in children, has not been assessed yet. The isoprostane (IP) 8-epi-F2α is considered as a reliable marker of in-vivo oxidation injury.

**Methods:** During a cholesterol preloading or in school children aged 6–14 years (n = 213), lipids and lipoproteins as well as 8-epi-PGF2α (in plasma, serum and urine) were determined after extraction immunochromatically.

**Results:** 8-epi-PGF2α in children of cigarette smoking parents was significantly elevated as compared to controls and children with non-smoking parents. IP-values were higher when mothers were smoking and lower if only the father was smoking. A trend, however, no significant correlation was found to the number of packs of cigarettes in total smoked by the parents. The urinary IP-determination was more predictive as compared to plasma and serum measurements.

**Conclusions:** These findings indicate for the first time that PS causes a significant oxidation injury in school children. This may well result in a proatherogenic action.

**ThP10:W29**

Atherosclerosis in EC-SOD null mutant mice

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**Objective:** To investigate the development of atherosclerosis in mice lacking extracellular superoxide dismutase (EC-SOD).

**Methods:** EC-SOD null mutant mice (Carlsson et al., PNAS 92, 1995, 6264) were bred to an apolipoprotein E (Apo E) null mutant mice strain, known to spontaneously develop atherosclerosis (Pendrathitha et al., PNAS 89:4471, (1992)). The resulting offspring were kept on normal chow for eight months or were put on an atherogenic diet for one, three or five months. The mice were examined for atherosclerotic lesions by staining of dissected and fixed aortas with Sudan IV or by staining of frozen sections of the aortic root with Oil Red-O. Serum cholesterol and triglycerides and bodyweight were monitored throughout the experiment. Urine isoprostane levels and serum TBA levels were determined at endpoint to evaluate the oxidative stress. Differences were evaluated with the Mann-Whitney U test.

**Results:** Mice fed atherogenic diet for one month differed significantly in percent of total aortic surface covered with lesions, (p < 0.05) between double knockout mice (1.0 ± 1.1%) and Apo-E single knock out mice (2.5 ± 2.1%). Serum cholesterol levels also differed significantly (p < 0.001) with double knockout mice showing higher levels (117± 30.5 mmol/l) than Apo E knockout mice (79.0 ± 24.1 mmol/l). After three or five month on the
atherogenic diet and with normal diet we detected no differences in percentage of lesions and serum cholesterol levels between EC-SOD/Apo E and Apo E knockout mice. TBARs and isoprostanes did not differ between the genotypes at any time.

**Conclusion:** Absence of EC-SOD delays development of atherosclerotic lesions in Apo E null mutant mice kept on atherogenic diet, in spite of the expected elevated oxidative stress in the aortic vessel wall and a higher serum cholesterol level. The superoxide radical may protect against atherosclerosis.

**ThP11.W29** Effect of extracellular superoxide dismutase on LDL oxidation

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**Objective:** The aim of the study was to show that rabbit extracellular superoxide dismutase (EC-SOD) is capable to protect LDL from B4 rabbit aortic endothelial cell-mediated oxidative modification.

**Methods:** Recombinant EC-SOD (10 ng/ml, 15 ng/ml and 75 ng/ml) was incubated on B4 endothelial cells with 10 μg/ml of 125I-labelled LDL in Ham’s F-10 medium containing 1% FBS for 2.5, 6, and 21 h. The antioxidative capacity was determined by agarose gel electrophoresis, TBARS analysis and degradation in macrophages.

**Results:** Agarose gel electrophoresis showed 30% reduced migration of 125I-LDL after 6 h incubation with 10 ng/ml EC-SOD which indicates reduced modification of LDL. Similarly, the degradation rate of 125I-LDL incubated with 10 ng/ml EC-SOD on RAW 264 macrophages was reduced by 28%–36% when compared to controls. TBARS analysis of LDL incubated with EC-SOD concentrations 75 ng/ml, 25 ng/ml and 15 ng/ml showed 74%, 56% and 41% protection as compared to controls, respectively.

**Conclusions:** Low concentration (10 ng/ml) of recombinant rabbit EC-SOD clearly reduced oxidation of LDL by endothelial cell. This indicates that EC-SOD can modulate LDL oxidation in vitro and that in vivo it likely plays an important role in the attenuation of free radical damage to LDL and cells. EC-SOD may also be one of the enzymes with a significant potential to inhibit the development of atherosclerosis.

**ThP12.W29** Adenovirus mediated gene transfer of human 15-lipooxygenase in vitro and in vivo

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**Objective:** To create recombinant adenovirus expressing human 15-lipooxygenase (15-LO) and study the effects of high expression of 15-LO in regard to atherosclerosis.

**Methods:** Recombinant adenovirus containing human 15-LO cDNA was produced in 293 cells by homologous recombination and was used for in vitro and in vivo gene transfers. Expression in vitro was verified by Northern hybridization and by gas chromatographic activity assay. Expression in vivo was analyzed by PCR, RT-PCR and immunocytochemistry.

**Results:** Northern hybridization showed that human 15-LO was expressed in infected rabbit aortic smooth muscle cells (RASM) and in RAW macrophages. Specific 15-LO enzyme activity could be detected in both infected cell lines with 25-fold increase in RASM and 10-fold increase in RAW cells as compared to lacZ adenovirus infected control cells. Further three-fold increase in activity was found in RAW cells by interleukin-4 induction, whereas no response to IL-4 could be detected in RASM. In vivo experiments are currently under study.

**Conclusions:** Adenovirus mediated gene transfer was successfully used to achieve high expression of human 15-LO and can thus be an efficient tool in studying the effects of 15-LO in the development of atherosclerosis.

**ThP13.W29** Reduced resistance of low density lipoprotein to oxidation in young healthy adult offspring of parents with type 2 diabetes compared with controls

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**Objectives:** Metabolic abnormalities including dyslipidaemias have been identified in close relatives of patients with type 2 diabetes. These subjects are at increased risk of diabetes and, by association, atherosclerosis. Oxidative modification of low density lipoprotein (LDL) is believed to be an initiating and central feature in atherosclerosis. We have investigated the susceptibility of LDL to oxidation in healthy, glucose tolerant adults (aged 18–38 years) with and without type 2 diabetic parents.

**Methods:** Fasting blood samples were obtained from 14 subjects (12 M/2 F) with at least one type 2 diabetic parent, and 14 subjects (12 M/2 F) with no family history of low density lipoprotein (LDL) is believed to be an initiating and central feature in atherosclerosis. We have investigated the susceptibility of LDL to oxidation in healthy, glucose tolerant adults (aged 18–38 years) with and without type 2 diabetic parents.

**Results:** Blood samples were obtained from 14 subjects (12 M/2 F) with at least one type 2 diabetic parent, and 14 subjects (12 M/2 F) with no family history of low density lipoprotein (LDL) is believed to be an initiating and central feature in atherosclerosis. We have investigated the susceptibility of LDL to oxidation in healthy, glucose tolerant adults (aged 18–38 years) with and without type 2 diabetic parents.

**Conclusions:** We report, for the first time, reduced resistance of LDL to oxidation in young healthy adult offspring of parents with type 2 diabetes. This may indicate an increased risk of atherosclerosis in these subjects.

**ThP14.W29** Oxidation of low density lipoprotein under physiologically relevant oxidising conditions and the effect of dietary antioxidants

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**Objective:** To develop techniques to assess the oxidative resistance of LDL in vitro, using physiologically relevant oxidising agents.

**Methods:** We have developed methods to assess oxidative resistance of LDL using hemin, myeloperoxidase (MPO), type V soybean lipoxidase (LPO) and the peroxyxirite-generating compound 3-morpholinolinidnythonine (SIN-1) alone or in combination and compared them with Cu2+ mediated oxidation over a range of pH values and antioxidant concentrations. LDL (50 μg/ml) was isolated from heparinised plasma from fasting individuals by rapid single spin ultracentrifugation (541,000 g, 1 h, 4°C) and incubated with each catalyst at 37°C. Conjugated diene formation was measured by absorbance at 234 nm.

**Results:** Oxidation of LDL by hemin, lipoxidase, SIN-1 and Cu2+ was less efficient at the acidic pH likely to be present in atherosclerotic plaques in vivo. As pH decreased within the range 4.5–7.0, myeloperoxidase becomes a more efficient catalyst of LDL modification. α-Tocopherol and ascorbate effectively inhibit the oxidation of LDL at the optimum pH of oxidation, for each substrate tested.

**Table 1. Inhibition of oxidation by α-tocopherol (35 μM) and ascorbate (Sem).**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>α-Tocopherol (35 μM)</th>
<th>Control</th>
<th>α-Tocopherol (35 μM)</th>
<th>Control</th>
<th>α-Tocopherol (35 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propagation rate</td>
<td>45.6</td>
<td>49.6</td>
<td>65.2</td>
<td>78.2</td>
<td>78.2</td>
<td>81</td>
</tr>
<tr>
<td>Control</td>
<td>44.9</td>
<td>23.5</td>
<td>45.6</td>
<td>23.5</td>
<td>45.6</td>
<td>23.5</td>
</tr>
<tr>
<td>Error</td>
<td>± 6.2</td>
<td>± 6.2</td>
<td>± 6.2</td>
<td>± 6.2</td>
<td>± 6.2</td>
<td>± 6.2</td>
</tr>
</tbody>
</table>

**Conclusions:** These results confirm that α-tocopherol and ascorbate effectively inhibit LDL oxidation catalysed by physiologically relevant oxidising agents. At pHs likely to occur in atherosclerotic plaques MPO is a more efficient catalyst of oxidation than Cu, Hm, SIN-1 or LPO.

**ThP15.W29** The influence of folate supplementation and MTHFR polymorphism on oxidative stress markers in patient with mild hyperhomocysteinemia


**Introduction:** Homocysteine (Hcy) is an established risk factors for both, atherosclerotic and thrombotic vascular diseases. The Hcy depends on the interaction of folates and polymorphism of enzyme methylenetetrahydrofolate reductase (MTHFR). One of the important pathophysiological mechanisms of Hcy is its influence on oxidative status and free radical formation. The aim of the study was to evaluate the efficacy of Hcy lowering by folate supplementation on oxidative status and whether this effect is dependent on polymorphism of MTHFR in individual patients.
Methods: the study included a series of 46 subjects (30 m, 16 f, mean age 62.3 ± 1 y) with Hcy levels ≥20 µmol/L. After 1 month placebo period, 2 month folate treatment period (10 mg daily) followed. At the end of placebo and treatment period, total Hcy (tHcy) was estimated and following oxidative stress markers as well: malondialdehyde (MDA), erythrocyte glutathione (GSH), and glutathione peroxidase (GSHpx), superoxidismutase (SOD) and the whole antioxidative capacity (AOC), all using commercial kits. Genetic polymorphism of MTHFR was estimated using PCR.

Results:

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Treatment</th>
<th>C/C</th>
<th>C/T</th>
<th>T/T</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy</td>
<td>28.3 (1.87)</td>
<td>20.3 (1.43)</td>
<td>* <strong>8.5-14.7</strong></td>
<td>* <strong>10.3-15.7</strong></td>
<td>4.0</td>
</tr>
<tr>
<td>MDA</td>
<td>2.38 (0.13)</td>
<td>2.91 (0.18)</td>
<td>0.53</td>
<td>0.26</td>
<td>0.05</td>
</tr>
<tr>
<td>GSHpx</td>
<td>41.2 (1.97)</td>
<td>55.7 (1.47)</td>
<td>* <strong>18.5-24.7</strong></td>
<td>* <strong>22.0-26.0</strong></td>
<td>0.49</td>
</tr>
<tr>
<td>SOD</td>
<td>11.29 (2.99)</td>
<td>12.24 (3.56)</td>
<td>0.98</td>
<td>0.64</td>
<td>0.19</td>
</tr>
<tr>
<td>AOC</td>
<td>1.36 (0.03)</td>
<td>1.03 (0.03)</td>
<td>0.05</td>
<td>0.04</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Wilcoxon's paired test: **p < 0.001; *p < 0.01; t < 0.05**.

Summary: Folate supplementation resulted in a significant decrease of tHcy, decrease of MDA, AOC and increase of GSHpx, GSH and SOD (table). No significant differences (ANOVA) in the response of these variables to folate treatment appeared among MTHFR polymorphism groups (C/C, C/T or T/T).

In conclusion, folate treatment resulted in our series in improvement of antioxidative capacity, this effect was independent of MTHFR polymorphism.

ThP16:W29

Expression of pro-apoptotic proteins and anti-apoptotic proteins in atherosclerotic lesions from cholesterol-fed rabbits: colocalization with ox-LDL

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Evidence for apoptosis has been found in advanced atherosclerotic lesions from humans and experimental animal models. Yet, factors triggering apoptosis in atherosclerotic lesions are poorly understood. Oxidised LDL are cytotoxic to a variety of cells and induce apoptosis of smooth muscle cells, fibroblast, macrophages and endothelial cells in vitro. Purpose of this study was to investigate the presence of apoptotic cell death in early atherosclerotic lesions in rabbits and to address whether oxidised LDL colocalize ex vivo with apoptotic cells. Male New Zealand albino rabbits were fed a standard diet (n = 6) or a diet containing 1.2% cholesterol (n = 6) for 60 days. The aortic arch from each animal was sectioned and stained with antibodies against smooth muscle cells, endothelial cells, macrophages and oxidized LDL or for proteins involved in apoptotic pathways such as Fas, bax, bcl-2, and caspase-3. Nuclei in adjacent sections were stained with Hoechst 33258 or TUNEL. Early atherosclerotic lesions were characterised by intimal thickening and the presence of abundant smooth muscle cells and macrophages. The percent of apoptotic cells, evaluated as the ratio of apoptotic nuclei (detected with TUNEL) vs. total nuclei (detected with Hoechst 33258), approached 31% in the lesions and was greater than 55% in the endothelium. Fas, bax and caspase-3 signals were mainly located in the endothelium and smooth muscle cells proximal to the lumen, while bcl-2 colocalised with macrophages and with smooth muscle cells deeper in the lesions. Abundant epitopes of oxidized LDL were detected in areas of lipid accumulation, mostly near TUNEL-positive cells. We conclude that early atherosclerotic lesion from cholesterol-fed rabbits contain several cells undergoing apoptosis, especially in the endothelial layer, and that these cells colocalise with oxidised LDL. This pattern may represent a hallmark of early atherosclerotic lesions and account, at least in part, for the endothelial dysfunction that is associated with hypercholesterolemia.

ThP17:W29

Circulating oxidized LDL and the assessment of cardiovascular risk

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Background: Major and independent risk factors for CAD are advancing age, hypertension, hypercholesterolemia, dyslipidemia, obesity, diabetes mellitus, and cigarette smoking. Previously, elevated levels of circulating oxidized LDL have been detected in CAD patients. The aims of the present study were to assess the relation between circulating oxidized LDL and major risk factors and to compare the diagnostic value of circulating oxidized LDL with that of known risk factors.

Methods and Results: In a blinded cohort study, levels of oxidized LDL were determined in plasma samples obtained from 352 patients: 106 patients with clinically diagnosed CAD and 246 patients without clinical evidence of CAD. The Global Risk Assessment Scoring (GRAS) was calculated on the basis of age, total cholesterol, HDL cholesterol, blood pressure, diabetes and smoking. The predictive value of GRAS for CAD was 73%. Adding circulating oxidized LDL to GRAS increased the predictive value to 87% as a result of the increase of the sensitivity from 42% to 75%. In a multivariate model not including circulating oxidized LDL, age (p < 0.001), hypercholesterolemia (p < 0.001), dyslipidemia (p = 0.0035), male gender (p = 0.0015) and hypertension (p = 0.019) predicted CAD. In a model containing circulating oxidized LDL, plasma levels of oxidized LDL (p < 0.001), age (p = 0.001), and dyslipidemia (p = 0.0024) predicted CAD. Receiver operating curve analysis revealed a stronger relation of CAD with oxidized LDL (Area Under Curve 0.86) than with the total cholesterol/HDL cholesterol ratio (0.68) and triglycerides (0.35). After adjustment for CAD, hypercholesterolemia (p < 0.001) and obesity (p < 0.001) were the strongest predictors of circulating oxidized LDL.

Conclusion: Adding circulating oxidized LDL to the risk factors included in the Global Risk Assessment Scoring increases the sensitivity of diagnosis of CAD.

ThP18:W29

Highly expressed cathepsins B/L and caspase-3 in human atheroma are involved in macrophage-death induced by 7β-hydroxycholesterol and 7-ketocholesterol

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We earlier reported that exposure of macrophages to ox-LDL leads to leakage of lysosomal contents into the cytosol, and that this process is accompanied by apoptotic cell death. Oxyesters are present in atheroma/ox-LDL and have been suggested as the main toxic components responsible for the toxic effects of ox-LDL. We hypothesized that oxyesters might be responsible for the lysosomal damage to macrophages following exposure to ox-LDL. We have here investigated the toxicity of two oxyesters, 7β-hydroxycholesterol (7β-OH) and 7-ketocholesterol (7-keto), to U937 and THP-1 cells. We found that both these oxyesters induced marked lysosomal rupture followed by the activation of caspase 3-like proteases, DNA fragmentation, and the appearance of apoptotic morphology. The importance of the relocation of lysosomal proteases to the cytosol in these events was supported by the observation that an inhibitor (E64) of the lysosomal cysteine cathepsins B, H and L suppresses caspase activation and apoptosis. To explore the pathological relevance to our in vitro experiments we further examined apoptosis and cathepsins in normal and atherosclerotic arteries of humans. Compared with normal arterial tissues highly expressed cathepsins B/L, and caspase-3 like proteases was detected in atheroma plaques and co-localized at the areas rich in apoptotic macrophages. We conclude that 7β-OH and 7-keto may contribute to macrophage foam cell apoptosis through a pathway that involves lysosomal rupture, release of lysosomal contents, and subsequent caspase activation. The toxicity associated with these compounds, and the promotion of apoptotic/necrotic cell death by lysosomal enzymes may be important in atheroma formation and plaque destabilization.

ThP19:W29

The expression of autoantibody against oxidized LDL in patients with acute myocardial infarction

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Objective: To study the expression of autoantibody against two different sources of oxidized LDL in the patients with early stage acute myocardial infarction (AMI) and normal subjects.

Methods: The LDL were isolated and purified from two groups of sera by ultracentrifuge. One is with high-LDL serum (above 170 mg/dl) and the other is in normal LDL range. The purified LDL was oxidized by 10 µM CuSO4 for 18 h, then stopped by EDTA solution. The two groups of oxidized-LDL were coated with 5 µg/ml in ELISA plate. After the blocking procedure, the 50x diluted sera from AMI patients and normal subjects were integrated against two different groups of ox-LDL, respectively, then incubated for 1 h at 37°C. After the washing procedure, the 2nd antibody (conjugated with alkaline phosphatase) was added and incubated at 37°C for 1 h again. Finally,
the results were showed by absorbance at 405 nm wavelength under substrate integrated.

**Results:** The results showed that the titer of anti-oxidized LDL antibody against oxidized LDL purified from high-LDL serum in patients with AMI was 133% higher than that in normal subjects. But the titer of antibody in AMI was only 68% higher than that in normal subjects when the normal LDL serum was used.

**Conclusions:** We assume that the binding motif of oxidized-LDL from high-LDL serum is different from that of normal range LDL serum. Therefore, it may be important to choose the source of oxidized-LDL in the assay of autoantibody against oxidized LDL. And we found that the titer of autoantibody against oxidized LDL was higher in AMI patients than in normal subjects. This result indicated that the expression of this autoantibody could be related with the process of acute myocardial infarction.

**ThP22:W29**

**LDL oxides in presence of plasma antioxidants after aggregation with chondroitin-4-sulfate**

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Although the oxidation of low-density lipoprotein (LDL) appears to be an important event in the development of atherosclerosis, it is unclear to date, how extensive lipid peroxidation could occur in the arterial intima. It has been shown that in the presence of high concentrations of water-soluble antioxidants, such as urate and ascorbate in plasma and endothelial lining fluid the oxidation of isolated LDL particles is inhibited very efficiently, both by metal-catalysed oxidation and by lipid peroxidation initiated by other radicals, e.g. water-soluble peroxyl radicals. Therefore, it was of interest whether aggregation by constituents of the arterial extracellular matrix could facilitate oxidation under these conditions. We incubated human LDL with 300 μM urate and 20 μM ascorbate, chondroitin-4-sulfate (C4S), chondroitin-6-sulfate (0.25 to 10 mg/mL each) and sphingomyelinase (SMase, 50–150 μM/mL), and monitored copper catalysed LDL oxidation by low-level cholinuminescence. Oxidation was inhibited in the control containing only urate and ascorbate, and in the incubations with C6S and SMase. However, in presence of C4S, oxidation proceeded nearly as rapidly as in an antioxidant-free control, and was considerably enhanced by pre-incubation of LDL with 15-hydroxyeicosanone (15-LOX). C4S, C6S and SMase have been reported to induce aggregation of LDL, in addition, C4S is able to bind CuI-ions very efficiently. The prooxidant effect of C4S in Cu-catalysed oxidation can explain be inclusion of copper ions into the aggregates, which can then oxidise LDL very efficiently, in the absence of water-soluble antioxidants. Together with the prooxidant action of 15-LOX, this constitutes a possible way of LDL oxidation in the presence of plasma antioxidants in vivo.

**ThP23:W29**

**Modulation of basal and IL-1-induced adhesion molecule expression by PHGFP and 15-lipoxygenase in rabbit aortic smooth muscle cells**

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**Objective:** Expression of cell adhesion molecules is an early event during atherogenesis. They are induced by cytokines and also by conditions of oxidative stress. We recently found that 13-HPODE was able to induce ICAM-1 in HUVEC. Surprisingly 13-HODE, the reduction product of 13-HPODE was even more effective. Both compounds worked additively with IL-1 and TNF. We were therefore interested in the contribution of 15-LO to which produces 13-HPODE and PHGFp which reduces 13-HPODE to 13-HODE in the induction of both ICAM-1 and ICAM-1 by IL-1 in smooth muscle cells overexpressing either PHGFP (SMCPhgfp) or 15-LO (SMCLO).

**Methods:** Cells were cultured for 48 h without FCS and stimulated with IL-1 for 4 h. Thereafter, total cellular RNA was prepared, analysed by RT-PCR and standardized to GAPDH mRNA.

**Results:** RT-PCR showed a decreased basal expression of ICAM-1 and VCAM-1 in both SMCPhgfps and SMCLO. Based on the high basal expression an induction of ICAM-1 by IL-1 was not observed in controls (SMC). In contrast, ICAM-1 was induced by IL-1 in SMCLO cells but not in SMCPhgfps cells. Constitutive expression of VCAM-1 in SMC was almost abolished in SMCPhgfps and SMCLO. VCAM-1 was not enhanced in controls whereas in transfected cells VCAM-1 could be induced by IL-1. The effect was much higher in SMCPhgfps than in SMCLO.

**Conclusions:** The modulation of basal and IL-1-induced expression of adhesion molecules by PHGFp and 15-LO will be discussed in the context of the interference of these enzymes with a balanced cellular redox state.

**ThP24:W29**

**Association between LDL constituents and its resistance against copper induced oxidation**

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**Objective:** Diabetes patients have an increased risk of developing cardiovascular disease. Oxidation of LDL is supposed to play a role in the development of atherosclerosis. The aim of this study was to explore how different LDL constituents contribute to the resistance of LDL to oxidation.

**Methods:** In this study 101 nonobese type 2 diabetes patients with acceptable glycemic control were included. The resistance of LDL to in vitro oxidation, expressed as lag time, was measured by monitoring the formation of conjugated dienes. The explained variability in lag time was estimated using stepwise multiple linear regression models with the lag time as the dependent variable.
Results: The mean lag time in this group of patients was 49.0 ± 6.5 min (range 36.1–68.5). The best fitted linear regression model was able to account for 49% of the variation in lag time, and is shown in the table.

<table>
<thead>
<tr>
<th>LDL constituents</th>
<th>β</th>
<th>SE of β</th>
<th>t-value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUPA with ≥3 double bonds</td>
<td>−0.058</td>
<td>0.006</td>
<td>−0.010</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.362</td>
<td>0.001</td>
<td>24.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mono unsaturated fatty acids</td>
<td>0.015</td>
<td>0.004</td>
<td>3.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>0.007</td>
<td>0.003</td>
<td>0.168</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Conclusions: The major determinants of oxidation susceptibility were vitamin E and the fatty acid composition. LDL rich in fatty acids with 3 or more double bonds seemed more prone to oxidation, while LDL rich in saturated – or mono unsaturated fatty acids was less susceptible to oxidation. We were able to predict 49% of the variation in lag time of LDL from its composition.

**ThP25-W29**

**Proinflammatory cytokines in atherosclerotic plaques are induced by LysoPC and PAF-like lipids**

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**Objective:** To characterize and identify causes of the inflammation in atherosclerosis.

**Methods:** Cytokines in plaques from human carotid arteries were determined by intracellular cytokine staining. Cytokine production at the single cell level from blood or plaque cells was determined by ELISPOT and ELISA.

**Results:** Among twenty cytokines studied, proinflammatory cytokines including TNF, IFN-γ, IL-1, IL-2 and IL-6 dominated. Also TGF-beta was abundant, while TH2-cytokines were present at lower levels. OxLDL induced TNF and IFN-γ from blood and plaque mononuclear leukocytes at 1–10 μg/ml, but tended to be inhibitory and also toxic at concentrations above that. The effects were mimicked by PAF-like lipids, including lysoPC, and inhibited by PAF-receptor antagonists. PAF itself could also induce TH2 cytokines.

**Conclusions:** Atherosclerotic lesions are characterized by a significant production of proinflammatory, TH1 cytokines, which can be induced by oxLDL and PAF-like lipids. LysoPC functions as one and is produced during lipid oxidation (as in oxLDL) but also by enzymes such as phospholipase A2. LysoPC and other PAF-like lipids may play an important role in atherosclerosis, by inducing proinflammatory cytokines. PAF and PLA2-inhibitors represent possible therapeutic strategies to treat this inflammatory disease.

**ThP26-W29**

**Regular exercise improves lipid and antioxidant profile**

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**Objective:** The aims of the present study were: a) to evaluate the lipid and antioxidant profile in sportmen under regular training in comparison to sedentary controls; b) to explore the distribution of α-tocopherol among the main lipoprotein fractions in the above mentioned populations.

**Methods:** We studied a group of well-trained rugby players (n = 15; 23 ± 3 years old) who were compared to sedentary controls (n = 15; 23 ± 3 years old). Both groups showed similar body mass index and waist/hip ratio. Participants were not taking antioxidants or any drug known to affect lipid metabolism. The following parameters were evaluated: triglycerides, total cholesterol (C), LDL-C, HDL-C, apo A-I, apo B, total radical trapping antioxidant potential (TRAP), superoxide dismutase (SOD), paraoxonase (PON), α-tocopherol in plasma and in lipoprotein fractions isolated by ultracentrifugation.

**Results:** The lipoprotein profile was similar in both groups except for a significant increase in HDL-C levels in sportmen (58 ± 16 vs. 48 ± 8 mg/dl, p < 0.05). Rugby players also exhibited an improved antioxidant status evidenced by: higher TRAP (363 ± 33 vs. 306 ± 42 μM Trolox, p < 0.01), SOD (12 ± 3 vs. 8 ± 3 U/A, p < 0.01) and PON (213 ± 36 vs. 181 ± 20 μmol/ml/min, p < 0.01) activities, and plasma α-tocopherol concentration (1.2 ± 0.2 vs. 0.9 ± 0.3 μmol/dl). This lipophilic antioxidant was significantly increased in IDL+LDL fraction from rugbers (p < 0.005), while no differences were observed in the other lipoprotein fractions.

**Conclusions:** Regular training in rugby players is associated with increase in the atherogenic lipoprotein, HDL, and in hydrolysolable, enzymatic and liposoluble antioxidants. Moreover, the most atherogenic fraction, IDL+LDL, would be better protected from oxidative damage in sportmen than controls. All these modifications could be in response to oxidative stress suffered during aerobic exercise and could confer additional protection against cardiovascular disease.

**ThP27-W29**

**Oxidized LDL induces intracellular reactive oxygen species production in endothelial cells: Comparative responses of different dihydrofolate reductases of different dihydrofolate reductase inhibitors**

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**Objective:** Dihydrofolate-reducing membrane (DHFRs) influence many of the early pathophysiological processes of atherosclerosis. Lacidipine has been demonstrated to reduce the intracellular production of reactive oxygen species (ROS); to verify if this effect is a peculiarity of this molecule, or belongs to other DHFRs, the activity of lacidipine was compared with that of amiodipine, lercanidipine, nilodipine and nifedipine.

**Design and Methods:** The compounds were incorporated in human umbilical vein endothelial cells (HUVECs) using native low-density lipoprotein (LDL) as carrier for 18 h at 37°C. The intracellular drug concentrations were measured by mass spectrometry. 5 μM Cu²⁺-oxidized LDL (oxLDL) were incubated with HUVECs for 5 min. 2,7-dichlorofluorescein (DCF) as a probe of intracellular ROS production, was measured by flow cytometry.

**Results:** The cellular amount of lacidipine, lercanidipine and amiodipine was similar (fmol/cell), while nilodipine and nifedipine were almost undetectable. Similar results were obtained using plasma as carrier. Ox-LDL induced an early, significant and dose-dependent increase in DCF production (p < 0.001). Lacidipine, at any concentration, determined a significant reduction in ox-LDL-induced DCF production (p < 0.001), while a slightly and non significant decrease was found only with amiodipine and lercanidipine.

**Conclusions:** We conclude that the inhibitory effect of lacidipine on ox-LDL-induced ROS production in endothelial cells is a peculiarity of this molecule and is determined both by its lipophilicity and its antioxidant activity.

**ThP28-W29**

**Gender specific induction of aortic catalase by exercise-induced oxidative stress in C57BL mice**

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**Objective:** To determine whether acute and chronic exercise induce the expression of catalase in the aortic wall, in both genders.

**Methods:** 6-week old LDL C57BL mice were fed with regular mouse chow and the exercised groups were trained on a treadmill (15 meters/min.) for 30 minutes during 8 consecutive days (1 week-exercise group) or 5 days per week during 6 weeks (6 week-exercise group). After sacrifice, blood was drawn by heart puncture, the aortic trunk was washed by perfusion of saline. The microdissection of the aorta was performed from the iliac bifurcation up to the heart and the adventitia was carefully removed under microscope. A 2-mm cross-section was fixed for immunohistochemical analysis whereas the remaining aorta was used for protein extraction. Catalase level was examined in the aortic wall by immunohistochemistry and western blot and in red cell blood by determination of catalase activity. Antibodies against oxidatively modified proteins were detected in plasma using ELISA method.

**Results:** In males, but not in females, exercise induced the expression of catalase in the aortic wall, accompanied by a decrease of oxidatively modified proteins in plasma.

**Conclusions:** We recently described that exercise-induced oxidative stress might be beneficial. Oxidation in plasma, till now thought to be deleterious for atherosclerosis, could be in fact beneficial by inducing tissue antioxidant enzymes(s).

**ThP29-W29**

**Vitamin E reduces aortic lipid peroxidation and restores nitric oxide availability in an experimental model of insulin resistance syndrome**

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**Hypertriglyceridemia and the accompanying insulin resistance are associated with an increased risk for cardiovascular disease, but the pathophysiological mechanisms and therapy are not completely understood. In this study, we examined aortic lipid peroxidation, lipoprotein susceptibility to oxidative modification and aortic eNOS content, which is the indicator of biologically active nitric oxide (NO), in adult male hereditary hypertriglyceridemic rats (HHTG) before and during vitamin E treatment (HHTG+VE, α-tocopherol, 500 mg/kg diet) for 4 weeks. The HHTG rats and randombred Wistar**
controls were fed a high carbohydrate diet (70% of energy as sucrose) ad libitum. Compared with controls, the HHTG rats exhibited markedly elevated serum triglyceride concentration (4.25 ± 0.28 vs 1.23 ± 0.37 mmol/l, p < 0.001), higher aortic intima-media lipid peroxidation (0.18 ± 0.02 vs 0.26 ± 0.03 MDA equivalents/mg protein, p < 0.05), enhanced susceptibility of LDL+LDL to copper-induced oxidation (lag time 75 ± 7 vs 88 ± 9 min, p < 0.05) and reduced aortic content of cGMP (0.38 ± 0.74 vs 13.91 ± 2.55 pmol/mg protein, p < 0.05). Vitamin E administration decreased aortic lipid peroxidation (~12%, p < 0.05), increased the resistance of lipoproteins to in vitro oxidation (~22%, p < 0.02) and increased aortic cGMP concentrations to those found in control animals. Results indicate that vitamin E, by inhibiting aortic lipid peroxidation, lowering the susceptibility of lipoprotein to oxidative modification and restoring NO availability, might beneficially influence some metabolic disturbances associated with the insulin resistance syndrome.

**ThP32:W29** Exercise decreases the formation of atherosclerotic lesion in LDL receptor-deficient mice

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**Objective:** To determine whether chronic exercise prevents the formation of atherosclerotic lesions in the aorta.

**Methods:** 6-week old LDL receptor-deficient male mice were divided into 4 groups: exercisers (Ex) or sedentary (S) on normal (N) or atherogenic diet (Ath) (S/N n = 5, S/Ath n = 8, Ex/N n = 6, Ex/Ath n = 11). Ex/N and Ex/Ath groups were trained during 12 consecutive weeks, 5 days per week for 30 min a day, on a treadmill (15 meters/min). After sacrifice, the heart was perfused with saline then the dissection of the aorta was performed from the iliac bifurcation up to the heart including the beginning of carotid and subclavian arteries. The adventitia was carefully removed under microscope, and the aorta was opened-up and pinned up on black wax for "en face" observation. Computerized determination of the lesion area was then performed.

**Results:** Exercise induces a 40% decrease of early atherosclerotic lesion formation in LDL receptor-deficient male mice fed with high fat diet (p = 0.017). No lesions were observed in normal diet fed groups.

**Conclusions:** Based on these data and on a previous work showing that exercise induces the expression of catalase in the aorta (in mice), we propose that exercise might prevent atherosclerosis by inducing antioxidant enzymes in the artery.

**ThP33:W29** Involvement of transplasma membrane electron transport in macrophage-mediated LDL oxidation

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Oxidation of low density lipoprotein (LDL) in the arterial intima has been implicated in atherosclerosis. Macrophages promote LDL oxidation in vitro via a transition metal-dependent process, but the exact mechanism remains unclear. We have shown that macrophages reduce extracellular transition metals by a direct transplasma membrane electron transport system (TPMET) (1). In the present study we investigated the role of TPMET in macrophage-mediated LDL oxidation.

Mouse J774A.1 macrophages reduced the impermeable oxidant ferricyanide (FEC) via direct transmembrane electron transport, but not by cell-derived reductants. The rate of FEC reduction was strongly enhanced by preloading cells with 100 μM ascorbic acid (AA) or dehydroascorbic acid (DHA). 2.5-fold enhanced reductase activity of the cells was observed for at least 5 hours after DHA was removed from culture medium, and the activity returned to normal by 24 hours after DHA-loading. Preincubation of cells with DHA or AA enhanced the ability of the cells to oxidise LDL in Ham’s F-10 medium compared to control cells. The stimulatory effect of DHA- or AA-pretreatment on cellular TPMET activity and LDL oxidation could not be explained by the release of AA into the medium. This conclusion is based on results from experiments in which we assessed the effect of ascorbate oxidase on the two processes, analysed the amounts of AA and DHA secreted by cells, and measured the ability of cell-conditioned medium from control and DHA- or AA-enriched cells to reduce FEC. In conclusion, the correlation between enhanced TPMET activity of macrophages and their ability to oxidise LDL in response to increase of the intracellular AA or DHA level supports our proposal that TPMET plays a significant role in macrophage-mediated LDL oxidation via reduction of extracellular transition metals.

**References**

**ThP34:W29** Effects of methionine load on oxidative modification of low density lipoprotein

**Objective:** Mild hyperhomocyst(e)inemia (HHcy) is an independent risk factor for atherosclerotic vascular diseases. Mechanisms by which HHcy accelerate atherothrombosis are supposed to be multifactorial. Homocyst(e)in (Hcy) and other thiols are able to generate several cytotoxic reactive oxygen species (ROS). Increased generation of ROS initiates lipid peroxidation. Oxidatively modified LDL formed during this process may play important role in the early stages of atherogenesis. Methionine (Met) load leads to a rise in plasma Hcy levels.

**Methods:** Fasting (0 hrs) and postmethionine loading (6 hrs, 0.1 g/kg bw) plasma levels of total Hcy (tHcy) were determined by the HPLC method (Araki and Sakó, 1997). Lipoperoxidation of LDL was measured by Cu²⁺-catalyzed conjugated diene formation (A₂₃₄nm) in ultracentrifugally isolated LDL (Estherbauer et al, 1989). Electromobility of LDL before and after in vitro Cu²⁺-catalyzed oxidation before and after Met load was measured on agarose (Sebia). Studied group (15 M/8 F) consisted of 8 healthy persons, 9 patients with atherosclerotic vascular disease, and 6 patients with mixed hyperlipidaemia.

**Results:** Post-Met loading changes in levels of tHcy (+152%, P < 0.001), were associated with an increase of concentrations in CD in LDL (+58%, P < 0.10), decrease in duration of lag time (~12%), P < 0.01 and increased oxidation rate of LDL (+11%, P < 0.05). Similarly, Met load led to increased electrophoretic mobility of "in vivo" Cu²⁺-oxidatively modified LDL (+20%, P < 0.05).

**Conclusion:** Met load, through induced mild HHcy, led to increased amount of CD in LDL, that implicated rise in concentrations of mm- and LDL. After Met load decreased duration of lag time, increased oxidation rate of LDL and increased electrophoretic mobility of LDL were observed as well. The data implies enhanced oxidation and increased susceptibility of LDL to lipid peroxidization after methionine load.

**ThP35:W29** Relation of LDL-cholesterol, LDL oxidability and antioxidants to endothelium dependent vasodilatation in hypercholesterolaemia and hypertension
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**Objectives:** The purpose of this study was to examine the relationship of LDL-cholesterol, oxidative susceptibility of LDL and antioxidants with the impairment of endothelium dependent vasodilatation in hypercholesterolaemia (HC), hypertensive (H), hypercholesterolaemia/hypertensive (HH) and normolipidemic normotensive subjects (N) (n = 11).

**Methods:** Ascorbate, urate, α-tocopherol, lycopene and β-carotene were determined by HPLC. The kinetics of LDL oxidation was monitored by incubating LDL with Cu²⁺ and following the absorbance in 234 nm for analyzing the lag time, rate, lag rate and peak time. Mode B ultrasonography was used to measure the blood flow and diameter in response to reactive hyperemia of brachial artery.

**Results:** Plasma ascorbate, lipid soluble antioxidants, the lag time and peak time were lower in H, HC, and HH than in N. The diameter increase percentages in response to hyperemia were 20.4, 12.9, 9.9 and 8.1 for N, H, HC and HH, respectively. LDL cholesterol was negatively correlated to the percentage of diameter increase in response to hyperemia. Positive correlations were found for the concentration of plasma lipid soluble antioxidants and blood flow increase, as well as, between the lag time of LDL oxidation and the percentage of diameter increase in response to hyperemia.

**Conclusions:** These data suggest that the cholesterol content and oxidability of LDL as well as, the lipid soluble antioxidants may be important for the impairment of endothelial dependent vasodilatation in hypercholesterolaemia and hypertension. Supported by FAPESP

**ThP36:W29** The effect of different intensities of exercise on α-tocopherol levels in the plasma and liver of rats
S. Kinoshita, E. Tsuji. Graduate School of Medical Professions, Kawasaki University of Medical Welfare, Okayama, Japan

**Objective:** Vitamin E is the major lipid soluble anti-oxidant and may play an important protective role against free radicals produced during exercise. Exercise induces free radical formation in muscles and liver, resulting in oxidative damage, such as lipid peroxidation. The amount of damage depends on exercise intensity, training state and the tissue examined. The present study investigated the effects of exercise intensities on α-tocopherol levels in the plasma and liver of rats.

**Methods:** Male Wistar rats were divided into a sedentary control group and exercise groups. The exercise groups were forced to exercise by treadmill running at 30%, 60% and 80% of maximum oxygen uptake (VO₂max) for 120 minutes. One group was sacrificed immediately and others at 6 hours after the exercise period. α-Tocopherol levels in the plasma and liver were analyzed by HPLC.

**Results:** In the group sacrificed immediately after the exercise period, plasma α-tocopherol levels significantly decreased in the 60% and 80%VO₂max groups compared to the control. In all exercise groups, plasma α-tocopherol levels were significantly higher 6 hours after the exercise period than in those sacrificed immediately after the exercise period. On the other hand, liver α-tocopherol levels decreased in all exercise groups 6 hours after the exercise period compared with those sacrificed immediately after the exercise period. The decrease was especially significant in the 80 VO₂max exercise group.

**Conclusions:** Although regular physical exercise is widely accepted to be a protective factor against the development of atherosclerosis, these results indicate that strenuous exercise raises the consumption of α-tocopherol and induces oxidative stress in the liver of rats.

**ThP37:W29** Micronised fenofibrate effect on serum paraoxonase activity in patients with coronary heart disease

**Objectives:** To evaluate the effect of micronised fenofibrate on the serum paraoxonase and lipoprotein levels in CHD patients with type II hyperlipidemia.

**Methods:** 52 patients were involved in the study (36 males, 16 females). The mean BMI was 27.13 ± 5.37 kg/m². The effects of daily 200 mg micronised fenofibrate (Lipidil®) on serum cholesterol, lipoproteins, triglyceride, apolipoproteins and fibrinogen levels as well as on liver and kidney function were determined. The serum paraoxonase activity was measured spectrophotometrically using paraoxon as substrate.

**Results:** During the three month study it was observed that following the effect of micronised fenofibrate, the serum triglyceride level (from 3.25 ± 0.66 mmol/l to 1.86 ± 0.89 mmol/l, p < 0.001) cholesterol level (from 6.7 ± 1.88 mmol/l to 5.83 ± 1.4 mmol/l, p < 0.001) were significantly decreased, while the protective high-density lipoprotein (from 1.05 ± 0.23 mmol/l to 1.29 ± 0.51 mmol/l; p = 0.005) was significantly increased. The low-density lipoprotein (from 4.22 ± 1.83 mmol/l to 3.7 ± 1.31 mmol/l; p = 0.028) and apolipoprotein B-100 (from 1.44 ± 0.44 G/l to 1.26 ± 0.40 G/l; p < 0.008) were also significantly decreased while HDL associated apolipoprotein A1 (from 1.98 ± 0.23 G/l to 1.60 ± 0.26 G/l; p = 0.05) was significantly increased. Body mass index and the standardized values for HDL (PON/HDL) were significantly increased (from 162 ± 75 to 191 ± 78, p < 0.05) while the PON/apoA ratio (from 131 ± 73 to 161 ± 64 p = 0.39) was not changed significantly.

**Conclusion:** The serum paraoxonase activity was lower in CHD patients with type IIb hyperlipoproteinemia compared to age matched healthy control subjects. The three month treatment with daily 200 mg micronised fenofibrate is thought to normalize the lipid profile and improve the antioxidant status by increasing serum paraoxonase activity in these patients.

**ThP38:W29** Potential role of oysterst and lysolceitin in the oxidised LDL-induced decrease in albumin synthesis in HepG2 cells
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**Objectives:** Oxidant stress is increasingly thought to be a key element in atherosclerosis especially due to the occurrence of oxidised LDL (OxLDL). Plasma albumin concentration is inversely correlated with the mortality risk and albumin can therefore considered as an antiatherogenic protein, due in part to its antioxidant properties. In the present work, we aimed to study the influences of OxLDL on albumin synthesis in HepG2 cells.

Methods: Albumin secretion and synthesis were evaluated by enzyme immunoassay and radiolabeled leucine incorporation followed by specific immunoprecipitation. Pulse-chase and Northern blot experiments were also carried out to determine synthesis and degradation of the protein.

Results: OxLDL lead to a dose-dependent decrease in albumin secretion. Moreover, protein synthesis and mRNA levels were decreased in the presence of OxLDL. The inhibitory effects of OxLDL on albumin secretion were reproduced only with the phospholipid and the sterol fractions extracted from these modified LDL. In addition, major oxysterols (7-oxogenated cholesterol) and lysolignol found in OxLDL reproduced the OxLDL-induced inhibition of albumin secretion.

Conclusions: From our in vitro data, we propose that the OxLDL-induced impairment of albumin synthesis, possibly due to oxysterols and lysolignols, may partly explain epidemiological data indicating that reduced levels of serum albumin are associated with an increase mortality.

ThP39: W29

Antithrombotic and antioxidative effects of lacidipine in apolipoprotein E-deficient mice

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Objective: Lacidipine a long-lasting calcium antagonist has been demonstrated to possess antioxidant activity and in biological membranes it showed an activity comparable to reference antioxidant compounds like vitamin E. Oxidation of low density lipoprotein (LDL) is believed to play an important role in the pathogenesis of atherosclerosis. The effect of lacidipine, a with antioxidant properties, on the development of atherosclerotic lesions in relation to the susceptibility of LDL to oxidation was studied in atherosclerotic apolipoprotein E-deficient mice.

Summary: Apolipoprotein E deficient mice received either placebo or 3 mg/kg/day of lacidipine. After 12 weeks of treatment, lacidipine reduced the aortic lesion area by 56% compared with the control group (p < 0.01). After the same period of treatment, the resistance of the mice LDL to undergo lipid peroxidation after its incubation with either CuSO4 or human umbilical vein endothelial cells (HUVECs), was significantly increased in the apo E-deficient mice treated with lacidipine compared with the placebo group (p < 0.01). The aortic lesion area resulted inversely correlated with the susceptibility of LDL to copper oxidation (measured as lag phase in minutes) (r = -0.85, p < 0.01). The native mice LDL-like particle derived from apo E-deficient mice treated with lacidipine contained significantly lower concentrations of triolein, oleate, different substances (TBARS) than the placebo group (p < 0.01) and there was a relationship between the lag phases and the corresponding concentrations of TBARS of both groups (r = 0.97, p < 0.01). After exposure to HUVECs, LDL-like particle vitamin E decreased significantly, however vitamin E concentrations were systematically and significantly higher in the apo E-deficient mice treated with lacidipine than in the placebo group (p < 0.01).

Conclusions: We demonstrated that lacidipine reduced the aortic lesion area in apo-E deficient mice and that this reduction is associated with decreased LDL susceptibility to oxidation induced by copper ions and endothelial cells.

P:W30 GENETICS OF LIPOPROTEIN METABOLISM

ThP1: W30

Structure of human ACYL-COA: Cholesterol acyltransferase (ACAT)-2 gene

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Objective: ACAT catalyzes the cholesterol esterification in mammalian cells. Two isoforms of ACAT have been reported to date (ACAT-1 and ACAT-2). ACAT-1 is ubiquitously expressed in tissues except intestine. In contrast, ACAT-2 is expressed mainly in the liver and intestine. The limited distribution of ACAT-2 suggests that it may contribute to the cholesterol esterification and lipoprotein synthesis in the liver and intestine. The purposes of this study were: 1) to identify the structure of human ACAT-2 gene and 2) to establish the methods to analyze ACAT-2 gene for investigating the relation between ACAT-2 and dyslipidemia.

Methods: A human PAC genomic library was screened by PCR-based procedures using a set of primers: 3′F: AACACCTCGATCTGTTGCTCC-3′R: GGAATGCAGACAGGAGCT-3′R. Both were designed upon the human ACAT-2 cDNA sequence. Isolated PAC clones were completely digested with BamHI and subcloned into plasmid vector. Subclones that contained exons were screened by dot blot hybridization using partial ACAT-2 cDNA fragments. The screened clones were sequenced to identify exons of the ACAT-2 gene.

Results: The coding region of ACAT-2 gene was encoded in 15 exons that existed on the 21 kilo base span of genomic DNA. The exonic sequences coincided completely with that of the reported ACAT-2 cDNA, and each exon-intron junction conserved splicing consensus sequences. The exon sizes ranged from 46 to 265 base pair. Based on the genomic sequence data, all 15 exons were successfully amplified with PCR from genomic DNA.

Conclusions: In the present study, we clarified the gene structure of human ACAT-2 and the results obtained might be useful for further investigation of the relation between various dyslipidemias and ACAT-2.

ThP2: W30

A common polymorphism of the cett gene predicts the increase in HDL-C in response to supplementation with dietary fish or fish oils

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Objective: To determine whether the B1 allele of the cholesterol ester transfer protein gene (CETP/TaqB) which has previously been associated with higher CETP levels and lower HDL-C levels, influences the increase in HDL-C seen with fish or fish oil supplementation in man.

Methods: One hundred and sixty nine subjects from 3 previous studies of fish or fish oil supplementation provided samples for genotyping. Subjects in those studies were recruited on the basis of either being overweight, having high normal BP, mild dyslipidemia and/or Type II diabetes mellitus. Dietary fish supplementation (providing approximately 3.2 to 4.1 g/day of n3 fatty acids) or fish oil (providing approximately 2.6 to 5.2 g/day of n3 fatty acids) were given to 101 subjects while the remaining 68 subjects acted as controls. Before and after 8 to 16 weeks of intervention fasting serum lipids were measured. Genomic DNA was extracted from EDTA anticoagulated blood by a standard Triton X-100 procedure and genotyped for the CETP/TaqB polymorphism.

Results: The B1 and B2 allele frequencies were 0.41 and 0.59, respectively. Compared to controls, ω fatty acids decreased triglycerides by 0.57 ± 0.13 mmol/L (P < 0.001) and increased HDL-C by 0.074 ± 0.018 mmol/L (P < 0.001). General linear modelling, after control for effects of age, gender, changes in weight, physical activity and alcohol intake revealed a significant interaction between CETP genotype and ω3 fatty acid supplementation (P = 0.046), with B1B1 homozygotes showing the largest increase in HDL-C, B2B2 homozygotes showing the smallest increase and B1B2 heterozygotes having an intermediate response.

Conclusion: The B1 variant of the CETP gene dictates a significantly greater increase in HDL-C after supplementation with ω3 fatty acids.

ThP3: W30

Apolipoprotein B gene polymorphisms: Prevalence and impact on serum lipid levels in hypercholesterolemic individuals from Brazil

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Objective: To investigate the prevalence and impact of four polymorphisms (Mplb, Xbal, Ins/Del and 3’HVR) of the apolipoprotein B (apo B) gene on serum lipid levels in 177 Brazilian hypercholesterolemic individuals (HG) and 100 controls (CG).

Methods: Genomic DNA was extracted from blood leukocytes by a salting out method. Mplb and Xbal polymorphisms (exon 26) were detected by PCR, RFLP and Ins/Del (signal peptide) and 3’HVR polymorphisms were analyzed by size separation in gel electrophoresis. Chi-square analysis was used to test Hardy-Weinberg equilibrium, and for comparison of allele frequencies between the studied groups.

Results: The genotype distribution and allele frequency for the polymorphisms Mplb, Xbal and Ins/Del were similar between HG and CG individuals. For 3’HVR polymorphism, we observed a higher frequency of the alleles.
smaller than 43 repeats (±43) in HG when compared to CG (16.4% vs. 8.5%, P = 0.0133). Moreover, these alleles were associated to higher total cholesterol in serum (P = 0.0288) in HG subjects. In addition, this study demonstrates an association between Ins/Dei and 3'HRV polymorphisms, the alleles ±43 and Dei are more frequent in the HG when compared to CG individuals (15.8% vs. 6.0%, P = 0.0270).

**Conclusion:** Our data indicate that 3'HRV polymorphism in apo B gene is associated with differences on serum cholesterol levels in hypercholesterolemic individuals, indicating that this polymorphism can be an useful genetic marker to evaluate atherosclerotic disease risk.

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**ThP4/W30** Lipoprotein lipase N9 stable cell lines show enhanced binding and internalisation of LDL: Disease in N9 carriers

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**Objective:** To clarify the functional basis for the association of the common lipoprotein lipase (LPL) gene variant D9N with plasma triglyceride (TG), reduced HDL-cholesterol concentrations and increased risk of coronary heart disease (CAD).

**Methods and Results:** CHO K1 cells were stably transfected with wild type D9 or mutant N9 LPL cDNA and similar levels of LPL-D9 and -N9 expression were observed. The ratios of monomeric to dimeric LPL secreted by D9 and N9 cells were no different and the purified dimers had the same specific activities (0.1 μU/μg). Defective secretion of LPL-N9 was confirmed. In addition, N9 cells showed enhanced binding (4.6 fold) and internalisation (2.6 fold) of 125I-LDL compared to D9 cells, which were eradicated by pre-treatment with either heparin or heparanase, confirming the importance of cell surface proteoglycans in these processes. Similarly, N9 cells bound and internalised 3.8 and 4.4 fold more oxidised 125I-LDL respectively, than D9 cells.

**Conclusions:** This suggests that LPL-N9 is secretion defective, but once on the cell surface it shows enhanced binding and internalisation of LDL. This augmented "bridging" function of LPL-N9 may explain the reported increased CAD in carriers of this LPL variant.

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**ThP5/W30** A rare Apo E variant, Apo E3 AL149, associated with familial hypercholesterolemia

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**Methods:** Apo E genotype was determined by PCR amplification and restriction isotyping in a group of 142 clinically diagnosed familial hypercholesterolemia (FH) patients. Four unrelated subjects showed a non-habitual DNA fragments pattern. DNA sequencing revealed a 3 nucleotide deletion between positions 4040 and 4042 of the apo E genomic sequence (Paik YK et al). Haplotypes of the LDL receptor (LDLR) gene were determined in probands' relatives in order to perform linkage analysis of the observed FH phenotype. PCR-SSCP analysis of the coding LDLR gene sequences was performed using fluorescently labelled primers and ALF Express System. Computer modelling of the apo E variant was performed using the Swiss-Pdb Viewer v3.5 over a previously described structure (PDB: 1pe, Wilson C et al).

**Results:** The in frame 3 bp deletion causes the dispensability of Leu at position 149 of apo E mature peptide. FH phenotype correlated with the apo E3 AL149 carrier condition in relatives of the probands. LDLR gene haplotype analysis did not revealed linkage with the hypercholesterolemia trait and SSCP analysis of the LDLR coding sequences did not show any change that could lead to a defective LDLR. The computer modelling of the apo E3 AL149 variant showed an accumulation of positive charge along the LDLR binding region of apo E.

**Conclusions:** Apo E3 AL149 seems to be associated with the expression of a FH phenotype. The probable effect of the mutation could be to increase the positive charge density in the apo E receptor binding region leading to perturbation in the interaction of apo E containing lipoproteins with LDLR family receptors.

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**ThP6/W30** A novel molecular defect in the LCAT gene associated with fish eye disease in a Spanish family

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Fish eye disease (FED) is a familial syndrome characterized by the absence of lecithin:cholesterol acyltransferase (LCAT) activity toward high density lipoproteins (HDL), which is caused by defects in the gene coding for LCAT. The most striking features in FED are pronounced corneal opacification and marked reduction in HDL cholesterol.

**Objective:** To identify the molecular basis of a Spanish FED family.

**Methods:** The proband and his sister had corneal opacities, severely reduced concentrations of HDL cholesterol and diminished LCAT activity toward apo A-I-containing lipoposomes. We analyzed the entire LCAT gene of both subjects by the single strand conformation polymorphism (SSCP) method, and the underlying mutation was identified by DNA sequencing and confirmed by restriction analysis.

**Results:** We found a novel mutation in exon 5 of the LCAT gene, in homozygosity in both patients with FED, consisting in a T→C transition at 598 position. This point mutation results in substitution of isoleucine for threonine at amino acid 178 of the LCAT enzyme. This Ile 178 is adjacent to the structural motif Gly-X-Ser-Gly, a binding and catalytic site in several lipases.

**Conclusions:** The I178T mutation identified in exon 5 of the LCAT gene seems to be responsible for dyslipidemia and corneal opacity in this Spanish FED family.

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**ThP7/W30** Genes control the coordinated covariation of HDL and LDL size phenotypes

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**Objective:** To identify factors responsible for the positive correlation of LDL and HDL size phenotypes.

**Methods:** Using gradient gel electrophoresis and a gel format that permitted evaluation of both phenotypes in the same gel lane, we measured LDL and HDL size phenotypes in samples from 1121 participants in the San Antonio Family Heart Study. We generated two size-related variables from the absorbance profiles, ΔHDL and ΔLDL, where absorbance in small particles was subtracted from absorbance in large particles. Quantitative genetic analyses, using maximum likelihood methods, were employed to identify significant covariates and to evaluate the additive effects of genes on HDL and LDL size phenotypes and their correlations.

**Results:** Detailed correlation analyses revealed two LDL size intervals (24.4–26.0 and 27.2–28.4 nm) that were strongly, and oppositely, correlated with two HDL size intervals (7.7–8.2 and 9.6–10.7 nm). Absorbance in these relatively limited intervals was used to construct two global measures of size variation, ΔHDL and ΔLDL, each showing strong heritabilities (h2HDL = 0.42 ± 0.06 and h2LDL = 0.35 ± 0.06). Significant covariates for both traits included sex, age, smoking and diabetes status, and they accounted for 13 and 10% of total trait variation, respectively. LDL and HDL sizes were strongly correlated (r = 0.52) and bivariate quantitative genetic analyses revealed a genetic correlation of 0.65, suggesting that a gene or group of genes is responsible for approximately 42% of the covariation in the two size traits.

**Conclusion:** LDL and HDL size phenotypes are positively correlated and in part this correlation is mediated by a common set of genes. Support: grant HL-45522 from the National Institutes of Health.

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**ThP8/W30** Additive effect of common polymorphisms in apolipoprotein E and B genes on plasma lipid levels

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**Objective:** To determine the simultaneous effect of polymorphisms in apoE (e2, e3 and e4) and B (Insertion/Deletion in signal peptide) genes on lipid parameters in 8 years cohort of the representative selected Czech population sample (255 unrelated individuals).

**Methods:** Polymorphisms have been measured by PCR and restriction analysis (apo E). Lipid parameters have been evaluated enzymatically in WHO
controlled laboratory. Statistical analysis was performed using the ANOVA. Analysis was carried out in men and women together.

**Results:** The effects of apoE and apoB polymorphisms were as expected. Gender differences have been observed, but no significant gene-gender interaction was detected. The carriers of the apoC2 and apoBII alleles have the lowest, and the carriers of the apoE4 and apoBDD alleles have the highest levels of plasma lipids (see table).

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>N</th>
<th>Apolipoprotein B (g/l)</th>
<th>LDL cholesterol (mmol/l)</th>
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<tr>
<td>apo E</td>
<td>apo B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+allele 2</td>
<td>II</td>
<td>22</td>
<td>1.02 ± 0.24</td>
</tr>
<tr>
<td>-allele 2</td>
<td>+allele D</td>
<td>14</td>
<td>1.08 ± 0.38</td>
</tr>
<tr>
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<td>80</td>
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</tr>
<tr>
<td>-allele D</td>
<td>+allele D</td>
<td>100</td>
<td>1.20 ± 0.28</td>
</tr>
<tr>
<td>+allele4</td>
<td>II</td>
<td>18</td>
<td>1.28 ± 0.27</td>
</tr>
<tr>
<td>-allele4</td>
<td>+allele D</td>
<td>21</td>
<td>1.35 ± 0.26</td>
</tr>
<tr>
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<td></td>
<td>0.002</td>
<td>0.005</td>
</tr>
</tbody>
</table>

**Conclusions:** In this sample, haplotype analysis of the apo E and apo B polymorphisms emphasizes the importance of combined and additive effect of more genes in plasma lipid parameters determination.

**ThP9-W30**

**Fluorescence based detection of the CETP TaqIB polymorphism: Identification of a new C77ST polymorphism in intron 1 by melting curve analysis**

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**Objective:** Cholesterol ester transfer protein (CETP) plays a crucial role in reverse cholesterol transport. It facilitates the transfer of cholesteryl esters from HDL to apoB-containing lipoproteins. The TaqIB polymorphism of the CETP gene has an influence on plasma CETP and HDL levels and the progression of CAD. The aim of the present study was to determine the performance of two new fluorescence based detection systems in the analysis of the TaqIB genotype in CAD patients.

**Methods:** The TaqIB genotype was determined by RFLP analysis using TaqI. A total of 150 patients, 50 patients each carrying the B1/B1, B1/B2 and B2/B2 genotypes were selected. Subsequently, the genotype was also analyzed by fluorescence based detection assays established in our laboratory with the SDS 7700 (TaqMan) and the LightCycler. DNA sequencing was performed with a ABI PRISM 377 sequence.

**Results:** The TaqIB genotypes obtained with the fluorescence assays corresponded to those of the RFLP analysis with the exception of three samples from heterozygous patients (B1/B2), that were misclassified as homozygous for the B2 allele with the TaqMan system. Melting curve analysis of these samples in the LightCycler revealed a distinct melting pattern with an additional melting point at 50°C. The same melting point also occurred in samples from four patients homozygous for the B1 allele. DNA-sequencing revealed a new, previously unknown C77ST nucleotide exchange in intron 1 of the CETP gene just 8 basepairs apart from the TaqIB nucleotide exchange.

**Conclusion:** Determination of the TaqIB polymorphism in the Sds 7700 resulted in unexpected misclassifications due to a previously unknown C77ST polymorphism of the CETP gene. However, the new polymorphism could be detected with the LightCycler due to the occurrence of a different melting point. Ongoing studies indicate, that the C77ST polymorphism may be an additional risk factor for the progression of CAD.

**ThP10-W30**

**A common hepatic lipase gene promoter variant determines clinical response to intensive lipid lowering treatment**

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Our understanding is limited as to why lipid and coronary artery disease (CAD) response to lipid-lowering therapy varies among different subjects. The common −514 C→T promoter polymorphism of the hepatic lipase (HL) gene affects HL activity. The C allele is associated with higher HL activity and a more atherogenic lipid profile: more dense LDL particles and lower levels of HDL2-cholesterol. Intensive lipid-lowering therapy lowers HL activity, increases LDL and HDL buoyancy and promotes CAD regression. We tested the hypothesis that subjects who experience the greatest CAD regression from these favorable effects are those with the CC genotype, associated with a more atherogenic lipid profile.

Forty middle-aged men with dyslipidemia and established CAD, undergoing intensive lipid-lowering therapy, were selected for the study of the influence of the HL gene promoter polymorphism on changes in HL activity, LDL buoyancy and lipoprotein levels and its association with CAD regression. The change in coronary stenosis was assessed by repeat quantitative angiography, the HL polymorphism by PCR amplification, HL activity by the use of 14C-labeled substrate and LDL buoyancy by plasma density gradient ultracentrifugation. Subjects with the CC genotype had the greatest decrease in HL activity (p < 0.005 vs. TC and TT by ANOVA) and the greatest improvement in LDL density (p < 0.005) and HDL-C (p < 0.05) with therapy. These subjects had the greatest CAD angiographic improvement, with 95% of them experiencing regression of coronary stenosis, while 63% of TC and none of the TT patients had disease regression (χ2 = 16.43; p < 0.001).

In patients with established CAD and dyslipidemia, the HL gene −514 C→T polymorphism predicts 16% of the change in coronary stenosis with lipid lowering treatment. This association appears to be accounted for by the modulating effect of this polymorphism on HDL-associated steps in LDL and HDL metabolism. This study identifies a gene polymorphism that strongly influences the lipid and clinical response to lipid-lowering drugs.

**ThP11-W30**

**Use of SNP technology to define disease-bearing haplotypes of the apo AI-ChI-AIV gene region in familial combined hyperlipidemia**

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In previous studies we have reported linkage and association in the apo AI-ChI-AIV gene region on chromosome 11 with Familial Combined Hyperlipidemia (FCHL). Using sequence analysis we identified five new SNP variants in this gene region, a C317T variant in intron 2 of the apoAI gene and four variants in the intragenic region between apo AI and apo CI, T3213C, A3235C, T3287C and A5132C. Haplotypes consisting of 7 SNPs, including MspI in promoter of apo AI gene and SstI in non-coding region of apo CIH, were assigned using parsimony of inheritance in 33 FCHL families. The distribution of these haplotypes was significantly different between the probands (n = 33) and spouse controls (n = 230) (p = 0.0005). Three major haplotypes were observed in both groups. The haplotype with only wild type alleles was significantly less frequent in probands (0.46 vs 0.62, p = 0.013), whereas the haplotype with the M1, 317T, 3213T, 3235C, 3287C, 5232C and S2 alleles had a frequency of 15% in probands vs 4% in spouse controls (p = 0.0005). Also the third haplotype consisting of the M2, 317T, 3213C, 3225A, 3287C, 5232C and S1 alleles was significantly more frequent in probands (26% vs 11%, p = 0.001). The MspI marker and T3213C variant were in complete linkage disequilibrium and are exclusively present on the same haplotype. Similarly the S2 allele and the A3235C variant reside on one haplotype. The C317T, T3287C and A5132C markers occurred in both haplotypes.

In conclusion, successful application of SNP technology provides evidence that two truly distinct haplotypes independently predispose to FCHL and they account together with the wild type for 90% of the occurring haplotypes in these FCHL families.

**ThP12-W30**

**Identification of novel cardiovascular susceptibility genes by mouse genetics and gene expression profiling**

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In order to identify new genes involved in the development of hyperlipidemia and atherosclerosis, we use transgenic APOE3-Leiden (E3L) mice. In contrast to non-transgenic mice, these E3L mice have high lipid levels due to a defect in the uptake of remnant lipoproteins from the plasma, allowing the analysis of genetic factors involved in VLDL metabolism. We use two different, complementary approaches.

1) **Genetic mapping:** We tested female F1 hybrids and backcross mice between strain E3L and 7 inbred strains (CBA, C3H, NZB, FVB, 129, BALB/c, AKR) for their lipid levels and susceptibility to atherosclerosis to look for the presence of dominant and recessive modifiers, after feeding several diets. Notably, FVB carries modifiers that increase the levels of cholesteryl as well triglycerides on all diets and also influence the susceptibility to atherosclerosis. These modifiers are currently being mapped in (E3L×FVB)F2 crosses.
(2) Gene expression profiling using microarray to identify genes that are differentially regulated under different (genetic or dietary) conditions. Of special interest are genes that are differentially expressed (a) during over-expression of APOE b in E3 mice on different dietary diets (c) in (FVB x E3L) backcross mice with high lipid levels compared to backcross mice with low lipid levels. Initial experiments showed that among 26 differentially expressed genes, 5 were absent or downregulated in backcross mice with low cholesterol levels compared to backcross mice with high cholesterol levels, whereas 21 were upregulated. Combining the two genetic approaches will facilitate the identification of novel (candidate) genes and pathways involved in lipid metabolism and atherosclerosis.

**ThP13-W3O**
Identification of (dietary response) genes involved in lipid metabolism and susceptibility to atherosclerosis in mice

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Serial Analysis of Gene Expression (SAGE) is used to identify new genes involved in lipid metabolism and susceptibility to atherosclerosis. As a model system we use APOE3-LEiden (E3L) transgenic mice on a C57BL/6 background. These mice show a profound diet-induced hyperlipidemia and are susceptible to the cholesterol-raising factor cafestol. SAGE allows a qualitative and quantitative analysis of thousands of transcripts simultaneously. Currently the first liver expression profiles have been obtained from the E3L mice and of C57BL/6 on standard chow. Over 11,000 transcripts have been analyzed of both mice. These 11,000 transcriptions represent 4000 different genes. Of these 4000 different genes, about 100 genes were differentially expressed. Forty-one percent of these genes are significantly upregulated in E3L mice, while forty-two percent are downregulated and seventeen percent are completely absent in E3L compared to C57BL/6. The differences in expression levels found are currently being validated using Northern blot analysis. High abundant genes are involved in several metabolic processes such as the synthesis of lipoprotein particles, glycolysis and gluconeogenesis and detoxification processes. Currently two more SAGE expression profiles are being generated to find genes responding to dietary factors e.g. E3L on moderate high fat diet and E3L on moderate high fat diet containing cafestol.

**ThP14-W3O**
Characterisation of apolipoprotein E sendai, an apolipoprotein E variant associated with lipoprotein glomerulopathy

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It has been well-known that nephrotic syndrome and chronic renal failure are associated with lipid and lipoprotein abnormalities. It has only recently become obvious that structural variants of apolipoprotein E may be responsible for the development of a new glomerular disease, lipoprotein glomerulopathy (LGP). A major hallmark of this disease is the deposition of lipoproteins and apolipoproteins in the mesangium. In addition several patients show a lipid profile reminiscent of type III hyperlipoproteinemia. The aim of our study was to obtain a more precise insight into the molecular mechanisms linking apoE to the pathogenesis of LGP. Purification of apoE from a single patient’s plasma is hardly feasible, especially because all carriers of apoE Sendai are heterozygous for this variation. Thus, we introduced the Arg145->Pro exchange into the wild type apoE3 cDNA using site-specific mutagenesis. ApoE2, apoE3 and apoE Sendai were expressed in the baculo virus system. LDL receptor binding was studied using recombinant apoE-complexed with phospholipid particles and with VLDL from a patient with familiar apoE deficiency. We examined cellular binding, uptake and degradation of these lipoproteins in human fibroblasts. ApoE2 and apoE Sendai displayed clearly reduced receptor binding activity compared to apoE3. Heparin binding of apoE Sendai was lower compared to apoE3 but higher than that of apoE2. Further studies will have to address the question in which way apoE Sendai lead to LGP. Taking into account that several carriers of apo E Sendai without any LGP symptoms exist, it is likely that renal factors cooperate with apoE variants in the development of lipoprotein glomerulopathy.

**ThP15-W3O**
A373P/R451Q CETP mutations, HDL cholesterol, and risk of ischemic heart disease. The Copenhagen city heart study

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**Objective:** We studied two common mutations in cholesteryl ester transfer protein (CETP) gene, A373P and R451Q; CETP mediates transfer of cholesteryl ester from high-density lipoprotein (HDL) in exchange for triglycerides in apolipoprotein B containing lipoproteins.

**Methods:** We genotyped 8467 healthy women and men from the Danish general population, and 1636 Danish women and men with ischemic heart disease.

**Results:** The prevalence of heterozygous carriers of 373P and 451Q was 0.10 and 0.07, and of homozygous carriers 0.003 and 0.002, respectively. All carriers of the 451Q allele also carried the 373P allele. Plasma levels of HDL cholesterol in women (mean ± SE in mmol/L) were 1.74 ± 0.01 for non-carriers of 373P, 1.62 ± 0.02 for heterozygous carriers, and 1.38 ± 0.09 for homozygous carriers (ANOVA: P < 0.001). In men, the equivalent values were 1.40 ± 0.01, 1.26 ± 0.02, and 1.19 ± 0.09 (ANOVA: P < 0.001). When we adjust for age alone, or a group of risk factors, CETP genotype did not predict risk of ischemic heart disease. When adjusting for a group of risk factors plus HDL cholesterol, women not treated with hormonal replacement therapy carrying the 373P allele had a decreased risk of ischemic heart disease (odds ratio: 0.64; 95%CI: 0.43–0.96), whereas the risk of ischemic heart disease in male or treated female carriers was statistically unaffected.

**Conclusions:** The A373P/R451Q polymorphism in CETP is associated with decreases in HDL cholesterol of 0.12–0.36 and 0.14–0.21 mmol/L in women and men, and possibly with a paradoxical 36% decrease in risk of ischemic heart disease in women.

**ThP16-W3O**
Familial recessive hypercholesterolemia (FH): Characterization of 10 homozygotes and 9 obligate heterozygotes

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**Objective:** We described an Italian family with several cases of hypercholesterolemia and sudden death. Probands had the clinical traits of homozygous familial hypercholesterolemia (FH), while parents were normal. The involvement of low density lipoprotein receptor (LDL-R) and apo B genes was excluded. LDL-R activity in fibroblasts, and LDL binding to LDL-receptor were normal in probands. We investigated the metabolic mechanisms underlying this new recessive disorder, and found a reduction in the in vivo LDL catabolism caused by a selective reduction in hepatic LDL uptake. We report 3 new Italian families, and describe the characteristics of 10 probands (5 M and 5 F), and 9 parents (5 M and 4 F) from 5 different pedigrees.

**Methods:** Plasma lipids were measured by standardized methods. LDL-R activity was determined by evaluating 125I-LDL internalization/degradation in cultured fibroblasts. LDL binding to LDL-R was measured in vitro by competition experiments in cultured fibroblasts.

**Results:** Probands (age: 13-47 yrs): all had tendonous/tuberous xanthomas, 4 had xanthelasmas. Coronary angiography was pathological in 6 out of 6. Lipoprotein parameters were (range, mg/dL): Tot Chol (450-642), LDL-C (372-592), HDL-C (28-58), Triglycerides (49-208), apo B (207-365), apo A-I (83-137), LDL-R activity in vitro was (range, % of normal): uptake (70-140), degradation (70-150). LDL binding activity in vitro was 84-100% of normal. Parents (age 51-83 yrs): 2 had type 2 diabetes, 2 had hyperension. Nobody had xanthomas or xanthelasmas. Lipoprotein parameters were (range, mg/dL): Tot Chol (172-237), LDL-C (103-153), HDL-C (40-70), Triglycerides (64-180), apo B (88-165), apo A-I (119-173).

**Conclusions:** 1) all FH homozygotes have the clinical and laboratory features of FH homozygotes despite having normal LDL-R activity in fibroblasts and normal LDL binding activity; 2) all parents (FRH obligate heterozygotes) show normal phenotype, supporting the hypothesis of an autosomal recessive transmission of this disease.
**ThP17:W30**

**A large deletion of ABC1 gene in a Japanese patient with Tangier disease**

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**Objective:** To determine defective type of ABC1 transporter gene in a Japanese patient with Tangier disease.

**Methods:** The patient is a 57 years old male and was diagnosed as coronary artery disease (LAD 90% stenosis) with heart failure. His clinical features are yellow tonsils, hepatomegaly, splenomegaly and CHOL 22 mg/dL, TG 88, HDL-C 4, APO A-I 3.2, A-II 3.5, B 40, C-I 0.1, C-II 0.7, E 1.3. His sister has a very low HDL-C of 2 mg/dL and a history of splenectomy. In order to determine whether the patient has a defect on ABC1 gene, we firstly examined each exon by PCR method. A long range PCR was performed to confirm the length of large deletion. Homozygosity was assessed by Southern blot.

**Results:** No PCR product was amplified in each of exon16, exon18, exon22 and exon30 in the patient. Using long range PCR, we examined ABC1 gene from exon14 to exon41 and confirmed a 26 kb deletion containing exon 16–exon30, which encodes 6th transmembrane region – linker region – 7th transmembrane region of the putative secondary structure. Southern blot using EcoR I digestion confirmed the patient is homozygous for the deletion by the probe of cDNA encoding exon14–exon41.

**Conclusion:** The large deletion of ABC1 gene is the cause of this patient with Tangier disease.

**ThP18:W30**

**Studies on strains of mice susceptible and resistant to cholesterol induced atherosclerosis**

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**Objective:** Strains of mice differ in their propensity to develop atherosclerosis when fed a cholesterol enriched diet. The aim of the study was to unravel the putative mechanisms responsible for these differences.

**Methods:** Female mice susceptible (C57Bl) or resistant (C3H and Balb/c) to diet-induced atherosclerosis were fed a chow containing atherogenic diet. Resident peritoneal macrophages were obtained by PBS lavage; elicited macrophages after thioglycollate injection. Cholesterol esterification was determined after exposure of cells to acylated LDL or βVLDL. Apolipoprotein E (apo E) was determined by Western blotting; apo E and scavenger receptor mRNA was determined by RT-PCR.

**Results:** In resident macrophages from all 3 strains, acLDL enhanced cholesterol esterification (CE) more than rabbit βVLDL. In elicited macrophages the extent of CE in presence of acylated LDL was lower in C57Bl strain and higher in presence of rabbit βVLDL than in C3H and Balb/c strains. In presence of acLDL higher amounts of apo E were recovered in the cultures in all 3 strains. When fed an atherogenic diet an increase in apo E mRNA occurred in Balb/c and in C3H macrophages. Scavenger receptor AI/II (SR-A/II) mRNA was higher in macrophages from C3H and Balb/c than from C57Bl.

**Conclusions:** It appears that increase in apo E and SR-A/II might contribute towards the resistance of C3H and Balb/c to diet-induced atherosclerosis.

**ThP19:W30**

**Population based screening for familial combined hyperlipidemia**

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**Objective:** To develop a population based method for screening of familial combined hyperlipidemia (FCH) in Iceland as an approach to primary prevention of ischemic heart disease (IHD) and genetic and epidemiological research of FCH.

**Methods:** For identification of index cases the Icelandic Heart Association population based database was used (approx. 53 thousand individuals). Index cases were identified by cholesterol level above 90th percentile and triglyceride level above 95th percentile for age and sex. A genetic tracing was performed in the Icelandic genealogy database (Decode Genetics). Pedigrees were identified for common ancestors, four generations back, and lists of descendants produced. Descendants with cholesterol or triglyceride or apoB levels above 90th percentile for age and sex were considered affected by FCH.

**Results:** Total number of index cases was 1045. Genealogical tracing resulted in 25 extended pedigrees. 726 individuals from the 16 most promising pedigrees are currently being recruited for examination. To date 396 individuals have been examined, 185 males and 211 females. 72 (39%) males are affected and 75 females (36%). This 40% detection rate is a 2-fold enrichment of the estimated frequency of individuals with cholesterol, triglyceride or apoB levels above 90th percentile for age and sex, which is 23% in the Icelandic population. Of those individuals affected, 18% were untreated at diagnosis.

**Conclusion:** Population based screening for FCH is feasible where genetic information is available. The large proportion of untreated FCH cases identified, emphasize the usefulness of this approach for both clinical purpose as well as for epidemiological and genetic studies of FCH.

**ThP20:W30**

**The role of a common variant in the CETP gene on size distribution of LDL subfractions**

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**Objective:** To study the influence of CETP genetic variability on plasma LDL particle size heterogeneity and on intima-media thickness (IMT) of the common carotid artery (CCA).

**Methods:** 240 healthy, 50-year old men were recruited after a population-based, randomized screening. The study population was divided into subgroups of individuals with high (>1.5 mmol/L) and low (<1.5 mmol/L) plasma triglyceride (TG) levels, respectively. LDL was characterized by a novel gradient gel electrophoresis method and CCA IMT was quantified by B-mode ultrasound. The CETP gene variability was analyzed by PCR and restriction length cleavage with the Taq1B enzyme within intron 1.

**Results:** In the subgroup of low TG subjects presence of the CETP Taq1B restriction site (B1) was associated with significantly lower plasma concentration of small LDL particles (215 mmol/L vs. 174 mmol/L vs. 172 mmol/L, p < 0.05). Furthermore, in this subgroup subjects homozygous for the Taq1B allele (B1B1) had significantly lower IMT of the CCA compared with subjects lacking this DNA variation (B2B2) (0.81 mm vs. 0.89 mm, p < 0.05).

In the subgroup of high TG levels, presence of the Taq1B allele was associated with higher VLDL triglyceride levels (1.65 mmol/L vs. 1.66 mmol/L vs. 2.20 mmol/L, p > 0.05), but had no significant influence on plasma LDL subtraction distribution, nor on CCA IMT.

**Conclusions:** This study suggests that the Taq1B polymorphism in the CETP gene is associated with changes in LDL subfraction distribution and is influenced by plasma TG levels. It is also suggested that subjects having a CETP-dependent genetic disposition for elevated small LDL particles show signs of increased CCA IMT.

**ThP21:W30**

**Apo e2 allele is a modulator of dysbetalipoproteinemia (type III) expression in individuals heterozygous for familial lipoprotein lipase deficiency**

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**Objective:** To examine the interactions of e2 allele with LPL gene mutations D9N (partial LPL activity deficiency in homozygotes) G118E & P207L (total LPL activity deficiency in homozygotes) on plasma lipoprotein profiles and expression of type III dyslipidemia.

**Method:** Studied individuals were divided into two groups: carriers of apo e3 allele (apoE 3/3 & 3/4) (n = 126) and carriers of apo e2 allele (apoE 2/2, 3/2 & 4/2) (n = 78). Within each apoE group, male and female heterozygote carriers of LPL gene mutations leading to total or partial LPL deficiency were compared.

**Results:** In both males and females carrying an e2 or e3 alleles, the total LPL deficiency further deteriorates the lipoprotein profile compared to partial deficiency. In individuals with apo e3 allele, the expression of type III dyslipidemia varied between 10% and 19% and was not statistically different between males and females as well as between the two types of LPL gene mutations. However, the frequency of type III expression in females with an e2 allele increased from 58 to 88% (p < 0.05) in partial versus total LPL deficiency. Finally, LPL deficiency (partial and total) induced the expression of type III in all apo E2/2 subjects.

**Conclusions:** The expression of dysbetalipoproteinemia in heterozygotes for both LPL gene mutation carrying an e2 allele indicates the importance of...
gene-gene interactions in the regulation of plasma lipoprotein metabolism, and that individuals with apo e2 allele and LPL deficiency are prone to potentially atherogenic dyslipoproteinemia.

Identification of novel missense variants in the coding region of peroxisome proliferator-activated receptor alpha in the patients with primary hyperlipidemia

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Objective: Peroxisome proliferator activated receptor (PPAR) α is activated by hypolipidemic drugs and is thought to play an important role in lipid metabolism. PPAR γ, also, may influence lipid levels as well as adipocyte differentiation. We studied variants of PPAR α and γ genes in primary hyperlipidemia.

Methods: Screens for mutations in the entire coding region of the PPAR α gene and PPAR γ 2 gene were performed by PCR-DGGE method. One hundred subjects with primary hyperlipidemia were investigated, and forty of them were unrelated patients with familial combined hyperlipidemia (FCHL).

Results: Two of the 40 FCHL subjects and one primary hyperlipidemic patients without familial history of hyperlipidemia had a missense mutation in the gene for PPAR α that resulted in the conversion of glycine to glutamic acid at position 395. In these families, G395E heterozygotes (n = 6) tend to have higher lipid levels (TC 264 ± 20, TG 134 ± 43, HDL-C 49 ± 11 mg/dl ± SD) than wild-type subjects (n = 6, TC 171 ± 36, TG 106 ± 33, HDL-C 50 ± 12). Gly395 is located in ligand-binding domain of PPAR α. One of the 40 FCHL subjects had a missense mutation in the gene for PPAR α that resulted in the conversion of asparagine to aspartate at position 140. Asp140 is located in DNA binding domain of PPAR α. No missense mutation was found in the gene for PPAR γ 2, but three silent polymorphisms were identified.

Conclusions: Variants of PPAR α gene may play important roles in lipid metabolism. This study suggests that PPAR α is one of candidate genes for dyslipidemia which can explain part of FCHL.

SR-BI gene polymorphism does not affect cholesterol absorption, but is related with serum HDL cholesterol levels and endogenous cholesterol synthesis

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Objective: The scavenger receptor class B type I (SR-BI), identified as the HDL receptor, is also involved in intestinal cholesterol absorption. This may suggest that SR-BI genotype modifies the effects of plant stanol esters, which lower intestinal cholesterol absorption.

Methods: 112 non-hypercholesterolemic subjects consumed for four weeks a low erucic acid rapeseed oil based margarine and shortening. For the next 8 weeks, the control group (n = 42) consumed the same products. The experimental groups were given similar products with in stand esters (3.8-4.0 g/day) (n = 70). Levels of campesterol and stanoloster were used as markers for intestinal cholesterol absorption and endogenous cholesterol synthesis.

Results: The frequency of the SR-BI-2 allele was 48.8% in the control and 50.7% in the experimental group. At the end of the control period, the SR-BI 1/2 & 2/2 subjects showed lower LDL cholesterol (P = 0.070) and cholesterol-standardized lathosterol (P = 0.002) concentrations compared to SR-BI-1/1 subjects. After plant stanol ester consumption, the observed LDL cholesterol reduction in SR-BI-1/2 & 2/2 subjects was −0.44 ± 0.34 mmol/l and −0.41 ± 0.31 mmol/l in SR-BI-1/1 subjects (P = 0.754). Also, changes in serum cholesterol-standardized campesterol and lathosterol concentrations were similar for both genotypes. Effects were adjusted for sex, age, and body mass index.

Conclusion: This polymorphic site in the SR-BI gene is not a functional polymorphism with respect to intestinal cholesterol absorption. The association of this SR-BI polymorphism with HDL cholesterol concentrations and endogenous cholesterol synthesis, however, deserves further study.

The influence of mutations in apolipoprotein c3, lipoprotein lipase and hepatic lipase on the expression of type III hyperlipoproteinemia

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Objective: Type III hyperlipoproteinemia (HLP) is a multifactorial metabolic disorder that is mostly associated with homozygosity for apolipoprotein (apo) E2 (Arg158 → Cys). Additional environmental and/or genetic factors are necessary for the expression of this disorder. In the present study, the genetic factors contributing to the expression of type III HLP were determined in a population of E2 homozygous subjects.

Methods and Results: DNA of 126 type III HLP patients (cholesterol ≥90th age- and sex-related percentiles) and 68 normalplidemic E2/2 subjects (controls) were screened for the APOC3 Serf, lipoprotein lipase (LPL) D9N, S447X and N291S, hepatic lipase (HL) V73M, L334F, T320I and -C480T and LDL receptor-related protein (LRP) C200T (exon 22) mutation. Some of the E2/2 subjects were family members. To adjust for the effect of family relationships, a new statistical analysis was designed that compares for each related individual the observed with the expected genotype frequency based on the Dutch population frequency. In patients, the analysis showed a significant difference between the observed and expected frequency of the APOC3 Serf (x^2 = 17.7, P < 0.001), LPL S447X (x^2 = 8.4, P < 0.01), HL V73M (x^2 = 6.9, P = 0.01) and HL -C480T (x^2 = 13.8, P < 0.001) mutation, whereas no differences were found in controls. 89 of 126 patients (71%) were carrier of one or more of these 5 mutations. Patients with the Serf polymorphism showed a more severe hyperlipidemia as compared to patients without this polymorphism (total cholesterol levels: 13.1 ± 5.6 vs. 10.02 ± 2.81 mmol/l, P < 0.05).

Conclusions: Our data indicate that mutations in genes that are involved in the lipolytic conversion of lipoproteins play an important role in the expression of type III HLP.

The apoprotein-a4-1/2 polymorphism does not affect the response of serum lipids to dietary cholesterol in humans

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Objective: We studied the effect of the APOA4-1/2 polymorphism on the response of serum lipids to a change in cholesterol intake.

Methods: We fed 33 subjects with the APOA4-1/1 genotype and 17 subjects with the APOA4-1/2 or 2/2 genotype two controlled diets, one supplying 140 mg/day of cholesterol and one supplying 950 mg/day. Each diet was fed for 29 days in cross-over design.

Results: The mean response of serum total, low-density lipoprotein and high-density lipoprotein cholesterol was similar in subjects with the APOA4-1/1 genotype and subjects with the APOA4-2 allele. The results of this study are in contrast with those of McCombs (1994) and Mata (1994), but agree with results of more recent research (Schafer, 1997; Jensen, 1997; Dardenne, 1997; Carmena-Ramon, 1998).

Conclusion: The APOA4-1/2 polymorphism does not affect the response of serum lipids to a change in cholesterol intake. Knowledge of the APOA4-1/2 polymorphism is probably not a useful tool for the identification of subjects who respond to a change in cholesterol intake.
Thursday June 29, 2000: Poster Abstracts
P-W30  Genetics of Lipoprotein Metabolism

ThP28-W30  Visceral obesity modulates the impact of the MTP - 493G/T polymorphism on plasma total and LDL-cholesterol levels
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Background: The dyslipidemic state of visceral obesity is characterised by increased plasma triglyceride (TG) levels, low HDL-cholesterol (C) concentrations and alterations in LDL composition and concentration. A functional - 493GT polymorphism detected in the promoter region of the microsomal triglyceride transfer protein (MTP) has been related to variations in LDL-C levels.

Objective: To study the effect of the MTP - 493G/T polymorphism on lipoprotein levels in visceral obesity.

Methods: A total of 178 men were assigned into two groups on the basis of their MTP - 493GT polymorphism: 1) 94 GG/HMZ and 2) 84 carriers of the T allele including 75 G/T HTZ and 9 T/T HNZ.

Results: The two genotype groups did not differ for age, BMI or visceral adipose tissue (AT) accumulation measured by computed tomography nor for their lipoprotein profile. Among non-obese, normoinsulinemic men with low levels of visceral AT, the T allele was associated with significantly lower plasma total and LDL-C levels (p = 0.002 and p = 0.01, respectively). However, this apparently protective effect of the T allele was lost in visceral obesity/insulin-resistant men.

Conclusion: These results suggest that visceral obesity modulates the impact of the MTP - 493G/T polymorphism on plasma total and LDL-C concentrations.

ThP29-W30  Effect of the -514T allele in the hepatic lipase gene on postheparin plasma hepatic lipase activity in Chinese subjects
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Objective: The common -514C to T polymorphism in the hepatic lipase (HL) gene has been shown to be associated with lower HL activity in Caucasians. We have examined the frequency of this polymorphism and its effect on postheparin plasma HL activity and lipids in 205 healthy Chinese subjects in Hong Kong.

Methods: The -514 polymorphism was assayed by polymerase chain reaction amplification and restriction enzyme digestion with NlaIII. Total lipolytic activity in postheparin plasma was measured using an emulsion of triolein and gum arabic as substrate. Hepatic lipase activity was determined as the activity of the salt-resistant lipase in the presence of 1 M NaCl.

Results: The frequency of the rare T allele was 0.361 and the genotype frequencies did not deviate significantly from those predicted by the Hardy-Weinberg equation. Subjects with the TT genotype had the lowest HL activity (TT 14.3 ± 5.8, CT 18.2 ± 7.8, CC 20.8 ± 8.2 μmol free fatty acid released/ml/h, p < 0.05). There were no significant differences in plasma total cholesterol, triglyceride and LDL cholesterol between the different genotypes. HDL cholesterol was significantly lower in those subjects with the CC genotype (TT 1.34 ± 0.40, CT 1.38 ± 0.37, CC 1.28 ± 0.34 mmol/l, p < 0.05).

Conclusion: The frequency of the rare T allele was higher in Chinese subjects than that reported in Caucasians and there were significant associations between the -514 polymorphism with HL activity and HDL cholesterol.

ThP30-W30  A-C (L334F) polymorphism of hepatic lipase gene is associated with plasma HDL-C and apolipoprotein AI in Chinese men
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Objective: To investigate the association of A-C (L334F) polymorphism with plasma lipids, lipoproteins and apolipoproteins in Chinese.

Methods: 583 Chinese including 345 men and 238 women aged over 40 were enrolled. A-C (L334F) polymorphism was determined by PCR amplification and restriction analysis. Plasma lipids and HDL-C were determined by routine techniques. Plasma apolipoproteins (apo) AI, AII, B100, CII, CIII and E were determined by radial immunodiffusion.

Results: A-C (L334F) polymorphism of hepatic lipase (HL) gene had an allele frequency of about 0.05 in Chinese, both men and women. When totally explored in the studied population, HDL-C was statistically different among the subjects with the three genotypes (P = 0.007). Plasma HDL-C levels were 62 ± 13, 54 ± 17 and 49 ± 14 mg/dl respectively in subjects with genotype CC (homozygote), AC (heterozygote) and AA (wild-type). When separately explored in women, no statistically significant difference of age, BMI, TG, TC; HDL-C; apo AI, apo AII, B100, CII, CIII and E was found among the wild-type, heterozygote and homozygote (P value ranging from 0.138 to 0.390). But in men, plasma HDL-C (P = 0.025) and apo AI (p = 0.048) were statistically different among the wild-type, heterozygote and homozygote subjects, which had plasma HDL-C of 45 ± 12, 50 ± 16 and 63 ± 12 mg/dl,
The hospital de Bellvitge atherosclerosis secondary preventive program. Main results and predictors of clinical course


Objective: To describe the results of the Hospital de Bellvitge Atherosclerosis Secondary Preventive Program (HBASPP)

Methods: Since January 1992 until December 1996, 882 patients with acute coronary artery disease (CAD) entered in the HBASPP 6-12 weeks after discharge from the hospital. Patients were treated according to a secondary preventive strategy in at least two occasions at the Lipid and Atherosclerosis Unit, during a mean follow-up period of 10.4 ± 3.8 months. A telephone interview was performed in 753 patients (636 men) after a mean follow-up since the first visit of 34 ± 16 months.

Results: Overall, lipid therapeutic goals were reached by more than 60% of patients. Smoking was given up by 29% of current smokers (15/51) and was resumed by 3% (15/461). Non-significant changes in blood pressure levels and body mass index were observed. After a mean follow-up of 33.7 ± 15.9 months, 41 patients (5.4%) had died (all causes) and 115 patients (15%) were hospitalized because of cardiovascular disease. To not met the goal of HDLc/LDLc ratio (HDLc/LDLc ≥ 0.27 or an increase ≥ 15%) was the main independent predictor of unfavorable clinical course (OR 2.1: 95% CI 1.1–4.03). The non-HDLc goal was the only additional independent predictor of clinical course.

Conclusions: The majority of patients admitted to the HBASPP achieve an adequate control of dyslipidemia. Patients with CAD who reach the HDLc/LDLc therapeutic goal have less than half the risk of hospitalization due to cardiovascular disease or death for any cause.

Passive smoking and atherosclerosis: The lesion is "daughter" of high carboxyhaemoglobin concentration

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Background: Acute exposure to passive smoking has been associated with cardiac and endothelial dysfunction unequivocally recognized as factors of atherosclerosis.

Objective: To assess whether there were parameters associated with both cardiac and endothelial dysfunction through a re-analysis of our previous studies.

Material: Two groups of subjects undergone different experimental procedures. 1. 9 healthy people (mean age: 30.5 ± 8.5 yrs), and 10 subjects with a previous myocardial infarction (mean age: 53.8 ± 8.5 yrs) underwent exercise stress testing twice: in a smoking environment (carbon monoxide concentration 30 to 35 ppm reached by a method of cigarette combustion described elsewhere) and then in the same atmosphere not polluted by cigarette smoking. 2. 11 subjects (mean age: 30.5 ± 7.3 yrs) assessed their endothelial function by ultrasonography and measuring the brachial-artery diameter under baseline condition, during reactive hyperemia, and after sublingual administration of nitroglycerin. All subjects had routine blood samples examination before and after smoking exposure as well as measuring carboxyhaemoglobin concentration. It was seen in a smoking exposure. Statistically increased carboxyhaemoglobin concentration in a smoking atmosphere was measured (mean: 2.4% ± 0.4 vs 1.2% ± 0.2 with a P < 0.01). It was markedly higher in those subjects who had a greater cardiac impairment. No change in other parameters was seen.

Conclusion: Atherosclerotic lesions could be "daughters" of chronic exposure to passive smoking and related to higher carboxyhaemoglobin concentrations due to exposure.
metric (Body Mass Index-BMI, arterial blood pressure-BP) and biochemical measurements: triglycerides (TG), HDL-cholesterol and glucose (gle). The prevalence of PS elements in the studied group: obesity (BMI > 30.0) – 15.7% (232 men); AH-17.7% (261 men); DM-1.6% (24 men); hyperglycaemia-2.0% (30 men); hypertriglyceridemia-17.4% (256 men); HDL-cholesterol concentrations below 35 mg/dl-9.1% (134 men). We observed statistically significant (p < 0.01) correlations (r) between BML and mean values of systolic BP (r = 0.22), diastolic BP (r = 0.23), TG (r = 0.23) and HDL-cholesterol (r = 0.14).

Most frequent elements of polymetabolic syndrome in the studied group are: AH, hypertriglyceridemia and obesity. In men with diagnosed obesity we found statistically significant higher values of BP and unprofitable shifts in lipid and carbohydrate metabolism parameters.

**ThP6.W31**  
A Mediterranean diet (MED) vs a low fat (LF) diet improves depression independent of cholesterol in coronary heart disease patients (CHD)

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A low saturated fat diet (LF) has failed to improve CHD outcomes in most randomised trials. LF may impact mood. Depression worsens the prognosis of patients with CHD independent of biological risk factors. CHD mortality for depression RR varies 1.94 to 3.5 over two years.

**Objective**: To compare the effect of a low fat diet to a Mediterranean diet on depression and cholesterol levels in patients with CHD.

**Method**: This is a randomised diet study of patients with CHD documented by coronary angiography. 64 patients were randomised to either a low fat (NCEP-Step II) or a high fat Mediterranean (>35% E, 50% MUFA). Patients were assessed for depressive symptoms at baseline and at three-month using the Beck Depression Inventory (BDI-II).

**Results**: Depression at baseline and 3 months was 33% and 20% (p = 0.603) for LF. For MED 32% and 5% (p < 0.01). Mean BDI at baseline and 3 months was 7.6 and 6.6 for LF (p = 0.343) and 7.8 and 4.6 for MED (p < 0.02). A 13% and 46% decrease respectively. Mean cholesterol at baseline for LF and MED are 4.57 mmol/L and 4.44 mmol/L, with no change at 3 months.

**Conclusions**: A MED but not a LF diet improves depression independent of cholesterol levels.

**ThP7.W31**  
Can the Australian food system cope with a Mediterranean diet?

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Mediterranean diet (MD) describes traditional dietary patterns bordering the Mediterranean Sea. Pasta, coarse breads, beans, nuts, seeds, olive oil, wine, and seasonal fruits and vegetables predominate. MD is sustained by cultural factors and is ecologically sustainable. The MD may be an appropriate strategy to combat coronary heart disease (CHD) in non-Mediterranean countries like Australia. Prescriptive interpretation of MD simply transfers lists of foods to other regions, without reference to climatic, agricultural or social factors. It may be difficult for the Australian food system to adopt MD at the present time. However, there are major changes in agriculture with 4000 hectares of new olive trees throughout Australia and projected production being 10,000 tonnes (t). Requirements (t per annum) of a Cretan type MD diet in Australia are:

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<tbody>
<tr>
<td>(13.6 ml pop)</td>
<td>(500 000 pop)</td>
<td>Current production</td>
<td></td>
</tr>
<tr>
<td>Fats/Oils</td>
<td>175 000 t</td>
<td>6 400 t</td>
<td>842 t olive oil</td>
</tr>
<tr>
<td>Meat/Seafood</td>
<td>300 000 t</td>
<td>11 000 t</td>
<td>(1 700 000 t beef)</td>
</tr>
<tr>
<td>Fruit</td>
<td>2 000 000 t</td>
<td>73 000 t</td>
<td>442 000 t orange</td>
</tr>
</tbody>
</table>

**Conclusion**: Current local food production is not adequate for widespread adoption of Cretan type MD for 1st or 2nd prevention of CHD. Within a decade MD may be feasible for 2nd prevention. But climate and geography may limit adequate production of these foods for adoption of the Cretan type MD for the entire population.

**ThP8.W31**  
Dietary misconceptions and missed opportunities to help patients lower coronary heart disease (CHD) risk

**D. Colquhoun**1, **A. Tonkin**2, **B. Schrapnell**2, **S. Somerset**3. 1 Core Research; 2 National Heart Foundation; 3 Griffith University, Brisbane, Australia

**Objective**: To assess community knowledge of blood cholesterol, diet, adherence to diet advice and to assess extent of screening and diet advice given by doctors.

**Method**: Two national telephone surveys were conducted throughout Australia of 1200 and 725 respondents. Stratified random sampling was used. Data were analysed according to gender, age, and geographical area. Data was weighted to reflect population density.

**Results**: There was high awareness of blood cholesterol as a risk factor (88.5%). But 45.1% did not know the safe upper limit. 32.4% had blood cholesterol measured in last 12 months but 33.5% had never had it measured by their doctor. Only 29.3% with high cholesterol were given diet advice by their doctor. 75.5% believed that diet had major impact on cholesterol. 74% correctly identified the need to lower saturated fats, but incorrectly 40% believed they needed to lower unsaturated fats. 19.7% found diet adherence difficult. Reasons for difficulty: no time (25.1%), need to give up favourite foods (45.7%), healthy food not readily available (23.2%), interferes with social activities (26.1%).

**Conclusions**: These data suggest high community awareness of blood cholesterol but dietary misconceptions still exist. Screening by doctors and diet advice remains unacceptably low. More public and medical education is required.

**ThP9.W31**  
Effects of short and long term cardiac rehabilitation on physical capacity, blood lipids, behavioral characteristics in men with ischemic heart disease

**J. Kukacka**, **J. Tylka**, **M. Kowalska**, **S. Rudnicki**, **K. Mazurek**, Clinic and Department of Cardiac Rehabilitation National Institute of Cardiology, Warsaw, Poland

The aim of the study was evaluation of short and long-term rehabilitation and its influence on physical capacity (PWc), blood lipids, recurrence of illness, behavioral characteristics and return to work of coronary patients.

**Material and Methods**: 70 men with IHD, after MI and by-pass surgery (x age 55.5 years) were divided into two groups: A – consist of 35 pts rehabilitated systemically (± 5 years), B – 35 pts, which were physical trained during 6 months and after than were occasionally treated. All of them were medically investigated when taking into account exercise test (ExT) and blood lipids profile.

Psychological investigation were made when taking into account type of behaviour, mood and psychosomatic disturbances in patients.

**Results**: After 5 years follow up rehabilitation, the patients from A group presented better PWc in form of duration (665 ± 526.7 s, p < 0.01) an loading (900 W/min vs 695.7 W/min, p < 0.001) of Ext. Blood lipids: Group A TChol 212 vs 201 mg/dL, HDL 45 vs 49 mg/dL p < 0.01, LDL 139 v 129 mg/dL, TG 155 vs 119 mg/dL (p = 0.001), % HDL 21 vs 25 p = 0.001. Group B TChol 224 vs 206 mg/dL (p < 0.01), HDL 43 vs 46 mg/dL, LDL 15 vs 133 mg/dL (p < 0.01), TG 158 vs 124 mg/dL (p = 0.01), % HDL 20 vs 24.9 p = 0.05). Psychical tension and sensibility to stress presented more subjects (68%) from group B in relation to 55% from group A. 77.1% of pts from A and 65.7% from B group returned to work.

**Conclusions**: (1) Systematic and supervised physical training have a good effect on physical capacity and blood lipids profile of coronary patients.

(2) Systematically trained patients presented better psychological profile and in higher per cent than others return to work.

**ThP10.W31**  
Is the number of coronary risk factors a predictor of the severity of coronary disease?

**A. Batalla**1, **G.L. Cabero**2, **J.J.R. Reguero**2, **S. Hervia**2, **S. Braga**2, **E. Bustillo**1, **A. Cortina**2. 1 Department of Cardiology; 2 Hospital de Cabanes (Gijon); 3 Hospital Central de Asturias Oviedo, Spain

**Purpose**: To determine whether the number of coronary risk factors is related to the severity of coronary disease in males under 50 years of age with coronary heart disease.

**Methods**: Consecutively, 230 male patients before 50 years of age (mean age 43 ± 5 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease, were prospectively studied. In the acute phase by
measures of a structured questionnaire the presence of smoking habits, hypertension, diabetes and dyslipidemia were determined. A physical exam and fasting analysis were also made. Due to clinical inestability or persistent myocardial ischemia 132 patients underwent a catheter catheterism. The number of coronary risk factors and the number of affected vessels were considered. Due to the small number of subjects in some of the groups, some of them were grouped together. Those subjects with no risk factor or only one were studied (Group I). Those who presented more than one risk factor were also studied (Group II). Those patients with normal coronary angiography and single-vessel disease were also grouped. For statistical analysis, the Chi-square test was applied.

Results:

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 26</td>
<td>n = 106</td>
<td></td>
</tr>
<tr>
<td>No multivessel</td>
<td>6 (23%)</td>
<td>7 (7%)</td>
<td>0.01</td>
</tr>
<tr>
<td>disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivessel</td>
<td>20 (77%)</td>
<td>99 (93%)</td>
<td></td>
</tr>
<tr>
<td>disease</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: Patients with early-onset coronary disease and with more than one coronary risk factor have a greater frequency of multi-vessel disease than those patients who present none or only one coronary risk factor.

ThP11:W31 Comparative study of the efficacy and tolerance of policosanol (10 mg/d) and lovastatin (20 mg/d) on patients with type II hypercholesterolemia and non insulin dependent diabetes mellitus


This randomized, double blind study was undertaken to compare the efficacy and tolerance of policosanol (10 mg/d) and lovastatin (20 mg/d) on patients with type II hypercholesterolemia and non insulin dependent diabetes mellitus. After 6 weeks on lipid lowering diet, 53 patients were randomized to receive under double-blind conditions, policosanol or lovastatin tablets that were taken o.d. for 8 weeks. Both groups were similar at randomization. Policosanol significantly (p < 0.001) lowered LDL-C (20.4%), total cholesterol (TC) (14.2%) and the ratio of LDL-C/HDL-C (23.7%). Lovastatin significantly (p < 0.01) lowered LDL-C (18.6%), TC (14%) and the ratio (p < 0.05) of LDL-C/HDL-C (14.9%). Policosanol, but not lovastatin, significantly increased (p < 0.01) levels of HDL-C (7.5%). Comparisons between groups showed that changes on HDL-C induced by policosanol were significantly larger than those induced by lovastatin (p < 0.01). Both treatments were safe and well tolerated. Lovastatin moderate but significantly (p < 0.05) increased aspartato alanino transaminase, alkaline phosphatase and creatinina phosphokinase levels. The frequency of AE in lovastatin group was higher (p < 0.01) than in policosanol group. It is concluded that policosanol administered at 10 mg/d shows advantages compared with lovastatin 20 mg/d regarding to the changes on HDL-C and a better safety and tolerability profile.

ThP12:W31 A dedicated clinic for management of hypercholesterolemia

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Objective: In our region the prevalence of hypercholesterolemia it’s about 38%. Among these subjects, 32% are treated, but only 9% experience adequate blood lipid reduction. Aim of our study was to evaluate the control of lipidic parameters before and a 6 months period of treatment in hyperlipidemic patients referred to a dedicated clinic. Our clinic is participating to a regional network for diagnosis and treatment of hyperlipidemia.

Methods and Results: We report data of the first 100 hyperlipidemic patients referred to our clinic. The baseline and after 6 months levels of lipidic parameters are showed in the tables.

Lipidic parameters | Levels at baseline (mg/dL) | Levels after 6 months (mg/dL) | Variation (%)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>271.7</td>
<td>192.6</td>
<td>-25.4</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>167.5</td>
<td>114.1</td>
<td>-31.9</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>212.8</td>
<td>174</td>
<td>-18.3</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>45.1</td>
<td>47</td>
<td>+4.4</td>
</tr>
</tbody>
</table>

The patients receiving only dietetic treatment obtained the following reduction TC: 10.8%, TG: 35.4%, LDL: 23.5%. Fifty-six patients underwent pharmacological therapy. We used statins to achieve LDL levels <140 mg/dL or <90 mg/dL respectively in primary or in secondary prevention. We did not registered increased levels of AST, AST and/or CPK during therapy. At baseline, 62.5% of patients showed moderate-severe hypercholesterolemia (TC ≥ 250 mg/dL) and 8% severe hypertriglyceridemia (T ≥ 500 mg/dL). After 6 months of treatment, 11.4% showed moderate-severe hypercholesterolemia and 4.5% severe hypertriglyceridemia.

Conclusions: The patients followed in our clinic showed a better control of lipidic parameters respect all treated hyperlipidemic subjects of our region. This is probably due to a better valuation of patients and to a better use of therapy in a dedicated clinic.

ThP13:W31 Plasma α-tocopherol is not the predictor of repeat revascularization in coronary artery disease patients

A.R. Tomaski, W. Jacheć, J. Wodniecki, B. Skrzep-Poloczek1, C. Wojciechowska1, E. Widera1, R. Rarnowski1, K. Szczurek-Katafalski, A. Węgiel, E. Borawska, I. Światańska, II Dept of Cardiology; 1 Dept of Biochemistry, Silesian Sch. of Medicine, Zabrze, Poland

Objective: The CHAOS study revealed that oral supplementation with α-tocopherol (Toc) is associated with significant reduction of non fatal myocardial infarction and cardiovascular death. We aimed to assess the relationship between plasma level of Toc and rate of repeat coronary revascularization (RCR) in a population of young coronary artery disease patients (aged <55 years).

Methods: 195 pts were enrolled into this retrospective study. Detailed history of previous coronary revascularization procedures (either bypass grafting or angioplasty), repeat revascularizations and their dates were obtained. Toc was measured in venous blood plasma using HPLC method.

Results: Toc levels ranged from 7 µg/mL to 41.85 µg/mL, with lower quartile 14.34, median 17.4, and upper quartile 21.44 µg/mL. The number of patients with subsequent RCR and number of patients with myocardial infarction (AMI) in follow-up are presented in table.

<table>
<thead>
<tr>
<th>RCR% (n)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>p (chi²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMI% (n)</td>
<td>7</td>
<td>14.3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

where 1 is the lowest and 4 is the highest quartile of Toc level. Additionally, Toc does not affect the RCR free survival time in Cox proportional hazard analysis.

Conclusion: Low Toc levels account for higher number of myocardial infarction, although Toc is not the predictor of repeat revascularization in coronary artery disease patients.

ThP14:W31 Role of age at onset of smoking on peripheral artery disease development


The potential effects of age at onset of smoking on cardiovascular diseases (CVD) have been studied little, in contrast to the mounting evidence supporting a causal role of cigarette smoking in these diseases.

Objective: To analyze the relationship between age at smoking onset and development of symptomatic peripheral arterial occlusive disease (PAOD).

Methods: Design: Case-control study. Setting: Urban neighborhood of Barcelona (Spain); Participants: A population-based sample of 573 active or former male smokers aged 55 to 74 years; Main outcome measure: Present or previous symptomatic PAOD confirmed by non-invasive tests.

Results: Sixty-one subjects (10.6%) had symptomatic PAOD. Prevalence of disease increased the earlier the starting age (15.6% if ≤ 16 years vs. 5.4% if > 16 years) of smoking. After controlling for risk factors that meet conventional factor criteria (i.e. subject age and number of pack-years), men who started smoking at 16 or earlier had a substantially higher risk of developing PAOD (odds ratio = 2.19; 95% confidence interval 1.15-4.15) than those who began later.

Conclusions: A starting age for smoking of 16 years or earlier more than doubles the risk of future symptomatic PAOD regardless of the amount of exposure to cigarette smoking.
ThP15:W31 Predicting individual risk of coronary heart disease (CHD) on an Italian sample of patients with hypercholesterolemia

L. Denis, A. Cecchetti, F. Merli, R. Benedetti, G. Pasolini, G. Valenti. Chair of Gerontology and Geriatrics - University of Parma, Parma, Italy

Objective: To assess the agreement between CHD prediction algorithm, developed from the Framingham risk data, and NCEP risk stratification system in selecting patients that deserve lipid-lowering drug.

Methods: 802 patients consecutively referred to an outpatient Center for metabolic diseases, which had been previously classified according to NCEP risk stratification system, have been re-evaluated for the assessment of their CHD risk, using the Framingham prediction algorithm. The model has been applied only to 573 patients, after exclusion of patients in secondary prevention or with excessively high triglyceride levels (>400 mg/dl). Patients in primary prevention with a CHD risk exceeding 20% were considered as deserving drug treatment. In addition, according to indications from the American Heart Association and American College of Cardiology, therapy was considered as justified also for some of the patients with a CHD risk of 10 to 20%.

Results: While NCEP-II risk stratification indicated the need of drug treatment for 65.7% of patients in primary prevention, using risk estimates based on Framingham algorithm, aggressive treatment was warranted for a consistently lower percentage of patients (34%). Contingency table showed that among patients needing drug treatment by NCEP-II, the need of intervention was confirmed for 43.7% only, using Framingham risk estimates; furthermore, for a sizable percentage (15.8%) of patients not deserving aggressive intervention by NCEP-II, therapy turned out to be warranted.

Conclusions: In the selection of patients who need drug treatment for hypercholesterolemia, changing the procedure for CHD risk assessment can consistently modify the risk-reduction strategy for individual patients.

ThP16:W31 Relationship of obesity distribution and peripheral arterial occlusive disease in elderly men


The potential association of obesity and its distribution with peripheral arterial occlusive disease has been studied little.

Objective: to examine the relationships between total body fatness and abdominal fat distribution with peripheral arterial disease.

Methods: Design: cross-sectional; Subjects: population-based sample of 708 men aged 55 to 74. Measurements: body mass index to estimate total body fatness and waist-to-hip ratio for abdominal fat distribution; peripheral arterial disease defined by ankle/brachial index <0.9; cardiovascular risk factors.

Results: Peripheral arterial disease was observed in 13.4% of subjects. Body mass index did not correlate with peripheral arterial disease, whereas an increased waist-to-hip ratio over 0.966 (median value) doubled the prevalence of arterial disease. After controlling for smoking, diabetes, hypertension, high-density lipoprotein cholesterol and triglycerides, increased waist-to-hip ratio was independently associated with peripheral arterial disease (odds ratio: 1.68; 95% confidence interval 1.05–2.70).

Conclusion: Abdominal fat distribution, but not total body fatness, is associated with peripheral arterial occlusive disease, independently of concurrent cardiovascular risk factors.


M. Rafaei, M. Boshnham, N. Sarraf-Zadegan, F.A. Sayed Tabahabaei, A. Jalali. Isfahan Cardiovascular Research Center, Isfahan, Iran

Objective: Cerebral artery diseases (CAD) are still considered as the first killer in Iran as well as other parts of the world.

Methods: In 1994, the 1st Isfahan CVD Risk Factor Survey aimed identifying the mean and prevalence of CAD risk factors on 2200 subjects randomly selected from Isfahan clusters, was carried out. The second one was conducted in 1998 on 1200 men and women of this population. In the two projects, the same questionnaire was completed for each subject. Also, fasting blood specimens were taken from each one and were analyzed by autoanalyzer ELAN 2000 for the lipids. The blood pressure was measured by random-zero sphygmomanometer after at least five minutes of rest from right arm in sitting position. Height and weight were measured by Seca scale in light cloths and without shoes.

Results: As the tables show, hypertension and hypercholesterolemia prevalence didn’t change during these 5 years. In this period, the prevalence of hypertriglyceridemia and obesity have increased while high LDL cholesterol and low HDL cholesterol have decreased. The mean values of triglycerides and HDL cholesterol have increased but the results for other factors in opposite.

Table 1. Trends in mean values of CAD risk factors

<table>
<thead>
<tr>
<th>Factors</th>
<th>1994 Mean ± SD</th>
<th>1998 Mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tcho (mg/dl)</td>
<td>197 ± 27.7</td>
<td>194 ± 30.5</td>
<td>0.04</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>134.1 ± 36.2</td>
<td>150.1 ± 36.8</td>
<td>0.00</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>124 ± 24.1</td>
<td>120 ± 24.7</td>
<td>0.00</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>40.3 ± 5.2</td>
<td>44.3 ± 7.8</td>
<td>0.00</td>
</tr>
<tr>
<td>BMI</td>
<td>23.5 ± 2.5</td>
<td>22.4 ± 2.2</td>
<td>0.00</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>126 ± 13.9</td>
<td>114 ± 10.0</td>
<td>0.00</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79.9 ± 8.4</td>
<td>74.3 ± 5.1</td>
<td>0.00</td>
</tr>
</tbody>
</table>

ThP18:W31 Vasomotor response to acetylcholine and its correlates with serum lipid levels in early postmenopausal women

T. Haraki1, K. Ueda1, K. O-c1, M. Noto1, H. Mabuchi2. 1Komatsu Municipal Hospital, 2The Second Department of Internal Medicine, Konazawa University, Komatsu, Japan

Objective: The incidence of cardiovascular disease (CVD) increases in women after menopause. We investigated the response of coronary artery to intracoronary injection of acetylcholine (Ach) in early postmenopausal women and age-matched men showing normal coronary angiogram.

Methods: Early postmenopausal women (n = 23, Mean ± SD age, 54 ± 3.8 years) and age-matched men (n = 15, 54.4 ± 6.1 years) without suffering from definite diabetes mellitus nor hypertension were enrolled. To evaluated the changes in coronary diameter in response to Ach (25 to 50 μg/ml), and followed by isosorbide dinitrate infusion, the luminal diameters in the segments of proximal and distal regions were determined by quantitatively with a computerized analysis system.

Results: Serum cholesterol levels (215 ± 39 mg/dl) in postmenopausal women were relatively higher than those (203 ± 37 mg/dl) in men. Then, was a dose-dependent vasoconstricting response of proximal and distal segments to Ach in both postmenopausal women and men. A vasomotor response in proximal segments to Ach was negatively correlated with age (p < 0.05), serum cholesterol (p < 0.05) and LDL-cholesterol levels (p < 0.05) in postmenopausal women, however, these correlation was not found in men. Serum cholesterol levels were increased more rapidly by age in postmenopausal women (p < 0.05) than in men.

Conclusions: Associations between the endothelial dysfunction of coronary arteries and serum cholesterol levels, and age in early postmenopausal women were stronger than those in men. These results could provide further evidence to support beneficial effects of lipid-lowering and hormone replacement therapy for CVD prevention in postmenopausal women.

ThP19:W31 Secondary prevention measures profoundly lower total mortality in high risk czech population

J. Pitha1, I. Podraskal1, P.H. Frost2, R. Polede1, R.J. Havell1. 1Institute for Clinical and Experimental Medicine, Prague, Czech Rep.; 2Cardiovascular Research Institute, University of California, San Francisco, USA

To evaluate the effects of an intensive secondary prevention program on mortality after myocardial infarction MI in a population not previously treated with cholesterol-lowering drugs, we enrolled all MI survivors under age 60 (men) or age 65 (women) admitted to a coronary care unit responsible for an entire district in Northern Bohemia during 18 months in 1992–93. All subjects were invited to participate in the program, which emphasized smoking
cessation and dietary/pharmacological control of hyperlipidemia, instituted before hospital discharge. More than 90% of eligible patients (70 men and 38 women) participated. In 1999, total mortality of survivors was ascertained (100% follow-up). The same data were obtained in 1999 for a comparable cohort of MI survivors (control group: 70 men and 33 women) at the same coronary care unit during the previous 18 months (1991–92). The principally modified risk factors in the intensive as compared with the control group were smoking and serum total cholesterol.

At entry, the intensive and control groups were comparable in average age (59.5 and 59.4 years), recent smoking (46 and 48%), and diabetes mellitus (30 and 30%). Body weight (86.1 and 81.9 kg; p < 0.05), total cholesterol (6.95 and 6.49 mEq/l; p < 0.05), and prevalence of hypertension (55 and 30%; p < 0.001) were higher in the intensively treated group. During follow-up the all-cause mortality per year was 2.3% in intensively treated and 4.2% in control patients (p < 0.05). The substantial reduction in all-cause mortality observed here suggests that increasingly effective application of secondary prevention measures has contributed to the progressive fall in total mortality in the Czech Republic during the 1990s.

**ThP20-W31** Selenium as an anti-risk factor of atherosclerosis in thyroid dysfunction

M. Ami1, M. Samsam-Shariati1, N. Sarraf-Zadegan2, G. Naderi2, S. Asgary2, 1Dept. Clinical Biochem., Isfahan Univ. of Med. Sci.; 2Isfahan Cardiovascular Research Center, Isfahan, Iran

**Objective:** To study the effect of selenium on plasma levels of lipid fractions and lipoprotein lipase (LPL) activity in hypo- and hyperthyroidism.

**Methods:** Male wistar rats were made hypo- or hyperthyroid by administration of methimazole or levothyroxine, respectively. Measuring serum T4 levels, 39% decrease in hypothryoid animals and 7 fold increase in hyperthyroid were observed. Selenium in the form of Na2SeO3 was injected (ip) for 30 days together with methimazole or levothyroxine.

**Results:** In methimazole treated group, LPL activity was decreased but plasma triglycerides, total cholesterol, VLDL, LDL and HDL increased. Selenium administration increased LPL activity and decreased triglycerides, cholesterol and lipoproteins. Liver phospholipids which were increased in hyperthyroid animals decreased following selenium treatment. No significant change in triglyceride content of liver was observed.

In hyperthyroid animals, LPL activity was increased but plasma levels of triglycerides, cholesterol and lipoproteins decreased. In this group selenium treatment led to a further increase in LPL activity. HDL-C was also increased in selenium treated animals which is reflected in the increase in total cholesterol. Liver phospholipids decreased in hyperthyroid condition which was reversed by selenium. Malondialdehyde (MDA), an index of lipid peroxidation, increased in hyperthyroidism but selenium in high doses decreased MDA markedly.

**Conclusion:** It is concluded that selenium not only could be considered as an antioxidant agent but also changes the pattern of plasma lipoprotein fractions in hypo- and hyperthyroidism in a way that is favourable to reducing the risk of atherosclerosis.


R. Kelishadi, M. Hushemi pour, N. Sarraf-Zadegan, M. Amiri Isfahan. Cardiovascular Research Center, Isfahan, Iran

**Objective:** To achieve the status and the trend of CHD risk factors in young population.

**Methods:** For the first time in Iran, the prevalence of major coronary heart disease (CHD) risk factors has been evaluated in 1993 on 4500 samples aged between 2–18 years selected randomly from different clusters of Isfahan city. The same study was carried out in 1999 and the prevalence of risk factors have been evaluated under standards of W.H.O.

**Results:** The serum total cholesterol, LDL-C and triglyceride were significantly higher in both sexes and in different groups in 1993 and 1999 studies (p < 0.05). Furthermore, a significant increase in serum lipid levels was shown during this period of time especially in teenage group. (p < 0.05). Although HDL-C was significantly lower than standard values (p < 0.05) in both studies in 6–18 years girls and boys, but there was no significant change in HDL-C value between 1993 and 1999 (p > 0.05).

There was a significant difference between the blood pressure percentiles and the prevalence of hypertension in our study and standard values in 1993 with no significant change in these values in 1999 (p > 0.05).

No case of diabetes mellitus was found randomly in 1993 and 1999 studies. Although the prevalence of obesity was low in these studies (0.2% in 1993 and 0.35% in 1999), a double fold rise was shown in the prevalence of overweight (4% in 1993 and 8% in 1999. P < 0.05) especially in school aged and adolescent girls.

**Conclusion:** Regarding the increase in the prevalence of hyperlipidemia and overweight in our children and adolescents and the increasing incidence of CHD among young people, special attention should be paid to primary prevention from childhood by both individualized and population approaches.

**ThP22-W31** Effect of exercise training for subjects with coronary risk factors on the oxidative susceptibility of LDL and serum

Y. Takamani1, T. Shimomitsu1, Y. Kawai1, Y. Kimura1, K. Shida1, Y. Iwasaki1, T. Katsumura2, 1Tokyo Medical University; 2Tokyo, Kansai Medical University, Osaka; 4Women’s University, Tokyo, Japan

Many studies have reported on the importance of regular physical exercise to improve the risk factors for atherosclerosis. Paradoxically, it has been pointed out that exercise may increase free radicals in the body, but lipid peroxidation may cause various health disorders including atherosclerosis. The purpose of this study was to clarify whether aerobic exercise training for subjects with coronary risk factors can modify the oxidative susceptibility of LDL and serum. We examined 42 subjects (15 males and 27 females; 55.4 ± 9.2 yr) who participated in the exercise training program which consisted of a 30 min aerobic exercise using treadmill or ergometer, twice a week for three months. We evaluated the lag time for the initiation of conjugated diene formation in LDL or serum exposed to CuSO4 in vitro, which was used as an index of oxidative susceptibility of LDL or serum. LDL-lag time prolonged, and α-tocopherol in LDL increased significantly after the exercise training (9.2% and 44%, respectively). Serum-lag time was extended following the exercise training (9.9%); however, no significant changes in serum α-tocopherol and uric acid, both of which are well-accepted antioxidants, were detected. Although we could not find a change in the serum HDL-cholesterol (HDL-C) on average, there was a correlation between the change in serum-lag time and the change in HDL-C (r = 0.419, p < 0.05). In conclusion, LDL and serum could become more resistant to oxidation by aerobic exercise training even in subjects with coronary risk factors. The change in the level of HDL, which is perceived to have antioxidative properties, may be partially relevant to the change in the oxidative resistance of the serum through the exercise training.

**ThP23-W31** The reduced oxidative susceptibility of LDL and serum following prolonged physical exercise is relevant to exercise-induced increase in antioxidants

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The purpose of this study was to elucidate the effects of prolonged exercise-induced oxidative stress on indices of susceptibility to atherosclerosis, such as the oxidative susceptibility of LDL and serum. We examined thirty-three male athletes (36.7 ± 9.1 yr) who participated in the 1997 Ironman Japan Triathlon race (average: 9 hrs 40 min). Blood samples were taken before and after the race. The lag time for the initiation of conjugated diene formation in diluted LDL (LDL-lag time) and serum (serum-lag time) exposed to CuSO4 in vitro was determined as an index of oxidative susceptibility of LDL and serum. We also measured antioxidant vitamins such as vitamin C and α-tocopherol. Both LDL-lag time and serum-lag time were significantly prolonged immediately after the race (+9.0% and +18.5%, respectively, p < 0.01), indicating that LDL and serum oxidation susceptibility were reduced through the prolonged bout of exercise. However, no relationship was observed between the change in LDL-lag time and the change in serum-lag time following the race. A significant increase in serum-α-tocopherol (+20.7%, p < 0.01), and a significant correlation between the change in serum-α-tocopherol and the change in LDL-lag time were observed immediately after the race. Similarly, there was a significant increase in serum vitamin C (+20.1%, p < 0.001), and this change in serum vitamin C was significantly correlated with the change in serum-lag time immediately after the race. We conclude that the susceptibility of LDL to oxidation might be reduced in relation to an increase in serum-α-tocopherol, and total serum susceptibility to oxidation could be reduced in connection with an increase in serum vitamin C after a single bout of prolonged exercise as an acute phase defense against exercise-induced oxidative stress.
Antiphospholipid syndrome is not an independent risk factor for acute stroke

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Objectives: The relevance of antiphospholipid-antibodies (APab), β2-glycoprotein-antibodies (β2GParb), lupus anticoagulant (LA), factor V G1691A and factor II G20210A as acquired or hereditary risk factors for the development of stroke still remains unclear.

Methods: In a case control study, we examined 230 consecutive patients admitted to our hospital with acute stroke (68% males; mean age: 55 years and 230 age and sex-matched controls for these five parameters. The diagnosis of stroke was confirmed by CT or MRT. Blood was drawn 1–10 days after the acute event. The control group of 230 healthy volunteers was recruited from the local blood donor center.

Results: CAab were found in 10 patients (4.3%) vs. 2 controls (p = 0.036). 6 patients had β2GParb compared to only one in the control group (p = 0.22). In 2 patients both antibody-types were found. Only one of these patients with detected antibodies was younger than 45y. All controls with positive antibody-screening were older than 45y. The number of heterozygotes for factor V G1691A (14 [% = 6.1%] vs 12 [% = 5.2%]) and factor II G20210A (9 [% = 3.9%] vs 9 [% = 3.9%]) detected in both groups was not significantly different. All patients had additional independent risk factors for stroke (hypertension, obesity, smoking, diabetes).

Conclusion: We did not find any significant association between the incidence of stroke and the presence of Factor V Leiden and Prothrombin G20210A polymorphism. Although CAab were significantly more frequently found in older stroke patients than in the healthy controls, the antiphospholipid syndrome was not an independent risk factor for the development of stroke.

Assessment of coronary heart disease risk prediction in general practice—which table?

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Objective: To compare the relative accuracy of published risk assessment methods based on the Framingham equation.

Methods: Clinical and laboratory risk factor data from 691 patients attending 12 general practices was used for coronary heart disease (CHD) risk estimation using all eight of the published tables/charts based on the Framingham equation. CHD risks were also calculated using the same risk factor data by the full Framingham equation programmed onto the laboratory computer. The sensitivity and specificity of each table to predict CHD risk was calculated compared to the computer calculated risk.

Results: At equivalent risk levels, the original Sheffield tables and New Zealand tables have similar sensitivities (40.0% vs. 41.2%) and specificities (98.6% vs. 98.8%). Modification of the Sheffield tables to include HDL cholesterol improve sensitivity (91.4%) but reduce specificity (95.8%). The Joint British Guidelines charts have high specificity (99.7%) and moderate sensitivity (69.5%).

Conclusions: If tables are to be used for risk prediction the Joint British Guidelines perform the best, however computer calculation remains the most accurate method and is practical even in general practice.

Lipi-Goal: A treat-to-target study with atorvastatin in patients with hypercholesterolemia

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Objective: Lipi-Goal was a multicenter, open label, non-comparative treat-to-target study, that was conducted from March 1998 to May 1999. It has assessed the percentage of patients reaching 1992 EAS LDL-C targets with atorvastatin 10–80 mg/day in subjects with hypercholesterolemia, defined as LDL-C > 160 mg/dl after a 12-week step I diet.

Methods: Patients were treated towards the following LDL-C goals: ≤135 mg/dl in patients with atherosclerotic disease and/or CHD risk ≥40%/10 yrs; ≤155 mg/dl in all others. All patients started treatment with atorvastatin 10 mg/day for 6 weeks. The dose was doubled every 6 weeks, to 20, 40, 80 mg at weeks 12, 18, 24 respectively, if target levels were not reached.

Results: Of the 587 patients screened for participation, 468 were enrolled (80%), and 428 (84% male; 42% females; mean age 61 yrs) were considered for efficacy evaluation. 54% had atherosclerotic disease and/or CHD risk >40%/10 yrs. Dose titration was not needed in 302 (71%) patients, who reached target with 10 mg/day. At weeks 12, 18 and 24, 350 (82%), 350 (85%) and 371 (87%) patients, respectively, reached LDL-C goal. Atorvastatin was generally well tolerated.

Conclusion: Most patients at high risk for CHD reached LDL-C goals with atorvastatin 10–80 mg/day. 72% of patients reached target without the need of titration from 10 mg/day. This simplifies clinical management and should improve adherence to recommendations.

High prevalence of overweight and metabolic syndrome among 60 year old women and men in Stockholm, Sweden

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Objective: To study the cardiovascular risk factor pattern and the occurrence of the metabolic syndrome in 60 year old women and men in Stockholm, Sweden.

Methods: Every third woman and man in Stockholm County were invited to a baseline investigation in an epidemiological cohort study concerning risk factors for cardiovascular disease. The investigation included a physical examination, fasting blood samples and an extensive questionnaire. The metabolic syndrome was defined as having at least three of the criteria: Waist-hr-ratio (WHR) > 0.85 and ≥ 0.95, HDL-cholesterol < 1.1 and < 1.0 mmol/l, f-insulin > 11.7 and ≥ 13.5 mU/l in women and respectively, triglycerides ≥ 1.7 mmol/l, f-glucose ≥ 6.1 mmol/l, blood pressures diastolic ≥ 90 and systolic ≥ 140 mmHg.

Results: In total 4232 individuals participated (78%), 2193 women and 2039 men. The occurrence of cardiovascular risk factors are presented in the table. The metabolic syndrome was present among 15% and 30% of the women and men respectively. The most common combination of risk factors was central obesity plus hypertriglyceridemia plus hyperinsulinemia.

Plasma levels of total homocysteine are elevated in overweight children and adolescents


Objectives: Childhood and adolescent obesity, independently to adult weight, is proposed to predict, among a broad range of adverse health effects, atherosclerosis. Abundant epidemiologic evidence has now led to the wide acceptance that even mild to moderate elevation of plasma total homocysteine (tHcy) must be regarded as an independent risk factor for coronary, cerebral, and peripheral vascular disease. Aim of our study was to establish levels of tHcy in plasma of children and adolescents that lack the traditional risk factors.

Methods: Twenty four overweight children (14/24, 9–12 years, 13 females and 11 males) and adolescents (10/24, 12–14 years, 5 females and 5 males), defined as those with body mass index (BMI) greater than the 95th percentile on the growth charts from the National Center of Health Statistics, were eval-

uated for EDTA-plasma levels of tHCY, measured on the Abbott IMX analyzer using fluorescence polarization immunonassay technology, and compared to 14 age-, sex-, and BMI-matched, lean children and adolescents, defined as those with a BMI less than the 75th percentile. Subjects with hypothyroidism, anemia, renal disorders, diabetes or hyperlipidemia were excluded from the study.

**Results:** Plasma levels of tHCY were found to be significantly higher in overweight than in lean children and adolescents (8.4 ± 0.5 vs 7.7 ± 0.3 μmol/L, plasma levels of tHCY were found neither between children and adolescents in both overweight (7.8 ± 0.4 and 9.2 ± 1 μmol/L, p = 0.2 (NS), respectively) and lean [6.8 ± 0.9 and 7.4 ± 0.8 μmol/L, p = 0.8 (NS), respectively] groups, nor between females and males in both overweight [8.3 ± 0.5 and 8.4 ± 0.9 μmol/L, p = 0.9 (NS), respectively] and lean [6.6 ± 0.5 and 7.2 ± 0.5 μmol/L, p = 0.9 (NS), respectively] groups.

**Conclusions:** It is suggested that an unrelated to age and sex elevation of plasma tHCY is noticed in overweight children and adolescents and this may confer an independent risk for atherosclerosis.

**ThP29:W31 The effect of body weight change on the multiple risk factor (MRF) syndrome**

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**Objective:** Weight reduction is one of the most important factors to improve the condition of cardiovascular risk factors. In this longitudinal study, the effect of weight change in 5 years on the MRF syndrome was evaluated.

**Methods:** Subjects were apparently healthy Japanese males who visited our clinic to medical check up. We selected following clinically relevant markers as RFs and each markers were measured after an overnight fast: systolic/diastolic blood pressure (SBP, DBP), total cholesterol (TC), triglycerides (TG), HDL-cholesterol (HDL-C) and glucose (FFG). Their respective clinical thresholds were as follows; 1) elevated BP (SBP > 140 and/or DBP > 90), 2) TC > 220 mg/dl, 3) TG > 150 mg/dl, 4) HDL-C < 40, 5) FFG > 110 mg/dl. The measurement was performed in 1993 and re-performed in 1998. According to the results of the first measurement, we selected the subjects with at least 2 RFs as MRF syndrome (n = 167, age 45±5.3, BMI 23.0±2.6).

**Results:** The change of BMI (dBMI) during 5 years was 0.3 ± 1.4. The relationship between dBMI and dTC, dTG and dHDL were r = 0.3 (< P < 0.01), r = 0.48 (p < 0.01), r = -0.28 (p < 0.01), respectively. Subjects were divided into following 4 groups according to the change of RF in 5 years. G1; increase or unchanged the number of RFs, G2; decrease 1 RF, G3; decrease 2 RFs, G4; decrease 3 or more RFs. dBMI of each group were +0.8 ± 1.3, -0.1 ± 1.1, -0.6 ± 1.2, and 0.6 ± 0.6 (p < 0.05), respectively.

**Conclusions:** 1) Long term body weight change has a great impact on the condition of RFs. 2) In terms of the treatment strategy of MRF syndrome in our population, weight reduction is very practical method even if the change is a few.

**ThP30:W31 Comparison of schoolchildren with high or low risk lipid profile**


A random sample of 511 schoolchildren of Tallinn aged 11–15 years were studied on cardiovascular risk factors. Objective statuses of pupils, lifestyle risk factors, nutrition habits and pedigree data were registered. Serum cholesterol (C), triglycerides, HDL-C, apo A-I and B were determined, LDL-C was calculated. Three parallel high- and low-risk groups were composed basing on blood levels of LDL-C or apo B or apo A-I. Children having values of those parameters more than 85th percentile were considered being at high risk and those with the values lower than 15th percentile, at low risk. Special interest was to clear out in which high-risk group the prevalence of other cardiovascular risk factors is higher, i.e. which lipid parameter is the best risk indicator.

**Results:** In the high-risk groups composed by LDL-C level or apo B/apo A-I ratio, the mean number of myocardial infarctions and strokes in child I and II degree relatives was 2-fold higher than in responsible low-risk groups (P < 0.01). The difference in number of those diseases was not significant, if the serum apo B level was used as determinant of risk. In the group of children 10–12 years the number of atherosclerotic diseases in family history correlated positively with child apo B/A-I ratio and apo B level (P < 0.05), but in 13–15-year old children with LDL-C (P < 0.05). The characteristic feature of dietary pattern of all high-risk groups was significantly lower sucrose intake and higher food cholesterol content as compared to corresponding low-risk groups.

**Conclusion:** Expression of risk indices was rather similar in all high-risk groups. However, integrated indices, apo B/apo A-I and LDL-C, seemed to be somewhat better indicators to find out children with familial aggregation of atherosclerotic vascular diseases.

**ThP31:W31 Comparing treatment success with statins: Results from the atorvastatin comparative cholesterol efficacy and safety study (ACCESS)**

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**Background:** Studies suggest that many patients are not treated to National Cholesterol Education Program (NCEP) LDL-C goals. The ACCESS study evaluated the efficacy and safety of atorvastatin versus other statins and compared their ability to treat patients to NCEP goals.

**Methods:** At 154 US sites, patients (n = 3816) with or without CHD and/or peripheral vascular disease who met NCEP drug treatment criteria were randomized to either atorvastatin (10–80 mg), simvastatin (10–40 mg), pravastatin (10–40 mg), fluvastatin (20–80 mg) or lovastatin (20–80 mg). Patients were randomized in a 4 to 1 ratio (atorvastatin, n = 1958; simvastatin, n = 482; pravastatin, n = 481; fluvastatin, n = 497; lovastatin, n = 498) and received initial doses for 6 weeks. Thereafter, patients were titrated if they did not reach their NCEP LDL-C goal and were followed to 54 weeks. Primary efficacy parameters were the % of patients achieving NCEP goal at initial dose and % change from baseline in LDL-C at Week 6.

**Results:** The main findings after 6 weeks of treatment at starting doses are given in the Table. These differences were maintained at Week 54 (p < 0.0001, atorvastatin vs other statins).

<table>
<thead>
<tr>
<th>Week 6</th>
<th>Atorvastatin</th>
<th>Simvastatin</th>
<th>Pravastatin</th>
<th>Fluvastatin</th>
<th>Lovastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C reduction, %</td>
<td>36.1</td>
<td>29.5*</td>
<td>19.6*</td>
<td>26.7*</td>
<td>18.5*</td>
</tr>
<tr>
<td>Patients at NCEP goal, %</td>
<td>52.4</td>
<td>37.7*</td>
<td>13.4*</td>
<td>26.4*</td>
<td>14.6*</td>
</tr>
</tbody>
</table>

*p < 0.0001 vs atorvastatin

Treatment differences were maintained at Week 54. All statins were well tolerated and persistent ALT/AST elevations >3 × ULN were infrequent (<0.51%).

**Conclusion:** A significantly greater proportion of atorvastatin-treated patients achieved NCEP goals, both at starting dose and over the full dose range, compared with patients treated with the other statins tested.

**ThP32:W31 Primary prevention of atherosclerosis with long-acting garlic powder tablets Allcor**

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**Objective:** To assess the overall risk of coronary artery disease (CAD) and myocardial infarction (MI) in Moscow population and to explore the efficacy of garlic powder tablets Allcor administration for the prevention of atherosclerosis-related diseases.

**Methods:** The programs utilizing the multilogistic function for calculating statistical risk of MI and CHD have been used. The total group of examined Moscow inhabitants consisted of 1850 men, aged 40–64, free of severe arterial hypertension, diabetes mellitus and cardiovascular diseases at the baseline. In half of participants, the one-year treatment with Allcor (600 mg per day) was performed. After one-year follow up, the assessment of the overall risk was performed again in the same cohort.

**Results:** At the baseline, the 4-years risk of MI was almost by 40% higher than the statistical risk for age-matched subjects from PROCAM Study (p < 0.0001). The 10-years risk of CAD was by 15% higher than the statistical for age-matched subjects from Framingham Study (p < 0.0001). In men treated with Allcor, the estimated risk of MI as well as CAD was significantly reduced and became equal to the theoretical risk for men from Western population. In control group the further statistically significant increase in both estimated MI and CAD risks was observed. Additional data on direct antithrombotic effects of Allcor were obtained from limited clinical study with ultrasonic imaging of carotid arteries in men.
Conclusions: The results of this study demonstrated that Allicor (garlic powder tablets) is an effective drug for atherosclerosis primary prevention.

**ThP33:W31** Treatment goals achieved in patients at high risk for atherosclerotic disease treated in general practice

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Objective: To evaluate the achievement of the European treatment guidelines (total cholesterol <5 mmol/l and LDL cholesterol <3 mmol/l, blood pressure <140/90 mm Hg, in diabetes <130/85 mm Hg) in patients treated with a statin.

Methods: In 1999 a total of 3 935 patients treated with a statin were screened in 412 general practices in Norway for their blood lipids and blood pressure. The inclusion criterion was ongoing medication with a statin independent of the indication.

Results: Two-thirds of the patients were in secondary prevention. Before treatment there were high levels of total cholesterol (mean 7.9 mmol/l), LDL cholesterol (5.7 mmol/l) and triglycerides (2.4 mmol/l). In the total material 36% of the patients achieved the treatment goal of total cholesterol and LDL cholesterol. 14% had satisfactory values of total cholesterol, LDL cholesterol and blood pressure, in patients with diabetes only 7%. More patients in secondary prevention than in primary prevention achieved the treatment goal of blood lipids (44% vs 17%), more patients with than without diabetes (45% vs 34%), and more men than women (42% vs 27%). This was mainly due to differences in baseline levels of total cholesterol and LDL cholesterol and not to different statin doses. In the total material the combined goal of total cholesterol and LDL cholesterol was obtained in 38% with atorvastatin or simvastatin, 26% with pravastatin and even lower with the other statins.

Conclusions: Too low statin doses were used, especially in patients in primary prevention and in women, in whom the lipid profile often remained unfavourable. General practitioners in Norway have to intensify the lipid lowering therapy both in primary and secondary prevention.

**ThP34:W31** How is the diet in patients with atherosclerotic disease compared with the population?

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Objective: Primary to compare the quality of the diet in patients with atherosclerotic disease with the diet in the general population. Secondary to compare the patients’ lipid levels and fulfilled European treatment goals (total cholesterol <5 mmol/l and LDL cholesterol <3 mmol/l) and tertiary to observe the doctors’ judgement of the diet with the patients’ own characteristics.

Methods: In a survey in 363 general practices in the period 1997–1999 we examined the diet of 3 160 patients with established atherosclerosis and given a lipid lowering agent, and compared it with the diet of 1 009 persons interviewed in a Scan-Fast omnibus, representative for the general Norwegian population. A simple diet questionnaire was used for diet registration.

Results: Our queries showed that the patients seemed to have a more healthy diet than the general population as a greater proportion of the patients used skimmed milk, polyunsaturated margarine and vegetable oil, and they ate less cheese with high fat content and less fat meat compared to the other group. There were only small differences in the use of fish, fruit and vegetables. In both the patients and the population fewer women than men were smokers and the diet information indicated a lower saturated fat content and the use of more fruit and vegetables. In those patients who had a healthy diet the treatment goals for blood lipids were more easily achieved. Overall the treatment goals was satisfactory in only 40% of the men and 28% in women.

Conclusions: The patients had changed their diet habits, but the use of fruit and vegetables has to be encouraged. A more healthy diet in the patients reflected a better achievement of the treatment goals for blood lipids. The lipid lowering medication was unsatisfactorily.

**ThP35:W31** Apolipoprotein B-100 kinetics during treatment with carbamazepine – A prospective study in healthy males

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Objective: The antiepileptic drug carbamazepine (CBZ) has been shown to increase apolipoproteinB(a)apoB)-100-containing lipoproteins. In a prospective study, we investigated the effect of CBZ treatment on the kinetics of apoB metabolism in 13 healthy male volunteers (mean ± SD age: 27 ± 3 years, BMI: 23 ± 2 kg/m² with a normal baseline lipid profile. CBZ was given at 800 or 1200 mg/day for 69 ± 19 days and a mean serum concentration of 6.6 ± 0.6 μg/dl was achieved.

Methods: Metabolic parameters of apoB-containing lipoprotein fractions were determined twice, with and without treatment, using a primed, constant infusion of L-[3H]labellecine. Lipoprotein fractions were separated by sequential density ultracentrifugation. Isotopic enrichment was measured by gaschromatography mass spectrometry and kinetic parameters were estimated using a multicompartamental model.

Results: VLDL apoB increased by 29 ± 23% during treatment, IDL apoB by 33 ± 36% and LDL apoB by 13 ± 14% (all p < 0.03). The changes in the fractional catabolic rates of VLDL, IDL and LDL apoB showed marked individual differences. For VLDL apoB, r were > 3 ± 56%, > 5 ± 88% and > 9 ± 25%, respectively (n.s.), production rates changed by > 34 ± 87%, > 83 ± 154% and > 2 ± 46%, respectively (n.s.). Multiple regression analyses indicated that changes in LDL apoB were due to changes in IDL fractional catabolic rates.

Conclusion: We conclude that increased LDL apoB is not due to increased apoB production or decreased LDL catabolism but rather due to changes in conversion of IDL particles.

Supported by a grant of BMBF (01EC9402).

**ThP36:W31** Human cholesterol metabolism is modified independently by restriction of dietary energy versus dietary fat

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Objective: The study objective was to determine whether effects of energy restriction on circulating lipoprotein cholesterol levels and synthesis differ from those of reduced fat intake.

Methods: Thirteen hypercholesterolemic males (LDL > 3.6 mmol/l) participated in a randomized, crossover study. Subjects consumed 4 prepared diets, each for 4 wk containing either typical fat and energy (TFE), low fat but adequate energy (LE), low fat but reduced energy (LFE), or typical fat but reduced in energy through carbohydrate restriction (LE). A 6 wk washout period separated each diet. Energy restricted diets were 30% calorie-reduced.

Results: Body weights (BW) declined (p < 0.001) on LE and LFE diets. Total cholesterol (TC) decreased (p < 0.05) only for LF (7.9%) and LE (10.9%) diets. Diet-induced shifts in LDL-C were not observed, although high density lipoprotein cholesterol values declined (p < 0.05) during LF (13.8%) diet. Only LE (31.7%) and TFE (16.6%) diets reduced (p < 0.05) plasma triglyceride (TG). Cholesterolgenesis rates at 4 wk were lower (p < 0.05) for all diets compared with TFE. When subjects within the LFE group who failed to lose more than 1.8 kg BW over the feeding period were excluded from the analysis, declines in TC (8.2%) (p < 0.05) and TG (23.4%) (p < 0.05) relative to day 0 were observed.

Conclusion: These results demonstrate that energy and fat independently reduce TC by lowering cholesterol biosynthesis; however, the most favorable plasma lipid profile with both reduced TC and TG was achieved through energy restriction accompanied by weight loss, regardless of dietary fat level. Supported by Medical Research Council of Canada.

**ThP37:W31** Predictors of premature coronary artery disease

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Objective: To examine the lipid profile and cardiovascular risk factors related to premature coronary artery disease (CAD).

Methods: Eighty-nine patients with CAD (men <45, women <55 yr) were compared to 92 healthy controls. Lipid profile was obtained by automated enzymatic method after washout of hypolipidemic drugs and under phase I AHA diet. Risk factors were evaluated according to NCEP II guidelines.

Results:

<table>
<thead>
<tr>
<th>Group</th>
<th>CT</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>TG</th>
<th>Apo A</th>
<th>Apo B</th>
<th>Lip (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD+</td>
<td>237 ± 8*</td>
<td>38 ± 1*</td>
<td>154 ± 6*</td>
<td>200 ± 12*</td>
<td>131 ± 3*</td>
<td>123 ± 4*</td>
<td>39 ± 5</td>
</tr>
<tr>
<td>CAD−</td>
<td>213 ± 5</td>
<td>45 ± 2</td>
<td>142 ± 5</td>
<td>130 ± 11</td>
<td>142 ± 3</td>
<td>97 ± 3</td>
<td>32 ± 4</td>
</tr>
<tr>
<td>*CADs vs. CAD−, t-test, p &lt; 0.05: Lipid variables expressed in mg/dl, ± sem</td>
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</table>
ThP38:W31 Achieving greater event reduction – the need for more aggressive lipido-lowering therapy

W. V. Brown. For the Averstatin Versus Resvascularization Treatments (AVERT) Trial Investigators, USA

Background: Subgroup analysis of the AVERT study has been carried out to assess whether aggressive lipid-lowering treatment with atorvastatin to LDL-C levels <100 mg/dL (2.6 mmol/L) can provide additional clinical benefits, in terms of reducing ischemic events, compared with a more moderate treatment strategy.

Methods: 341 patients with stable coronary artery disease (CAD) referred for revascularization were randomized to 18 months of atorvastatin 80 mg/day (n = 164), or to undergo angioplasty followed by usual care (n = 177). The primary efficacy parameter was the incidence of ischemic events.

Results: The groups were well balanced at baseline. After 18 months, 6 (22%) of the 27 patients with LDL-C > 100 mg/dL treated with atorvastatin had an ischemic event vs 15 (11%) of the 135 patients with LDL-C ≤ 100 mg/dL. Moreover, event rates continued to decrease in atorvastatin-treated patients as LDL-C levels were reduced. Of patients achieving LDL-C levels >100 mg/dL (2.6 mmol/L), 75–100 mg/dL (1.9–2.6 mmol/L), and <75 mg/dL (1.9 mmol/L), 22%, 13% and 10%, respectively, had an ischemic event.

Conclusions: The incidence of ischemic events in the angioplasty/usual care group was unaffected by the degree of LDL-C reduction.

ThP39:W31 Estimation of nourishment way and physical training influence on changes of blood serum lipid indicators of pilots from group of increased atherosclerosis threat

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Objective: The aim of the work was estimation of the cholesterol (Ch), triglyceride (Tg) high-density lipoprotein (HDL) and low-density lipoprotein (LDL) fraction changes in the Polish pilots’ blood serum during staying 3 weeks in the special training camp.

Methods: Total of 127 pilots, from the group of increased atherosclerosis threat, was examined. The cholesterol levels exceeding 6.09 mmol/L and triglyceride content of 2.02 mmol/L was found during preliminary examinations of m/a pilots. During staying in the training camp pilots participated in specially planned physical workout including 8 hours’ schooling daily. Moreover they were fed special diet with food rations energy value of 17.19 ± 1.75 MJ (4236 ± 1.75 kcal). Used of that kind food rations delivered limited amount of animal fat and contained no pork. The vegetable fat was of main source of fat. Total of 10.5% of daily food ration energy came from polyunsaturated fatty acids. Huge amount of fruits and vegetables was used for pilots’ alimentation

Results: It was found that following parameters decreased in the blood serum: Ch from 8.31 ± 0.84 to 5.77 ± 0.78 mmol/L, Tg from 2.26 ± 0.26 to 1.55 ± 0.02 mmol/L and LDL from 3.86 ± 0.82 to 3.43 ± 0.86 mmol/L, while HDL value did not change. The most Ch level decrease was found in the following age groups: up to 30 and 31–40: Tg 31–40 and 41–50 but LDL value was most decreased in the groups: up to 30 and 31–40. Increase of HDL level in the age group 31–40 was found.

References:

ThP40:W31 The relationship of daily physical activity and serum lipid profiles in elderly patients with coronary heart disease

E. Seki, Y. Watanabe, Y. Iwama, H. Satoh, H. Daida, H. Yamaguchi. Department of Cardiology, Juntendo University, Tokyo, Japan

Objectives: Sedentary life style avoid the successful prevention of coronary heart disease (CHD). Due to the methodological difficulties of the quantitative measurement of daily physical activity, little is known about the effect of daily physical activity on serum lipid profile in elderly. In this study we used the Life Corder³ (Kenz, Nagoya, Japan), which is a pedometer that can memorize patient’s daily physical activity for up to 42 days semi quantitatively.

Methods: We analyzed 25 elderly (over 65 year-old) men with coronary heart disease who visit in our outpatient clinic. We measured the daily physical activity (mean step count/day (S), calculated mean energy of physical activity/day and calculated mean total energy expenditure/day (TE)), and exercise tolerance (peak VO2/kg, AT VO2/kg) using treadmill. Serum lipid profiles were analyzed enzymatically at least 12 hours fasting.

Results: The levels of serum total cholesterol was inversely correlated with TE: r = −0.47, P = 0.017. The levels of serum HDL cholesterol was significantly correlated with S: r = 0.45, p = 0.024. Triglyceride did not correlate with the parameter of daily physical activity. Exercise tolerance did not correlate with serum lipid profiles.

Conclusions: In elderly patients, daily physical activity was related to the serum lipid profile. This result suggested that improvement of daily physical activity could support the secondary prevention by improving the lipid profiles in elderly patients.

ThP41:W31 LDL-receptor mRNA in lymphocytes of normo- and hypercholesterolemic patients

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Objectives: LDL-receptor mRNA of lymphocytes was measured to determine differences between normo- and hypercholesterolemia subjects.

Methods: A qualitative RT-PCR for LDL-receptor mRNA was developed and applied to normo- and polygenic or familial hypercholesterolemia subjects.

Results: In polygenic, but not in familial hypercholesterolemia LDL cholesterol correlated negatively with LDL-receptor mRNA. Concentrations of LDL-receptor mRNA overlapped largely in polygenic and familial hypercholesterolemia. LDL-receptor mRNA was significantly lower in hyper- than in normocholesterolemia subjects. LDL-receptor mRNA continuously decreased up to an LDL-cholesterol of 100 mg/dL and was suppressed at higher cholesterol concentrations. Age, gender and medication or diet did not seem to affect the LDL-receptor mRNA in lymphocytes.

Conclusions: The LDL-receptor of lymphocytes is suppressed above an LDL-cholesterol of 100 mg/dL. Possibly this is the reason why lower levels have to be reached to stop atherogenesis. LDL-receptor defects do not lead to up-regulation of the LDL-receptor mRNA, so that this assay cannot serve as a diagnostic test.

ThP42:W31 Prevalence of smoking among CHD patients in the Asia-Pacific: the ASPAC study

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Objective: To determine the extent to which cigarette smoking is prevalent in those individuals developing CHD in the Asia-Pacific region.

Methods: The ASPAC study evaluated the measurement rates of smoking status, and the prevalence and treatment rates of smoking among 4,112 patients with CHD during 6 months follow-up.

Results: The rate of smoking assessment, prevalence and treatment are presented in the table.
**Conclusions:** A higher prevalence of smoking among men with CHD was found than in many Western countries, in Korea almost triple that in New Zealand. Female rates are considerably lower. In Indonesia no females were reported to be current smokers. Smoking status was mostly well documented. Generally, low levels of documentation of anti-smoking advice or treatment were found. A systematic approach to tobacco control policies and advice for patients is needed.

**ThP43:W31 Evaluation of a direct method to determine LDL-cholesterol levels**

A. Gayarré, E. Berlanga, F. Campos, F. Salceda, M. Roselló. Corporación Sanitaria Parc Tauli. Laboratory Department, Sabadell, Barcelona, SPAIN

**Objective:** In recent years, LDL-Cholesterol has become an important tool to assess an individual risk of developing Coronary heart disease (CHD), since a strong positive relationship between LDL-Cholesterol levels and the incidence of CHD was reported. The aim of this work is to evaluate a new direct method to determine LDL-Cholesterol serum levels, without any preparatory steps, to be used for routine testing.

**Methods:** We have evaluated this new method called LDL-plus (Roche Diagnostics), based in the selective micellar solubilization of LDL particles by a nonionic surfactant and the interaction of a sugar compound with other lipoproteins like VLDL and Chylomicrons. The evaluation has been performed in an Hitachi 717 Autoanalyzer, studying the within-run imprecision, using a commercial control (Precipath HLD/LDL) and processing it 26 times; between-day imprecision has also been studied, processing the same control every day during 20 consecutive days. We have evaluated the comparison between this method and the calculation of LDL-C by the Friedewald Formula, using 165 serum samples of patients of our Hospital, determining LDL-C levels.

**Results:** Within run: N = 26; x = 91.962; d.s = 3.206; C.V = 3.48 Between day: N = 20; x = 89.75; d.s = 2.8; C.V = 3.11

The correlation coefficient of Pearson between LDL-plus and Friedewald calculation was r = 0.986.

**Conclusion:** The obtained results show a very good analytical quality, being a good alternative to the reference method (Ultracentrifugation), to be used as a routine test in many clinical laboratories.

**ThP44:W31 Gender differences in plasma Lp A-I and Lp A-I-A-I levels in the heritage family study**

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**Objective:** To examine the differences in Lp A-I and Lp A-I-A-I levels between men and women and their potential associations with body fatness, abdominal adipose tissue (AT) accumulation and metabolic risk profile variables.

**Methods:** Fasting plasma Lp A-I and Lp A-I-A-I concentrations were assayed in a sample of 217 white men and 166 white women. Body fatness (underwater weighing), abdominal AT accumulation (computed tomography) and plasma lipoprotein- lipid profile variables were also measured.

**Results:** Women had higher Lp A-I (p < 0.0001) and lower Lp A-I-A-I (p < 0.05) concentrations compared to men. Significant negative correlations between adiposity, abdominal AT accumulation and Lp A-I levels were noted in women but not in men. Increased Lp A-I concentrations were not associated with a more favorable lipoprotein-lipid profile in either groups, with the exception of increased HDL-cholesterol levels (men = 0.47, p < 0.0001 and women; r = 0.73, p < 0.0001). Finally, the difference in Lp A-I-A-I levels between men and women was no longer significant after statistical adjustment for the well-known gender difference in HDL-cholesterol levels.

**Conclusions:** Our results suggest that the increased Lp A-I levels found in women compared to men are explained, at least to a certain extent, by their higher HDL-cholesterol concentrations compared to men.

**ThP45:W31 Smoking-associated deaths in Reykjavik; twice as many due to cardiovascular diseases as cancer**

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**Objective:** To assess the attributable risk associated with smoking with regard to all deaths and cardiovascular deaths in the Reykjavik Study.

**Methods:** We have studied the relative and absolute death rate of different causes in the prospective Reykjavik Study 1967–1997, in the smoking group compared to those who have never smoked. A stratified sample of the Reykjavik population, born 1907–1934, has been followed up for a mean of 19 years. Of 9326 men 3179 have died and of 10062 women 2255 are dead. The causes of deaths were verified by death certificate. The attributable (excess risk) deaths associated with smoking have been calculated from the observed relative risk (RR) in the smokers/never smokers and the current prevalence of smoking in Reykjavik. The RR was closely similar in univariate and multivariate analysis including other well-known risk factors.

**Results:** Excess deaths associated with smoking:

<table>
<thead>
<tr>
<th>Age groups (both sexes)</th>
<th>% of total deaths</th>
<th>% of coronary heart disease</th>
<th>% of other vascular diseases</th>
<th>% of all cancers</th>
<th>% of other causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;35 years</td>
<td>24</td>
<td>31</td>
<td>20</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>35-69 years</td>
<td>39</td>
<td>47</td>
<td>21</td>
<td>39</td>
<td>38</td>
</tr>
</tbody>
</table>

*Cerebral stroke, aortic aneurysm etc.

**Conclusions:** About half of all deaths associated with smoking was due to cardiovascular diseases, twice as many as all cancer deaths associated with smoking. Thirty-nine percent of all deaths in the age group 35–69 years were associated with smoking. Smoking is therefore one of the main health problems in Iceland.

**ThP46:W31 The effect of palmitic acid on endogenous synthesis of cholesterol and on lipoprotein cholesterol levels in humans after feeding low or high fat diets**

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**Objective:** To assess the effect of high versus low intakes of palmitic acid on plasma lipoprotein cholesterol levels and rate of endogenous synthesis of cholesterol in normal subjects.

**Methods:** Two experiments were conducted in which subjects were provided with diets containing a low (30% energy) or high (38% energy) fat level. In each experiment four diets were formulated to provide a high vs low 16:0 intake at a low vs high intake of 18:2n-6. Each diet was consumed for 21 days followed by a washout period. On day 21 of each diet treatment a fasting blood sample was drawn for lipoprotein determination and to provide a background measure of deuterium. A priming dose of D2O was consumed and a second blood sample was drawn 24 hrs later to measure the fractional synthesis rate for cholesterol after deuterium labeling.

**Results:** Total cholesterol and LDL cholesterol was not altered by a high dietary intake of 16:0 when the diet also contained a high level of 18:2n-6. At the low fat intake level a high intake of 16:0 increased LDL cholesterol levels when the diet was low in 18:2n-6. The dietary level of 16:0 did not alter the fractional synthesis rate of cholesterol.

**Conclusions:** 16:0 has no effect on lipoprotein cholesterol level in the presence of recommended intakes for 18:2n-6.

**ThP47:W31 The incidence of coronary artery disease and arterial hypertension in the variable types of genetic dyslipidemias**


The coexistence of arterial hypertension (AH) or other risk factors increase the incidence of coronary artery disease (CAD) in the genetic dyslipidemias. The purpose of this study was to evaluate the factors that play an important role at the appearance of CAD and AH in these dyslipidemias.
Method: We studied 941 patients (pts), without treatment for their dyslipidemia. 402 with familial combined hyperlipidemia (FCH), 386 with heterozygous familial hypercholesterolemia (fHfH) and 153 with familial hypertriglyceridemia (HTGL), mean age: 46.32 ± 8.76, 45.7 ± 10.4, 45.59 ± 10.50 years respectively, p = NS. Lipid plasma levels, thrombogenic factors [fibrinogen (FB) and lipoprotein a (Lp[a])] and somatomeric factors [body mass index (BMI) and waist to hip ratio (WHR)] were measured. We also evaluated the incidence of CAD, and AH.

Results: The incidence of CAD and AH is shown at the table.

<table>
<thead>
<tr>
<th></th>
<th>FCH</th>
<th>fHfH</th>
<th>HTGL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD</td>
<td>61/402 (15.17%)</td>
<td>84/386 (21.76%)</td>
<td>18/153 (11.76%)</td>
<td>0.031</td>
</tr>
<tr>
<td>AH</td>
<td>142/402 (35.32%)</td>
<td>50/386 (12.95%)</td>
<td>20/153 (12.98%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Logistic regression analysis using as dependent factor CAD showed that CAD depended of W/H (p = 0.0007, b = 8.075) and Lp(a) (p = 0.047, b = 0.007) in pts with FCH, of age (p = 0.006, b = 0.053) and WH/H (p = 0.0001, b = 8.47) in pts with fHfH and of W/H in p = 0.0019, b = 26.04) and of Lp(a) (p = 0.028, b = 0.028) in pts with HTGL. Logistic regression analysis using as dependent factor AH showed that AH depended of W/H (p = 0.0004, b = 5.57) in pts with FCH and of age (p = 0.0001, b = 0.110 and p = 0.0005, b = 0.1385) in pts with fHfH and HTGL respectively.

Conclusions: The incidence of CAD was higher in pts with fHfH and lower in pts with HTGL. The incidence of AH was much higher in FCH pts (35.32%) and lower in HTGL pts (12.95%). The incidence of CAD depends of W/H and Lp(a) in FCH and HTGL pts but not of Lp(a) in fHfH pts. AH depends of age in fHfH and HTGL pts and of W/H in FCH pts.

**ThP48-W31** Exercise training accelerates low-density lipoprotein catabolism as evaluated by a cholesterol-rich emulsion

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Objective: High physical fitness is associated with better plasma lipid profile. Here, the removal from plasma of a non-protein emulsion composed mainly of high-density oleate and phosphatidylcholine shown to bind to LDL receptors through apo E it acquires in the circulation was determined in exercise-trained men to evaluate the LDL catabolism status.

Methods: 18 cyclists from Bike Brazil Association (31 ± 9 yr, 4 times/week average training load) and 10 sedentary controls aged 37 ± 6 yr underwent a maximal cardiopulmonary exercise test on a bicycle ergometer to measure peak oxygen uptake (VO2peak). The emulsion labeled with 3H-cholesterol oleate was injected intravenously, and blood samples were collected at 5 min, 1, 2, 4, 6, 10 and 24 h to determine the plasma decay curve (expressed by fractional clearance rate (FCR) of the emulsion label).

Results:

<table>
<thead>
<tr>
<th></th>
<th>Cycles</th>
<th>Sedentary</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2peak (ml/kg/min)</td>
<td>56 ± 6</td>
<td>31 ± 4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FCR (h^-1)</td>
<td>0.175 ± 0.019</td>
<td>0.0975 ± 0.033</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol Total</td>
<td>177 ± 34</td>
<td>184 ± 14</td>
<td>0.42</td>
</tr>
<tr>
<td>(mg/dl) LDL</td>
<td>111 ± 27</td>
<td>124 ± 14</td>
<td>0.07</td>
</tr>
<tr>
<td>VLDL</td>
<td>48 ± 10</td>
<td>38 ± 7</td>
<td>0.04</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>18 ± 11</td>
<td>20 ± 6</td>
<td>0.60</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24 ± 2</td>
<td>24 ± 2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

FCR positively correlated with VO2peak (Spearman rank test, p = 0.03). Conclusions: Greater emulsion FCR in trained subjects indicates that exercise increases LDL catabolism, probably by increasing LDL receptor expression. Increased LDL removal may be beneficial, since LDL with greater turnover is conceivably less prone to pro-atherogenic changes.

**ThP49-W31** Influence of nutrition on lipid profile in adolescents with atherosclerosis risk factors

E. Rapacki1, M. Kozlowska-Wojcieszowska2, J. Baszczyński1, J. Kaczmarek3, B. Zbigniewa2.1. Dpt of Pediatrics; 2. Dpt of Informatics, M. Medical Academy, Lodz; 3. National Food and Nutrition Institute, Warsaw, Poland

Atherosclerosis and coronary heart disease (CHD) are two of the most important among the circulatory system diseases caused by improper nutrition. Atherosclerosis is a slow and latent process, which is not visible for many years, though it may start in childhood. Long period of the disease's development creates a possibility of halting the process or slowing down the formation of atherosclerotic lesions. The occurrence of the CHD at still earlier age makes conducting studies on the risk of atherosclerosis in the young necessary. Prevention of atherosclerosis in adolescents should be based on altering the nutritional habits through the modification of the regular diet, especially in the case of highcholesterolemia and obesity.

The study comprised adolescents aged 15–18, both sexes, with CHD or atherosclerosis risk factors mainly abnormal lipid profile. The study was dynamic and was carried out in two stages. The analysis of dietary habits showed abnormalities caused by excessive intake of fats, and especially of animal fats. After the results of the first stage had been obtained, the adolescents were given instructions modifying their dietary habits, according to the principles of proper nutrition. It should fulfil the 24-hours energetic demands which should be supplied by: 10–15% of proteins, 15–30% of fats (including 6–7% saturated and 8% polyunsaturated fatty acids) and 55–75% of carbohydrates.

After 10 months diet a tendency towards normalization of the level of lipids was observed. The obtained results demonstrate that modification of the dietary habits of adolescents is the environmental factor relatively easy to assess.

**ThP50-W31** Comparison of lipid profile and fibrinogen levels in male middle aged and elderly coronary patients and healthy individuals

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It has been suggested that patients who have sustained premature MI prior to the age of 55 have a more unfavorable lipid profile compared to elderly coronary patients aged 55–64 years. We compared lipid and fibrinogen levels in male middle aged (45–54 years) with male elderly coronary patients (65–74 years). We also compared healthy middle aged and elderly individuals. We used baseline determinations of the BIP study and healthy subjects from the THOS study.

<table>
<thead>
<tr>
<th>Patients with CAD</th>
<th>Healthy Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age 45–54</strong></td>
<td><strong>Age 65–74</strong></td>
</tr>
<tr>
<td>n Mean (SD)</td>
<td>n Mean (SD)</td>
</tr>
<tr>
<td>Ldl-cholesterol</td>
<td>29 ± 7</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>15 ± 8</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25 ± 2</td>
</tr>
</tbody>
</table>

Male elderly coronary patients had significantly lower levels of cholesterol, LDL, triglycerides, Apo B and higher in HLD than middle aged coronary patients. Male elderly healthy individuals had significantly higher cholesterol, LDL, ApoB, fibrinogen, HDL and Apo A1 levels than middle aged individuals. One has to take into account that the elderly patients may be a selective group, high risk lipid patients may die earlier.

**ThP51-W31** Prognosis of cardiovascular risk in dyslipidaemia patents

N. Doncheva1, Z. Kunova2, A. Mladenova2. 1. Center of Hygiene; Ecology & Nutrition; 2. Alexandria Hospital, Sofia, Bulgaria

Objective: To establish a combination of lipid and haemostasis indices with highest predictive value about cardiovascular risk in dyslipidaemia (DLD) patients.

Methods: Twelve lipid and 23 haemostasis parameters are estimated in 39 DLP patients and 39 DLP patients with clinical manifested cardiovascular disease (CVD). Enzyme, colorimetric, chromogen, ELISA, immune precipitation, turbidimetric, coagulation, visual-optic and agglutination methods are applied. Data are analyzed by step discriminative analysis.

**Results:** Four of all 35 variables evaluated, high density lipoproteins cholesterol (HDL-C), circulating thromocyte aggregates (CTA), fibrinogen (Fg) and factor II (FII), showed to be enough informative to distinguish DLP patients from patients with DLP plus CVD (percent of correct classification 87.18). Fg demonstrated highest predictive value (elevation by 1 g/L increases CVD risk 6.02 times). A prognostic cardiovascular risk index is promoted: (LDL-C + Fg/HDL-C). Values of this index higher than 6.66 are estimated as risky. Analysis of HDL-C, CTA, Fg and FII and calculation of the discriminative significance enables evaluation of the individual risk for each patient by formula and his real enrolment into one of the two groups. Seven DLP patients without CVD were assessed as high cardiovascular risk patients.

**Conclusion:** Computation of the individual risk and the lipid-fibrinogen index proposed by this study, may be useful in practice for assessment of the cardiovascular risk in DLP patients.

**ThP52-W31** Coronary lesion morphology and prognosis in young myocardial infarction males with or without familial hypercholesterolemia

T. Yasuda1, M. Shimizu1, H. Iino1, K. Oke1, M. Yamaguchi1, N. Fuji1, H. Fujii1, T. Mabuchi1, H. Mabuchi1, S. Mizuno2, The Second Department of Internal Medicine, School of Medicine, Kanazawa University, 1Kanazawa, Cardiology Division, Fukui Cardiovascular Center, 2Fukui, Japan

**Objectives:** We examined the angiographic characteristics and prognosis of young men with myocardial infarction and FH, compared with similar patients with myocardial infarction and without FH.

**Subjects:** We analyzed the coronary, angiograms of 43 male patients under 40 years with myocardial infarction, who were divided into an FH group (n = 16) and a non-FH group (n = 27). Overall 26 patients were followed up for an average of 9.4 years. Patients with a history of preintervention therapy were excluded.

**Results:** The mean age and the prevalence of hypertension, diabetes and smoking did not differ significantly between the groups. The mean serum total cholesterol concentration in the FH group was greater than in the non-FH group (FH 294 ± 69 mg/dl vs non-FH 196 ± 42 mg/dl, P < 0.001). The frequency of angiographic normal or non-obstructive culprit lesions was significantly higher in non-FH (FH 0% vs non-FH 37%, P < 0.01), and the incidence of totally occlusive lesions was higher in the FH group (FH 56% vs non-FH 11%, P < 0.01). There were also more patients with complex morphology at the culprit lesions of recanalized sites in the FH group (FH 86% vs non-FH 77%, P < 0.01). At 10-year follow-up, survival rate from cardiac death (FH 85% vs non-FH 100%, P < 0.06), survival from acute myocardial infarction (FH 83% vs non-FH 89%, P < 0.05), and survival from any ischemic event at a new lesion (FH 95% vs non-FH 67%, P < 0.01) were reduced in the FH group.

**Conclusion:** The mechanism of myocardial infarction in young patients with FH differs from that in similar patients without FH, and the overall prognosis of these patients is less favorable.

**ThP53-W31** The assessment of cardiovascular risk in type 2 diabetes mellitus

N. Hancu, Anca Cergizhan. Diabetes Center and Clinic, Cluj-Napoca, Romania

One of the main objective of the St. Vincent Declaration is to reduce morbidity and mortality from coronary heart disease (CHD) in diabetics by risk factor control.

**Objective:** To assess the global cardiovascular risk status (CVRS) in type 2 diabetes mellitus (DM) with a method that could be recommended for practical purposes.

**Method:** We used two charts: one proposed in 1998 by European Task Force called Euro '98 and one proposed by J. S. Yudkin and N. Chaturredvi from University College London in 1999 and called UK '99. Both charts provide estimates of the 10 year risk of coronary heart disease events and both are using risk coefficients derived from the Framingham Study. We have applied these two charts on 494 type 2 DM persons, 158 with microalbuminuria.

**Results:** CVRS score (%) has been greater using UK '99 chart, which takes in account the presence of microalbuminuria: 18.48 ± 0.5 vs 12.34 ± 1.1 with Euro '98. Also the segregation on three CVR groups (low, moderate, high) is different. We found out a difference between the UK '99 and Euro '98 charts concerning the segregation of the three CVR groups (low, moderate, high): with Euro '98 8.2% of the subjects had a high risk compared to 36% using K '99.

**Conclusion:** The assessment of CVRS is mandatory in prevention of CHD in DM. The UK '99 is more precise, is useful and accessible and can be recommended in diabetes care.

**ThP54-W31** Relationship between arterial blood pressure and lipid cardiovascular risk factors among professional soldiers of Polish Army: "CORO" program

R. Grabys1, A. Grabysa1, J. Adamus2, Dept. of Internal Diseases; 1Military Hospital, Olszyn; 2Clinic of Cardiology, Central Military Clinical Hospital, Warsaw, Poland

The "CORO" Program is prophylactic program realized among professional soldiers of Polish Army. The purpose of this program is first of all primary prevention and early diagnosis and treatment of coronary heart disease (CHD).

The aim of the study was to evaluate the relationship between some metabolic risk factors to CHD and arterial blood pressure values in the population of Polish Army professional soldiers living in the area of Warsaw Military Region (North East Poland).

In 1477 men (mean age 43 ± 5.0; range 35-65 yrs) we evaluated clinical, anthropometric (a.o. arterial blood pressure) and biochemical parameters (total cholesterol-TC; LDL-cholesterol by Friedwald’s equation, HDL-cholesterol, TC/HDL-chol index ang triglycerides-TG).

Among men with diagnosed arterial hypertension (AH) we observed statistically significant (p < 0.01) shifts of mean concentrations of TC, LDL-cholesterol, TG to a higher values. Mean values of TC/HDL-chol index were statistically significantly (p < 0.01) higher in group with AH. Mean concentrations of HDL-cholesterol were lower (p < 0.05) in AH group.

The present data suggest the existence of a significant relationship between blood pressure values and lipid metabolism parameters.

**ThP55-W31** Unstable angina with persistent T wave inversion: Different signs due to different duration of persistence

A. Batalla1, J. Mayordomo2, J.C. Sammartino2, J. Gutiérrez1, T. Raviña1, 1Department of Cardiology; 2Hospital de Cabueñas (Gijón); 2Hospital Central de Asturias (Oviedo), Gijón, Spain

**Purpose:** To study the significance of T waves associated with unstable angina.

**Methods:** We prospectively studied 109 patients (pts) admitted to the coronary care unit for prolonged chest pain (>20 minutes) while at rest, who developed T wave inversion in at least two precordial electrocardiogram (ECG) leads, without enzymatic rise or evidence of previous infarction. All patients were given a daily ECG and coronary angiography and echocardiogram on the seventh day or their hospital stay.

**Results:** The T waves inversion persisted less than 7 days in 19 pts (Group A) and more than 7 days in 90 pts (Group B). There were 85 males and 24 females. The results are shown in the following table:

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>P</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>90 (82.5%)</td>
<td>91 (17.5%)</td>
<td></td>
</tr>
<tr>
<td>Hyper-Tcho</td>
<td>55 (39%)</td>
<td>0.06</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>No. Vessels</td>
<td>1.7 ± 0.8</td>
<td>&lt;0.05</td>
<td>1.2 ± 0.8</td>
</tr>
<tr>
<td>LAD</td>
<td>83 (92%)</td>
<td>&lt;0.05</td>
<td>14 (73%)</td>
</tr>
<tr>
<td>Complex</td>
<td>80 (90%)</td>
<td>&lt;0.05</td>
<td>12 (66%)</td>
</tr>
<tr>
<td>No CAD</td>
<td>5 (6%)</td>
<td>&lt;0.05</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>Distressery</td>
<td>49 (55%)</td>
<td>&lt;0.05</td>
<td>4 (21%)</td>
</tr>
</tbody>
</table>

*CAD = Coronary artery disease; *Hyper-Tcho = Hypercholesterolemia *LAD = Left anterior descending; *No. Vessels = Number of vessels

**Conclusions:** The T wave inversion persisted during the hospital stay in patients with unstable angina is significantly associated to segmental dissection and to culprit complex lesion in LAD.

**ThP56-W31** Chronic treatment with lipid lowering drugs in young coronary patients and clinical events

A. Batalla1, G.I. Cabero2, J.J.R. Reguero2, S. Hevia3, F. Merino3, E. Bustillo2, A. Cortina2, 1Department of Cardiology; 2Hospital de Cabueñas (Gijón); 3Hospital Central de Asturias (Oviedo), Spain

**Purpose:** To determine whether differences exist in the appearance of coronary events in patients with onset of coronary disease before 50 years of age according to whether they receive treatment or not with lipid lowering drugs.

**Methods:** Consecutively, 227 male patients (pts) before 50 years of age (mean age 42 ± 4 years), who were admitted to our Coronary Care Unit as
result of an episode of coronary disease were prospectively studied. In order to determine new coronary events, a mean follow-up of 32 ± 13 months was carried out. Patients were divided into 2 groups according to whether they were receiving (Group A) treatment with lipid lowering drugs during follow-up or not (Group B).

### Results:

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart failure</th>
<th>OR</th>
<th>P</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1 (1%)</td>
<td>1.255</td>
<td>0.0083</td>
<td>1.204</td>
</tr>
<tr>
<td>B</td>
<td>2.143</td>
<td>0.018</td>
<td>1.437</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

No differences were found in mortality, appearance of angina, myocardial infarction and the need for coronary revascularisation.

### Conclusions:

Patients with early-onset of coronary heart disease and chronically treated with lipid lowering drugs present less frequently heart failure than patients without lipid lowering drugs.

### ThP57:W31

**Effectiveness of atorvastatin 10 mg in reaching EAS LDL-c target in secondary prevention**


**Objectives:** To carry out a descriptive study of our patients in secondary prevention of coronary heart disease and to analyze the evolution of their lipid profile during 6 weeks of treatment with atorvastatin 10 mg/day.

**Methods:** One hundred and twenty-eight patients with stable coronary artery disease were evaluated in our Cardiology Dpt. from July ‘98 to September ‘99.

**Results:** The baseline characteristics of this population were the following: Age 61 ± 9 year-old, males 91%, smokers 13%, past-smokers 64%, obesity 23%, hypertension 48%, diabetes 19%, angina 66%, prior AMI 70%, PTCA 27%, stent 29%, CABG 9%, CVA 5%. At baseline, 56% of them were treated with statins (78% did not reach the NCEP therapeutic targets (LDL-c < 100 mg/dl), and 47% did not comply with the EAS guidelines (LDL-c < 115 mg/dl)). From the 44% that were not treated with statins, only 9% and 4% achieved the EAS and NCEP therapeutic targets, respectively. After 6 weeks of treatment with atorvastatin 10 mg/day, LDL-c decreased from 156 ± 23 to 96 ± 22 mg/dl, triglycerides from 137 ± 76 to 110 ± 60 mg/dl and HDL-c increased from 49 ± 11 to 50 ± 11 mg/dl. Ninety-two percent of the patients reached the EAS objectives. Multivariant analysis showed that the only factors independently associated to a larger reduction of LDL-c were age and baseline LDL-c.

**Conclusion:** Treatment with atorvastatin 10 mg/day reaches LDL-c EAS/ECS target in 92% of patients in secondary prevention, easily achieving LDL-c levels < 100–115 mg/dl, in accordance with the scientific societies recommendations.

### ThP58:W31

**Hypertriglyceridemia contributed factors in Iranian people**

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**Objective:** Hyperlipidemia is known as a major risk factor for cardiovascular diseases (CVD). Recently, it has been mentioned that serum TG level can be influenced CVD independently. Therefore, as the hypertriglyceridemia is most prevalent type of hyperlipidemia we decided to determine the contributed factors of hypertriglyceridemia in Isfahan (Iran) population on the data from the 2nd Isfahan CVD Risk Factor Survey.

**Methods:** This survey has been conducted on 1200 citizens of Isfahan aged 20–70 years who were randomly selected from clusters of population. A fasting blood sample was taken from each participant and analyzed for lipids. Also, study questionnaires were filled for all subjects. Blood pressures were measured according to WHO criteria. Height and weight were measured in light clothes and without shoes by Seca scale. Nutrition intakes were calculated by three 24-hr recall method using FCP-HC software. Logistic regression was performed to define the contributed factors for hypertriglyceridemia. Also, four factors were created by factor analysis including factor-1 (dietary cholesterol, PUFA, protein, CSI), factor-2 (LDL and HDL cholesterol), factor-3 (age, systolic and diastolic blood pressures), factor-4 (sex, occupation, smoking) which were used in logistic regression.

**Results:** Based on the data of the table, it’s found that in total population, CSI index, protein intake, LDL and HDL cholesterol, systolic and diastolic blood pressures and age are related to hypertriglyceridemia in this society while the pooled data for history of CVD indicates that the subjects with negative history of CVD showed the same results but in those who had positive history, none of the dietary factors can change hypertriglyceridemia.

**Conclusion:** Therefore, we conclude that in Iranian people, dietary protein, cholesterol and saturated fatty acids are the major dietary factors associated with hypertriglyceridemia.

### ThP59:W31

**Plasma lipoproteins in gaucher disease: Effects of enzyme replacement therapy**

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**Background:** Gaucher disease (GD) is an inherited lipid storage disorder which can be effectively treated by enzyme replacement-therapy (ERT). GD patients have decreased plasma total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) levels, as well as apolipoproteins AI (apo AI) and B (apo B). Conversely, plasma apolipoprotein E (apoE), synthesized by liver and activated macrophages, is increased.

**Objective:** To evaluate the effect of ERT on plasma lipoproteins in GD patients.

**Methods:** Plasma lipids, lipoprotein, and apolipoprotein concentrations, and chitotriosidase activity, an enzyme synthesized by activated macrophages, were measured in 51 GD patients before and after 18 months of follow-up of ERT. Seventeen patients who did not receive ERT were followed for a similar time period.

**Results:** ERT resulted in significant increases in HDL-C (+35%) and apo AI (+17%) levels in GD patients, whereas no changes were observed in LDL-C and apo B levels. Conversely, chitotriosidase activity, plasma total and HDL- apoE levels were dramatically reduced (~27%) after ERT.

**Conclusion:** Our data indicate that ERT in GD patients has significant effects on composition and concentration of plasma lipoproteins, resulting in an apparently less atherogenic lipid profile.

### ThP60:W31

**Reduction of serum lipid fractions following aluminum administration**

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**Objective:** To find out whether aluminium is able to affect lipid Metabolism

**Methods:** Male wistar rats weighing 200–250 g were used in this project. At standard conditions animals were injected with varying amount of aluminium, and sera were collected. Lipid related parameters were determined using reliable laboratory methods.

**Results:** Aluminium, as aluminium chloride led to an elevation of lipoprotein lipase (LPL) activity by 26% in wistar rat. Addition of aluminium in complex with citric acid (1:2) or with 20 mM bicarbonate increased the enzyme activity by 26 or 35 percent, respectively.

Daily intraperitoneal administration of aluminium (15 mg/kg BW) for 15 days reduced serum triglyceride, cholesterol, VLDL and HDL concentration by 78, 14, 77 and 39, respectively.

**Conclusion:** It seems that therefore aluminium may use to prevent lipid accumulation in atherosclerosis patients, as it is routinely used for the treatment of other patients particularly as an anciadid.
Thursday June 29, 2000: Poster Abstracts

P:W31 Prevention of CVD

ThP61:W31 Screening and registration program for patients with familial hypercholesterolemia in Spain

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Background: Familial hypercholesterolemia (FH) is an autosomal disorder caused by defects in the low-density lipoprotein receptor (LDLR) gene. Patients with FH have an increased risk for premature coronary artery disease (CAD).

Objective: Prevention of CAD by early identification and treatment of patients with FH.

Methods: 1) The Spanish Familial Hypercholesterolemia Foundation coordinates active case-finding by a toll-free telephone helpline and a mail questionnaire. 2) Identified patients are referred to one of the 35 National Lipid Clinic Network Centers. 3) Lipid analysis and DNA-diagnosis are performed in a Central Laboratory. 4) Optimal treatment is initiated and patients are registered for a long-term follow-up.

Results: Up to December 1999, 1224 contacts to the helpline were recorded. Of these, 954 were interactive calls and 270 were requests for the questionnaire. Three hundred and seventy five people were clinically and biochemically diagnosed with FH. Of these, 22% were shown to be positive for one of the 37 FH known causes for LDLR mutation present in Spain. Only 48% of these patients received some form of lipid-lowering therapy previously. Conversely, after referral, 95% adheres to a statin therapy.

Conclusion: 1) Toll-free telephone helpline is very effective to identify FH patients. 2) Referral to a Lipid Clinic, early diagnosis and initiation of optimal therapy may reduce cardiovascular morbidity and mortality.

ThP62:W31 Comparison of simvastatin and gaur gum in the treatment hyperlipidemic patients with diabetes mellitus II type

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Diabetes mellitus II type is characterized by the increased risk CHD mortality. For reduce cardiovascular risk, it is necessary to treat the patients with hypolipidemic drugs. We investigate the effect of simvastatin and guar gum was in diabetic patients with hyperlipidemia (basal level TC 7.78 mmol/L, TG 2.94 mmol/L).

Twenty patients have been treated with simvastatin 10 mg once a day, and sixteen patients was treated by guar gum 5 g three times daily.

After 4 week treatment simvastatin decreased TC by 17%, LDL-C by 21%, TG by 13%, and increased HDL-C by 6%. In the guar gum group TC, LDL-C, TG decreased by 14%, 7%, 21%, and HDL-C increased by 3%. Fasting blood sugar was reduced by guar gum (P < 0.001), and didn’t changed by simvastatin. Glycated hemoglobin remained similar to the basal level in both groups.

Two patients stopped the treatment by guar gum because of gastrointestinal side effects.

Conclusion: 1) Simvastatin and guar gum are effective in reducing atherogenic lipids in diabetic patients with dyslipidemia. 2) Guar gum appears to be an effective hypoglycemic agent, and demonstrated better hypotriglyceridemic efficacy than simvastatin. 3) Simvastatin was more effective in lowering TC, LDL-C and increasing antithromogenic HDL-C, and better tolerated than guar gum.

ThP63:W31 Vitamin supplementation in epileptic patients with hyperhomocysteinemia

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Objective: Hyperhomocysteinemia is a risk factor of atherosclerosis and homeostasis is an objective convulsant. Patients suffering from different types of epilepsy with hyperhomocysteinemia were treated with vitamins.

Methods: From a group of 85 patients on long-term antiepileptic treatment we selected 18 patients with hyperhomocysteinemia /total plasma homocysteine (tHcy) > 16 umol/L, confirmed by the positive L-methionine loading test. We started the vitamin mono- or combination therapy according to the vitamin levels. The daily doses were 1 to 5 mg folic acid, 5 to 10 mg vitamin B6, and 0.3 mg vitamin B12 per week, duration from 9 to 18 months.

Results: After treatment with vitamins tHcy decreased to the levels in reference ranges in all the cases. Neither negative clinical or EEG symptom occurred in these patients. Our findings indicate that treatment with a single vitamin may negatively influence the status of other vitamins.

Conclusions: Antiepileptic drug treatment may increase plasma concentrations of homocysteine. Vitamin therapy in patients on AED with hyperhomocysteinemia decreases total homocysteine levels to reference ranges.

ThP64:W31 Fat, cholesterol and fatty acids intake in the diet as a factor of atherosclerosis treatment among people at advanced age

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Objective: The aim of the work was estimation intake of fat, cholesterol (Ch) and fatty acids (FA) in the diet and cholesterol, triglyceride (TG), high density lipoproteins (HDL) in the blood serum of people aged 60–90, living in the pensioner house for few years.

Methods: Using software "FOOD" contents of Ch and FA were calculated and fat content was estimated analytically. In serum m/a elements were estimated in 26 pensioners.

Results: It was stated that average fat content in 40 analytical examined daily food rations was 133.1 ± 24.9 g and covered 30.4% of daily energy value. Moreover meals delivered 524.6 ± 121.7 g of Ch, 56.2 ± 10.1 g of saturated, 57.0 ± 14.1 g monounsaturated and 31.5 ± 12.8 g of polyunsaturated fatty acids. Ratio of polyunsaturated to saturated fatty acids P:S was in average 0.59. Analysed daily food rations contained in an average 29.4 ± 13.0 linoleic acid, 2.2 ± 0.35 g g-linolenic acid and 0.259 ± 0.17 g arachidonic acid. Mean Ch level in the examined blood serum was follows: 236.2 ± 66.1 mg/dl. TG content in the blood serum was in average 147.9 ± 61.3 mg/dl. There was a significant difference in the HDL level in the women’s blood serum of 70.0 ± 13.9 mg/dl but among men 49.4 ± 12.3 mg/dl.

Conclusion: Performed examinations showed necessity to implement proper food ration planning, with cholesterol content not exceeding 300 mg and bigger contribution of vegetable fat containing polyunsaturated fatty acids, as a atherosclerosis prophylactic factor.

ThP65:W31 Primary prevention program on atherosclerosis and cardiovascular disease in youth

M. Wosik-Frenbek, A. Sierakowska-Fijalka, K. Sieniwicki, M. Medical University of Lodz, Lodz, Poland

Objective: To study the influence of the primary prevention program in some anthropometric parameters in school girls and boys.

Methods: Our primary prevention program based on regular physical activity, health education, and life style modification was performed on 70 school teenagers. We have used some anthropometric indices and compared them before and after the prevention program implementation. Examined parameters included body mass (m), height (h), skinfolds, waist and hip circumferences. Using these parameters we calculated: the body mass index (BMI), ponderal index (PI), waist/hip ratio (WHR), body fat percentage (BF%) (according to the Siri’s formula), subscapular/triceps skin folds ratio (Ss/TR), and biiceps/triceps/suprailiac + subscapular skinfolds ratio (BT/SSSR).

Results: Based on our primary prevention program we found positive changes in the body fat distribution, heart health knowledge, and health self-monitoring in the investigated subjects.

Conclusions: It is beneficial to use our school-based primary prevention program as a part of regular educational programs. In addition, its implementation is very easy.

ThP66:W31 Comparison of two systems for LDL-apheresis: Immunoadsorption and hole blood perfusion

S. Pokrokovsky, I. Adamova, O. Afanasieva, H. Borberg1. Cardiology Research Center, Moscow, Russia; 1 German Haemapheresis Centre, Cologne, Germany

Extracorporeal LDL elimination called LDL apheresis for the treatment of FH patients have been successfully used since 1981 year. Currently several systems for LDL apheresis are available: immunoadsorption, chemoadsorption by dextran sulfate, heparin-induced extracorporeal LDL precipitation all this systems was designed for the plasmatherapy and requires step at plasmaperfusion, couple years ago new system - Direct Adsorption of Lipids (DALI) for whole blood perfusion have been developed. We performed comparison
study of the immunoadsorption system LDL Lipopak® (Pocar, Russia) and DALI® (Fresenius, Germany). LDL Lipopak® columns contain 400 and 200 ml gel with sheep polyclonal antibodies as a ligand; usually used as twin technology – pair of multiple used columns for one patient. DALI system include single use columns which contain 500, 750 or 1000 ml gel with modified polyacrylate as a ligand. We state that sufficient procedure must have post treatment value of LDL cholesterol 30–70 mg/dl. Small LDL Lipopak® columns were mainly developed to improve the economy and efficacy of LDL apheresis. Both LDL Lipopak® and DALI® systems have been used for the treatment of homozygous FH patients. Under long-term therapy 400 ml and 200 ml LD Lipopak® columns showed lower average and post-treatment TC and LDL-C values as the DALI system used for comparative reasons.

We conclude, that repetitive cycling LDL-apheresis in general as demonstrated with our improved, economical device is most efficient as compared to whole blood perfusion.

ThP67:W31  Prediction of angiographic stabilization/reversal of coronary atherosclerosis by a risk factor graph
W.E. Feeman, Jr., J. Niekhuwer. Bowling Green, Ohio, Leipzig, Germany
The only reason to treat dyslipidemia is the prevention of atherosclerosis as if already evident, reversal of atherosclerosis. Angiographic studies of reversal of atherosclerosis (STARS, LCAS, POSCH, NHLBI, LOCAT PLAC-1, FAT, and the Heidelberg Study) have been analyzed in terms of a risk factor graph. This risk factor graph relates the cholesterol retention fraction (CRF) or [LDL-HDL]/[LDL] on the ordinate and systolic blood pressure (SBP) on the abscissa. A threshold value, termed the angiographic stabilization/regression line, with defining loci of (0.74, 100) and (0.49, 140), has been determined empirically. In the cited studies, if the patient’s CRF-SBP plot was brought below the threshold line, angiographic stabilization/regression of coronary atherosclerosis occurred in 75% of cases. It is suggested that the graph can act as a goal for therapy for the treatment of dyslipidemia in patients with atherosclerosis.

P:W32  INTRACELLULAR LIPID METABOLISM

ThP1:W32  Role of fatty acid metabolism in hepatocyte proliferation
T. Maeda, K. Kojima, E. Horie, Y. Fujimaki, N. Shimazu, M. Fujita, M. Kinoshita, T. Teraomoto. Internal medicine, Teikyo University school of medicine, Tokyo, Japan
Objective: It has been reported that hepatic steatosis is observed transiently after partial hepatectomy (PH). Both increase in plasma free fatty acid and regeneration of hepatocytes occur coincidentally with the hepatic steatosis. The present study examined the relationship between cell proliferation and fatty acid metabolism in rat liver.

Methods: The activities of β-oxidation and fatty acid synthetase (FAS) in the liver were determined at 1, 2, 7 days after PH. The effect of inhibitors of FAS, acyl-CoA synthetase (ACS), diacylglycerol acyltransferase (DGAT) and acyl-CoA cholesterol acyltransferase (ACAT) on DNA synthesis was studied with use of primary cultured rat hepatocytes in the presence or absence of fatty acid. DNA synthesis in the cells was estimated by pulse-labeling with [3H]-thymidine.

Results: FAS activity increased significantly, but the increase was observed later than the appearance of hepatic steatosis. The β-oxidation activity did not change both in hepatic mitochondria and peroxisome after PH. Although FAS inhibitor and ACS inhibitor suppressed the DNA synthesis of hepatocyte, supplementation of 1 mM oleic acid or linoleic acid reversed the effects. On the other hand, either DGAT inhibitor or ACAT inhibitor did not affect the DNA synthesis.

Conclusion: The role of fatty acid in hepatocyte or the acyl-CoA plays a pivotal role in hepatocyte proliferation.

ThP2:W32  Macrophage reversal of LDL aggregation by plasminogen activation
H.S. Kruth, W.-Y. Zhang, I. Ishii. Section of Experimental Atherosclerosis, NHLBI, NIH, Bethesda, MD, USA
Aggregation of low density lipoproteins (LDL) is believed to contribute to their retention in atherosclerotic lesions. Previously, we showed that aggregated LDL (AgLDL) induces and enters surface-connected compartments (SCC) in human monocyte-derived macrophages by a process we have named patocytosis. Most aggregated LDL taken up by these macrophages is stored in SCC and remains undegraded. We now show that macrophages released AgLDL (prepared by vortexing or treatment with phospholipase C or sphingomyelinase) from their SCC when exposed to 10% lipoprotein-deficient serum (LPDS) for 1 d. Importantly, the released AgLDL was degraded. <1% of original TCA-insoluble [14C]-AgLDL could pass through a 0.45 μm filter, but >60% could pass after release from macrophages. Disaggredation of AgLDL was verified by electron microscopy. The factor in serum that mediated AgLDL release and disaggredation was plasmid generated from plasmidogen by macrophage uPA. AgLDL release was decreased >90% by inhibitors of plasmin (EACA and an anti-plasmin Mab), and also by inhibitors of uPA (PAI-1 and an anti-uPA Mab). Moreover, plasminogen could substitute for LPDS and produce similar macrophage release and disaggredation of AgLDL. Although plasmin could dissociate LDL in the absence of macrophages, interaction of AgLDL with macrophages was necessary for reversal of its aggregation by LPDS. This was because only plasmin bound to the macrophage surface is protected from serum plasmin inhibitors. Aggregation of LDL causes it to fuse forming lipid particles larger than LDL. Similar-sized disaggredated lipid particles have been observed in and isolated from atherosclerotic lesions. Plasmin-mediated reversal of LDL aggregation can account for these lesion lipid particles that are larger than LDL. Also, reversal of LDL aggregation could facilitate removal of LDL aggregates from atherosclerotic lesions because efflux of lipoproteins from the vessel wall is inversely proportional to their size.

ThP3:W32  In vivo metabolism of apo B, apo AI and VLDL triglycerides in a form of familial hypobetalipoproteinemia not linked to the apo B gene
N. Elias, B.W. Patterson, G. Schonfeld. Washington University School of Medicine, St. Louis, MO, USA
Objectives and Methods: Familial hypobetalipoproteinemia (FHBL) is an autosomal codominant disorder that results from different mutations in the apoB gene. We have identified a 40-member FHBL kindred in which the hypobeta phenotype segregates as an autosomal dominant trait and linkage to the apoB gene on chromosome 2 was ruled out. Linkage to FHBL susceptibility region on chromosome 3p21-1.2 was found. Some FHBL affected subjects also had low HDL cholesterol and apoAI levels compared to unaffected relatives. Our aim was to define the metabolic bases of this disorder. Therefore, we studied the in vivo kinetics of apoB, apoAI and VLDL triglycerides (TG) in 4 affected subjects and 5 normolipidemic subjects of this kindred. Deuterated leucine and deuterated glycerol were used to label the apoproteins and TG, respectively. Compartmental modeling was used to obtain the kinetic parameters.

Results: Affected subjects had normal fractional catabolic rates (FCR) for VLDL apoB but increased LDI apoB FCRs compared to normal controls (0.050 ± 0.009 vs 0.030 ± 0.006 pools/hr, p < 0.005). Decreased production rates for apoB and VLDL apoB (11.4 ± 1.7 vs 25.6 ± 4.6 mg/kg/d, p < 0.003), LDI apoB (7.8 ± 1.3 vs 12.7 ± 3.7 mg/kg/d, p = 0.04) and VLDL TG (8.2 ± 4.5 vs 19.6 ± 10.8 μmol/kg/hr, p = 0.09) were demonstrated in affected subjects. Low HDL cholesterol and apoAI levels were caused by higher apoAI FCRs (0.035 ± 0.005 vs 0.018 ± 0.005 pools/hr, p < 0.01) without significant decrease in apoAI production rates.

In conclusion, both decreased secretion of apoB containing lipoproteins and hypercatabolism of LDI cholesterol levels in apoB and cholesterol levels in this novel form of FHBL.

ThP4:W32  Dietery regulation of diacylglycerol acyltransferase (DGAT) activity and mRNA levels
Objective: DGAT catalyzes the committed step in triglyceride (TG) synthesis. Therefore, an understanding of DGAT regulation may lead to effective control of TG levels. This study was undertaken to investigate the influence of various diets on DGAT activity and mRNA abundance.

Methods: Rats, six per group (n = 6), were fed a chow (CH), 68% sucrose (SU), or 10% olive oil (High Fat) diet for 2 weeks. For the fasted studies, a second group of animals on the chow diet for 23 days were fasted 24 hours before ending the experiment. Lipids, DGAT activity and mRNA levels were analyzed by routine methods.
**ThP5.W32**  
**Nitric oxide induces apoptosis in vascular smooth muscle**  
E. LaBelle, C. Pflane. MCP Hahnemann University, Philadelphia, PA, USA

**Objective:** To determine the role of arachidonate and ceramide during apoptosis induced by nitric oxide (NO) and UV light.

**Methods:** Cells cultured from rabbit aortal smooth muscle (SMC) were grown to confluence and treated with either UV light, sodium nitroprusside (SNP), or S-nitrosyl-N-acetylpenicillamine (SNAP) to induce reactive nitrogen species (RNS). Cellular ceramide was measured by either extracting DNA and separating fragments on agarose gels or by TUNEL analysis. The cells were also preloaded with [3H] arachidonic acid (AA) in order to label the phospholipids and then induced to undergo apoptosis while AA release from the cells was measured. Ceramide levels within the cells were also measured by means of the diallylglycerol kinase method, and cPLA2 levels within the cells were measured by immunoblotting. The concentration of NO released from the SNAP and the SNP was determined by the Gress reaction.

**Results:** Both UV light and NO induced apoptosis in the SMC. Both UV light and NO stimulated AA release from the cells with kinetics similar to the kinetics of apoptosis. They also increased ceramide levels within the cells together with levels of cPLA2. We determined that the concentration of NO released from SNAP and SNP that was effective in stimulating apoptosis was about 20 μM.

**Conclusions:** Both NO and UV light appear to stimulate apoptosis in SMC via a mechanism that might involve both AA release and ceramide increase. (Supported by Grant HL 37413 from NHLBI)

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**ThP6.W32**  
**Hepatic oversecretion of small, cholesterol-rich very low density lipoproteins (VLDLs; Sp 20–60) in familial combined hyperlipidemia (FCHL)**  
J. Pitzchek, U. Julius, M. Hanefeld. Institute of Clinical Metabolic Research, Medical Faculty, Dresden, Germany

The diagnosis FCHL is based on the occurrence of different types of primary hyperlipidemia (elevated cholesterol and/or triglycerides) in several individuals within the same pedigree. The aim of this stable isotope study was to differentiate to what extent hepatic overproduction of apolipoprotein (apo) B-100 or a disorder of catabolism of apoB-100 containing lipoproteins give rise to the hypertriglyceridemic condition. We compared the *in vivo* kinetics of apoB-100 in 7 hypertriglyceridemic subjects (selected from 3 FCHL families without defects in their lipase genes) with 6 normolipidemic controls using a tracer of [13C2]-phenylalanine. Isotopic enrichment of apoB-100 in VLDLs (Sp 60–400), LDLs (Sp 400–1000), and chylomicrons (Sp 1000–3000) was measured by gas chromatography-mass spectrometry. From tracer mass data the rates of production, catabolism, and transfer were estimated by model-based analysis. FCHL subjects showed a discrete 1.5-fold increase in direct hepatic VLDL2 apoB-100 secretion when compared with controls (307 ± 25 vs. 256 ± 23 μg/dl/p, < 0.01). In FCHL subjects catabolism of VLDL, LDL, and LDI was not substantially impaired. Furthermore, in FCHL plasma mevalonate and lathosterol levels, two precursors of cholesterol biosynthesis, were significantly increased (6.3 ± 1.4 vs. 2.8 ± 0.6 μg/ml, and 1850 ± 240 vs. 674 ± 91 nmol/l, resp.) and well correlated with VLDL2 secretion (r = 0.558, p = 0.000 and r = 0.720, p = 0.000, resp.). In conclusion, this study indicates that in the FCHL subjects selected an increased number of exclusively VLDL2 particles are being secreted, leading to their hypertriglyceridemia. Furthermore, VLDL2 oversecretion seems to be strongly regulated by increased hepatic availability of cholesterol substrate.

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**ThP7.W32**  
**Temporal gene expression in macrophages exposed to oxidised low density lipoprotein (LDL)**  
D.J. Freeman 1, A. Galazar 1, S. Blakemore 1, M. Walker 2, D. Wallace 2, D. Hassall 2, 1 University of Durham, Durham; 2 GlaxoWellcome Medicines Research Centre, Stevenage, UK

**Objective:** To study genes regulated in the monocytic cell line THP-1, on exposure to native, acLDL (AcLDL) and oxidised LDL (oxLDL).

**Methods:** A differential gene expression system comprising an arrayed custom library of atheroma-related genes was utilised. THP-1 cells were differentiated into macrophages using phorbol myristate acetate (PMA). Cells were then exposed to a) 0.2 mg/ml native, acLDL or oxLDL for 24 hours or b) 0.2 mg/ml oxLDL for 0, 4, 8, 16, 24, 48 and 72 hours. Messenger RNA was isolated and radiolabelled cDNA probes generated. Gene expression was assessed by hybridisation to the atheroma library cDNA array and quantitation by phosphorimaging.

**Results:** Out of the 770 atheroma library genes, 70 genes/ESTs on exposure to acLDL and 258 genes/ESTs on exposure to oxLDL were differentially expressed compared to native LDL exposure. Collagenase Type IV (3 fold increase), β-tubulin (7 fold increase) and CD36 (5 fold increase) were specifically regulated by oxLDL exposure. Using multivariate cluster analyses, nine clusters of genes showing similar temporal patterns of gene expression were identified. For example IL-1β and TIMP-1 had correlated gene expression.

**Conclusions:** Lipid loading per se, as represented by acLDL treatment, presents a different challenge to the macrophage than the pro-inflammatory oxLDL. Identification of genes regulated in the macrophage to foam cell transformation may offer insights into the underlying mechanisms of atherosclerotic plaque formation.

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**ThP8.W32**  
**Sterol regulation of chylomicron synthesis and secretion in an intestinal epithelial cell model (CaCo-2)**  
Emma Allister 1, Sebely Pa 1, John Mamo 2, 1 Department of Nutrition, Dietetics and Food Science, Curtin University; 2 Department of Medicine, University of Western Australia, Australia

**Objective:** The aim of this study was to investigate whether chylomicron production could be regulated by altering sterol availability.

**Methods:** In this study we used a transformed human intestinal epithelial cell line (CaCo-2). The relative amounts of apo B-48 in the cells and media was quantified by Western blotting using an apo B antibody and enhanced chemiluminescence. Cholesterol concentrations in the cells and media were determined by gas chromatography mass spectrometry.

**Results:** Initially we explored the impact of substrate supply on apo B-48 production. Intracellular apo B-48 increased as substrate availability increased; however, the effects on secretion were variable and there was no net change in the secretion of CM apo B48. The effects of atorvastatin were determined in the presence of exogenous lipid. The intracellular and media concentration of unesterified cholesterol decreased suggesting that atorvastatin was inhibiting cholesterol biosynthesis. Cellular apo B-48 decreased however this did not translate to decreased secretion into the media. To explore the putative role of endogenous sterols on apo B-48 secretion, we deprived the CaCo-2 cells of exogenous lipid and blocked endogenous cholesterol synthesis with atorvastatin. Apo B-48 secretion was not attenuated by atorvastatin however intracellular apo B-48 was increased. CaCo-2 cells incubated with ACAT inhibitor in the presence of exogenous sterol displayed an increase in the intracellular and media concentrations of free cholesterol suggesting that esterification was inhibited. Concurrently there was a decrease in apo B-48 concentration in cells, which also translated to a decrease in secretion.

**Conclusion:** Collectively the data suggests that the availability of cholesteryl ester in CaCo-2 cells regulates the secretion of apo B-48.

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**ThP9.W32**  
**Pulse-chase studies: Estimating apolipoprotein secretion and post-translational degradation rates**  
P.H.R. Barrett. Department of Medicine, University Western Australia, Perth, Australia

**Objective:** To determine rates of protein synthesis, secretion and degradation using cell and media apolipoprotein B (apoB) radioactivity data collected during a pulse-chase experiment on cultured hepatocytes using a compartmental model. Values estimated by the model are then compared to the traditional method for estimating such rates.

**Methods:** A compartmental model was developed to describe the process of apoB synthesis using 35S-methionine as the tracer amino acid. The model
includes compartments that describe the building of the polypeptide chain to yield full-length apoB. Three additional compartments are included describing the transport of apoB through the cell and into the media. Loss pathways are included from these pathways to account for apoB degradation. The model is fit simultaneously to the cell and media apoB radioactivity data and secretion and degradation rates are estimated. The model incorporates all aspects of the experimental protocol including tracer pulse and chase with excess cold amino acid. Using a typical cell-apoB tracer curve, media apoB curves were simulated assuming different secretion rates. Secretion rates were also determined by comparing peak cell radioactivity to the plateau media radioactivity, the traditional method.

**Results:** Compartmental model secretion rates were 43% of those estimated using the traditional method. The percent difference in secretion rates varies as a function of the protein (size and time to secretion).

**Conclusions:** The compartmental model describes the process of protein synthesis and secretion and thus provides accurate estimates of these rates. The traditional method significantly underestimates protein degradation, and therefore overestimates secretion. Compartmental modeling provides a functional and quantitative description of protein synthesis and secretion that can be tested under different experimental conditions and interventions.

**ThP10:W32** The effect of peroxisome proliferator-activated receptor-alpha (PPARα) on the activity of the human cholesterol 7-α-hydroxylase (C-7OH) gene

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**Objective:** Cholesterol 7-α-hydroxylase plays a central role in the regulation of bile acid and cholesterol metabolism, and transcription of the gene is controlled by bile acids and hormones acting through a number of steroid hormone binding sites. The effect of PPARα on the transcription of the C-7OH gene has been investigated.

**Methods:** Reporter gene constructs containing regions of the C-7OH promoter upstream of the luciferase gene were transfected into HepG2 cells together with expression vectors for PPARα, retinoid X receptor (RXR) and other hormone receptors.

**Results:** Co-transfection of PPARα lowered the activity of C-7OH promoter constructs in HepG2 cells. The effect was augmented by RXR and activators of PPARα to give a maximum reduction of approx. 70%. Hepatic nuclear factor 4 (HNF4) increased promoter activity and reduced the effect of PPARα. Gel shift assays failed to detect any direct binding of PPARα/RXR heterodimers to regions of the promoter containing potential binding sites. Also, the diurnal peak of hepatic C-7OH mRNA observed in mice during the light phase was essentially unaffected by the disruption of the PPARα gene.

**Conclusion:** PPARα could affect C-7OH gene transcription through an indirect action, possibly by competing for co-factors, but this is unlikely to be a major influence on C-7OH activity in vivo.

**ThP11:W32** Inhibition of sterol regulatory element-binding protein cleavage by the prodomain of human SKI-1 protease


**Objectives:** The protein convertase SKI-1 (or S1P) governs the transposition of multiple genes that regulate cholesterol and fatty acid biosynthesis. This process is initiated within the secretory pathway by SKI-1 specific proteolysis of sterol regulatory element binding proteins (SREBPs). We asked whether the N-terminal domain of SKI-1 could act as a specific inhibitor of this protease, thus diminishing the levels of cholesterol/fatty acid metabolism biosynthesis.

**Methods:** A cDNA corresponding to the preprosequence of human SKI-1 (PROSKI-1) was ligated into the pCDNA3.1 (Zeo+) expression vector and used to generate stable transfecants in HK293 cells. The levels of SREBP processing in these cells were determined by Western blotting, whereas the levels of gene transcription involved in cholesterol/fatty acid metabolism were assessed via Northern blotting. In parallel experiments, SREBP-1 was overexpressed along with PROSKI-1 to enhance the detection of SREBP cleavage.

**Results:** Immunocytochemical studies demonstrated that overexpressed PROSKI-1 is mostly localized in the ER and Golgi, similar to the distribution pattern of SREBP-1. Immunoblotting experiments showed that PROSKI-1 inhibits the accumulation of nuclear SREBP when cells are deprived of sterols. In addition, in cells overexpressing SREBP-1, we were able to demonstrate a significant reduction of SREBP-1 processing in membrane fractions. In agreement with these immunoblot results, mRNA levels of SREBP1 target genes declined substantially in cells overexpressing PROSKI-1.

**Conclusion:** We have demonstrated that the N-terminal domain of SKI1 can function as a specific inhibitor of this protein convertase, leading to a substantial reduction in the specific proteolysis of SREBPs as well as in the transcription of genes governing cholesterol/fatty acid biosynthesis. Our results suggest that this approach to proteolytic regulation may be a useful tool in the quest to understand the progression of lipid-related diseases.

**ThP12:W32** Scavenger receptor A1, co-expressed in COS cells with acyl-CoA:acyl-CoA transferase (ACAT), enhances cholesteryl ester accumulation in response to acetylated LDL (AcLDL)

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**Objective:** Evaluation of the effect of AcLDL on the cellular distribution of scavenger receptor A1 (SRAI) and on cholesteryl ester deposition.

**Methods:** Cos cells were cotransfected with a plasmid containing the full length sequence of SRA1 fused N-terminally to enhanced green fluorescent protein (EGFP) and a plasmid bearing the full length sequence of hamster ACAT. On average, 25% of the cells were fluorescent 24 h after initiation of transfection. The cells were then incubated with 50 microgram of AcLDL per ml of medium 24 h after transfection. Another 24 h later, cells were harvested and lysed, or they were analysed for SRA1 distribution by immunofluorescence. Analysis of cholesteryl ester content was performed according to established procedures (Asmis et al., 1997, J Chroniatogt, 691, 59-66). All determinations were performed in triplicates.

**Results:** Immunofluorescence analysis revealed that SRA1, when expressed as an EGFP fusion product in COS cells, stained only weakly in non-permeabilized cells. Upon addition of AcLDL, the antigentic sites of SRA1 became exposed on the surface of the cells as revealed by SRA1 staining of the non-permeabilized cells. 2) AcLDL administration to COS cells that were cotransfected with SRA1 and ACAT resulted in a two fold higher cholesteryl ester accumulation than the same treatment in COS cells that were either mock-, or single-transfected.

**Conclusions:** 1) AcLDL-binding increases SRA1 expression on the cell surface. 2) SRA1 is involved in foam cell formation.

**ThP13:W32** Influence of ursolesoxycholic acid on late steps of cholesterol synthesis

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**Objective:** The influence of Ursodeoxycholic Acid (UDCA) on hepatic sterol metabolism is not completely elucidated. Here we investigated the influence of UDCA on late steps of cholesterol synthesis.

**Methods:** Rat liver slices and HepG2 cells were incubated with different concentrations of UDCA and cholesterol precursors were measured by gas-chromatography/mass-spectrometry. Incubations with 13C-acetate and isotopomer spectral analysis (ISA) on different cholesterol precursors were performed.

**Results:** Dimethyl- and monomethylsperos in rat liver slices increased dose-dependent (2-15-fold) after incubations with UDCA (40-160 μM) for 12-h. In contrast, cholesterol precursors later in the biosynthetic pathway (e.g. lathosterol, desmosterol) declined. ISA analysis of lathosterol revealed a lower fractional synthesis rate for lathosterol. Interestingly, incubations of HepG2 cells with UDCA led to the accumulation of desmosterol.

**Conclusions:** Our results show that UDCA affects cholesterol synthesis by reducing demethylation of cholesterol precursors in rat liver slices, leading to a reduced synthesis of sterols after demethylation. Since the human hepatoma HepG2 cell line is known to have a reduced uptake of bile acids, the observed accumulation of desmosterol might be explained by a second, possible cell membrane mediated effect of UDCA on cholesterol synthesis.

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ThP14:W32  Inhibitory effect of squalene synthase inhibitors on triglyceride synthesis

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Objective: Squalene synthase (SQS), a key enzyme in cholesterol (Chol) synthetic pathway, has been reported to be a novel target to treat hyperlipidemia. We found that oral administration of ER-27856, a potent SQS inhibitor (SQSI), but not atorvastatin (ATOV) decreased plasma triglyceride (TG) in Watanabe heritable hyperlipidemic rabbits. To determine the mechanism how SQSIs decreased plasma TG, we focused on the effect of SQSIs (ER-27856 and RPR-107393) on de novo TG synthesis in rat hepatocytes.

Methods: Cells were incubated with William’s medium E containing 10% fetal bovine serum (FBS) or lipoprotein deficient FBS (LPDS). Cells were incubated with SQSIs for 2-24 hr in the presence or absence of 2 mM mevalonolactone (MVL) or 1 μM ATOV prior to addition of [14C]acetate. After 2 hr incubation with [14C]acetate, cellular [14C]Chol and [14C]TG were measured.

Results: In the time course study, SQSIs inhibited Chol synthesis in 2-24 hr incubation and suppressed TG synthesis in 6-24 hr incubation, but not in 2 hr incubation. ATOV potently inhibited Chol synthesis, but non TG synthesis. Importantly, MVL or LPDS potentiated the inhibitory activity of SQSIs to TG synthesis. In contrast, the effect was diminished in the presence of ATOV:

<table>
<thead>
<tr>
<th>IC50 of TG synthesis (nM)</th>
<th>FCS</th>
<th>FCS, 2 mM MVL</th>
<th>LPDS</th>
<th>FCS, 1 μM ATOV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-27856</td>
<td>100</td>
<td>3.5</td>
<td>4.8</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>RPR-107393</td>
<td>1040</td>
<td>47</td>
<td>130</td>
<td>&gt;10,000</td>
</tr>
</tbody>
</table>

Conclusions: We provided the first evidence that the continuous inhibition of SQS suppressed TG synthesis by increasing mevalonate-derived non-sterol product. SQSIs, which suppress both Chol and TG synthesis, may be a novel therapeutic agents to prevent atherosclerosis.

ThP15:W32  Deglycosylation is an atherogenic modification of ApoB-containing lipoproteins

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Objective: Several years ago we have found and isolated a subfraction of LDL with low level of sialic acid. In contrast to native styalized LDL, desialylated LDL was capable to accumulate cholesteryl esters in human aortic intimal smooth muscle cells and macrophages. In vitro treatment of VLDL and IDL with 2.6-and 2.3-specific sialidases causes an appearance of lipoprotein atherogenicity. Moreover, it was observed that demannosylation of VLDL, IDL and LDL with alpha-mannosidase is accompanied with an increase of lipoprotein atherogenic potential. Strong atherogenic effect was produced by the removal of lipoprotein high mannose and biantenary carbohydrate chains using endoglycosidases F1 and F2 and peptide-N-glycosidase F. In all cases, deglycosylation of VLDL, IDL and LDL was accompanied by aggregation of lipoprotein particles. The filtration of deglycosylated lipoprotein samples prevented intracellular lipid accumulation.

Results: Like desialylated LDL, in vivo desialylated VLDL and IDL stimulated free and esterified cholesterol content in human intimal smooth muscle cells and macrophages. In vitro treatment of VLDL and IDL with 2.6- and 2.3-specific sialidases causes an appearance of lipoprotein atherogenicity. Moreover, it was observed that demannosylation of VLDL, IDL and LDL with alpha-mannosidase is accompanied with an increase of lipoprotein atherogenic potential. Strong atherosclerotic effect was produced by the removal of lipoprotein high mannose and biantenary carbohydrate chains using endoglycosidases F1 and F2 and peptide-N-glycosidase F. In all cases, deglycosylation of VLDL, IDL and LDL was accompanied by aggregation of lipoprotein particles. The filtration of deglycosylated lipoprotein samples prevented intracellular lipid accumulation.

Conclusions: Deglycosylation-mediated lipoprotein aggregation is the reason for lipid accumulation in human intimal smooth muscle cells and macrophages.

ThP16:W32  Human plasma trans-sialidase affects LDL metabolism in aortic intimal smooth muscle cells

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Objective: Earlier we have found that incubation of human low density lipoprotein (LDL) with autologous blood plasma-derived serum leads to loss of sialic acid by lipoprotein particles. Later we have established that this modification is produced by trans-sialidase of human blood plasma. The goal of this study is to investigate the properties of plasma trans-sialidase and to elucidate an effect of this enzyme on the intracellular LDL metabolism.

Methods: Trans-sialidase was isolated from lipoprotein deficient serum by the affinity chromatography on polyclonal acid-asepharose. Results: Isolated trans-sialidase removes sialic acid from apolipoproteins and ganglycosides of LDL, VLDL, IDL, and HLDL particles (in order to decrease of rate) as well as from plasma glycoproteins (fetuin, transferrin, ferritin). Removed molecule of sialic acid can be bound to galactose or N-acetyl-galactosamine of acceptors by the alpha-2,3 and alpha-2,6 linkage. The lipoprotein particles, plasma proteins and glycosphingolipids may serve as the acceptors of trans-sialidase and sialic acid. Trans-sialidase treatment of native LDL leads to lipoprotein aggregation. Aggregates of trans-sialidase treated LDL is taken up by human aortic intimal smooth muscle cells via phagocytosis that leads to cholesteryl ester accumulation.

Conclusions: Trans-sialidase may be involved in earlier stages of atherogenesis.

ThP17:W32  The proteasome mediates the degradation of endocytosed lipoproteins

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Objective: Lipoprotein particles are receptor mediated endocytosed and then intracellularly degraded in the lysosomes. We now present data which indicate that in addition to the lysosomal enzymes, the proteasome contributes to the degradation of apolipoproteins.

Methods: In cultured human skin fibroblasts degradation of endocytosed 125I-VLDL was measured in the presence of proteasine inhibitors. In addition subcellular fractionation and gel filtration was included in the study.

Results: E64d, an inhibitor of cysteine proteases that penetrates into cells caused 24% inhibition. In combination with the pepidyl aldehydes MG132 or P113 inhibition increase up to 36 and 43%, respectively. MG132 and P113 are good inhibitors of the proteasome but have also some effect on cystein proteasines. We therefore used them in combination with E64d. In contrast lactacystin is considered specific for the proteasome and suitable for the use in cell culture. We measured 16% inhibition with lactacystin. In subcellular fractionation we detected undegraded 125I-VLDL in the cytoplasm.

Conclusion: It has previously been reported that de novo synthesized apoB and apoE are degraded by the proteasome. It is also known that virus are receptor mediated endocytosed and then released into the cytoplasm and there hydrolyzed by the proteasome. Up to our knowledge the results presented here indicate for the first time that the proteasome mediates the degradation of endocytosed lipoproteins.

ThP18:W32  Effect of atorvastatin on the metabolism of cholesterol and triacylglycerides in HepG2 cells

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Atorvastatin is a synthetic hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitor that effectively reduces plasma cholesterol and triacylglyceride levels. The effect of atorvastatin on plasma lipids in vivo is stronger compared to other HMG-CoA reductase inhibitors known so far. The aim of this study was to evaluate the effect of atorvastatin on the metabolism of cholesterol and triacylglycerides in vivo in comparison to lovastatin and simvastatin.

In HepG2 cells all drugs were equally effective in inhibiting cholesterol synthesis. The IC50 values of the three drugs were only slightly different (atorvastatin 68 nM, lovastatin 31 nM, simvastatin 93 nM). All compounds increased triacylglyceride synthesis by about 50%. Atorvastatin increased the receptor mediated endocytosis of LDL in a dose-dependent manner. At a concentration of 10-6 M, binding, uptake and degradation of 125I-labeled LDL was enhanced 1.6 to 2.1 fold. The effect of atorvastatin on LDL receptor expression was not significantly different compared to simvastatin and lovastatin. In transfection experiments using a plasmid coding for firefly-luciferase coupled to the LDL receptor promoter gene, atorvastatin increased the expression of the LDL receptor gene. As determined by reporter gene assays and Northern blot analysis all HMG-CoA reductase inhibitors significantly induced expression of fatty acid synthase (FAS). These data suggest that
atorvastatin does not significantly differ from other HMG-CoA reductase inhibitors in regard to its effect on cholesterol metabolism in vitro. We conclude that the higher efficacy of atorvastatin in reducing plasma lipids compared to lovastatin and simvastatin is likely due to pharmacokinetic differences rather than explained by different modes of action. We further demonstrate that the inhibition of sterol biosynthesis caused by statins is accompanied by an induced triacylglyceride synthesis in vitro.

**ThP19-W32**
Modifying THP-1 cell cholesterol and triglyceride content to mimick macrophage foam cells

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Macrophage foam cells from atherosclerotic plaques are enriched in neutral lipids, primarily cholesteryl esters (EC). Triglycerides (TG) generally account for <15% of the neutral lipid. In attempts to develop an in vitro macrophage foam cell model we incubated differentiated THP-1 cells, a human monoocyte/macrophage cell line, for up to 48 hours with acetylated LDL (acLDL) or acetylated rabbit β-VLDL (acβ-VLDL) to load them with cholesterol. Before loading, THP-1 macrophages had 12, <1, and 65 µg of free cholesterol (FC), EC, and TG per mg cell protein respectively. After incubation with acLDL or acβ-VLDL for 48 h the cellular cholesterol content was 28 µg/mg FC and 18 µg/mg EC for acLDL and 24 µg/mg FC and 33 µg/mg EC for acβ-VLDL. However, the acβ-VLDL induced a much greater increase in TG (284 µg/mg) than did the acLDL (104 µg/mg). Although EC levels of over 100 µg/mg can be achieved by extending the loading period, even at a lower level of loading, both of these liproproteins produced a much higher TG concentration than what is seen in vivo. TG content can be reduced by at least 50% without any loss of EC by incubation of loaded THP-1 cells in the presence of 400 μm albumin. Lower levels still need to be reached in order to produce cells which do not exceed more than 15% of the neutral lipid as TG, and additional techniques such as inhibition of TG synthesis coupled with removal of TG with albumin will likely be required.

**ThP20-W32**
Mechanisms involved in the triglyceride-lowering effect of atorvastatin in fructose-fed rats


**Objective:** To determine the effect of atorvastatin (ATV) in fructose-fed rats on key enzymes involved in the hepatic synthesis of triglyceride (TG) and the free fatty acid (FFA) turnover in adipose tissue.

**Methods:** Male Sprague-Dawley rats were fed 10% fructose for two weeks and treated with 5 and 30 mg/kg of ATV or drug vehicle alone (fructose group), administered by oral gavage. Control rats did not receive either fructose or drug. After sacrifice liver and adipose tissue samples were taken for analysis of: a) phosphate diester phospholipase (PAP), 3-hydroxy-3-methylglutaryl-CoA reductase (HMG- CR) and microsomal triglyceride transfer protein (MTP) enzyme activities; b) HMG-CR, acetyl-CoA carboxylase (ACC) and acylation stimulating protein (ASP) mRNA levels; and c) cholesterol (C) and TG hepatic concentrations. Further, plasma apoB and lipid concentrations, comprising C, HDL-C, apoB-100, TG and FFA, were determined.

**Results:** ATV at 30 mg/kg increased HMG-CR mRNA levels and activity (134% and 240%, respectively, vs fructose group), without modifying PAP and MTP activities. Further, ASP mRNA levels were reduced (38% vs fructose group), while no significant changes in ACC mRNA levels were observed. Fructose feeding caused an increase of plasma and liver TG concentration (100% and 146% respectively, vs control) and both were reduced by ATV treatment (48% and 46%, respectively, vs fructose group, at 30 mg/kg). Neither plasma cholesterol nor apoB concentration were changed by ATV, whereas FFA levels were reduced (49% vs fructose group, at 30 mg/kg).

**Conclusions:** ATV reduced the hypertriglyceridemia induced by fructose feeding without affecting PAP or MTP activity. Moreover, ATV was able to reduce markedly liver TG content, probably due to a decreased flow of FFA into the liver, as plasma FFA levels were reduced. FFA turnover in adipose tissue seems to be involved in the reduction of plasma FFA, since ASP mRNA levels were decreased by ATV treatment. This work was supported partly by FPCNL, Parke-Davis (project nº 981-421-07) and CICYT grants SAF97-0215 and SAF98-0105.

**ThP21-W32**
Role of ganglioside GM3 in foam cells formation

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**Objective:** Formation of foam cells in arterial intima is associated with cell differentiation under influence of large range of stimulators. Ganglioside GM3 is known to accumulate in atherosclerotic lesions. Gangliosides, are located at the cell surface and involved in cell differentiation.

**Results:** An average of fivefold increase in ganglioside GM3, a major intimal ganglioside, was demonstrated in intimal atherosclerotic plaques by contrast to uninvoluted intimal areas of the same aorta. This agrees with immunohistochemical finding that sections of intimal atherosclerotic plaques contained cells specifically and very intensive stained with antibodies against ganglioside GM3 in 100 µM concentration as well as antibody-negative cells. A low but specific expression of GM3 by SMC in the regions bordering atherosclerotic plaques without histological signs of atherosclerosis, could be demonstrated with antibody to GM3 in a 285 µM concentration. Double immunostaining procedures with anti-GM3 antibodies and antibodies against ox-LDL showed that both GM3 and ox-LDL were located at areas occupied with macrophage origin foam cells (CD68 positive). Ultrastructural analysis of intimal areas with GM3 overexpression in cells and extracellular space showed that part of the foam cells are macrophage origin. Another the foam cells were drawn from VSMC.

**Conclusions:** These results provide evidence that GM3 accumulation is a prerogative of foam cells independently from their origin. It was found that significant GM3’s incorporated in human myelogenous leukemia cells during their differentiation into monocyte/macrophage type, and GM3 itself was highly potent in the induction of monocyte/macrophage differentiation (Saito 1989). Similar cell differentiation are suggested to take place in arterial intima during atherosclerotic lesion formation.

**ThP22-W32**
Naringenin, a citrus flavonoid, inhibits apoB secretion from HepG2 cells, an effect associated with selective inhibition of ACAT-2 expression

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Naringenin, the principle flavonoid in grapefruit, has been shown to dramatically reduce both net apoB secretion and cholesterol esterification in HepG2 cells. The present study was designed to test the hypothesis that the fructose-induced decrease in apoB secretion was due to decreased expression of ACAT-1, ACAT-2 or MTP and an increase in intracellular apoB degradation. The relationships between apoB100 secretion and intracellular degradation were examined using pulse-chase protocols and the data analyzed by multicompartamental modeling. The kinetic model describes an initial compartment from which apoB can be secreted, via a second intracellular compartment, or degraded - both rapid and slow pathways. Olate (100 mM) stimulated apoB secretion 1.4 fold (p < 0.01) and decreased degradation via the rapid pathway (p < 0.05). Pre-incubation (24 h) of oleate-treated cells with naringenin (10 to 200 μM) resulted in a dose-dependent inhibition of apoB secretion (up to 58% at 200 μM, p < 0.001). Naringenin enhanced apoB degradation by increasing the proportion removed via the rapid pathway (p < 0.01). The secretion of apoA1 and apoE were unaffected. Naringenin decreased cholesterol esterification in oleate-treated cells by 75% (p < 0.05). Cellular mRNA content for ACAT-2 was decreased by 47% (p < 0.0001) whereas ACAT-1 mRNA was unaffected. This finding is consistent with the hypothesis that ACAT-2 primarily provides cholesterol esters for lipoprotein assembly. Naringenin also inhibited MTP activity by 30% (p < 0.05) and decreased MTP mRNA by 31% (p < 0.02). We conclude that naringenin reduces oleate-stimulated apoB secretion by activating a rapid degradation pathway. This effect may be due to reduced apoB lipiddiation mediated by the selective inhibition of both ACAT-2 and MTP expression.

**ThP23-W32**
Characterization of cleavage enzymes for sterol regulatory element binding protein

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**Objective:** Sterol Regulatory Element Binding Proteins (SREBPs) are key...
transcription factors for the regulation of the cellular cholesterol level. Cleavage enzymes regulate maturation and degradation of SREBPs. To study these enzymes, we developed an in vitro assay system and characterized them.

Methods: A fluorogenic peptide substrate, MOCaC-GRSVLFSK (Dnp)pr-NH₂, was synthesized according to the proposed cleavage site of human SREBP-2. The hamster liver microsome proteins were solubilized and separated by series of chromatographies. Purified proteins were subjected to amino acid sequence analysis. Human S1P (site 1 protease) or SREBP-2 DNA was inserted into pBlueBac plasmid and each recombinant virus was obtained by co-transfection with baculo virus to S9 cell.

Results: In microsome fractions from hamster liver, we found a peptide activity inhabitable by the synthetic inhibitor Ac-GRSVL-aldheyde with an IC₅₀ of 40 nM. This peptidease separated into several peaks upon gel permeation chromatography. Partial amino acid sequence analysis revealed that they are thepepsein B and nephrilysin. Membrane bound S1P expressed on baculo virus cleaved SREBP-2 in vitro.

Conclusions: By using synthetic peptide substrate, we purified cathepsin B and nephrilysin from hamster liver microsome. These findings suggest several degradative pathways for SREBP.

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Intrahepatic trafficking of free and remnant-bound fatty acids

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Objectives: The compartments involved in the trafficking of free and remnant-bound fatty acids of different chain length and degrees of saturation in rat hepatocytes were to identify.

Methods: After intravenous injection of free or chylomicron remnant-bound ³H-palmitic, ³H-oleic or ⁴H-eicosapentaenoic acid, ⁴H in plasma membranes, early and late endosomes, lysosomes, mitochondria and peroxisomes was determined.

Results: Within 60 min ³H-free fatty acids were up to 40-fold enriched in multivesicular bodies, and fatty acids of remnant > 100-fold similar to LDL. At any time point up to 15% of fatty acids were in mitochondria, and up to 10% of ⁴H-eicosapentaenoic acid in peroxisomes.

Conclusions: In hepatocytes free fatty acids are at least in part transported via the same endosomal pathway as remnant-bound fatty acids. Fatty acids do not enter lysosomes, but mitochondria. Longer chain fatty acids reach also peroxisomes.

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Expression of cholesterol regulatory proteins on extracellular baculoviruses

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Objective: Cholesterol regulatory proteins, such as SREBPs, SCAP and HMG-CoA reductase are membrane proteins located in the endoplasmic reticulum. The transmembrane domain of these proteins are postulated to be important for cellular cholesterol regulation. To investigate the functions and roles of these membrane proteins, we have developed an efficient expression and purification system using baculovirus.

Methods: Each pBlueBac plasmid containing human SREBP-2, SCAP or HMG-CoA reductase cDNA was cotransfected with wild-type baculovirus DNA into S9 cells. Recombinant viruses were transfected to S9 cells or Hi-Five Cells. After 24 hr to 96 hr, cells and culture supernatant were collected and subjected to western blot analysis using specific antibodies.

Results: Recombinant proteins were appeared at 24 hr in the cell lysate and at 48 hr in the supernatant fraction. In cell lysate, degradation of SREBP-2 was observed at 48 hr, which was less prominent in supernatant fraction. With ultracentrifugation of this supernatant, SREBP-2 was recovered in 40,000 g pellet representing extracellular virus (ECV). Also SCAP and HMG-CoA reductase were detected ECV fractions. SREBP-2 was efficiently solubilized and purified from ECV fraction.

Conclusion: We found that the ER membrane protein has expressed on the ECV. This system will be very useful for studying of cellular regulatory mechanism.

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Primary human hepatocytes are more suitable than HepG2 cells for the study of catabolism of cholesterol to bile acids

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Objective: Hepatic catabolism of cholesterol to bile acids is of major importance for the excretion of cholesterol from the human body. Impaired bile acid formation may lead to hypercholesterolaemia and, finally, atherosclerosis. The present study aimed at comparing bile acid metabolism in primary cultures of human hepatocytes and the human hepatoblastoma cell line HepG2.

Methods: Primary human hepatocytes (PHH), prepared from organ donor liver, and HepG2 were grown under established conditions. For the study of bile acid de novo synthesis, media was renewed and collected every 24 hours for five days. For the study of glycosidic bile acid conjugation, media were supplemented with 100 µM of bile acids and 1–2 µM of UDP-glucose, -glucuronic acid, -N-acylglucosamine, and -octyl-glucoside, resp. Media were extracted using GC/MS.

Results: PHH secreted 71% cholic acid (CA), 24% chenodeoxycholic acid (CDCA) and 5% bile acid precursors, compared to 25% CA, 26% CDCA and 49% precursors in case of Hep G2 cells. In PHH, 95% of the bile acids were conjugated with glycine or taurine whereas only 30% of bile acids and 14% of precursors were conjugated in case of Hep G2 cells. In contrast to Hep G2 cells, PHH were also capable of forming glycosidic bile acid conjugates.

Conclusions: Cultured human hepatocytes resemble intact liver as they are able to convert cholesterol almost completely to conjugated cholic and chenodeoxycholic acids. This is in contrast to cultured Hep G2 cells which release large amounts of bile acid precursors and are defective in several bile acid conjugation reactions.

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PPARs regulate genes involved in cholesterol homeostasis in human monocyte/macrophages

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PPARs are transcription factors regulating the expression of genes implicated in lipid metabolism, cellular differentiation and inflammation. However, the functions of PPARs in lipid metabolism in macrophages is still unknown. Here, we investigated the regulation of two genes involved in intracellular cholesterol metabolism in human macrophages. CLA-1/SR-BI is a scavenger receptor which binds HDL with high affinity and LPL is the major enzyme responsible for the hydrolysis of lipoprotein triglycerides. Here we show that CLA-1 is induced upon differentiation of monocytes into macrophages. Immunohistochemical analysis on human atherosclerotic lesions showed high expression of CLA-1 in macrophages of the lipid core colonizing with PPARα and PPARγ. Activation of PPARα and PPARγ resulted in the induction of CLA-1 protein expression in differentiated macrophages. In addition, we demonstrate that incubation of monocytes or differentiated macrophages with PPAR ligands resulted in an increase of LPL protein and mRNA levels. The induction of LPL expression by PPAR activators was associated with an increased LPL mass secreted in the medium but, surprisingly, LPL enzyme activity was significantly reduced. Furthermore, PPARs increased the secretion of apoB, a known inhibitor of LPL activity, thus providing a possible explanation for the decreased LPL activity. Finally, immunohistochemical analysis of aortas of apoE deficient mice showed the presence of SR-BI and LPL proteins in atherosclerotic plaques which was furthermore increased after treatment with the PPAR ligands. In conclusion, our results demonstrate that PPAR activators modulate cholesterol homeostasis in atherosclerotic lesion macrophages through the CLA-1/SR-BI and LPL pathways.

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Structure and promoter analysis of the human PPARα gene

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PPARα is a key regulator of lipid and lipoprotein metabolism and exerts anti-inflammatory activities in the vascular wall. Thus PPARα may play an important modulatory role in the development of atherosclerosis. Identification of factors regulating PPARα expression is of major interest to understand PPARα physiology in humans. However, the regulation of human PPARα gene
expression remains largely unknown. In this work, the genomic structure of the human PPARα was determined and its 5' flanking region was functionally characterized. Based on available cDNA sequence, the intron-exon boundaries of the human PPARα gene were mapped. Similar to its mouse homologue, the human PPARα gene is composed of eight exons. The 5' untranslated region is encoded by the first two exons and part of exon 3. The remainder of exon 3 plus exons four to eight code for the protein. By RT-PCR analysis we identified an alternatively spliced form of exon 1 in both human liver and adipose tissue samples. This genomic organization is similar to the 5' region of the mouse PPARα gene. 5th RACE was performed on human liver RNA in order to map the transcription initiation site (TIS). The proximal promoter of the human PPARα gene contains no typical TATA or CAAT boxes boxes that have a high GC content. These features are also present in the mouse PPARα gene. However, both the 5' untranslated region and the promoter region show no significant degree of sequence conservation between species. The transcription factor 3.5 kb in front of the TIS was cloned into a Luciferase reporter vector this construct was shown to possess significant PPARα promoter activity in HepG2 cells. Future identification of metabolic factors governing human PPARα gene expression and characterization of the regulatory elements within the human PPARα promoter involved in its regulation will help to elucidate the role PPARα plays in human metabolic disorders.

**ThP29.W32** Metabolism of 7-ketocholesterol by sterol 27-hydroxylase in human hepatic cells

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Recently we reported that radiolabeled 7-ketocholesterol delivered to rats in a modified lipoprotein and cleared mainly by the liver was rapidly excreted into the intestine as aqueous-soluble products, presumably bile acids (1). In the present study we aimed to elucidate the primary route by which 7-ketocholesterol is metabolised. We hypothesised that 27-hydroxylase, a mitochondrial cytochrome P450 and the first enzyme of the acidic bile acid pathway, is responsible for the initial metabolism of 7-ketocholesterol. HepG2 cells, a human hepatic cell-line, were used to investigate the metabolism of 7-ketocholesterol. Indeed, 27-hydroxylated 7-ketocholesterol was shown to be the initial, lipid-soluble product of 7-ketocholesterol metabolism. It was produced in mitochondrial incubations and cells, and was mostly released into the media from the cells. Intact cells generated aqueous-soluble products early and extensively and their production was ablated with Cyclosporin A, a sterol 27-hydroxylase inhibitor. Furthermore, we have demonstrated the effectiveness of a second, potent inhibitor of this enzyme. This new inhibitor also ablated the production of aqueous-soluble products in cells and decreased the production of 27-hydroxylated 7-ketocholesterol in mitochondrial preparations. This is the first study to demonstrate that sterol 27-hydroxylase plays an important role in the hepatic metabolism of oxysterols.

References


**ThP30.W32** Accumulation of oxidised esters in lysomes of macrophage foam cells

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**Objective:** Cholesterol- and cholesteryl ester-rich macrophage foam cells, characteristic of atherosclerotic lesions, are often generated in vitro using oxidized low-density lipoprotein (OxLDL). However, relatively little is known of the nature and extent of sterol deposition in these cells, nor of its relevance to the foam cells formed in atherosclerotic lesions.

**Methods:** We determined the content and subcellular processing of sterols in OxLDL-loaded macrophages, and compared this with cells loaded with acetylated LDL or 7-ketocholesterol-enriched acetylated LDL (7KCAcLDL; cholesteryl ester-loaded cells selectively supplemented with 7-ketocholesterol (7KC), the major oxysterol present in OxLDL).

**Results:** Both cholesterol and 7KC and their esters were measured in cells after uptake of these modified lipoproteins. Oxysterols comprised ~50% of total sterol in OxLDL-loaded cells. Free 7KC and cholesterol partitioned into cell membranes, with no specific retention in lysosomes. The cells also contained cytosolic, ACAT-derived, cholesteryl and 7KC esters. Esterification of free cholesterol and 7KC was 10-fold less in OxLDL-loaded cells than in AcLDL or 7KCAcLDL-loaded cells, partly due to fatty acid limitation. OxLDL-loaded macrophages also contained large (~40–50% total cell sterol content) pools of oxidized esters, containing cholesterol or 7KC esterified to oxidized fatty acids. These were insensitive to ACAT inhibition, very stable and located in lysosomes, indicating resistance to lysosomal esterases.

**Conclusions:** Macrophages loaded with OxLDL do not accumulate free sterols in their lysosomal compartment, but do accumulate lysosomal deposits of OxLDL-derived cholesterol and 7-ketocholesterol esterified to oxidized fatty acids. The presence of similar deposits in lesions foam cell would represent a pool of sterols which is particularly resistant to removal.

**ThP31.W32** Sterol 27-hydroxylase acts on 7-ketocholesterol in human atherosclerotic lesions and macrophages in culture

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**Objective:** 27-Hydroxycholesterol (27OH) is the major oxysterol in human atherosclerotic lesions followed by 7-ketocholesterol (7KC). Whereas 7KC is believed to originate non-enzymically, 27OH arises by the action of sterol 27-hydroxylase, a cytochrome P450 enzyme that is expressed at particularly high levels in the macrophage and proposed to represent an important pathway by which macrophages eliminate excess cholesterol. We hypothesised that 27-hydroxylated 7-ketocholesterol (27OH-7KC) may also be present in human lesions, generated by the action of sterol 27-hydroxylase on 7KC.

**Method and Results:** Analyses of 10 samples obtained from carotid endarterectomies indicated the presence of 27OH-7KC (mean ± SD: 17.5 ± 8.5 mmol/mmol 7KC; 38 ± 46 µmol/mmol cholesterol). Levels of 27OH-7KC were not correlated with 27OH but were strongly associated with 7KC levels (r = 0.90; p < 0.0005), suggesting that this oxysterol is largely produced by the action of sterol 27-hydroxylase on 7KC rather than non-enzymic oxidation of 27OH. Moreover, 27OH-7KC was produced by human macrophages in culture supplied with 7KC and was predominantly secreted into the medium. The majority of 7KC was metabolised to aqueous soluble products by human macrophages in culture and this metabolism was ablated by a sterol 27-hydroxylase inhibitor.

**Conclusions:** Sterol 27-hydroxylase therefore appears to represent an important pathway by which macrophages eliminate not only cholesterol but also oxysterols such as 7KC. The fact that 7KC (and cholesterol) still accumulates in lesions and foam cells indicates that this pathway may be perturbed in atherosclerosis.

**ThP32.W32** Glycosylation mutants of human lysosomal acid lipase

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**Objective:** To analyze the six potential N-glycosylation sites of human lysosomal acid lipase (LAL), the key enzyme in lysosomal lipid metabolism.

**Methods:** A baculovirus vector was utilized for high-level expression of LAL in cultured insect cells

- a carboxyterminal polyhistidine tag for Nickel-NTA affinity chromatography allowed enzyme purification by a single step method from tissue culture supernatants
- site-directed mutagenesis of the six potential glycosylation sites using PCR overlap strategy to obtain Asn- > Gln mutants
- measurement of wildtype and mutant LAL activities in supernatant with tritium-labeled triacylglycerol

**Results:** To investigate which of the six potential glycosylation sites are used in LAL, single mutants were produced. Two mutants at positions Asn134 and Asn246 showed no activity and were not detectable on a SDS-PAGE stained with silver nitrate. These two mutants were also not detectable in Western blot analysis with a monoclonal antibody recognizing the carboxyterminal polyhistidine tract. The mutant at position Asn9 showed a significantly reduced activity in comparison to wildtype LAL. Double mutants in all possible combinations except for positions 134 and 246 were produced. Double mutants in combination with position 9 showed a reduced lipolytic activity compared to the others.

**Conclusions:** The data indicate that three of the six N-linked glycosylation sites are used in the LAL protein. Asn134 and Asn246 are essential for LAL activity. Asn9 is glycosylated, but glycosylation is not important for LAL activity.
**P:W33** PROLIFERATION AND DIFFERENTIATION OF SMOOTH MUSCLE CELLS

**ThP1:W33**

**Jun protein expression is required for serum induced smooth muscle cells proliferation - Regulated by p38 and BMK1 map kinases cooperatively**

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**Objective:** To explore the relationship between p38 and BMK1 MAP kinases on up-regulating the c-jun gene transcription through phosphorylation of MEF2 family transcription factors, and its importance on serum-induced smooth muscle cells (SMC) proliferation.

**Methods:** In vitro kinase assay, reporter gene assay, western blot, immunostaining, [3H]-thymidine incorporation.

**Results:** MEF2A, MEF2C and MEF2D, but not MEF2B, are preferred substrates for Big MAP kinase 1 (BMK1), while MEF2A and MEF2C only, are preferred substrates for p38 MAP kinase. Both p38 and BMK1 MAP kinases trans-activate MEF2 family transcription factors, and they are in different pathways. Like p38, BMK1 up-regulates c-jun transcription through phosphorylation of MEF2 proteins, and these two MAP kinases have cooperative effect on it. Inhibition of p38 and BMK1 MAP kinase pathways may result in inhibition of serum-induced c-jun transcription, and furthermore, inhibition of serum-induced SMC proliferation.

**Conclusions:** Jun protein expression is required for SMC proliferation. The p38 and BMK1 MAP kinases are the crucial pathways for serum-induced signal transduction in SMC proliferation.

**ThP2:W33**

**A novel adipocyte-derived plasma protein, adiponectin, inhibits vascular smooth muscle cell proliferation**

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**Objective:** Obesity is the most common nutritional disorder and one of the major risk factors of atherosclerosis. However the molecular basis for the link between obesity and atherosclerosis has not been fully elucidated. We found an adipocyte-specific secretory protein, adiponectin, and developed an enzyme-linked immunosorbent assay system to determine adiponectin concentrations.

**Methods:** Plasma adiponectin level negatively correlated with body mass index (Arita et. al. BBRC 1999) and was significantly low in patients with coronary artery disease compared with body mass index-adjusted control subjects (Ouchi et. al. Circulation 1999). Vascular smooth muscle cell proliferation in the vascular wall is considered crucial for the development of atherosclerotic intimal thickening as well as restenosis after percutaneous transluminal coronary angioplasty. Here we investigated the effects of adiponectin on proliferation of human aortic smooth muscle cell.

**Results:** Cell proliferation was analyzed by [3H]thymidine uptake and cell count. Growth signals were quantified by immunoprecipitation and western blot.

**Conclusions:** Adiponectin acts as an endogenous modulator of smooth muscle cell proliferation through MAP kinase pathway.

**ThP3:W33**

**Scavenger receptors are expressed on pseudoendothelium composed of smooth muscle cells in NZW rabbit aorta after balloon denudation**

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**Objective:** Expression of SR-A in macrophages (M0s) has been well-defined in atherogenesis, host defense and calcium-independent adhesion of monocytes. However, the expression of SR-A in smooth muscle cells (SMCs) in vivo has remained controversial. The aim was to study the expression of SR-A in various types of New Zealand White (NZW) rabbit atherosclerotic lesions.

**Methods:** A new high-titer antisera (SRKO 8) produced in knockout SR-A-mice was used to detect SR-A expression in balloon-denuded NZW
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rabbit aortas. Fifteen rabbits with and without cholesterol diet (0.5%) were balloon-damaged. Aorta was studied with immunocytochemistry one and two weeks after the damage.

Results: Cell types were identified with the following Mabs: SMCs: HHP-5, Mogs: RAM-11, endothelial cells: CD-31. It was found that SRKO 8 antisem recognizes the luminal layer of SMCs devoided of CD-31 positive endothelial cells in the deendothelialized rabbit aorta.

Conclusions: It is concluded that in the deendothelialized aortas the luminal layer consisting of SMCs expresses SR-A. It is possible that this may give SMCs pseudendothelium-like properties in the absence of intact endothelium.

ThP4.W33  Endogenous opioids have an inhibitory effect on arterial smooth muscle cell proliferation after intimal injury

Objective: How stress influence the process of arteriosclerosis is unknown. We investigated the role of endogenous opioids (the major stress-protecting hormone) on the rat model of arteriosclerosis both in vivo and in vitro.

Methods: In 28 rats the intima of descending aorta was injured with 2 F baloon catheter. Naltrexone (Nal: 2 mg/kg/d x 7 days), Met-enkephalin (ENK: 10 ng/kg/d, n = 7), beta-endorphin (END: 10 ng/kg/d, n = 7) or phosphate buffered saline (PBS, n = 7) were administered intraperitoneally by infusion with osmotic pump. Three days after the medial SMC were examined for expression of proliferating cell nuclear antigen (PCNA) by immunohistochemistry. Influences of Nal (0.002 mg/ml), ENK (10 pg/ml), END (10 pg/ml) on PDGF induced proliferating activities of cultured rat aortic SMC were measured by tritiated thymidine incorporation method.

Results: PCNA labeled nuclei were significantly decreased in the ENK group (63%, p < 0.01) and in the END group (-32%, p, 0.05) compared with PBS. But Nal did'n have a significant effect. Increase in tritiated thymidine incorporation with PDGF was significantly inhibited by ENK (50%, p < 0.01) and by END (-56%, p < 0.01), but not by Nal.

Conclusion: Endogenous opioids had the inhibitory effect on the SMC proliferation induced by both intimal injury of aorta and PDGF.

ThP5.W33  Differential spatial distribution of activated MAP-kinases in smooth muscle cells after endothelin-1 stimulation: No nuclear translocation of ERK

Objective: Activation and inflammatory response of vascular smooth muscle cells (SMC), mediated by mitogen activated protein (MAP)-kinases, has been implicated in plaque destabilization. Therefore the effect of endothelin (ET-1) on activation of MAP-kinases c-Jun amino terminal kinase (JNK), p38MAPK and extracelluar signal regulated kinase (ERK) in SMC was studied.

Methods: SMC were exposed to ET-1 for different times at a concentration of 10^{-6} M. MAP-kinase activity was quantitated by Western Blot and immunocomplex kinase assay. Nuclear translocation of kinases was assessed by immunocytochemistry. Activity of transcription factors (TF) were studied by AP-1 gel shift assay.

Results: ET-1 stimulation resulted in an ET-1A receptor dependent activation of JNK, ERK and p38MAPK. In response to ET-1, ERK and p38MAPK showed the fastest response with a peak activation at 5 min. The kinase activity and the nuclear translocation of JNK was maximal at 30 min after addition of ET-1 and both went back to baseline at 60 min. Also for p38MAPK concomitant activation and translocation was observed. In contrast, kinase activity of ERK was maximal at 5 min after ET-1 stimulation, whereas nuclear presence of ERK was first detected at 30 min after ET-1 stimulation, when ERK kinase activity was already back to baseline. Accordingly the AP-1 complex almost exclusively consisted of c-Jun as evidenced by supershift assays. Different from ET-1, stimulation with platelet derived growth factor (PDGF) led to a concomitant maximum of ERK kinase activity and nuclear translocation at 5 min after stimulation. Phosphorylation of the nuclear TF Elk, which can be activated by ERK and JNK, was detected 5 min after PDGF stimulation, however, after ET-1 stimulation was most marked at 30 min, the peak of JNK translocation.

Conclusion: The data describe a new mechanism how MAP-kinases can lead to differential signal transduction. Whereas JNK was activated and readily translocated to the nucleus, activated ERK was exclusively found in the cytosol. Hence it is conceivable that after ET-1 stimulation JNK directly regulates transcription factors in the nucleus whereas ERK may affect cytosolic proteins rather than nuclear ones.

ThP6.W33  Senescent and activated pericyte-like cells in human aorta
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Objective: To identify pericyte-like cells in human aorta and to reveal the relationship between the different pericyte-like cells and the main atherosclerotic manifestations: lipidosis, fibrosis and proliferation.

Methods: Senescent pericycle-like cells (SP) were revealed with antibody against O-sialoglycoside. Activated pericyte-like cells (AP) were identified using antibody against high molecular weight melanoma associated antigen. SP and AP were colocalized with the sites of lipid deposition, procollagen type I (PC1) synthesis and proliferating cell nuclear antigen (PCNA) expression.

Results: SP were abundant in the subendothelial intima of grossly normal human aorta, where they comprised up to one third of total cell population and formed continuous subendothelial network. In atherosclerotic lesions the number of SP dramatically decreased. The negative correlation was found between the number of SP and the lipid deposition in the intima. We failed to find out PC1-synthesizing SP and PCNA-positive SP. On the contrary, AP were not found in grossly normal intima, but their number increased in atherosclerotic lesions, where it closely correlated with lipid deposition. Some of AP expressed PC1. But the most remarkable feature of AP was the expression of PCNA. The proliferative index of AP was about 20% in compare to 2-3% proliferative index of resident intimal cells.

Conclusions: We assumed that SP are bearing the homeostasis in normal intima similar to microcirculatory pericytes. In atherosclerotic lesions AP become predominant type of pericyte-like cells. With their high proliferating potency AP may participate in compensatory processes in atherosclerotic lesions serving as the source for renovation of resident cell population.

ThP7.W33  Fucoidan inhibits smooth muscle cell proliferation by reducing mitogen-activated protein kinase activity
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Objective: Fucoidan has been shown to inhibit the proliferation of arterial smooth muscle cells both in animal models and in vitro, however, the mechanisms behind the anti-proliferative effects of this polysulfated polysaccharide is not known in detail.

Methods: The inhibitory effect of fucoidan on rat smooth muscle cell proliferation was examined and compared with the effects of heparin after stimulation with fetal calf serum, platelet-derived growth factor BB, basic fibroblast growth factor, heparin-binding epithelial growth factor, and angiotensin II. The cultures were analysed with respect to DNA synthesis, expression of phosphorylated and activated mitogen-activated protein kinase, and nuclear translocation of phosphorylated and activated mitogen-activated protein kinase.

Results: Fucoidan was shown to inhibit smooth muscle cell proliferation in a similar manner as heparin, reduced growth factor-induced expression of phosphorylated mitogen-activated protein kinase and prevented nuclear translocation of phosphorylated and activated mitogen-activated protein kinase.

Conclusion: Fucoidan is a more potent anti-proliferative polysulfated sugar than heparin and appears to mediate its effects through the inhibition of mitogen-activated protein kinase in a similar manner as heparin.

ThP8.W33  Differential effects of lovastatin on cell cycle progression depending on the dose
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Objective: To determine the role of cholesterol and non-sterol mevalonate derivatives in cell cycle progression.

Methods: HL-60 and MOLT-4 human cell lines were cultured in a cholesterol-free medium and treated with increasing concentrations of lovastatin and their effects on cell counts, DNA synthesis, cell cycle distribution and BrDU incorporation were studied.

incorporation into DNA were studied. The cholesterol pathway was monitored by measuring the incorporation of \(^{14}C\)-acetate into organic acids and sterols, separately, by HPLC.

**Results:** At relatively low concentrations (up to 10 \(\mu M\)), lovastatin blocked cholesterol synthesis barely affecting the rate of \(^{14}C\)-mevalonate formation, which resulted in cell proliferation inhibition and accumulation of cells preferentially at G2; these effects were both prevented and reversed by LDL-cholesterol. At higher doses (50 \(\mu M\)), lovastatin significantly inhibited mevalonate synthesis and cell cycle was arrested at both G1 and G2. Both LDL-cholesterol and a minimal amount of mevalonate were required to prevent these effects of high lovastatin doses. Notably, mevalonate alone allowed cells at G1 to advance to G2, whereas LDL-cholesterol permitted cells at G2 to transit M phase, completing division.

**Conclusions:** The results show that mevalonate or some non-sterol mevalonate derivatives are required for the G1-S transition while cholesterol is specifically needed for the G2 traversal. Therefore, cell cycle is differently affected depending on the availability of these metabolites.

**ThP11:** Role of the cyclic AMP-dependent pathway in free radical-induced cholesterol accumulation in vascular smooth muscle cells
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**Objectives:** The transformation of macrophages and smooth muscle cells (SMC) into foam cells is a key event in atherogenesis. Oxidant stress and oxidized LDL are determinant in this process. We have chosen to study direct effects of free radicals on intracellular cholesterol homeostasis in vascular SMC.

**Methods:** Using an azo-type free radical generator and rat aorta SMC (A7R5), we have set up optimal conditions to study the effects of minimal oxidant stresses with no significant changes in cell viability.

**Results:** We found that oxidant stress lead to an increase in total cholesterol which appeared to be due to: 1/ an increase in cholesterol biosynthesis; 2/ a decrease in cholesterol ester hydrolysis; 3/ a reduced HDL-mediated cholesterol efflux. All these effects were opposed by antioxidants. Intracellular cyclic AMP concentration was also dose-dependently decreased by oxidant stress cell overrun. Experimental conditions, incubations in the presence of adenylate cyclase stimulators (carbachol, forskolin) did not restore the cAMP levels. Conversely, addition of dibutyryl cAMP normalized the cholesterol content through normalization of the oxidant stress-induced change of enzymes involved in cholesterol metabolism (HMG-CoA reductase, ACAT, cholesterol esterase).

**Conclusions:** Our data suggest that free radicals alter cellular cholesterol homeostasis by mechanisms leading to impairment of adenylate cyclase activity. The resulting intracellular low cAMP concentration seems to be determinant for the regulation of enzymes involved in cholesterol metabolism. Oxidant stress-mediated cholesterol loading of vascular SMC may favor the formation of foam cells leading to atherosclerotic lesions.

**ThP12:** Lipid liberated from dead macrophages is responsible for smooth muscle foam cell formation in a co-culture system
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Conversion of arterial smooth muscle cells (SMC) into foam cells contributes to the progression of atherosclerotic lesions. We have developed a co-culture system using macrophages and SMC to investigate smooth muscle-derived factors. J774 or mouse peritoneal macrophages were cholesterol enriched using acLDL and free cholesterol/cholesterol dispersions. A fluorescent fatty acid was also added to label the cholesterol esters in cytoplasmic inclusions in macrophages. The macrophage cells were then plated on one side and rabbit aorta SMC on the other side of a 2 well chamber slide in medium containing the ACAT inhibitor CP-113,818. Exposure of cholesterol-enriched macrophages to an ACAT inhibitor causes necrosis and apoptosis, resulting in the liberation of the cytoplasmic lipid inclusions as well as other cell debris. After a 24 hour incubation, the barrier between the wells was removed to allow all the two cell types to interact. Incorporation of fluorescently labeled macrophage cholesterol ester inclusions into SMC was monitored by fluorescent microscopy. After 2 days of incubation, the SMC phagocytized a substantial amount of lipid (total cholesterol is increased 4-fold) which resulted in a smooth muscle foam cell phenotype. In a parallel set of experiments we determined that the cholesterol ester taken up by the SMC was initially located in lysosomes, hydrolyzed by the acid lipase to free cholesterol, re-esterified by ACAT and deposited in a cytoplasmic compartment. Thus, we have produced a series of events, starting with macrophage foam cell formation, followed by macrophage foam cell death and ending with the production of smooth muscle-derived foam cells. This is consistent with the events thought to occur during atherosclerotic lesion progression.

**P:W34** LIPOPROTEIN RECEPTORS

**ThP11:** Biosynthesis and posttranslational processing of lectin-like oxidized LDL receptor-1 (LOX-1)
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**Objective:** To clarify the mechanism and role of posttranslational processing
of LOX-1, which can act as a cell surface receptor for atherogenic oxidized LDL.

Methods: TNF-α-activated BAEC and CHO cells stably expressing bovine LOX-1 (BLOX-1-CHO) were subjected to pulse-chase labeling and glycansese digestion to examine the mode of posttranslational processing of LOX-1. To see the role of N-linked glycosylation of LOX-1 in cell-surface expression, FACs analysis, cell ELISA and immunofluorescence confocal microscopic analysis were performed. Furthermore, the role of N-linked glycosylation in the ligand binding was examined by binding assay using radiolabeled Ox-LDL.

Results: LOX-1 is synthesized as a 40kDa precursor protein with N-linked high mannose carbohydrate chains (Pre-LOX-1), which is subsequently further glycosylated and processed into the 48kDa mature form. Deglycosylated form of LOX-1 is not efficiently transported to the cell-surface and retained in endoplasmic reticulum, in TNF-α-activated BAEC but not in BLOX-1-CHO. Deglycosylated form of LOX-1 had a reduced affinity for Ox-LDL binding.

Conclusion: N-linked glycosylation appears to play key roles in the cell-surface expression and ligand binding of LOX-1.

**ThP2.W34**

**Marked increased expression of I. R11, a mosaic LDL receptor family member, in atherosclerotic lesions**

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Objective: Receptors belonging to the low density lipoprotein receptor (LDLR) family are thought to play key roles in lipoprotein metabolism in a variety of tissues including the arterial wall. Here, we report that the expression of a 250-kDa mosaic LDLR family member, LR11, is markedly induced during the process of atherogenesis.

Methods and Results: Analysis by RT-PCR and RNase protection assays revealed that LR11 transcript levels rise in rabbit aorta expressing atheroma tus lesions following feeding a high-cholesterol diet. Immunohistochemistry demonstrated that the highest induction of LR11 occurs in intimal smooth muscle cells (SMCs), followed by medial SMCs close to the intimal border of the atheromatous lesions. Experimental intimal hyperplasia by endothelial denudation showed that LR11 mRNA levels were also increased in the arteries after balloon injury, with the highest level, transcripts localized primarily in the hyperplastic intimal layer. In agreement with the correlation of LR11 induction during increased cell proliferation, cultured SMCs showed an increase in LR11 expression in the proliferative phase. Furthermore, Northern and Western blot analyses showed that medium conditioned by the monocyte/macrophage cell line, THP-1, enhanced LR11 expression in cultured SMCs.

Conclusions: Up-regulation of LR11 might be contributing to the pathological roles of intimal and medial SMCs during atherosclerotic lesion development.

**ThP3.W34**

**GPI-anchored type HDL-binding protein on human monocyte-derived macrophages**

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Objective: High-density lipoprotein (HDL) plays a central role in reverse cholesterol transport, in which excess cholesterol is cleared from atheroma, particularly from macrophages. However, the HDL-binding proteins on human macrophages have not been fully characterized. To identify the binding proteins of HDL3 on human macrophages, we examined the involvement of glycosylphosphatidylinositol (GPI)-anchored protein in the binding of HDL, and tried to purify the HDL3-binding protein localized primarily in the hyperplastic intimal layer. In agreement with the correlation of LR11 induction during increased cell proliferation, cultured SMCs showed an increase in LR11 expression in the proliferative phase. Furthermore, Northern and Western blot analyses showed that medium conditioned by the monocyte/macrophage cell line, THP-1, enhanced LR11 expression in cultured SMCs.

Methods: Up-regulation of LR11 might be contributing to the pathological roles of intimal and medial SMCs during atherosclerotic lesion development.

Conclusions: Up-regulation of LR11 might be contributing to the pathological roles of intimal and medial SMCs during atherosclerotic lesion development.

Results: From membrane fractions of human macrophages, we obtained HDL3 binding proteins with a Mr of 80 and 130 kDa, respectively, by ligand blotting and the HDL3-binding protein was up-regulated by cholesterol loading. Ligand blotting demonstrated the enrichment of the 80 kDa HDL3-binding protein on detergent resistant membranes (DRMs). Treatment of human macrophages with PI-specific phospholipase C (PL-PLC) decreased the specific HDL3 binding in a dose dependent manner. Furthermore, treatment of cells with increasing concentrations of mannosamine, which blocks GPI-anchored protein function, decreased specific HDL3 binding in a dose dependent manner. PI-PLC treatment released from the cells the proteins with a Mr of 80 kDa, which could also bind HDL3. PI-PLC as well as mannoseamine treatment reduced cholesterol efflux from macrophages in association with the decreased HDL-binding. Using HDL3-affinity chromatography we purified the 80 kDa GPI-anchored type HDL3-binding protein.

Conclusion: We demonstrate the implication of the 80 kDa GPI-anchored protein in the binding of HDL3 to human macrophages, which might have some role in reverse cholesterol transport.

**ThP4.W34**

**Expression of high-density lipoprotein receptors, scavenger receptor class B type I in human central nervous system**

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Objectives: Lipoprotein particles the size of plasma high-density lipoproteins (HDL), are known to be present in the cerebrospinal fluid (CSF). Lipoproteins in the CSF might represent a potential source of lipids for cells of the nervous parenchyma. Some receptors for lipoproteins have been identified in the central nervous system (CNS), however, little is known about receptors for HDL in CNS. We have been reported that in humans, scavenger receptor class B type I (SR-BI) which is recognized as a receptor for HDL is expressed not only in liver but also in macrophages and in atherosclerotic plaques. In the current study, we clarified SR-BI was expressed in human CNS.

Methods: We examined immunohistochemically the expression of SR-BI in human CNS (spinal cord, cerebrum, and cerebellum) using tissues from autopsy cases. Furthermore, we investigated the regulation of expression of SR-BI in human glioma cell lines.

Results: Northern blot analysis of whole brain mRNA and Western blot analysis of protein extracted from whole brain showed SR-BI expressed in human brain. Immunoreactivity to anti-SR-BI antibodies was detected in all CNS tissues examined. Double staining for SR-BI and astrocyte or microglia revealed that astrocyte mainly expressed SR-BI protein. Some human glioma cell lines also expressed SR-BI. Glial expression of SR-BI protein was upregulated by incubating with lipoprotein deficient serum.

Conclusions: We clarified that SR-BI was expressed in human CNS and suggested that SR-BI might play a role in lipid homeostasis and transport in the CNS.

**ThP5.W34**

**Itavastatin inhibits modified LDL-induced foam cell formation and scavenger pathway, mediated with Rab, Rho and Rac small G proteins, in RAW264.7 macrophages**

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Objective: To elucidate the effect of Itavastatin and the molecular mechanism in modified LDL-induced foam cell formation and the scavenger pathway, lipid deposition and proteins in the modified LDL-associated membrane were determined in RAW 264.7 macrophages.

Methods: Oxidized LDL (oxLDL) and acetylated LDL (acLDL) were prepared by UV light irradiation and the method of Basu, respectively. Cells were incubated with Itavastatin, clofibrate (C), HMG-CoA reductase (H), Ado-arginine, Ado-arginine (A) and Rho GDI (GD). For 24-48 hrs before treatment with LDLs. The cellular lipids were extracted after 3 day incubation, and cholesterol and phospholipid were measured by enzymatic methods. Cellular low density membrane (LDM) fraction were prepared from cells incubated with DiI labeled oxLDL or acLDL and immunoprecipitated with antibodies for small G proteins (ras, Rab, rho and Rac).

Results: Itavastatin potently suppressed cellular cholesterol ester deposition at IC50: 56 nM (oxLDL) and 116 nM (acLDL), and inhibited uptake of 0x-DDL and DIL-acLDL. These effects were prevented by mevalonate or geranylerol. CD, J5, C3 and GDI suppressed cholesterol ester deposition and uptake of DiI-oxLDL and DiI-acLDL. DiI-oxLDL or DiI-acLDL associated LDM were co-precipitated with the immunoprecipitates of anti-Rab5 (A, B), Rho (A, B) and Rac (1, 2) antibodies and these were decreased by Itavastatin.

Conclusion: Actin assembly and small G proteins (Rho, Rab, Rac) are involved in scavenger pathway of modified LDLs and foam cell formation in macrophages. Itavastatin inhibits foam cell formation by inhibiting incorporation of small G proteins and may contribute to stabilization of atheromatous plaque.

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P-W34 Lipoprotein Receptors

**ThP6:W34** Regulation of low density lipoprotein receptor activity after retrovirus mediated gene transfer

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*Objective:* To study the regulation of recombiant low-density lipoprotein receptor (LDLR) after retrovirus mediated gene transfer to LDLR deficient fibroblasts.

*Methods:* In view of potential strategy for correcting complete or partial LDLR deficiency by gene therapy, a high titer VSV-G pseudotyped retrovirus containing a human full length LDLR-cDNA under transcriptional control of Moloney Murine Leukemia Virus (MMLV) long terminal repeat (LTR) was produced in 293GP cell line. The virus was used to transduce LDLR-negative Watanabe Heritable Hyperlipidemic (WHHL) rabbit fibroblasts in vitro. To characterize the LDLR activity of the transduced cells, uptake of 3,3'-dioctadecyldimethoxycarbonyl (Dil)- labeled LDL was studied by flow cytometry. Increase in the fluorescent intensity per cell was used as a measure of Dil-LDL uptake and as an indicator of the presence of functional LDLR on the cell surface. LDLR gene expression was studied by Northern blot.

*Results:* Normal LDLR is mainly regulated on transcriptional level by 5’ sterol regulatory element, which is not present in the virus vector. The transduced cells, deficient of native LDLR, showed significantly higher LDLR activity compared to untransduced control cells and were also affected by preincubation with or without lipoproteins. The LDLR mRNA levels of the transduced cells were not affected by sterol pre-treatments.

*Discussion:* These results indicate that the regulation of recombinant LDLR resembles that of the native LDLR but may involve another, yet unknown post transcriptional pathway. Understanding the regulation of the recombinant protein would be of significant importance for their potential use in human gene therapy.

**ThP7:W34** Secreted macrophage scavenger receptor gene transfer in LDL receptor knock-out mice reduces atherosclerotic lesion area

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*Objective:* Macrophage scavenger receptors (MSR) type A and II are trimeric membrane glycoproteins which are involved in atherogenesis and foam cell formation. It is conceivable that modulation of MSR activity has beneficial effects on atherogenesis. We have previously shown that a secreted decoy MSR (sMSR) inhibits the degradation of acetylated (Ac) and oxidized (Ox) LDL in RAW 264 macrophages and scavenger receptor knockout mouse macrophages by 70–90% and inhibits foam cell formation in vitro.

*Methods and Results:* To study in vivo effects of sMSR we cloned first generation adenoviruses which express sMSR under the control of CMV promoter. LDL receptor knock-out mice were kept on cholesteral diet for 1.5 months and transplanted with tail vein injection of 10^8 pfu sMSR and control LacZ adenoviruses. Effects of the transfection on Ac-LDL and Ox-LDL turnover were measured 3 days later and effects on atherogenesis were measured four weeks later.

It was found that intravenous injection of sMSR adenoviruses leads to the expression of sMSR mRNA in liver and spleen when measured with RT-PCR and slows down the clearance of injected, radiolabeled Ac-LDL and Ox-LDL by 50%. In addition, sMSR gene transfer shows an antiatherogenic effect in cholesterol-fed LDL receptor knock-out mice by reducing the aortic lesion area by 19%

*Conclusion:* sMSR is a potential new tool for gene therapy of atherosclerosis.

**ThP8:W34** Stable scavenger receptor gene transfer leads to increased susceptibility to aposis in rabbit aortic smooth muscle cells

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*Objective:* Macrophage (MQ) scavenger receptors (SR-A) are important in the pathogenesis of atherosclerosis. However, their role in non-MQ cell lines remains unknown. To test the hypothesis that SR-A activity leads to proatherogenic changes in non-MQ cell lines SR-A was stably transduced into rabbit aortic SMC.

*Methods:* Cells were incubated with 100–500 μg/ml acLDL, oxLDL or native LDL for 48 or 72 h and analysed for foam cell formation with lipid staining and for apoptosis with annexin-V binding using flow cytometry.

*Results:* Incubation with 350 μg/ml acLDL led to foam cell formation in SR-A transduced SMC. After a 72 h incubation of 200 μg/ml oxLDL 18% of the SR-ASMHC were early apoptotic as compared to 7% in control cells. A 48 h incubation with 500 μg/ml oxLDL increased Annexin V and PI positivity in the SR-ASMHC 3.6-fold and 4.3-fold, respectively. Higher concentration of ox-LDL also induced non-apoptotic cell death.

*Conclusion:* Expression of SR-A in SMC leads to proatherogenic changes by increasing lipid accumulation and predisposing cells to apoptosis. In addition to the significant role of SR-A in MQ, these receptors may participate in atherogenesis by predisposing non-MQ cells to proatherogenic changes.

**ThP9:W34** Identification of a polymorphic CA repeat near the apolipoprotein E receptor-2 (APOER2) gene on chromosome 1

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*Objective:* To identify a polymorphic marker can be used to investigate whether genetic variation at the APOER2 locus is associated with risk of neurodegenerative diseases.

*Methods:* Human genomic clones containing APOER2 were isolated from a gridded PAC library (HGMP Resource Centre, Cambridge) by hybridisation with a 32P labelled probe to exon 18 of APOER2, and their size determined by PFGE. Sau3A-digested fragments of the inserts were subcloned into pCDNA3 and hybridized with a labelledCA22 probe. Positive clones were sequenced.

*Results:* Two PAC clones containing APOER2 were obtained: a 16 (180 kb insert) contained a 27CA repeat and a 20 (190 kb insert) contained a 21 CA repeat. Primers were designed to amplify each from genomic DNA; multiple bands were always obtained with clone o-20. Further restriction digestion and hybridisation of clone o-16 showed that the 27CA repeat was less than 28 bp from the 5’ end of the APOER2 gene. Eleven different alleles were observed in a healthy population of 198 white males, with between 20 and 32 repeats (weighted mean = 26.9) and a heterozygosity index of 0.876. Co-dominant inheritance of alleles at this locus was demonstrated by genotyping 48 individuals from two 3-generation families and one 4-generation family. We have also identified a PsI polymorphism at the 5’ end of this gene, and have deduced haplotypes for the 2 loci in 116/198 individuals. 18/22 possible haplotypes were observed, with evidence for linkage disequilibrium (delta = 0.263).

*Conclusion:* A highly polymorphic CA repeat within 28kb of the ApoER2 gene and a PsI RFLP within the coding region have been characterised that will be useful for genetic association studies.

**ThP10:W34** Expression and characterization of a functionally active human low density lipoprotein receptor (LDLR)

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*Objective:* LDL are heterogeneous in size, lipid composition and protein structure and in their LDLR binding properties. In order to assess how LDL heterogeneity influences the kinetics of binding to the LDLR an active and soluble human LDLr was expressed in Chinese hamstring ovary (CHO) cells.

*Methods:* The N-terminal 692 amino acids of the LDLr encoded by a 2.1 kb fragment in pcmv5 was stably expressed in CHO cells. The receptor was purified by affinity chromatography using the anti-human LDLr monoclonal antibody, C7. Receptor functionality was assessed by ligand blotting and by an ELISA assay with biotinylated LDL. The kinetics of LDL binding to the LDLr were determined by surface plasmon resonance (SPR).

*Results:* CHO cells produced a homogeneous and active LDLr in quantities of 0.1 mg/ml. SDS-PAGE stained by Comassie blue showed a single band of correct size (76 kDa). Positive reactivity was obtained by immunoblot analysis using C7, an antibody which only binds to a partially folded receptor. The LDLr bound biotinylated LDL on a ligand blot and on an ELISA assay. SPR showed that LDL bound to the LDLr with high affinity (Kd = 2.9 nM). The specificity of the LDLr was shown with acetylated LDL which did not bind to the receptor.

*Conclusion:* A functionally active LDLr is secreted by CHO cells that is similar to a native receptor and therefore can be used for identifying parameters that modulate LDL binding to the LDLr.
The female plasma lipoprotein pattern induced by continuous growth hormone infusion in male rats is estrogren receptor-dependent

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Objective: In the rat, plasma total and HDL cholesterol levels are lower in males than in females. A gender-related difference is also present in the secretory pattern of growth hormone (GH). GH is important to maintain hepatic estrogen receptor (ER) expression. We have previously noted that GH-infusion in normal male rats increases HDL levels, leading to a feminized lipoprotein profile. The aim of the current study was to determine whether the GH-induced feminization of the plasma lipoprotein pattern in male rats is linked to modulation of hepatic ERs by GH.

Results: Infusion with GH for 6 days increased total plasma and HDL cholesterol levels. Concomitantly, hepatic ER expression increased by 2.5-fold. The GH-induced effects on plasma lipoproteins were abolished by the estrogen antagonist, tamoxifen. Tamoxifen treatment alone reduced plasma cholesterol levels by 30%, mainly due to reduced HDL cholesterol. Pulse injection twice daily with GH had no effect on HDL cholesterol or hepatic ER expression.

Conclusion: It is concluded that hepatic ERs likely play an important role in the regulation of HDL cholesterol in male rats, and that the feminization of plasma lipoprotein pattern induced by GH-infusion is dependent on ERs.

Regulation of the scavenger receptor type A in human blood-derived monocytes

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Objective: To understand what regulates basal Scavenger Receptor type A (SR-A) expression and what factors are responsible for the observed intra and inter individual variations in levels of expression.

Methods: Monocytes were extracted from the plasma of healthy normolipidemic volunteers and cultured in autologous serum for 7 days. Expression of SR-A I and II, c-Jun, Jun B, et2 and IP-10 was measured by RT-PCR. GAPDH and L27, a ribosomal protein, were used as internal standards. Binding to AP-1 and GAS sequences of the SR-A promoter was assessed by electrophoretic mobility shift assays.

Results: We show that the expression of the AP-1 transcription factors c-jun and Jun B, as well as et2, is correlated with SR-A induction in human blood-derived monocytes. The kinetics of binding of nuclear extracts to AP-1 sequences of the promoter parallels the kinetics of SR-A expression in these cells. Our results also show that treatment of the cells with 175U/ml of recombinant human IFN-γ, early after placing in autologous serum, is a potent activator of SR-A expression. The same treatment becomes inhibitory once the cells have started to mature into macrophages. The level of SR-A expression in freshly extracted monocytes varies widely not only among individuals, but also in the same individuals at different times. The extent of stimulation by IFN-γ is inversely proportional to the initial level of SR-A expressed by the monocytes. We also found a positive correlation between the level of SR-A in monocytes and the expression of IP-10 by the same monocytes. Since IP-10 is a reflection of the exposure of the cell to IFN-γ, our results suggest that inflammation plays an important role in determining SR-A basal levels.

Conclusions: Inflammatory-induced stimulation of SR-A expression in early monocytes could indicate a potential role of SR-A as an adhesion molecule stimulating the recruitment of monocytes to the arterial wall during very early steps in foam cell formation and atherosclerosis.

Identification of a γ-IFN responsive element in the promoter of the human macrophage receptor-A gene

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We have recently described the high expression and activity of the γ-IFN inducible transcription factor STAT1 in Scavenger receptor type A (SR-A) overexpressing monocytes from a normal lipemic patient with xanthomatosis. RNA expression studies now demonstrate γ-IFN inducible SR-A mRNA expression during early stages of monocyte differentiation which is predominately mediated by SR-A type II mRNA induction of the two SR-A isoforms. Increased degradation of acetylated LDL and accumulation of cholesteryl esters in γ-IFN activated THP1 monocytes also indicate elevated SR-A activity under these conditions. In contrast, and consistent with other reports, γ-IFN inhibits SR-A expression in mature macrophages as well as after prolonged incubation of THP1 monocytes with γ-IFN. Gel retardation assays identified γ-IFN inducible DNA binding activity in THP1 nuclear cell extracts to a potential STAT1 binding site (GAS) located between 30 and 25 nucleotides upstream from the transcription initiation site of the human SR-A gene. STAT1 binding activity to the SR-A GAS site correlates with the SR-A mRNA expression pattern in γ-IFN induced early monocytes. Furthermore, a 290 bp SR-A promoter fragment containing the SR-A GAS element confers γ-IFN inducible reporter gene expression after transient transfection of THP1 cells. Taken together these results suggest that γ-IFN induced expression of SR-A is mediated by STAT1 in early monocytes.

VLDL receptor modulates VLDL metabolism in LDL receptor knockout mice

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The effect of the VLDL receptor in VLDL triglyceride metabolism was studied using VLDL receptor knockout (Vldr−/−) mice and a newly generated transgenic mouse model that expresses the human VLDL receptor in endothelial and smooth muscle cells (PVL mouse). To investigate the role of the VLDL receptor under relatively high VLDL triglyceride levels, both mouse models were crossed on an LDL receptor knockout (Ldlr−/−) background. Plasma cholesterol and triglyceride levels were measured after 4 hours and after overnight fasting, both in mice on a chow diet and in mice on an atherogenic diet. On an atherogenic diet, plasma triglyceride levels were increased by a factor 2 in Vldr−/−; Ldlr−/− mice and decreased by a factor 2 in PVL. Ldlr−/− mice as compared to Ldlr−/− controls. On a chow diet, Vldr−/− Ldlr−/− mice that are fasted overnight show a more than twofold increase in plasma triglyceride levels. Lipoprotein profiles show that the effects on plasma triglycerides are mainly due to an effect on VLDL triglycerides. No significant differences in apolipoprotein B and triglyceride production rates were found between Vldr−/−; Ldlr−/− and Ldlr−/− mice. In conclusion, the data show that the VLDL receptor modulates VLDL metabolism in the Ldlr−/− mouse. While expression of an additional human VLDL receptor in the endothelium leads to a decrease in VLDL triglyceride levels, the absence of the VLDL receptor leads to an increase in VLDL triglyceride levels. Absence of the VLDL receptor does not lead to changes in VLDL production, suggesting a defect in clearance of triglycerides from the plasma. This is in line with our previous observations that imply a facilitating role for the VLDL receptor in lipolysis.

Expression of HB2, a candidate HDL receptor, in THP-1 cells

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Objective: We have purified and cloned HB2 as a candidate HDL receptor which structurally is quite different from SR-B1 or HB (HDL binding protein), also candidate HDL receptors. HB2 shows high sequence homology with the adhesion molecules ALCAM and BEN. Since the function of HB2 remains unknown, we have investigated factors that regulate the expression of HB2 in THP-1 cells to obtain clues to its physiological role.

Methods: THP-1 cells were incubated with RPMI-1640 medium containing 10% of fetal bovine serum. At confluence, cells were differentiated to macrophages by the addition of 200 nM of PMA and harvested at 1, 2, 4, 8, 16 and 24 h after addition of PMA. Total RNA was extracted by guanidine thiocyanate method. HB2 expression was determined by ligand blot using a ligand and Northern blot using an HB2 cDNA fragment as a probe. Levels of mRNA were measured by BAS system. We also determined the effect of bile acids, cytokines, and fat-soluble vitamins on HB2 mRNA expression in THP-1 cell without PMA treatment.

Results: THP-1 cells treated with PMA resulted in a time dependent increase in HB2 mRNA reaching maximum at 16 h after treatment when it was approximately 8 times higher than non-treatment cells. Correspondingly, expression in protein levels of HB2 increased in a time dependent manner as shown by ligand blots. This data shows that HB2 expression is regulated...
Effect of ACTH on the hepatic LDL receptors and plasma cholesterol in the rat

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Objective: The aim of the present study was to determine the effect of ACTH and hydrocortisone on lipoprotein metabolism in intact and adenorexized rats.

Plasma cholesterol decreases the days after myocardial infarction. The mechanism for this is unclear. It is well known that stress hormones are increased following myocardial infarction and it has been of interest to evaluate if such hormones have effects on lipoprotein metabolism. It is shown that ACTH reduces plasma cholesterol in humans and ACTH or hydrocortisone increases LDL receptor activity in vitro.

Results: We found that treatment with ACTH of normal rats (500 µg/ kg/day) for 3 days, suppressed hepatic LDL receptor expression while the LDL receptor mRNA was unaltered. Total plasma cholesterol increased, especially within HDL. These effects were not obtained following hydrocortisone treatment. The effects of ACTH were however absent in adenorexized rats.

Conclusions: Our results strongly suggest that the effects of ACTH on lipoprotein metabolism are likely mediated by a yet unidentified factor derived from the adrenals.

A radiolabeled commercial lipid emulsion traces human chylomicron metabolism

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Background: Chylomicron (chyl) triglyceride (TG) kinetics has been difficult to directly determine owing to their extremely short half-lives and the inability to isolate particles not already partially hydrolyzed.

Objective: To develop a simpler method for studying human chylomicron metabolism.

Methods: After an overnight fast, normal volunteers (n = 6) were asked to sip a cream-based drink at a rate of 175 mg fat/kg/hr for 7.5 hours to produce steady state chylomicronemia. A commercial 10% intravenous triglyceride (TG) emulsion was labeled with [3H] triglyceride, purified by HFLC and sterilized. The labeled emulsion (140 mg TG) was injected intravenously 30 min before (i.e., in the fasting state) and 5, 6 and 7 hours after sipping began (i.e., triplicate determinations in the fed state). Chylomers were isolated and washed ultracentrifugally in three spins, and the concentrations of TGs and radioactivity were measured. Chylom TG half lives were calculated from the mono-exponential decay curves, and apparent volumes of distribution were estimated by back-extrapolation.

Results: Plasma and chylom TG increased from 87 ± 10 to 270 ± 61 mg/dL (mean ± SEM) and from undetectable to 36 ± 18 mg/dL, respectively and reached a steady state by 5 h. The tracer determined chylom TG half-lives were 5.3 ± 0.6 and 6.6 ± 0.8 min during the fasting and fed states, respectively (p = 0.03). The apparent distribution volume was 27% larger than plasma volume (4,624 ± 327 vs. 3,631 ± 115 mL, p = 0.02), suggesting the margination of lipid emulsion particles.

Conclusions: The results suggest that a radiolabeled commercial lipid emulsion is metabolized in a fashion similar to native chylom. This technique is much simpler than endogenous labeling and may potentially be used to study chylom metabolism under a wide variety of disease, nutritional, physiological, and pharmacological conditions.

Measurement of endogenous plasma lipoprotein lipase activity using a non-isotopic method

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Background: Although the great majority of lipoprotein lipase (LpL) is bound to capillary endothelium, some does circulate with triacylglycerol (TAG)-rich lipoprotein remnants. The physiological significance of circulating (endogenous) LpL activity is uncertain.

Objective: The purpose of this study was to develop a new, non-isotopic assay for endogenous LpL activity using gas chromatography (GC).

Methods: Heparinized plasma (100 mL) was combined with 12 mg of gum-arabic-emasculated triolein, 5 mg of sodium dodecyl sulfate (SDS), and 54 mg of internal standard (C17:0) in a total volume of 700 mL. After incubation for 60 min at 30°C, the lipids were extracted, briefly methylated, and analyzed in 8-min by GC. The amount of liberated oleic acid was determined (after subtracting appropriate blanks) and activity was expressed as nmol oleic acid released/hr/mL plasma.

Results: SDS not only suppressed hepatic lipase activity but also markedly stimulated the activity of endogenous LpL (from about 0.65 to 1 unit, compared to about 8 units for postheparin samples). Activity was completely blocked by 0.5 M NaCl, 0.5 M guanidine chloride, 7 mg/mL protamine sulfate, 20 mg/mL paraxon and by 5 mg/mL tetrahydropiprolast. Activity was not different in serum, EDTA or heparin plasma, whether collected on iced or not, and was stable over 2 freeze-thaw cycles. In a group of 39 patients with hypertriglyceridemia, mean endogenous LpL activities were 1.27 ± 0.6 units, similar to that in a group of 6 normal subjects (1.40 ± 0.54 units). Values were stable in 13 patients studied before (1.23 ± 0.71) and after (1.20 ± 0.66 units) a 3 month placebo period.

Conclusions: This endogenous LpL assay is simple to perform, requires no isotopes, is sensitive and reproducible. This technique may facilitate future studies to understand the physiological significance of endogenous LpL activity.

A novel ACAT inhibitor (F12511) overexpresses liver SR-B1 in hamsters

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Objective: To investigate the effect of a novel ACAT inhibitor (F12511) on cholesterol metabolism in hamsters.

Methods: Four-week old male golden Syrian hamsters (LPN strain) were fed a semi-synthetic diet (cornstarch 42.5, saccharose 20, lard 9.2, walnut 0.8, casein 20, mineral mix 2.5) for 4 weeks. One group was treated daily with F12511 (10 mg/kg by stomach intubation) and the control group received a placebo. Absorption coefficient (AC) of dietary cholesterol was measured using an isotope balance method. Liver expression of LDL receptor (LDR) and HDL receptor (scavenger receptor SR-B1) was determined using specific antibodies. Hepatic and plasma cholesterol (-18%) and in the liver concentration of esterified cholesterol (-75%) occurred in treated hamsters. The lipoprotein profile was similar in the two groups. The expression of LDLr was not changed by the treatment, whereas that of SR-B1 was increased considerably (+142%, p = 0.01).

Conclusions: F12511 induced the two expected effects of an ACAT inhibitor on both cholesterol absorption and liver cholesterol storage. While an hypercholesteremic effect of F12511 has been reported previously with another strain of hamsters, no reduction in HDL-cholesterol was observed in the present study, despite the overexpression of SR-B1. Since SR-B1 is critical for reverse cholesterol transport, this ACAT inhibitor offers new perspectives for the prevention of atherosclerosis.

Scavenger receptor type B, class 1 (SR-B1) as a receptor for oxidized low density lipoprotein (OxLDL)

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Objective: A well-established function of SR- B1 is mediation of selective transfer of lipids between HDL and cells. However, it also binds other lipoproteins, including OxLDL. In the present studies we characterize this "scavenging" function and the nature of the ligands on OxLDL domains involved in binding.

Results: The specific binding of OxLDL to SR-B1-transfected CHO cells was 10-fold greater than to non-transfected cells. The affinity of OxLDL for SR-B1 was 20-fold greater than that of HDL (Kd values, 3.6 and 72 µg protein/mL, respectively). OxLDL was internalized and degraded to a degree comparable to the specific cell-association, whereas no degradation was detected in non-transfected cells. The protein and lipid moieties of OxLDL were both able to compete for a portion of the binding of intact OxLDL to SR-B1. In addition, the isolated OxLDL protein and lipid moieties each displayed specific binding to SR-B1 that was 6-fold and 2-fold greater, respectively, than
that to non-transfected cells. Furthermore, both monoclonal antibody EO6, which binds to the oxidized phospholipid POVPV, as well as a POVPV-BSA adduct were able to inhibit the binding of OxLDL to SR-B1.

**Conclusions:** In addition to its well-described "selective uptake" function, SR-B1 has the ability to "scavenge" OxLDL and, potentially, apoptotic cells as well. The interaction of OxLDL with SR-B1 is apparently mediated to some extent by both the protein moieties and the lipid moieties of the intact particle. In addition, the oxidized phospholipid POVPV is an important ligand on OxLDL that mediates recognition by SR-B1.

**ThP21:W34** Very low density lipoprotein (VLDL) receptor expression is activated by dexamethasone in a glucocorticoid receptor-dependent manner in adipocytic 3T3-L1 cells

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LRP and LDL receptors have previously been postulated to be important for the homeostasis of apoE-containing lipoproteins. VLDL receptors belong to the same gene family and binds multiple ligands, some of them related to lipoprotein metabolism. Due to a high expression of VLDL receptors in adipose tissue and muscle the VLDL receptor has been proposed to contribute to lipoprotein metabolism in extracellular tissues. These hypotheses are controversial and recent studies link the VLDL receptor to neuronal migration in mice possibly by interaction with non-lipoprotein ligands. Since VLDL receptor knockout mice demonstrated a relatively slower rate of adipose tissue accumulation compared to intact mice we investigated the expression and regulation of VLDL receptors in 3T3-L1 cells, a cell line able to transform from fibroblast to adipocyte-like phenotype by addition of dexamethasone, insulin and isobutyloxymethylxanthine in the culture medium. During the first 1–3 days of adipocytic formation 3T3-L1 cells showed an increased expression of VLDL receptors. This stimulation could be mimicked by dexamethasone (1 μM) alone in a time- and dosedependent manner. Inclusion of the GR blocker RU-486 (10 μM) inhibited the response. The mouse VLDL receptor promoter was isolated and sequenced. No classical DR-3/4 cis-acting element (GTGACANNTGTGC) could be demonstrated. Transfection of 3T3-L1 cells with reporter genes containing 1.6, 2.6 and 3.6 kb of upstream promoter sequence showed stimulation by dexamethasone and abrogation by inclusion of RU-486. We demonstrate a hormonal regulation by dexamethasone on the VLDL receptor that involves indirect or direct GR-mediated on the VLDL receptor promoter. Studies addressing the issue of dexamethasone regulation in vivo is ongoing.

**ThP22:W34** Lipoprotein receptors in extraembryonic tissues of the chicken

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Yolk is the major source of nutrients for the developing chicken embryo, but molecular details of the delivery mechanisms are largely unknown. During oogenesis in the chicken, the yolk components vitellogenin and very low density lipoprotein (VLDL) are taken up into the oocytes via the LDL receptor family member, LR8 (EMBO J. 13, 5165-5175). Endocytosis is accompanied by partial degradation of the yolk precursors’ protein moieties; however, fragmentation does not abolish binding of VLDL to LR8. This receptor exists in two isoforms that differ by a so-called O-linked sugar domain; the shorter form (LR8–) is the major form in oocytes, and the longer protein (LR8+) predominates in somatic cells. Here we show that both LR8 isoforms are expressed at ratios that vary with embryonic age, in the extraembryonic: yolk sac, which mobilizes yolk for utilization by the embryo, and in the allantois, the embryo’s catabolic sink. Stored yolk VLDL interacts with LR8 localized on the surface of the yolk sac endodermal endothelial cells (EEC), is internalized, and degraded, as demonstrated by the colorimetrically labelled VLDL in cultured EEC. Importantly, EEC express significant levels of microsomal triglyceride transfer protein and protein disulfide isomerase, key components required for lipoprotein synthesis. Since the apolipoprotein pattern of VLDL isolated from the yolk sac-effort omphalomesenteric vein is very different from that of yolk VLDL, these data strongly suggest that embryo plasma VLDL is re-synthesized in the EEC. LR8 is a key mediator of a two-step pathway, which effects the uptake of VLDL from the yolk sac and the subsequent delivery of its components to the growing embryo. (Supported by the Austrian Science Foundation FWF F-606, F-608).

**ThP23:W34** Early platelet activation by low density Lipoprotein

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Low density lipoprotein (LDL) is known to increase the sensitivity of human platelets for agonists. Little is known about the receptor and the signalling pathways involved. The platelet LDL receptor is different from the classical LDL receptor, described by Brown and Goldstein.

We have earlier reported how LDL might sensitize platelets. A first step is the activation of the enzyme p38 MAPK which is activated within 10 sec (1 g apoB100/L) after addition of LDL and at concentrations as low as 0.1 g apoB100/L (within 10 min). A second, equally rapid step is the activation of focal adhesion kinase (p125FAK), which is an essential step in the formation of focal adhesion plaques. p38 MAPK and p125FAK-activation are both upstream of most platelet signalling pathways and therefore close to the platelet LDL receptor.

It is still unclear which component of LDL is responsible for platelet sensitization. Modification of lysine residues of apo B100 via carbamylation reduces p38 MAPK activation by 80%, indicating that apoB100 is required for binding to the platelet surface and subsequent signal generation. Anti-human apoB100 monoclonal antibodies, 4G3 and 2D8 are both directed against regions of apoB100 which are susceptible for carbamylation. The antibody 4G3, directed against aa 2980–3084, which is described to inhibit LDL binding to the ‘classical’ LDL receptor, reduced LDL-platelet binding and subsequent p38 MAPK phosphorylation by 50%. In contrast, 2D8, directed against aa 1438–1481, showed no reduction. After carbamylation of apoB100, LDL is still able to phosphorylate p38 MAPK after extended incubation time (1–2 hrs), suggesting that the lipid moiety of LDL might also contribute to platelet activation.

These observations imply that specific domains of apoB100 are essential for targeting LDL to the platelet membrane, whereas other signal transduction pathways can be initiated by the lipid moiety.

**ThP24:W34** Identification of an HDL and apoAI binding site on baculovirus expressed extracellular domain of HBV

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**Objective:** To confirm the function of HBV as a candidate HDL receptor.

**Methods:** Constructs of the extracellular domain of HBV were engineered that coded for residues Ex (extracellular) 1–144, Ex 1–247, Ex 1–415 and 1–527, the respective extracellular domain. After transfection the vector (pBacPAK8) expression plasmid was transferred into Spodoptera frugiperda with BacPAK6 viral DNA and baculenv to generate recombinant baculovirus proteins with yields ranging from 7–12 mg/l culture medium. Ligand blots were performed using HDL, apoAI or LDL as ligands and binding was detected with specific antibodies.

**Results:** Mapping of HDL binding site. Truncated peptides 1–144, 1–247, 1–415 and 1–527 did not bind apoAI or HDL; however HDL binding to peptide 1–527 was similar in strength as binding to full length HBV. Thus a specific binding domain is located proximal (within 144 residues) of the membrane region. Additional experiments showed that the extracellular domain bound apoAI and apoAIL but not LDL, confirming the specificity reported previously for full length HBV.

**Conclusions:** Together with recent data demonstrating a connection between HBV and sterol synthesis, this designation of a precise extracellular binding domain is compelling evidence for a role of HBV in lipid metabolism, influenced by HDL apoprotein.

**F:W35 MONOGENIC HYPERLIPIDEMIA**

**ThP1:W35** Impaired TG removal in well-controlled type III hyperlipoproteinemia

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**Objective:** TG removal activity in patients with well-controlled type III hyperlipoproteinemia was assessed by IV-FTI.

**Methods:** The study was performed in ten normal subjects, and two brothers with type III hyperlipoproteinemia. The older brother showed mild renal
dysfunction due to hypertension and therefore was only given 200 mg/day of bezafibrate, which was half the dose amount. 10% intralipid (1 ml/kg weight) was intravenously injected, and blood samples were obtained before and 10, 20, 30, 45 and 60 minutes after injection. Reductions of chylomicon-triglyceride level were sequentially determined with the samples and K2 values (0.693±0) were calculated as the index of TG clearance.

**Results:** The TG values were 96 ±10 (mean ± SD) mg/dl, 150 mg/dl and 212 mg/dl in normal subjects, the younger brother with type III hyperlipoproteinemia and the older brother with type III hyperlipoproteinemia, respectively.

The K2 values were 0.0294 ± 0.088 (mean ± SD), 0.0231 and 0.0126 in normal subjects, the younger brother with type III hyperlipoproteinemia and the older brother with type III hyperlipoproteinemia, respectively.

**Conclusions:** Patients with type III hyperlipoproteinemia showed slight delay of TG-rich lipoprotein clearance even after the pathologic condition had been normalized by the treatment.

**ThP2:W35 A T3799M substitution in apolipoprotein B-100 in a familial hypobetalipoproteinemia kindred with no detectable chylomicronemia and truncation**

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**Objectives:** Familial hypobetalipoproteinemia (FHBL) is an autosomal co-dominant disorder characterised by reduced levels of cholesterol (CH) and apo B containing lipoproteins. FHBL may be due to mutations in the apo B gene or in other genes affecting the metabolism of apo B containing lipoproteins. Some forms of FHBL are due to the presence of truncated apo B that may or may not be detectable in plasma, whereas in other FHBL forms no truncated apo B have been demonstrated. We have searched for apo B gene mutations in a large FHBL kindred with no detectable truncated apo B form in plasma lipoproteins.

**Results:** The proband and 4 family members had low LDL-C (14–52 mg/dl) and apo B (17–34 mg/dl); their lipoprotein density profile was consistent with heterozygous FHBL. The proband and her mother had hepatic steatosis and mild malabsorption. No truncated apo B was found in plasma. The sequence of the apo B gene revealed that the proband was heterozygote for a C→T transition in the 3’ end of exon 26, that leads to the substitution of threonine at position 3799 with methionine (T3799M). This transition causes the insertion of a NalIII site in exon 26 which allows the rapid screening of the mutation. This mutation, that had not been previously reported, was not detected in a sample of 50 subjects randomly selected from the general population. It was found to co-segregate with FHBL lipoprotein phenotype in the kindred.

**Conclusions:** T3799M substitution, that is located in the amphipathic β2 strand domain of apo B-100 and is 400 aa downstream the LDL-receptor binding domain, might be the cause of FHBL in this kindred or be linked to a causative mutation located within or close to the apo B gene locus.

**ThP3:W35 Analysis of mutations in the LDLR gene in Spanish patients with familial hypercholesterolemia by SSCP**

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**Background:** Familial hypercholesterolemia (FH) is a common autosomal disorder that affects about one in 500 individuals in most Western populations and it is caused by a defect in the low-density-lipoprotein receptor (LDLR) gene.

**Objective:** To determine the molecular basis of FH in Spain.

**Methods:** One hundred and thirty three unrelated Spanish FH subjects were screened for mutations in the LDLR and apolipoprotein B (apoB) genes using single strand conformation polymorphism (SSCP) method, DNA sequencing and restriction analysis.

**Results:** None of the patients presented the apoB3500 mutation. We identified seventeen missense mutations, four nonsense mutations, seven frameshift mutations, eight splicing mutations and one silent mutation affecting the LDLR gene. In total, we detected thirty seven different mutations. Seventeen patients were found to carry two different mutations in the same allele. The E10X, Q71E/S131 + 1G→ C (located in the same allele) and 518delG mutations were the more frequent ones, which were present in 7.5%, 5% and 3.8% of the studied subjects, respectively.

**Conclusion:** These results demonstrate that there is a broad spectrum of mutations in the LDLR gene in the Spanish population.


**Objective:** To identify the genomic localisation of the defect in an inbred family with recessive phenotypic FH that is not caused by a defect in the genes for the low density lipoprotein (LDL) receptor or apolipoprotein B.

**Methods:** Fluorescence-labelled primer pairs flanking polymorphic markers were used to amplify genomic DNA in 10 family members. PCR products were sized and analysed using an ABI 377 sequencer with Genescan and Genotyper software. Allele frequencies in the population were estimated in unaffected unrelated individuals of the same racial origin. Linkage analysis was performed using Genehunter software.

**Results:** A preliminary scan for homozygosity with 254 markers at 10–30 cm intervals revealed no regions of shared homozygosity in only the three affected individuals. Thus recombination may have occurred in ancestral carriers of the defective gene. Several uninformative or potential candidate loci were excluded with additional markers. A second scan with 450 markers at 10-cm intervals has so far tentatively excluded linkage to chromosomes 16, 18, 19, 20 & X, and has revealed potential areas of interest on chromosomes 10, 11, 12 & 15. The data also exclude involvement of several candidate genes for the defect, which is manifested as impaired internalisation of the LDL receptor from the cell surface, including components of the clathrin-coated pit adaptor complex and other members of the LDL receptor gene family. Markers close to the gene on chromosome 17 for the ubiquitously expressed heavy chain of clathrin could not exclude this locus; sequencing of the 5kb coding region of clathrin mRNA, amplified by PCR from the patient’s cells, is underway.

**Conclusion:** Several candidate loci for a novel genetic defect causing phenotypic FH have been identified.

**ThP5:W35 Genetic deficiency of lipoprotein lipase is a frequent cause of chylomicronemia-associated acute pancreatitis in pregnancy**

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**Objective:** Hypertriglyceridaemia-associated acute pancreatitis is a rare but potentially fatal complication of pregnancy. The cause of this syndrome has not been systematically investigated.

**Methods:** We analyzed four pregnant women with pancreatitis and plasma triglyceride levels > 2000 mg/dl before the onset of symptoms. LPL concentration in post hepatic plasma was quantitated by ELISA, LPL activity was determined with an artificial emulsion. All nine coding exons of LPL were sequenced.

**Results:** Normal activator protein apoCII was demonstrated in all women. In 3 of the women, LPL activity and concentration in post hepatic plasma was lower than 10% of normal, proving LPL deficiency. In these patients, DNA sequencing revealed homozygote mutations for LPL. Two of these mutations (N435, G188E) had been previously shown to lead to a complete absence of LPL activity. The third mutation represents a novel deletion at position 1099 of the LPL cDNA resulting in a frameshift and an LPL protein lacking the entire carboxy-terminal domain. No mutation in the coding exons could be identified in the fourth woman who had LPL activity values consistent with heterozygote LPL deficiency. Her course was complicated by gestational diabetes. With an extreme low fat diet and in case with bezafibrate, all pregnancies resulted in uneventful childbirth.

**Conclusion:** 1) We have identified a novel LPL mutation in exon 6 (1099delE) leading to complete LPL deficiency. 2) Chylomicronemia-associated pancreatitis is not an indication for pregnancy termination. 3) Genetic LPL deficiency appears to be a frequent cause of severe hypertriglyceridaemia in pregnancy.
Enhanced postprandial RLP-Cholesterol decreased after simvastatin in heterozygous familial hypercholesterolaemia (FH) patients

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In FH the Low-Density-Lipoprotein Receptor (LDL-R) is defective and consequently a delayed chylomicron-remnant removal could be assumed. In a case-control intervention study the postprandial (PP) chylomicron-remnant metabolism was studied in heterozygous FH patients (n = 7; mean age 47, body mass index (BMI) 25.5; fasting plasma triglycerides (TG) 1.39) and 7 matched (for sex, age, BMI, TG and Apo-E genotype) control subjects. They underwent an oral Vitamin-A (RE) fat loading test (OFLT). FH patients were investigated before and after 3 months treatment with Simvastatin (80 mg once a day). Baseline TG did not change during treatment. In FH patients LDL-cholesterol decreased significantly after treatment (from 10.28 ± 1.60 to 4.78 ± 0.91 mmol/l; p < 0.05). In the untreated FH patients the RE (with a peak 4 hr PP) in the chylomicron-remnant fraction (SF < 1000) were higher (area under curve (AUC) SF < 1000 (RE): 24 ± 10 mg*hr/ml) than in controls (6.3 ± 5.9 mg*hr/ml; p < 0.05). After Simvastatin treatment the PP-RE response in the FH patients did not change significantly. Fasting RLP-C (non-precipitable fraction after immunoprecipation with anti ApoB100 and anti ApoAI) and the PP RLP-C (peaks at 2 hr) were significantly higher in the untreated FH patients (42 ± 19 mg/dl and AUC-RP-C: 415 ± 82 mg*hr/dl, respectively) than in the controls (7.3 ± 46 mg/dl and 101 ± 35 mg*hr/dl; p < 0.05). After treatment fasting RLP-C (13.3 ± 3 mg/dl and PP AUC-RP-C (136 ± 51 mg*hr/dl) decreased significantly (p < 0.05).

In conclusion, chylomicron-remnant levels were elevated in heterozygous FH patients. After Simvastatin treatment, larger (late) PP chylomicron-remnant (represented by RE), were not affected. However, the smaller (earlier) PP remnants (more atherogenic), reflected by RLP-C, decreased significantly and suggested to be mostly removed by the LDL-R.

A description of LDL receptor gene mutations found in France in 105 patients with FH

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Objective: To analyse the nature of LDL receptor (LDLR) gene mutations found in patients with Familial Hypercholesterolaemia (FH) in France.

Methods: The LDLR gene and promoter region were analysed by DGGE, DNA sequencing and Southern blotting, in 105 unrelated patients originating from 11 centres in France. A clinical presentation of homozygous FH was present in 18, while 87 were heterozygotes. Ten bi-allelic markers were used for haplotyping.

Results: Eighty-two different mutations were found in all excons except for exons 1, 2, 16, and 18. None were found in the promoter. Donor or acceptor splice site mutations (n = 8) were found in introns 2, 3, 9 and 13. There were 4 large gene rearrangements. Missense mutations were most prevalent 51% (n = 42), the remaining being nonsense (n = 9), and frameshift (n = 19) mutations. The only French Canadian mutations found were the W66G and the E207K. Novel mutations represented 38%, while 59% had been reported in European genetic background. Recurrence was found for 13 mutations, 9 sharing a common haplotype and geographic origin, suggesting they were more recent and specific to the country.

Conclusion: Numerous LDLR mutations cause FH in France, mostly on European genetic background, though with some local specificities.

Identification of an apolipoprotein E variant associated with type III hyperlipoproteinaemia in an Indigenous Australian

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Indigenous Australians have a high cardiovascular mortality rate. Cardiovascular risk factors include hypercholesterolaemia, non-insulin dependent diabetes mellitus, central obesity associated with hyperinsulinaemia of partial triglyceridaemia, and low high-density lipoprotein (HDL)-cholesterol levels. Apolipoprotein (apo) e allele frequency is likely to be another factor contributing to cardiovascular disease. The frequency of the apo e4 allele is significantly higher in indigenous Australians compared with Australians of European descent (0.227 versus 0.155). As a result of testing for lipid and apolipoprotein E phenotype status of an Indigenous Australian community, an apolipoprotein E variant associated with type III hyperlipoproteinaemia has been identified. Apolipoprotein E phenotype was determined by isoelectric focusing, and in DNA amplified by polymerase chain reaction, using two different restriction enzyme isopying assays. Phenotypes were discrepant in samples from two subjects and an abnormal sized restriction fragment was also observed in their isopying gel patterns. DNA sequencing studies further revealed a single nucleotide deletion, 3817delC, at amino acid 135 on apo E. This resulted in a new reading frame and the premature termination of the apo E protein due to a stop codon (TGA) at nucleotide 4105. This apo E variant is not functional because of the missing receptor-binding region and the C-terminal domain, which is involved in lipoprotein binding.

In summary, we discovered in indigenous Australians a new apo E variant (3817delC) that is associated with type III hyperlipoproteinaemia.

An analysis of clinical diagnostic criteria of FH in children, in light of the molecular diagnosis

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Objective: To identify clinical features specific to children with FH caused by LDL receptor (LDLR) gene mutations, as opposed to other children with dominantly inherited hypercholesterolaemia who are non-carriers of such mutations.
Methods: The LDLR gene was analysed by DGGE, gene sequencing, and Southern blotting in 55 prepupal children (age \( \geq 5 \pm 3 \) yrs; M/F = 22/33) who belonged to 38 families with dominantly inherited hypercholesterolemia. Definite and probable heterozygous FH was found in 47 and 8 children respectively. None had any xanthoma or cardiovascular symptoms. Family history was analysed.

Results: Thirty-one children had a heterozygous LDLR gene mutation (20 defective/11 negative alleles). Mutation carriers had higher basal TC (3.24 vs 2.98 g/L, \( p < 0.05 \)) than non carriers. Diet-induced lowering of TC and LDL-C was less pronounced (2.96 vs 2.82 g/L, \( p < 0.05 \), and 2.30 vs 2.09 g/L, \( p < 0.05 \)). Cardiovascular events were rare in the affected parent (18%) most of whom were in their fourth decade (age 35 ± 5 yrs). However in carrier parents, TC was 1.2 times and LDL-C was 1.5 times the 95th percentile, while these were 1.0 and 1.1 times this percentile in affected parents of non-carrier children (\( p < 0.02 \) and 0.05). Moreover, a premature cardiovascular event was found in 75% carrier grandparents (12/16), while these events were not found in most grandparents (64%) of non-carrier children (\( p < 0.02 \)).

Conclusion: Family history has high diagnostic value in children with FH caused by LDL receptor gene mutations, as compared to other forms of dominantly inherited hypercholesterolemia.

### ThP11:W35

Mutations in the LDL-receptor gene in Swedish patients with familial hypercholesterolemia

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Objective: To investigate the distribution of mutations in the LDL-receptor gene in Swedish patients with familial hypercholesterolemia. Methods: A material of 181 patients suffering from FH according to standardized criteria was collected from Huddinge University Hospital (111 patients) and Sahlgrens' University Hospital, Göteborg (70 patients). Genomic DNA were investigated with SSCP (Exons 1–18), Southern blotting and DNA sequence analysis. In some cases DNA fragments were cloned and sequenced.

Results: In 56 of the patients (33%) mutations were identified in the gene for LDL-receptor, while 3 of the patients were found to have a mutation for familial defect apoB. All patients were found to be heterozygotes. The mutations represented 17 different miss- or nonsense mutations, 6 mutations in splice sites and 3 deletions. The most frequent mutation was the deletion FH-Helsinki (10 patients). None of the other mutations occurred in more than 4 patients. In the promoter 4 cases with single base insertions were observed, but the significance of this finding for precipitation of disease is uncertain. In exons 2, 5, 7, 16 and 18 no point mutations could be detected.

In addition, a number of polymorphic variants were identified, which did not affect the polypeptide sequence. 21 of the patients displayed the polymorphism A370T in the peptide sequence.

Conclusions: The pattern in Swedish FH-patients differs compared to other Scandinavian countries, in which a restricted number of mutations is found in larger proportions of patients (i.e. up to 40% in Finland). The results show that Swedish FH-patients display a higher degree of heterogeneity.

### ThP12:W35

Regional difference of LDL receptor gene mutations in Japanese patients with familial hypercholesterolemia

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Objective: To characterize mutations in the low density lipoprotein (LDL) receptor gene causing familial hypercholesterolemia (FH) in Hokuriku district of Japan.

Methods: A total of 375 unrelated patients fulfilling clinical criteria for heterozygous FH were investigated. We examined all patients for four LDL receptor gene mutations that had been reported to be most common in the Japanese population (1845 + 2 T -> C, C317S, K790X, L547V) by PCR-restriction fragment length polymorphism. The negative samples were screened by Southern blotting and long-PCR analysis to look for the presence of large gene rearrangements.

Results: Mutations in the LDL receptor gene were identified in 92 of the 375 patients. Of these, 3 deletions (FH-Tonami-1, FH-Tonami-2, FH-Oyama) were found in 35 patients by Southern blotting analysis. The frequencies were: K790X 14.5% (56/375), L547V 0.3% (1/375), FH-Tonami-1 4.5% (17/375), FH-Tonami-2 3.7% (14/375), FH-Oyama 1.1% (4/375). The C317S and 1845 + 2 T -> C mutation were not identified. The five mutations accounted for 24.5% of this cohort.

Conclusions: The mutation pattern in FH patients of Hokuriku district differs considerably from that in other districts of Japan. The regional difference suggests that there is a broad spectrum of LDL receptor gene mutations in the Japanese population.

### ThP13:W35

Increased postprandial lipemia characterize a new hepatic iron overload dysmetabolic syndrome

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Objective: Recent studies suggested the existence of a new iron overload dysmetabolic syndrome (HFER), characterized by: normal transferrin saturation, hyperferritinemia and mild to moderate iron over load, associated with metabolic disorders such as: hyperlipidemia, abnormal glucose metabolism, obesity and hypertension. HFER might be a syndrome associated to atherosclerosis. Postprandial (PP) triglyceridemia (TG) is an independent risk factor for cardiovascular disease. Study aim was to evaluate: 1. fasting lipid profile of 16 HFER compared to 16 hyperlipidemic patients (HL); 2. TG response to an oral fat load (OFL) in HFER and HL compared to healthy controls; 3. PP ferritin profile.

Methods: All subjects were evaluated after 0, 3 and 6 hours from an OFL, performed according to Patch et al.

Results: 1. Fasting lipid profiles in patient’s groups were statistically different (Total Cholesterol: 216 ± 41 vs 246 ± 40 mg/dl; Triglycerides: 125 ± 42 vs 264 ± 140 mg/dl; HDL Cholesterol 53 ± 14 vs. 39 ± 16 mg/dl; LDL Cholesterol 137 ± 39 vs. 162 ± 35 mg/dl; Risk = Total Chol/HDL Chol: 4.38 ± 1.72 vs. 6.83 ± 1.87; Apo A-I: 149 ± 30 vs. 128 ± 31 mg/dl; Apo B: 106 ± 22 vs. 127 ± 28). Fasting lipid profiles of HFER and controls were not different. 2. When subjected to an OFL, the two patient’s groups, compared to healthy controls, had different TG responses at each time point of the PP test (\( p < 0.001 \)) and HFER patients showed a TG increase statistically higher, if compared to HL. 3. PP ferritin has a different concentration trend in HFER and HL patients (increases in HFER, stays stable in HL).

Conclusions: The analysis of our data suggest that in HFER patients the postprandial response is altered and may confer an increased cardiovascular risk.
Thursday June 29, 2000: Read by Title Abstracts

**T:W27 LIPOPROTEIN(a)**

Serum lipids and lipoprotein (a) [Lp(a)] in patients with diabetic uremia treated by maintenance hemodialysis

Vassa Arsova. Institute of Clinical Biochemistry, Clinical Center, R. Macedonia

Patients with diabetes and long-stage renal disease treated by hemodialysis have a tremendous risk for cardiovascular complications that cannot be explained by traditional atherosclerosis risk factors. The purpose of this study is to investigate the concentrations of lipids, lipoproteins and lipoprotein (a) in twenty night diabetic uremic patients and forty eight nondiabetic subjects on long term maintenance hemodialysis treatment and risk of cardiovascular disease. There was no significant difference in mean levels of serum cholesterol, triglycerides and high-density lipoproteins between diabetic and nondiabetic uremic patients treated by hemodialysis. But, there were significantly higher levels of apolipoprotein B (mean 121.4 ± 32.5 g/dl), Lp(a) (mean 47.8 ± 28.5 mg/dl) in patients with diabetic uremia compared with nondiabetic ones, being; apolipoprotein B (mean 108.3 ± 22.4 mg/dl), Lp(a) (mean 36.8 ± 19.4 mg/dl) and significant reduction of apolipoprotein A-I (mean 89.6 ± 22.7 mg/dl). It was conclude that serum apolipoprotein B and Lp(a) levels in uremic patients treated by hemodialysis were always higher if they were present diabetic cardiovascular complications. Serum Lp(a) levels have accelerated cardiovascular complications and mortality rates in diabetic uremic patients.

**T:W27 Are lipoprotein(a) levels predictors of clinical events in males with early coronary disease?**

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**Purpose:** To determine whether serum levels of Lp(a) out of acute phase predict the appearance of coronary events.

**Methods:** Consecutively, 209 male patients before 50 years of age (mean age 43 ± 5 years), who were admitted to our Cardiac Care Unit as result of an episode of coronary disease, were prospectively studied. Serum levels of Lp(a) were determined by means of ELISA after 12 hours of fasting. Levels in the acute phase were not considered as it is know that these increase in acute coronary syndromes. None of the patients were receiving treatment with niacin or nicotinic acid. A cut-off point of 30 mg/dl was established. Cardiac events were considered as the appearance of angina, myocardial infarction (MI), heart failure and the need for cardiac revascularisation (revasc). For statistical analysis, the Student’s t test was employed for the comparison of percentage of independent groups.

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>Lp(a) ≤ 30 mg/dl</th>
<th>Lp(a) &gt; 30 mg/dl</th>
<th>p</th>
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<tbody>
<tr>
<td>n = 114</td>
<td>n = 95</td>
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<tr>
<td>Angina</td>
<td>34 (30%)</td>
<td>42 (44%)</td>
<td>0.04</td>
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<tr>
<td>Heart failure</td>
<td>2 (2%)</td>
<td>2 (2%)</td>
<td>n as</td>
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<tr>
<td>MIO</td>
<td>5 (4%)</td>
<td>5 (5%)</td>
<td>n as</td>
</tr>
<tr>
<td>Revasc</td>
<td>34 (30%)</td>
<td>38 (40%)</td>
<td>n as</td>
</tr>
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</table>

**Conclusions:** The elevated levels of Lp(a) after the acute phase predict a greater number of episodes of angina in patients with established early-onset coronary disease.

**T:W27 Effects of statins on elevated lipoprotein(a) in primary hypercholesterolemia**

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**Introduction:** High lipoprotein(a) levels may increase coronary risk in hypercholesterolemia however, the opportunity of treating this risk factor is still under debate and the effect of drugs are not well defined.

**Objective:** To evaluate the effects of three different statins on elevated Lp(a) levels in patients affected with primary hypercholesterolemia.

**Methods:** 35 patients were evaluated in an open observational study (FH, n = 29, polygenic hypercholesterolemia, n = 4, PCHL, n = 2). Drug treatment (simvastatin 20 mg/day, n = 13, fluvastatin 40 mg/day, n = 2, pravastatin 20 mg/day, n = 8), was started after at least 3 months of AHA phase I diet. Lp(a) was determined by Sandwich-ELISA using the same batch of antibodies during the whole period.

**Results:** During fluvastatin treatment mean Lp(a) levels remained unchanged (but 50% of patients showed a decrease > 10%). Pravastatin did not affect Lp(a) values.

During simvastatin therapy mean Lp(a) levels increased by 16% (p = 0.09) and in 66% of subjects the increase was higher than 10%.

**Conclusion:** Individual response of elevated Lp(a) levels in hypercholesteremic subjects treated with statins should be accurately evaluated and may condition the choice of the drug in order to avoid possible harmful effects.

**T:W27 Spectroscopic correlation of structure and size of lipoproteins in normal subjects and coronary artery disease (CAD) patients**

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It is accepted that lipoproteins are involved in vascular diseases. Optical methods, Fourier transform infrared spectroscopy (FTIR) is traditional tool in the analysis of the structure and size of macromolecules in fluid suspension. It is found that plasma lipoproteins contain α-helical and β-form structural conformations which exhibit different optical and bioophysical properties. Human serum were obtained from NS and CAD patients. Lipoproteins were preisolated by ultracentrifugation. Spectroscopic studies of lipoprotein samples were analysed by FTIR.

A comparison of the curves from NS and CAD patients shows that α-helical structure is present in the lipoproteins only from NS and HDL class from intermediate patients and decrease in lipoproteins from advanced CAD Patients. β-form structures were almost fully dominated in the lipoproteins from patients with advanced CAD (confirmed by coronary-angiography).

Provide information that may be useful in observing the onset of oxidative process and in predicting the development of CAD before its actual clinical manifestation.

The project is still going on and under progress.

**T:W27 Effect of age on plasma lipoprotein(a) levels**

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**Objective & Methods:** In order to determine whether the age may affect on plasma lipoprotein(a) [Lp(a)] levels, we investigated the plasma Lp(a) levels and apolipoprotein(a) phenotypes in unrelated 552 subjects (238 men and 314 women, from 20 to 88 years old).

**Result:** The mean ± SD of plasma Lp(a) levels were elevated in proportion to aging, especially those for old age group over 60 years old were statistically significantly elevated in all apo(a) phenotyping groups compared with those for young age group under 39 years old (47.6 ± 25.8 mg/dl vs. 30.7 ± 19.7 in low molecular weight, F, B and S1, p < 0.05, 24.0 ± 12.6 vs. 13.7 ± 10.6 in S2 and 10.9 ± 6.8 vs. 6.5 ± 4.2 in high molecular weight, S3 and S4, P < 0.001, respectively).

**Conclusions:** These data suggest that the age is effect on plasma Lp(a) level and we guessed that high Lp(a) level would give the promotion of atherosclerosis diseases in middle and old people.


**THURSDAY**
**ThT6/W27**

**Association between lipoprotein(a), homocysteine and other risk factors for atherosclerosis**

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**Objective:** Coronary arthery disease (CAD) is the leading cause of mortality in middle age men in Poland. Elevated homocysteine (Hcy) and lipoprotein (a) [Lp(a)] have been described as independent risk factors for CAD, venous thrombosis and stroke. We investigated the occurrence of abnormal Hcy and Lp(a) in randomly selected men aged 20–50 years.

**Methods:** 250 subjects were divided into four groups according to Clin. Recommedation (Nutr. Metab. Card. Disease 1998). Serum Hcy by FPIA, lipoprotein(a) [Lp(a)] by immunonephelometric assay, cholesterol (TCH), HDL-Ch and triglycerides (TG) were measured.

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>Healthy (N = 36)</th>
<th>I (N = 55)</th>
<th>II (N = 117)</th>
<th>III (N = 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mg/dL)</td>
<td>102 ± 44</td>
<td>132 ± 71</td>
<td>165 ± 97</td>
<td>245 ± 126*</td>
</tr>
<tr>
<td>TCH (mg/dL)</td>
<td>171 ± 20</td>
<td>207 ± 30</td>
<td>229 ± 46*</td>
<td>270 ± 38*</td>
</tr>
<tr>
<td>Hcy (mg/dL)</td>
<td>53 ± 1.3</td>
<td>50 ± 1.4</td>
<td>51 ± 1.3</td>
<td>47 ± 1.0</td>
</tr>
<tr>
<td>Lp(a) (mg/dL)</td>
<td>317 ± 15.0</td>
<td>247 ± 28.3*</td>
<td>256 ± 32.9*</td>
<td>337 ± 44.7*</td>
</tr>
<tr>
<td>Hcy (nmol/L)</td>
<td>167 ± 4.7</td>
<td>9.6 ± 2.6</td>
<td>10.5 ± 4.3</td>
<td>9.6 ± 2.6</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. healthy subjects

No correlation was found between Hcy and lipid parameters, however there was a strong relationship between Lp(a) and TCH. Elevated Hcy was found in 20% of healthy and 17% of remaining subjects while increased Lp(a) was observed in 11% and 27% respectively.

**Conclusions:** Lp(a) seems to be more atherogenic risk factor than Hcy. Elevated Lp(a) was shown in 31% of III° subjects (positive family history of CAD), while abnormal Hcy was found only in 14.6%. High contribution of elevated Lp(a) may accelerate the development of atherosclerosis.

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**ThT7/W27**

**Serum lipoprotein (a) level in normolipidemia and different types of hyperlipoproteinemia**

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**Objective:** The aim of our study was to evaluate serum lipoprotein (a) level frequency distribution in normolipidemia and different types of hyperlipoproteinemia (HLP).

**Methods:** Research was performed in 213 healthy subjects and 3649 patients with increased risk for the development of premature atherosclerosis. Serum Lp(a) concentrations were measured by RID (Immuno AG), and the evaluation was carried out according to distributive categories of normal (up to 0.25 g/l) and increased levels (moderate 0.26–0.5 g/l; extremely > 0.5 g/l).

**Results:** In healthy subjects with HLP, comparing with normolipidemic subjects, serum Lp(a) levels were significantly higher only in the category of extremely elevated Lp(a) levels (p < 0.001). In patients group, however, Lp(a) levels were significantly higher in the category of increased Lp(a) levels (p < 0.001), in moderate (p < 0.001) and in extremely (p < 0.001) also. In IIa and IIb HLP type, high coincidence in findings was noticed, as well as in normolipidemia and type IV HLP, on the other hand. In healthy subjects with HLP, there is an insignificant increase of mean Lp(a) levels comparing with normolipidemic, while in patients group the difference is significant (p < 0.001), noticed in IIa (p < 0.01) and IIb (p 0.01) also.

**Conclusions:** Our data strongly supports important practical fact that an increased Lp(a) levels are more frequently found in hypercholesterolemia.

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**ThT8/W27**

**Lipoprotein(a) levels predict myocardial infarction in young men**

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**Objective:** Several studies have demonstrated that lipoprotein(a) [Lp(a)] level is a risk factor for premature coronary heart disease (CHD). The objective of the study was to establish the parameters predisposing to the development of myocardial infarction (MI) in young men.

**Methods:** Coronary atherosclerosis was verified by coronary angiography in 322 male CHD patients (aged 29 to 68 years). All known cardiac risk factors were recorded at clinical examination. Concentrations of total and HDL cholesterol, triglycerides, fibrinogen were determined. Lp(a) concentration was measured by ELISA.

**Results:** In this cohort the mean Lp(a) level was 32 ± 34 mg/dl. Two hundred and one (62%) patients suffered one or more MI. According to age we have divided all patients into the three groups: 1) before 45 (n = 68), II) 46–55 (n = 138) and III) over 55 (n = 116) years. The only significant difference was the total cholesterol concentration between I and III groups: 7.0 ± 1.7 versus 6.5 ± 1.4 mmol/l respectively, p = 0.01. Spearman’s correlation analysis revealed that in patients younger 45 years Lp(a) levels and family history of CHD correlated with history of MI whereas in patients over 55 years smoking and family history were associated with MI. Only Lp(a) and family history of CHD were correlated with occlusions in coronary arteries in both groups.

**Conclusion:** data from this study have shown that high Lp(a) level can be used as a predictor of myocardial infarction in men younger than 45 years old.

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**ThT1/W28**

**HYPERTENSION, KIDNEY DISEASE, AND ATHEROSCLEROSIS**

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**ThT1/W28**

**Treatment of hyperlipidemia in the nephrotic syndrome:**

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**Objective:** To test the efficacy and safety of a new HMG-CoA reductase inhibitor atorvastatin in the nephrotic syndrome.

**Methods:** The effects of atorvastatin as a hypolipidemic agent were evaluated in 15 adult patients with the nephrotic syndrome (defined as a urinary protein excretion > 2.5 g/d) due to biopsy proven primary glomerulopathies and fasting total cholesterol higher than 6.48 mmol/l. Atorvastatin was given as a single daily dose (10 mg) in the evening for a total of 3 months. Lipoprotein measurements were performed at the beginning and upon the completion of the study.

**Results:** Atorvastatin produced a prompt mean fall of 33% in total cholesterol from a baseline of 10.3 ± 2.19 mmol/l (p < 0.01) and 47% in LDL cholesterol from a baseline of 7.8 ± 1.9 mmol/l (p < 0.01) by the end of the study. HDL cholesterol increased by 24% from a baseline of 1.44 ± 0.52 mmol/l (p < 0.05). Total/HDL cholesterol ratio had fallen by 52% from a baseline of 7.89 ± 3.2 (p < 0.01), and LDL/HDL cholesterol ratio by 63% from a baseline of 6.02 ± 2.75 (p < 0.01). Apo B decreased by 37% from 2.17 ± 0.52 g/l (p < 0.05). Apo A increased by 24% from 1.57 ± 0.31 g/l (p < 0.05).

**Conclusions:** Atorvastatin was effective and safe in favorably altering the lipoprotein profile of patients with the nephrotic syndrome.

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**ThT2/W28**

**Magnesium and sodium sensitivity in hypertensive subjects**

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**Objective:** Several studies reported negative correlation of blood magnesium and blood pressure, and changes in magnesium metabolism during different sodium intake in hypertensive subjects.

**Methods:** The study group consisted of 50 untreated patients (27 woman and 23 men; average age 42 ± 9.2 years; average BMI 27.91 ± 4.6 kg/m²) with essential hypertension. All patients were put on a high sodium diet (200 mmol NaCl per day) for one week after a week on a low sodium diet (20 mmol NaCl per day). Sodium sensitivity was defined as 10 mmol Hg increase in mean blood pressure at the end of high vs. low sodium diet. Sodium-sensitive group consisted of 26 patients and sodium-insensitive of 24 patients.

**Results:** Average blood magnesium levels were within the normal range during different sodium regimes. Urinary magnesium decreased during low-sodium intake and increased during high-sodium intake although the difference was not significant. Our study showed significantly lower blood magnesium levels in sodium-sensitive subjects in comparison to sodium-insensitive subjects during low-sodium regime (p = 0.03), and non-significant difference during high-sodium regime.

**Conclusion:** Our study revealed lower blood magnesium levels in sodium-sensitive patients. These patients could benefit from higher magnesium intake.
Homocysteine, cystatin C, lipid levels and bioelectrical impedance analysis in dialysed and transplanted patients


The homocysteine and LDL-cholesterol elevation are strong risk factors for accelerated atherosclerosis, coronary heart disease and cardiovascular complications. We measured the serum homocysteine, cystatin C and lipid levels (CHO, LDL, HDL, ApoB, ApoA1), the bioelectrical impedance and ABPM + ECG monitoring in 74 haemodialysed (male 38, female 36 – mean 62 years) and renal transplanted pts. (male 115, female 77 – mean 43 years). The homocysteine levels (22.9 vs. 16.8 umol/l) and cystatin C levels (5.95 vs. 2.56 mg/l) were higher in dialysed pts. than in transplanted pts. The homocysteine levels were significantly higher at diabetic pts. or pts. after stroke (29.75 vs. 22.9 umol/l). In obese, dialysed and transplanted pts. were explicit dyslipidemic alterations (high CHO, LDL, ApoB levels). Haemodialysed pts. had mostly hypertriglyceridemia. After transplantation we found especially hypercholesterolaemia, high LDL and ApoB concentrations. After renal transplantation were good correlations between the serum creatinine, cystatin C (r = 0.6381) and homocysteine levels (r = 0.2936). In haemodialysed and transplanted pts. had different fat body weight (FBW %), total body water (TBW%) and ICV/ECV quotient (1.73 vs. 2.07).

HD patient | TRS patient
---|---
FBW % | 13.90 | 25.83 | 14.64 | 26.71
TBW % | 64.31 | 57.48 | 62.34 | 52.08
ICV/ECV | 2.03 | 2.12 | 1.67 | 1.79

It seems to be a good correlation with the total body weight increase, hyperpertension, hyperlipidemia, diurnal rhythm changing and ECG abnormality (silent ischaemic periods, rhythm alterations and left ventricular hypertrophy).

The effects of the addition of losartan or fenofibrate on the uric acid metabolism in patients receiving indapamide


Objective: A number of adverse metabolic effects are associated with indapamide administration, including an increase in serum uric acid levels. It has been reported that fenofibrate and losartan significantly decrease serum uric acid levels. However, there are no data on the effects of combination therapy of either of these drugs with indapamide on uric acid levels.

Methods: We studied 30 hypertensive patients in whom serum metabolic parameters, including uric acid levels in serum and urine, were studied before and after 8 weeks of indapamide administration (2.5 mg once daily) as well as 8 weeks after combination treatment with either losartan 50 mg/day (in 16 patients whose blood pressure was not controlled by monotherapy) or micronised fenofibrate (200 mg/day) (in 14 patients who were well controlled after indapamide administration but also had dyslipidaemia).

Results: A significant increase in serum uric acid levels was noticed after indapamide administration. Losartan addition was followed by a nearly offsetting of the indapamide hyperuricemic effect. The strong hypouricemic effect of micronised fenofibrate was associated with a significant decrease in serum uric acid levels in lower than the baseline ones. Losartan and mainly fenofibrate induced a substantial increase in uric acid excretion.

Conclusion: The addition of either losartan or mostly fenofibrate could offset the hyperuricemic effect of indapamide administration.

Analysis of the presence of subpopulation of oxidized low density lipoprotein (oxLDL) in pre and post-dialysis samples from renal insufficiency patients

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Objective: To analyze the effect of dialysis on the presence of oxLDL.

Methods: We have developed an ELISA that detects, in serum, LDL with various degrees of oxidative modifications (described in an accompanying abstract). After consent, sera were obtained from 80 patients where prepared from peripheral blood samples taken pre and post dialysis. These samples were simultaneously tested in ELISA for the presence of oxLDL.

Enhanced clearance of chylomicron remnants in hypertensive normolipidemic patients during nifedipine treatment is age related

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To evaluate the effect of nifedipine (30–60 mg qd) on postprandial (PP) lipemia in 16 normolipidemic patients (pts) had vitamin A-fat loading test before and during treatment. Nifedipine reduced systolic and diastolic blood pressure (bp) by 20% (p < 0.01), but had no metabolic effect when the entire group was evaluated. Yet, subanalysis showed that older pts (subgroup A, n = 8, age: 57–71; mean ± SD 62 ± 4) compared to younger pts (subgroup B, n = 8, age: 45–56; mean ± SD 51 ± 4) had significantly higher pretreatment levels of PP of triglycerides (TG) chylomicrons (CM) and chylomicron remnants (CMR) levels (1.7–1.9, 1.3-fold, respectively). Subgroup A compared to subgroup B also had 2.8-fold higher insulin increment (p = 0.04) and 0.7-fold lower glucose/insulin ratio (p = 0.04) following 75-g oral glucose load. In subgroup A, nifedipine treatment was associated with 22% (p = 0.032) reduction in CMR level. This was strongly related to the pretreatment CMR (r = 0.88, p = 0.025). It was also related to diastolic bp decrement (r = 0.78, p = 0.02) during treatment. Our result suggest that 1. Catabolic rate of PP TG-rich lipoproteins is age-related i.e. older pts with hypertension have delayed PP clearance of TG, CM and CMR in comparison to younger pts, and this may be associated with decreased insulin sensitivity 2. Clearance of CMR is enhanced by nifedipine and this enhancement is highest in those pts with the greatest disturbance in CMR catabolism i.e. older pts.

Plasma homocysteine determination by CE-CLIF in hemodialysed and ischemic subjects

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Objective: To determine whether hyperhomocysteinemia, associated with premature vascular disease (VD), is one of the factors that can increase the risk of VD in chronic renal failure/hemodialysis disease (HD) and coronary artery disease (CAD).

Methods: Fasting total plasma homocysteine (HCy) was determined by capillary electrophoresis/laser-induced fluorescence detection (CE-CLIF) in 51 HD patients, 40 CAD patients and 102 control subjects. Prevalence of VD was determined: i) after exercise test, and coronarography if positive exercise test, ii) by doppler examination to detect peripheral vascular disease.

Results: Plasma Hcy level was markedly elevated in all HD (25.23 ± 1.72 μM) and CAD (19.13 ± 0.95 μM) patients versus control subjects (13.9 ± 0.53 μM; p < 0.001). However, among the patients having coronary lesions (>70% stenosis) there was no difference in Hcy levels between HD and CAD individuals, suggesting that the risk of atherosclerosis is similar in these two populations. However, some HD patients have a high Hcy (>100 μM) without coronary lesions. These high Hcy levels were significantly associated with the presence of peripheral arterial disease in the lower limbs but not with carotid lesions.

Conclusion: Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease in hemodialysed and ischemic patients.

T:W29 OXIDATION AND AtherosGENESIS

TH1:W29
OX-LDL induced apoptosis of arterial vascular smooth muscle cells of rats


Objective: To investigate apoptosis of arterial vascular smooth muscle cells (VSMCs) of rats induced by OX-LDL.

Methods: Electron microscopy for cell morphology, terminal deoxynucleotidyl transferase deoxy UTP nick end labeling assay, flow cytometry and Western blotting were performed to examine the apoptosis of VSMCs induced by OX-LDL.

Results: VSMCs of rats treated with OX-LDL (50–200 μg/ml) underwent the arrest of cell mitosis at metaphase and induced apoptosis. The cells treated with OX-LDL were demonstrated shrinkage, condensation or fragmentation of chromatons, and apoptotic body appeared. TUNEL assay showed brown and positive particles in apoptotic chromatons and cytoplasm. The expression of p53 was increased, while expression of bcl-2 decreased.

Conclusion: Apoptosis of VSMCs induced by OX-LDL was closely associated with mitotic arrest of cells cycle, and alteration of p53 and bcl-2 gene may play an important role in regulating OX-LDL-induced apoptosis.

TH2:W29
Fluvastatin and antioxidants in acute coronary syndromes

A. Laukevičius, Z. Petrušiūnienė, V. Dženkėvičiūtė. Clinic of Cardiology, Vilnius University Hospital, Lithuania

Objective: Statins effect on immune function, macrophage metabolism, cell proliferation, endothelial function, vasomotion, platelet reactivity and humoral thrombogenic factors independent of changes in plasma LDL concentrations. They use in acute coronary syndromes (ACS) may influence on early clinical benefit.

Methods: The clinical pain syndrome, EKG changes as well as ultrasound asyngeric zones were investigated in-patients with acute coronary syndrome. One group of patients (n = 15) received fluvastatin (80 mg) and second group (n = 15) – fluvastatin (80 mg) plus vitamin E (400 UI) and vitamin C (1000 mg). In control group (n = 20) usual antithrombotic, cardioprotective treatment was used.

Results: After four weeks of treatment the pain, EKG and asyngeric zones markedly diminished in fluvastatin, fluvastatin plus vit. E and C as well as in control group of patients with acute coronary syndrome. No statistical differences were found in treatment benefit between fluvastatin, fluvastatin plus vit. E and C and control group.

Conclusions: Further investigations are necessary to assess the role of statins and antioxidants in acute coronary syndrome.

TH3:W29
Effect of insulin resistance on serum paraoxonase and diazoxanase activities in nondiabetic populations

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Objective: PON-1 is an esterase that protects against lipid peroxides accumulation in LDL. We investigated the possible change in PON-1 enzyme activities in insulin resistance.

Methods: We studied 238 nondiabetic subjects. PON-1 enzyme activity was measured using paraoxon (PON activity) and diazoxan (DZO activity) as substrates. PON-1 gene polymorphism was determined by PCR-RFLP method. Insulin resistance was assessed by homeostasis model assessment index (HOMA index).

Results: PON activity was QQ vs. QR vs. RR, whereas DZO activity was in the opposite order. PON and DZO activities correlated positively with HDL-c. PON/HDL and DZO/HDL ratios correlated positively with BMI, WHR, fasting plasma glucose, insulin and HOMA index. Multiple regression analysis indicated that HOMA index showed a significant association with both PON/HDL and DZO/HDL ratios independent of PON-1 genotype, age and obesity.

Conclusions: Insulin resistance as assessed by HOMA index is a significant factor affecting PON-1 in a nondiabetic population.

TH4:W29
Effects of glicazide on low-density lipoprotein oxidizability and atherosclerosis in cholesterol-fed rabbits

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Objective: Glicazide, a second generation sulfonurea that is widely used in the treatment of type II diabetes mellitus, has been shown to have free radical-scavenging activity in vitro. We studied the effects of glicazide on the oxidizability of low-density lipoprotein (LDL) and the development of experimental atherosclerosis in cholesterol-fed rabbits.

Methods: 16 rabbits were divided into two groups. The control group (n = 8) was given a standard diet containing 1% cholesterol. The glicazide group (n = 8) was given a standard diet containing 1% cholesterol and supplemented with glicazide (20 mg/kg/day).

Results: Glicazide did not affect diet-induced hyperlipidemia. 35.3 ± 16.4% of the aortic surface of the control rabbits were covered with atherosclerotic lesions. Glicazide treatment tended to result in a somewhat smaller area covered with atherosclerotic lesions (29.9 ± 16.1%), although this was not statistically significant. The administration of glicazide tended to inhibit the increase of serum thiobarbituric acid-reacting substances (TBARS) by cholesterol feeding, however this was not statistically significant. Supplementing the rabbit diet with glicazide for 10 weeks resulted in a 36.8% increase in lag time of the conjugated-diene formation in LDL subjected to in vitro oxidation by copper ion although without significance.

Conclusion: This study suggests that glicazide may have antioxidative properties in vivo, and have further beneficial effects for the treatment of diabetes mellitus by inhibiting the oxidation of LDL.

TH5:W29
Does alpha-tocopherol and beta-carotene supplementation prevent abdominal aortic aneurysm?

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Objective: To assess the effect of alpha-tocopherol and beta-carotene supplementation on incidence of abdominal aortic aneurysm (AAA) in a randomized, double-blind, placebo-controlled trial.

Methods: Subjects were 50- to 60-year-old male smokers, participants of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. They were randomized to receive 50 mg of alpha-tocopherol, or 20 mg of beta-carotene, or both, or placebo in one capsule per day. Incidence of AAA was monitored through hospital and mortality registers.

Results: Of the 29,133 trial subjects 181 were diagnosed to have ruptured AAA (n = 77) or nonruptured AAA with graft placement (n = 104) during a mean follow-up of 5.8 years. Relative risk for AAA was 0.83 (95% confidence interval CI: 0.62–1.11) among those who received alpha-tocopherol compared to those who did not, and 0.93 (95% CI: 0.69–1.24) among those who received beta-carotene compared to those who did not.

Conclusions: Neither antioxidant had significant preventive effect on abdominal aortic aneurysm among older male smokers.

TH6:W29
Inhibition of lipid peroxidation by magnesium tanshioate B in the heart

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Objective: The formation of oxidised lipids in a pathological setting may have damaging effects on the arterial wall. The objective of the present study was to investigate the anti-oxidant effect of magnesium tanshioate B (MTB), a bioactive compound isolated from Danshen.

Methods: The Langendorff-perfused isolated adult Sprague-Dawley rat hearts were used as a model system for study. Hearts were pretreated with MTB and subjected to 30 min of northermotic global ischaemia. At the end of the ischaemic period, the heart was reperfused with the same media containing MTB for an additional 20 min. The level of lipid peroxides, measured by the formation of malondialdehyde (MDA), was determined in the MTB-treated and untreated ischaemic/reperfused heart. Oxidative stress in the heart was also induced by perfusion with hydrogen peroxide in the absence or presence of MTB, followed by determination of MDA levels.

Results: The level of MDA was significantly elevated by 184 ± 12% (n = 5) in the ischaemic/reperfused rat hearts. Treatment with 1 μM MTB abolished this elevation in MDA levels. When the perfused heart was treated with 100 μM hydrogen peroxide, tissue MDA levels were elevated by 181 ± 19% (n =
5. In the presence of MTB, the elevation in MDA levels caused by hydrogen peroxide was abolished.

**Conclusion:** MTB may protect the arterial wall from oxidative damage. (Supported by the RGC and NSFC/RGC grants)

**ThT7W29**

**Probiol suppresses experimental atherosclerosis in rats**

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**Objectives:** Probiol is an antioxidant and has widely been used to treat hyperlipidemia. The aim of this study is to investigate the effects of probiol on atherosclerotic lesions in rats.

**Methods:** The carotid arteries of Wistar rats were injured with a balloon catheter, and the rats were allocated into 4 groups: 1) ND group fed with normal rat chow (ND), 2) AD group fed with atherogenic chow (AD) containing 2% cholesterol, 0.5% cholic-Na, 0.2% propylthiouracil, 10% lard and 5% sugar; 3) P-75 group given Probiol orally at 75 mg/kg body weight (bw) and fed with AD; and 4) P-150 group given Probiol orally at 150 mg/kg bw and fed with AD. After administration for 13 consecutive days, the status was assessed at 14 day after balloon injury.

**Results:** The lesions of carotid arteries showed intimal thickening with accumulated cholesterol esters and foam cells. The serum cholesterol in AD group increased to 995 mg/dl which was 15 times higher than that in ND group. In AD group, the accumulation of cholesterol esters in lesions increased significantly. Probiol suppressed the increase of cholesterol accumulation. Cholesterol esters in carotid lesions at 52.9% in P-75 and 51.3% in P-150 groups, respectively. Probiol also decreased intimal thickening. Probiol decreased serum total cholesterol levels by 35.3% in P-75 group and 33.0% in P-150 groups, respectively. Probiol prevented increase in cholesterol levels in carotid lesions more potently than increase in serum cholesterol levels in P-150 group.

**Conclusions:** Probiol suppressed the neo-intimal response to balloon injury in the AD-fed rat carotid artery by decreasing intimal thickening and cholesterol esters accumulation.

**ThT8W29**

**VLDL, LDL and HDL: Alterations in the oxidation profile and the formation of hydroperoxides induced by varying copper concentrations**

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**Objective:** When various concentrations of copper are used to promote the oxidation of LDL, conjugated diene (CD) production displays 3 very different profiles (A, B and C). We wished to investigate if similar oxidation profiles were apparent during copper oxidation of VLDL and HDL.

**Methods:** The 3 classes of lipoproteins were isolated by rapid ultracentrifugation: VLDL-1 h, LDL-1 h, LDL-2 h. Each lipoprotein was standardized for protein and then oxidized in the presence of various copper concentrations to mediate the 3 types of oxidation. VLDL-25 mg/ml protein, oxidised with either 0, 0.05, 0.2, 10 μM CuCl2 LDL-50 mg/l protein, oxidised with either 0, 0.05, 0.1 or 2 μM CuCl2 HDL-100 mg/l protein, oxidised with either 0, 0.1, 0.5 or 20 μM CuCl2. The formation of CDs and hydroperoxides (HPOs) were used to monitor oxidation.

**Results:** When CD and HPO production were followed for up to 2 h at 37° C, VLDL, LDL and HDL produced 3 characteristic types of oxidation. Type A occurred at high, type B at intermediate and type C at zero or low copper concentrations. However, when CD production was allowed to continue for up to 4 h, the type C oxidation of the zero or low copper concentrations became type B oxidation.

**Conclusions:** We have demonstrated that oxidation of VLDL, LDL and HDL is allowed to continue for 48 h at physiological temperature, instead of 30°C, type C oxidation becomes type B oxidation. These results suggest that in all classes of lipoproteins there may be 2, not 3, types of oxidation, so called type C oxidation merely being the initial slope of type B oxidation. Previous studies have demonstrated 1 that this type of oxidation is unaffected by the presence of endogenous antioxidants, unlike type A. The initial slope of type B oxidation which occurs both with zero or low copper concentrations may have a greater physiological impact on the development of atherosclerosis.

**References**


**ThT9W29**

**Protective effect of some herbal medicine against oxidative hemolysis of human erythrocytes**

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**Objective:** Free radical are very active molecule generated internally or externally react with biomolecules such as lipids and promoting peroxidation. Peroxidation of lipid membrane considered as an important destructive effect of free radicals damage. Therefore, it is necessary to block these compounds for preventing their effects.

**Methods:** The polyphenolic and total extracts of Matriaria chamomilla, Achillea millefolium and Morus nigra were prepared. For promoting lipid peroxidation on erythrocytes, AAPH was used and different concentrations of plant extracts (5, 2.5, 1, 0.5, 0.25) mg/ml were used. The level of hemolysis was measured by spectrophotometric method in 415 nm.

**Results:** The protective effect of these natural products on the occurrence of hemolysis were dose-dependent and in the highest concentration were as follows: Morus nigra (polyphenolic extract) (60.7%) > Achillea millefolium (polyphenolic extract) (50.7%) > Matricaria Chamomilla (total extract) (49%) > Morus nigra (total extract) (33.1%) > Matricaria Chamomilla (total extract) (21.7%) > Achillea millefolium (total extract prooxidant).

**Conclusions:** The results indicate that these natural products are protective agents for membrane and other cellerular structure against oxidant compounds but this protective effect is associated with the kind of extraction and is dose-dependent for each plant.

**ThT10W29**

**The serum levels of lipid hydroperoxide, conjugated dienes and glycosylated haemoglobin in smoker and non-smoker**

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**Objective:** Smoking is one of the important human health problems in recent years. Although there are several studies on the cigarette smoke, it is not clear how it causes damages pathophysiologically, biologically and physicochemically. This study was performed to determine the plasma levels of malondialdehyde (MDA), conjugated Dienes (CDs) and glycosylated haemoglobin (HbA1C) in smoker and non-smoker men.

**Methods:** Eighty two men were divided in two groups of smoker (n = 36) and non-smokers (n = 46). The measurements of Fast blood sugar (FBS), Triglycerides (TG), Total cholesterol (T.cho), MDA, CDs and HbA1C were determined for each subject. Data was analysed by ANOVA and t-test.

**Results:** The serum levels of MDA and HbA1C were significantly higher in smokers than non-smokers (P = 0.01 and P = 0.001, respectively) but CDs didn't show any significant difference (P = 0.35). Mean MDA were 1.09 ± 0.47 in smokers and 0.83 ± 0.44 in non-smoker. Mean HbA1C were 8.13 ± 1.42 in smokers and 7.29 ± 4.2 in non-smokers.

**Conclusions:** The obtained results indicate an acceleration in blood lipid peroxidation causing severe vascular damages in smokers. Mean HbA1C was higher in smokers than non-smokers. Although smokers had higher level of FBS than non-smokers, the likelihood role of advanced glycation products is confirmed for HbA1C increasing in smokers.

**ThT11W29**

**Total antioxidant status in young myocardial infarction survivors**

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Free oxygen radicals are involved in the endothelial lesion process which leads to the formation of the atheroma plaque and thrombosis. There’s some evidence that antioxidant therapy may be beneficial in coronary heart disease prevention.

**Objectives:** to study the plasma total antioxidant status in young acute myocardial infarction survivors.

**Methods:** population: 23 patients, mean age 35.2 ± 4.9 years (22-40) admitted for acute myocardial infarction since January 1995 until June 1998 (male: 20 patients). Risk factors: tobacco smoking 22/23, systemic arterial hypertension 4/23, hypercholesterolemia 17/23, positive family history for coronary heart disease 5 patients, previous cardiac history 4 patients, none of these patients had diabetes mellitus. The location of the infarct was anterior in 12 patients, inferior in 10 patients and non-Q wave in one patient.
Blood samples were drawn after overnight fasting and the plasma total antioxidant status (TAS) was determined by the modified trolox method (Trost equivalent). The mean time elapsed since the acute myocardial infarction until the samples collection was 16.5 ± 10.7 months.

**Results:** 18 patients had low TAS values, mean 1.23 ± 0.11 mmol/L (below the reference values: 1.3–1.77 mmol/L). There was a significant positive correlation between TAS and time since the infarction (p < 0.05).

**Conclusion:** In this group of patients the plasma total antioxidant capacity was globally decreased, which may constitute a risk factor for coronary heart disease and may help select those who might benefit the most from antioxidant therapy. There may be a recovery of the antioxidant capacity with time after an acute coronary syndrome.

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**ThT14-W29**

**Lipoperoxidation of LDL and VLDL in the different types of hyperlipoproteinaemia**


**Objective:** Much evidence was accumulated to suggest a role for the oxidation of both LDL and VLDL in the pathogenesis of atherosclerosis. Hyperlipoproteinaemia (HLP), an established risk factor for atherosclerosis, is supposed to be connected with an increased oxidative stress and different indices of the oxidative modification of LDL and VLDL (increased basal concentrations of conjugated dienes, shortened lag phase and faster propagation phase during the conjugated diene formation) were described.

**Method:** In this study we have analysed lipoperoxidation of VLDL and LDL in the different types of HLP by measuring the conjugated dienes formation (A 234 nm) catalysed by Cu²⁺. We have investigated 82 patients (48 M, 34 F) with hyperlipidaemia (24 IIA, 14 IIB and 44 IV and V phenotype) and compared them with the control group (53 M, 33 F).

**Results:** In the parameters of lipoperoxidation of LDL we have observed increased basal absorption in all HLP groups compared with C group. Lag phase was significantly (P < 0.01) shortened in the IIA (78.8 min) and IIB (85.5 min) but not in the IV and V group as compared with the C group (103.5 min). Propagation rate was significantly (P < 0.01) slower in the IV and V than in the both IIA and C group. Observing the parameters of liperoxidation of VLDL we have found increased (P < 0.05) basal absorbance in the IV and V group compared with the C group and significantly higher difference between maximal and basal absorbance in the IV and V group. Lag phase was shortened in all the HLP groups (P < 0.05).

**Conclusions:** We have observed unfavourably changes in the oxidability of VLDL and LDL and increased level of their lipoperoxidation in patients with both hypercholesterolaemia and hypertriglyceridaemia.

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**ThT15-W29**

**The antioxidative therapy of atherosclerosis with using of natural and synthetic antioxidants**

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**Objective:** The oxidative modification of LDL is thought to play an important role in atherogenesis.

**Methods:** We studied the influence of the vitamin E-reach diet (400 mg daily) on the Cu²+-mediated oxidizability of LDL from patients with atherosclerosis. So far as LDL is the main transport form of a-tocopherol we were surprised to find that during 4 mths a-tocopherol in vivo supplementation did not increase the oxidation resistance of LDL. These observations are consistent with the view that main natural antioxidant of LDL may be not a-tocopherol but reduced form of ubiquinon Q10 – ubiquinol Q10.

**Results:** Really, of mths treatment of patients with inhibitor of cholesterol and ubiquinon Q10 biosynthesis (BHG CoA reductase inhibitor) – pravastatin (40 mg daily) increased the level of lipohydroperoxides in the LDL of patients to about 30%. At the same time the therapy with pravastatine in combination with ubiquinon Q10 preparation (60 mg daily) in opposition decreased the concentration of lipid peroxides in LDL during 6 mths to 80% from initial level. The synthetic antioxidant probucol just as sharply decrease the lipoperoxides level in LDL after 6 mths treatment of patients with this drug in the daily dose 1000 mg as well as 250 mg. After oxidation of this probucol-contains LDL by C-15 reticulocyte lipoxgenase in these particles we identified the ESR signal of probucol phenoxyl radical that suggest the possible interaction of LDL-associated probucol with lipid radicals in vivo. Vitamin E increased the lag time of AIBN-initiated egg lecithin liposomes oxidation in two time lower than probucol.

**Conclusions:** Our findings show that probucol may act in the body as a trap for lipid free radicals and this synthetic antioxidant may be more effective in
the prevention of LDL peroxidation during atherogenesis than some natural antioxidants such as α-tocopherol but not ubiquinon Q10.

HDL₃ exerts more powerful anti-oxidative, protective effects against antioxidative modification of LDL than HDL₂

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Objective: To evaluate which HDL fraction, HDL₃ or HDL₂, exerts the greater preventive effect on antioxidative modification of LDL.

Methods: LDL was incubated for 96 hours in phosphate-buffered saline (PBS) without transition-metal ions alone, or in the presence of HDL₂ or HDL₃. Each sample was subjected to agarose gel electrophoresis.

Results: Both HDL₂ and HDL₃ significantly inhibited antioxidative modification of LDL, as assessed by electrophoretic mobility, but this effect was much more pronounced with HDL₃.

Conclusions: HDL₃ may play an important role in the prevention of atherosclerosis in vivo, more effectively inhibiting oxidation of LDL than HDL₂.

Mitogenic effects of oxidized low density lipoprotein

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Objective: To compare the mitogenicity of oxidized low density lipoprotein (oxLDL) to that of basic fibroblast growth factor (bFGF) and fetal bovine serum (FBS).

Methods: Cultured human fibroblasts were serum starved for five days before being treated with either 1) serum-free media and 0, 10, or 50 μg/ml oxLDL, 2) 10 ng/ml bFGF and 0, 10 or 50 μg/ml oxLDL, or 3) 5% FBS and 0, 10 or 50 μg/ml oxLDL for 24 or 48 hours. For quantification of the number of cells in culture following treatments, cells were trypsinized and counted in a hemacytometer.

Results: Incubating serum starved fibroblasts with serum-free media and oxLDL resulted in significant increases in cell numbers several orders of magnitude greater than in cells treated with native LDL. The mitogenic effect of oxLDL alone was significantly less than that of 5% FBS alone, but greater than that of 10 ng/ml bFGF alone at one concentration and time point. Treating cells with 10 ng/ml bFGF in combination with oxLDL resulted in increases in cell numbers which were greater than the effect of either bFGF or oxLDL alone. However, treating cells with 5% FBS in combination with oxLDL resulted in cell numbers which were lower than that of 5% FBS alone but still greater than that of oxLDL alone. Similar results were obtained in experiments using smooth muscle cells.

Conclusions: OxLDL is mitogenic on its own and acts synergistically with bFGF. OxLDL in combination with FBS exerts a mitogenic effect that is greater than that of oxLDL alone, but significantly less than that of FBS alone. OxLDL therefore has an unusual competitive, inhibitory effect on cell proliferation in the presence of FBS.

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The levels of serum sialidase in coronary heart disease

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Objective: Recently it was shown that LDL from atherosclerotic patients differ from LDL from healthy donors by a lower content of sialic acid. However the reason for the increased desialylation of lipoproteins is unknown. We have undertaken this study in order to investigate whether the levels of sialidase in patients with coronary heart disease (CHD) is changed.

Methods: The patient group consisted of 58 subjects and was divided into two subgroups according to their angiography results 28 subjects with no vessel disease and 30 subjects with double-triple vessel disease. The control group consisted of 30 healthy subjects. Serum lipid fractions were determined by enzymatically (Biocon).

Results: Serum total cholesterol, triglyceride, LDL and VLDL cholesterol levels in patients with no and double-triple vessel disease and also in total were found to be significantly lower than those in control group, however HDL cholesterol levels in patients with no and double-triple vessel disease and in total found to be significantly higher than control group. Also there was a significant difference between the levels of sialidase of patients with double-triple and no vessel disease.

Conclusion: We conclude that increased levels of serum sialidase may be responsible for the desialylation of lipoprotein in atherosclerosis.

Antioxidant properties of merlot wines in water soluble and lipid soluble free radical generating systems

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Objective: Oxidized low density lipoprotein (LDL) plays a role in atherogenesis. Red wine has been demonstrated to have antioxidant properties in vitro. This study examines the protective effects of catechin, epicatechin, rutin, transresveratrol, quercetin and Merlot wines from three different countries on LDL oxidation in water soluble (AAHP) and lipid soluble (AMVN) free radical generating systems (FRGS).

Methods: Isolated LDL was oxidized in AAHP or AMVN in the presence of catechin, epicatechin, rutin, transresveratrol, quercetin or alcohol of red wine. Conjugated diene assays were used to measure LDL oxidation.

Results: In an AAHP system, all polyphenolic compounds had an antioxidant effect. Change in absorbance decreased as concentration was increased. No protective effects were seen in an AMVN environment. LDL oxidation by AAHP was inhibited by red wine. Surprisingly, incubation of LDL with red wine in AMVN inhibited oxidation.

Conclusions: The five phenolic compounds and alcohols of Merlot wines displayed an antioxidant effect in a water soluble FRGS, but not in a lipid soluble one. Surprisingly, red wine inhibited LDL oxidation by a lipid soluble FRGS. Our data suggest red wines contain unidentified components that provide protection against LDL oxidation within the lipid phase.

This work was supported by the Medical Research Council of Canada.

Selective lowering of atherogenic lipoproteins and fibrinogen through hepatic-mediated extracorporeal LDL precipitation (HELP) normalizes basal formation of reactive oxygen species (ROS) and enhances bioconversion of nitroglycerin in patients with hypercholesterolemia

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Objective: Endothelial dysfunction is associated with reduced NO- and increased O2− release and leads to impaired vascular dilation. We analyzed the influence of hypercholesterolemia-induced oxidative stress and the change in low molecular thiol (LMT) plasma concentration on the bioconversion of nitroglycerin (GTN) to test the influence of HELP apheresis on formation of reactive oxygen species and GTN bioconversion.

Methods: This pilot study was carried out with 4 patients with hypercholesterolemia (total cholesterol 225 mg/dl, triglycerides 136 mg/dl) and with 8 healthy volunteers (total cholesterol 192 ± 11 mg/dl, triglycerides 90 ± 16 mg/dl). Basal and GTN-induced (0.5 mm) reactive oxygen species (ROS) formation and oxidized and reduced LMT concentrations were examined in whole blood using electron spin resonance spectrometry (ESR) and the spin trap CP-H or biradical respectively.

Results: Hypercholesterolemia enhanced the basal release of ROS in whole blood, which resulted in a 4 fold enhancement of oxidized LMT concentration and in a significant increase in reduced LMT in plasma. Basal ROS formation in whole blood, as parameter of the balance between prooxidative and antioxidative systems, was significantly (p < 0.001) increased in hypercholesterolemic patients compared to normocholesterolemic volunteers (0.47 ± 0.02 and 0.56 ± 0.03 nM/min respectively) but completely normalized after HELP-apheresis. GTN-induced ROS formation in whole blood, as parameter of oxidative stress and the bioconversion of GTN, was significantly (p < 0.001) diminished in hypercholesterolemic patients compared to normocholesterolemic volunteers (3.89 ± 0.30 and 5.48 ± 0.24 μM/min respectively). After apheresis we could observe a significant improvement (10 ± 0.3%) in nitroglycerine bioconversion.

Conclusion: Hypercholesterolemia results in an impairment of enzymatic bioconversion of organic nitrates in whole blood, which is not dependent on reduced low molecular thiol concentrations in plasma. This in turn leads to a limitation in the application of organic nitrates, which is effectively normalized
after apheresis. Further studies are needed to evaluate similar mechanisms of impaired intravascular bioavailability of NO in hypercholesterolemic patients.

**T-W30 GENETICS OF LIPOPROTEIN METABOLISM**

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**Objective:** It has been postulated that metabolic dysfunctions may be programmed in utero in thin newborn babies. Hypertension and Diabetes have been found to be related to intrauterine growth retardation (IUGR). Our aim was to measure serum lipid levels in newborns with IUGR.

**Methods:** Cord blood from 135 newborns with IUGR and 116 normal term babies was withdrawn to measure: Total Cholesterol, LDL-C, HDL-C, TG, ApoAI and ApoB with Boehringer Manheim reagents (Hitachi 717).

**Results:** Newborns with IUGR had higher serum TG levels than control ones (45.4 ± 27.2 versus 35.6 ± 19.2 mg/dl) (P < 0.01) and ApoB as well (31.1 ± 12.4 versus 28.1 ± 10.0 mg/dl) (P < 0.05).

**Conclusions:** Newborns with asymmetric IUGR already have dyslipidemia: higher TG and Apo-B levels.

**ThT22:W29 Kinetics study of LDL peroxidation induced by copper ions or by oxygen free radicals of γ-radiolysis**

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It is now well admitted that the oxidation of low-density lipoproteins constitutes a first step of a very complex process leading to atherosclerosis. The aim of this study was to compare the kinetics of low density lipoprotein oxidation induced by copper ions or by oxygen free radicals generated by 60Co γ-rays. The effects of copper concentration and irradiation dose-rate on LDL peroxidation kinetics were also studied. The oxidation of LDL was followed by the increase of conjugated dienes, hydroperoxides and thiobarbituric acid reactive substances formation as well as by α-tocopherol disappearance. In the case of gamma irradiation, the lag-phase before the onset of lipid peroxidation was inversely correlated to the radiation dose-rate. The radiation chemical yields (G-values) increased with dose-rate. Copper induced LDL peroxidation followed two kinetics: a low kinetics for copper concentrations ranged between 5 and 20 μM, and high kinetics for copper concentration of 40 μM. This difference in oxidation kinetics, according to the concentration, is in favor of the concept suggesting the existence of a saturable copper binding site on apo-B. When compared to γ-rays, copper ions react as a drastic and powerful oxidant only at higher concentrations (≥40 μM).

**ThT23:W29 Protective effects of *Craetesagus curvipesala* extracts against oxidative hemolysis of human erythrocytes**

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**Objectives:** The free radical-induced oxidative damages lead to various pathological events including atherosclerosis. Much interest exists in the possibility that antioxidant constituents of some medicinal plants reduce the risk of such diseases by inhibiting of these oxidative damages. *Craetesagus curvipesala* Lind, is one of the Iranian medicinal plants that used for treating of such disorders in cardiovascular diseases.

**Methods:** The polyphenolic and total extracts of dried flowers and leaves of *C. curvipesala* were prepared by pharmacopoeial methods. For evaluating of the actions of extracts as protective agents against oxidative damages mediated by lipid peroxidation in biomembranes, we used a method based on free radical induced deterioration of erythrocyte membranes and hemolysis. In the method, oxidative hemolysis was induced by 2,2'-azobis (2-amidinopropane)-dihydrochloride (AAPH) and in order to estimate the antihemolytic potency of herbal extracts, erythrocyte suspensions were coincubated with each extract and AAPH.

**Results:** These extracts retarded the occurrence of hemolysis and suppressed its percentage. The polyphenolic extract of the plant was much more effective than total extract in suppressing the hemolysis of erythrocytes in the experiment.

**Conclusions:** The polyphenolic extract of *C. curvipesala* acts as a protector against oxidative cell injury in human erythrocytes. Flavonoid compounds of the extract have been partly associated with these biochemical actions.

**ThT21:W29 Polysaturated fatty acids in lipoproteins and lipid peroxidation post LDL-apheresis**

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**Objective:** To investigate whether the extent of lipid peroxidation associates with the lipoprotein polysaturated fatty acid content pre- and post- low density lipoprotein (LDL)-apheresis.

**Methods:** Six hypertensive patients (3 men, 3 women, age 44–56, BMI 19.6–28.7) treated with diet, hypolipidemics and LDL-apheresis (LA) (Sepharose 4b columns carrying an anti-apolipoprotein B antibody) were studied. Blood samples were drawn pre and immediately post LDL-apheresis and analyzed for lipoprotein fractions by ultracentrifugation, and for fatty acids (FA) by capillary gas chromatography. Thioctic acid reactive substances (TBARS), which are products of lipid peroxidation, were determined after reaction with thiobarbituric acid spectrophotometrically.

**Results:** LA induced mean acute reduction of total cholesterol, and cholesterol in very low density lipoprotein (VLDL), LDL and high density lipoprotein by 52%, 56%, 48% and 64% respectively. Measurements of lipid peroxidation revealed decreased TBARS (4.6 ± 0.6 versus 3.7 ± 0.3 μmol/L, P < 0.05). Linoleic acid content increased in plasma (65.8 ± 25.5 versus 140.5 ± 91.9 μmol/L, P < 0.05) and decreased in LDL (30.8 ± 1.1 versus 27.2 ± 1.6%, P < 0.05). The content of arachidonic acid in plasma and lipoproteins did not change significantly. The content of docosahexaenoic acid decreased in plasma (4.7 ± 0.8 versus 4.1 ± 0.3, P < 0.05) and increased in LDL (1.1 ± 0.2 versus 1.3 ± 0.2, P = 0.08).

**Conclusion:** The decreased lipid peroxidation after LA may be explained by acute reduction of polysaturated fatty acids available for oxidation. Supported by grants IGA MH CR No. 4548-3, 5205-3.
increase levels of soluble cell adhesion molecules (sE-Selectin, sP-Selectin and sICAM-1) in overweight adults with combined hyperlipidemia

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Objective: Now is accepted that increase of soluble cell adhesion molecules (CAMs) is an early marker of coronary artery disease. The aim of this study was to determine whether combined hyperlipidemia in patients without clinical evidence of cardiovascular disease, diabetes mellitus or hypertension is associated with increased expression of CAMs.

Methods: We examined the levels of soluble cell adhesion molecules (sICAM-1, sE-Selectin and sP-Selectin) in patients (n = 40; 20 women and 20 men; age 49 ± 7 years) with combined hyperlipidemia (mean of total cholesterol (TC) level was 8.05 ± 1.53 mmol/l, serum triglycerides (TG) 4.51 ± 2.69 mmol/l, LDL cholesterol 5.25 ± 1.47, HDL cholesterol 1.37 ± 0.59). Mean of body mass index (BMI) was 27.27 ± 4.74 kg/m². Control group consisted of 40 healthy sex-and-age-matched persons (mean of BMI was 23.95 ± 3.62 kg/m²). Mean of TC was 5.30 ± 0.45 mmol/l, TG 2.13 ± 0.31 mmol/l, LDL 3.12 ± 0.32, HDL 1.34 ± 0.29). Patients were on a diet only for at least 6 weeks and none of the patients had clinical evidence of cardiovascular disease (by clinical history, physical examination, and ECG). Exclusion criteria for all subjects included renal insufficiency or proteinuria, altered hepatic function, smoking, diabetes mellitus and hypertension.

Results: Patients with combined hyperlipidemia had significantly higher serum levels of sE-Selectin (61.31 ± 9.15 ng/ml; versus 38.03 ± 13.25; P < 0.001, sICAM-1 (299.41 ± 47.63 ng/ml versus 223.63 ± 34.96 ng/ml; P < 0.001) and sP-Selectin (163.36 ± 25.91 ng/ml versus 104.68 ± 24.83 ng/ml; P < 0.001) compared with that in control subjects.

Conclusions: In our study patients with marked elevations of TC, LDL-C and TG had significantly increased levels of sICAM-1, sE-Selectin and sP-Selectin compared with control subjects. Patients were overweight. We predicted that if the increased levels of soluble CAMs were secondary to endothelial dysfunction resulting from hyperlipidemia, overweight might lead to exponentiation an increase the levels of soluble CAMs.

A point mutation in ABC1 gene in a patient with a severe premature clud and a mild phenotype of tangier disease

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The proband is a 50 years old woman born from a consangineous marriage. She has been suffering from angina pectoris since the age of 38 y. and underwent coronary bypass surgery for three- vessel disease at age 48 y. The presence of low plasma levels of total cholesterol and HDL cholesterol (2.4 and 0.1 mmol/L) and apo AI (<15 mg/dl), associated with corneal lesions and a mild splenomegaly suggested the diagnosis of Tangier disease (TD). However, none of the other features of TD, including hepatomegaly, anemia and peripheral neuropathy, were present. The analysis of the dinucleotide microsatellites located in chromosome 9q31 region showed that the proband was homozygote for the alleles of D9S53, D9S1784 and D9S1832. The mother and the son of the proband, both with low levels of HDL cholesterol, shared one of the proband’s haplotype, whereas none of these haplotypes was present in the nonmendipidic proband’s sister. The sequence of ABC1 CDNA obtained by RT-PCR of total RNA isolated from cultured fibroblasts showed that the proband was homozygote for a C > T transition in exon 13 which caused a tryptophane for arginine substitution (RS27W). This mutation was confirmed by direct sequence of exon 13 amplified from genomic DNA. It can be easily screened as the nucleotide change introduces a restriction site for the enzyme Alu III. RS27W substitution occurs in a highly conserved region of the NR_1G domain of ABC1 protein. RS27W co-segregates with the low HDL phenotype in the family and was not found in 200 chromosomes from normolipidemic individuals.

The independent correlation of the impact of lipoprotein(a) levels and apolipoprotein E polymorphism on carotid artery intima thickness

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Objective: Apolipoprotein E (apo E) plays a key role in lipoprotein metabolism. It occurs in three isoforms E2, E3 and E4. These isoforms have different impacts on plasma lipoprotein levels. Lipoprotein(a) (Lp(a)) is also atherogenic and its increased plasma concentration is presumed to be an independent risk factor for premature atherosclerosis. The aim of our study was to establish a relationship between common carotid artery intima thickness and two independent risk factors, the apoE polymorphism and the increase in plasma Lp(a) levels. A cross-sectional study was performed on 114 patients who were referred to the lipid clinic for primary hyperlipoproteinemia. The patients received no treatment prior to examination. Plasma levels of total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, apoA, apoB, Lp(a) and the apoE genotype were determined and the carotid artery intima thickness was measured using ultrasonography.

Conclusions: The equality of carotid intima thickness was tested using the Kruskal-Wallis test. Median of intima thickness in the subgroup with the allele E2 was 0.72 mm, in the subgroup with the E3/E3 genotype 0.70 mm and in the subgroup with the E4 allele 0.80 mm. The relationship between carotid intima thickness and Lp(a) levels was tested using Spearman’s correlation coefficient.

Post-LDL-apheresis data processing method: A physical interpretation


Objective: to interpret the post-apheresis experimental data trend as the physical process that produces the observed curve so that the fitting presupposed theoretical function is a direct consequence of the physic process.

Methods: For a given succession of times measured from the origin of curve natural co-ordinate (tj), the program finds the values of LDL elimination proportionality coefficient from plasma (k) and the asymptotic value of the concentration. The calculated through fitting in the times tj for t → ∞ which minimise the mean error (the research of the more appropriate k value is made through a progressive increase of this parameters); After comparing the mean error with the least one derived and saved from preceding calculations and after saving (or confirming) the value resulting more efficient in minimising the error, the succession of the tj is let vary and the previously described process is repeated; All the procedure is iterated until the error can no more be reduced.

Results: The time to analyse a curve that contains 15 experimental data is of a few seconds using a Quick Basic software on an Epson portable computer (486 microprocessor, 50 Mhz). Applying the proposed fitting method to a succession of 15 samples obtained from the mean of 6 plasma apheresis executed on 5 different subjects, small estimate standard error (5 mg/dl) and relative error (absolute mean error/mean LDL concentration = 1.7%) with a dispersion that appears to be evidently related to the experimental error were observed. Obviously, applying the same method to a single case, the dispersion is in general more marked (relative error until 5%), with a standard error varying from 10 to 13 mg/dl, even though the aspect of a casual phenomenon is conserved.

Conclusion: Our physical-mathematical interpretation of the experimentally obtainable data appears to be a practical model to understand the LDL-rebound kinetic of the single patient without using radioactive markers.

Triglyceride metabolism, microcirculation disturbance and atherosclerosis


Objective: We proposed perfusion metabolism theory as a cause of risk factor clustering syndrome. Triglyceride (TG) catabolism due to lipoprotein lipase effect is its main factor. From this view point, the lipase activity, triglyceride and HDL metabolism was investigated.
Methods: Postheparin lipoprotein lipase (LPL), hepatic lipase activity and serum lipid concentrations were examined in 67 male and female hyperlipidemic patients aged 50-70 years (60.1 years). In 52 patients the aortic wall thickening calcification volume (AWCV) was calculated from enhanced CT as atherosclerotic index (Ann J Hypertens. 1994). 

Results: HDL-C/apoA1, a parameter of cholesterol removal from macrophages and cells, was correlated positively with HDL-C and apoA1, and inversely with TG, apo B and CII, namely, cholesterol removal of HDL was lower in hyperlipemia. In these cases, higher increase of TG and higher decrease of FFA in serum after heparin injection were seen and AWCV inclined to be higher progression. We suggest that unless the presence of sufficient LPL in small vessels less substrate such as VLDL could touch to LPL because of perfusion disturbance.

Conclusion: Hemodynamics might reflect on metabolism and LPL effect in situ might be heterogeneous for familial substrate supply. HDL-C/apoA1 seems to be a parameter of microcirculation.

ThT8:W30 Srl1 polymorphism of the apo C3 gene and expression of dysbeta lipoproteinemia (type II) in individuals heterozygous for familial LPL deficiency

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Objective: To examine the interactions of Srl1 polymorphism of the apo C3 gene with apo e2 and apo e3 alleles on plasma lipoprotein profiles and expression of type III dyslipidemia in individuals heterozygous for familial LPL deficiency.

Method: Studied individuals were 24 heterozygotes for the LPL gene mutation P207L (total LPL activity deficiency in homozygotes) carriers an apo e2 allele (apos in 2/2, 2/3 and 2/4). They were divided in two body mass index (BMI) groups: low (BMI < 27) and high (BMI > 27). Within each BMI group, S1 (S1/S1, n = 14) and S2 (S1/S2 & S2/S2, n = 10) alleles of the Srl1 polymorphism of apo C3 gene were compared. Heterozygotes (n = 33) carrying an apo e3 allele (S3/S3 & S3/S4) were used as control group.

Results: The frequency of type III expression was 75% and 100% in individuals heterozygous for familial LPL deficiency carrying respectively one or two apo e2 alleles as compared to 9% in heterozygotes carrying an apo e3 (wildtype) allele. In carriers of apo e2 allele, the expression of type III dyslipidemia varied between 71 and 80% for low and high BMI and was not statistically different between males and females. Within each BMI group, the frequencies of type III expression were not influenced by the presence (S2) or absence (S1) of the Srl1 polymorphism of apo C3 gene. Finally, in heterozygotes carrying an apo e3 allele, the few cases of type III phenotype expression were not linked to the presence of a S2 allele.

Conclusion: The Srl1 polymorphism of the apo C3 gene is not associated to the expression of dysbeta lipoproteinemia (type III) in heterozygotes for LPL gene mutation P207L.

ThT9:W30 Hypercoagulability in centenarians-associations with hyperhomocysteinemia

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Epidemiological studies have demonstrated age associated changes of coagulation-fibrinolysis system. To assess the clinical significance of hypercoagulability in the oldest old, we investigated the associations of coagulation-fibrinolysis parameters with risk factors for atherosclerosis including lipid parameters and homocysteine (HCY) in 18 centenarians. As coagulation activation markers, the levels of fibrinogen, activated factor VII, activated factor X, antithrombin VIII antibody (anti-FVIII), thrombin-antithrombin complex, and activities of von Willebrand factor (vWF) were measured. As markers for platelet activation, beta-thromboglobulin (β-TG), platelet factor 4 (PF4), and as fibrinolysis markers, plasminogen and α2-Plasmin inhibitor (α2-Pi) were assayed. Plasma HCY concentration was measured by HPLC. In centenarians, increased levels of anti F VIII and TAT, elevated vWFα were observed. Increased levels of β-TG and PF4 were also demonstrated, indicating that endothelial injury and platelet activation may coexist in centenarians. Of great interest, HCY levels were highly correlated with both vWFα and anti FVIII (r² = 0.663, 0.803, respectively). These results suggested that hyperhomocysteinemia may cause endothelial dysfunction and hypercoagulabilities in centenarians.

ThT10:W30 Combination of lipoprotein lipase SER447-TAR and apo e2 may contribute to the development of hypertriglyceridemia

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Objective: The role of apo lipoprotein E (apo E) isoform in the development of hypertriglyceridemia in LPL, SER447-TAR (LPL446).

Subject and Method: Subjects were fifty-six patients with hypertriglyceridemia (TG > 500 mg/dl and seventy-one healthy normolipidemic control (TG < 150 mg/dl, T. Chol < 220 mg/dl). LPL446 were determined with PCR-RFLP methods. Apo E isoform was determined by isoelectric focussing/IEF-immunoblotting.

Results: The incidence of e 2 allele and e 4 allele in hypertriglyceridemia were significantly higher than in control. In control, distribution of e alleles did not change between LPL446 and LPL4Wild. However, In hypertriglyceridemia, the incidence of e 3 alleles were lower in LPL446 than LPL4Wild, whereas the incidence of e 2 alleles and e 4 allele were higher in LPL446 than LPL4Wild. Particularly, the incidence of e 2 allele was significantly higher in LPL446 than LPL4Wild.

Conclusion: These results suggested that the combination of LPL446 and apo e 2 allele might impair lipolytic activity, and may be involved in the development of hypertriglyceridemia.

ThT11:W30 Apo E phenotypes and plasma triglycerides in postmenopausal women with hormonal replacement therapy

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The objective of this study was to analyze the relationship of apo E phenotypes to plasma triglyceride levels in postmenopausal women before (basal) and during hormone replacement therapy (HRT).

Material and Methods: ApoE phenotypes (isoelectrofocusing) were determined in 149 postmenopausal women, non-smokers, non-diabetics, normolipemics, with alcohol intake < 20 g/day, TC < 250 mg/dl and TG < 200 mg/dl. Plasma TC, TG, HDL-C were determined before and during 3-6 months of HRT. Subjects were classified, according to their apo E phenotype, into three groups: 115/28 (E3/3) and E4/3 (25). All women received HRT, according to a similar protocol, during a 3-6 month period.

Results: At baseline, TC values were greater in the group with phenotype E3/3 (p < 0.0001) and plasma TG were greater in group E2/3 (p < 0.05) with respect to the other 2 groups. During HRT the maximal increase of plasma TG was observed in the group with phenotype E2/3 (p < 0.0001).

Conclusions: Women carrying the e 2 allele had highest plasma TG levels in basal situation and showed a greater increment during HRT.

ThT12:W30 Early onset mental retardation in a subject with cerebrotendinous xanthomatosis

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Cerebrotendinous Xanthomatosis (CTX) is an autosomal recessive disease linked to mitochondrial 27-hydroxylase gene mutations causing increased level of cholesterin in plasma and in different tissues. A 38-year-old male subject was evaluated for presence of severe tendon xanthomatosis and progressive mental impairment. The patient was classified as a mental retarded at age 7 and over the last decade a progressive alteration of mood and cognitive functions and movement disorders were noted. Family history was positive for early onset dementia and tendon xanthomatosis in a paternal aunt. Another paternal aunt, who died at the age of 45, was affected by an important mental retardation. The clinical evaluations showed: Achilles tendon xanthomas and xanthomatosis in other less typical sites. Radiologic features of osteoporosis and a mild cataract were noted. The measure of ventilatory function showed a restrictive pattern. Moreover EMG gave evidence of a sensorimotor neuropathy at the lower limbs. A picture of diffuse cortical atrophy without focal lesions emerged after brain MRI. From the biochemical point of view the patient had high cholesterol levels (TC 288 mg/dl, LDL 188 mg/dl) and cholesteral concentration tenfold the
normal values (3387 μg/dl), the 7αOH-cholesterol was 435 μg/dl whereas the 27 OH-cholesterol was not detectable.

In conclusion we describe here a new Italian subject with the CTX and some distinctive features that require e genetic study of the family.

ThT13:W30

Type III hyperlipidaemia in patient with apo E2*
(Arg136–Cys)/3 genotype

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Objective: Apolipoprotein E (apoE) is a polymorphic protein associated with plasma lipids. It occurs in three common isoforms apoE3, apoE4 and apoE2. Homozygous, genotype apoE2/2 can be found in 95% of patients with type III hyperlipidaemia (HLP). More than 12 structural mutants of apoE have been described up to now, some of them being associated with dominance of type III HLP. We have identified a proband with type III HLP carrying a heterozygous apoE2*(Arg136–Cys)/3 genotype.

Methods: After extraction of genomic DNA from peripheral leukocytes the PCR amplification of the DNA was performed. The PCR product was then cleaved with CfoI restriction endonuclease. As the Arg136–Cys mutation causes loss of the cleavage site for CfoI restriction endonuclease, in the proband an untypical 109 bp band on electrophoresis was observed. Therefore the whole sequence of PCR product was determined using an automated sequence analyser. The sequencing confirmed Arg136–Cys mutation.

Results: At the time of examination the proband was a 62-year-old woman with diagnosis of type III HLP established coronary heart disease and chronic pancreatitis. She had no signs of xanthomatosis. Her plasma total cholesterol was 11.17, triglycerides 8.58, HDL-cholesterol 1.00 (all in mmol/l), LDL-C was not calculated, apoB was 1.54 g/l, Lp(a) was 0.33 g/l. Her two sisters and a brother suffered from type III HLP and premature atherosclerosis, her son had type III HLP. None of the relatives was available for examination.

Conclusion: We have identified a proband with type III HLP carrying a heterozygous apoE2*(Arg136–Cys)/3 genotype. This finding lends support to previous observations of some authors concerning the association of this rare apoE mutation with late onset dominance of type III HLP.

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ThT1:W31

The levels of vitamins affecting homocysteine metabolism in different groups of patients

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Objective: The aim of this study was to investigate the differences in levels of vitamins affecting homocysteine metabolism.

Methods: 33 healthy persons (CG), 53 patients hospitalised for cardiac surgery (CS) and 52 patients without clinical evidence of atherosclerosis but with one of the atherosclerosis risk factors: hyperlipidaemia (HL), NIDDM and dialysis patients with chronic renal insufficiency (CRI) were examined. The level of vitamin B12, folate acid and vitamin B6 index in serum were determined.

Results: are shown as x ± SD:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CG (n = 33)</th>
<th>CS (n = 53)</th>
<th>HL (n = 13)</th>
<th>NIDDM (n = 20)</th>
<th>CRI (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12</td>
<td>292 ± 12</td>
<td>335 ± 13</td>
<td>553 ± 43</td>
<td>385 ± 55</td>
<td>434 ± 79</td>
</tr>
<tr>
<td>(μg/mL)</td>
<td>85</td>
<td>140</td>
<td>269</td>
<td>110</td>
<td>197</td>
</tr>
<tr>
<td>Folic acid</td>
<td>9.0 ± 0.4</td>
<td>9.4 ± 0.5</td>
<td>8.6 ± 0.4</td>
<td>8.2 ± 0.4</td>
<td>14.0 ± 0.4</td>
</tr>
<tr>
<td>(μg/mL)</td>
<td>2.8</td>
<td>3.7</td>
<td>3.9</td>
<td>3.7</td>
<td>7.0</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>12.5 ± 0.5</td>
<td>12.6 ± 0.5</td>
<td>36.0 ± 4.4</td>
<td>37.7 ± 1.7</td>
<td>46.0 ± 3.6</td>
</tr>
<tr>
<td>Index (%)</td>
<td>7.9</td>
<td>8.4</td>
<td>40.8</td>
<td>41.9</td>
<td>28.8</td>
</tr>
</tbody>
</table>

Conclusions: (1) Higher levels of vitamin B6 index, indicating vitamin B6 deficiency in dialysis patients is probably caused by low intake (dietary restricted) combined with the increased requirements of uremia in renal failure patient. (2) The careful planning of diet and the supplementation of vitamin B6 should be provided in patients with atherosclerosis risk factors.

ThT2:W31

Assessment of coronary heart disease risk

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Latvia has one of the highest total cardiovascular mortality rate in Europe. Effectiveness of the primary prevention strategy of Coronary heart disease (CHD) is based on the risk level estimation.

We have proposed scoring system for the risk assessment of CHD, which has based on utilizing Framingham risk equation, European Coronary Risk Chart and personal experience. Our scoring system has been based on number and significance of non-lipid risk factors and the level of total cholesterol (TH). Each of 12 utilized parameters has fixed score. Following two parameters give 1 point: (1) men in age 45–65 years or women over 55 years, (2) obesity (BMI ≥ 30 kg/m²). Following six parameters give 2 points: (1) men over 65 years, (2) family history of premature CHD, (3) smoking, (4) central obesity with waist circumference ≥ 102 cm in men or ≥ 88 cm in women, (5) arterial hypertension, (6) TH 5.0–5.9 mmol/l. TH level 6.0–6.9 mmol/l gives 3 points. Two parameters give 4 points: (1) diabetes mellitus, (2) TH 7.0–8.0 mmol/l. TH > 8.0 mmol/l gives 5 points.

Estimation of CHD risk level is done by counting up all points ≤ 2 points – mild risk, 3–6 points – moderate risk, 7–9 points – high risk ≥10 points – very high risk. Our scoring system is very convenient in practical use. It helps to separate patients with high CHD risk, who need intensive prevention strategy.

ThT3:W31

Smoking prevalence and its relations with lipid cardiovascular risk factors among professional soldiers of Polish Army: "CORO" program

R. Grabska¹, A. Grabsy¹, J. Adamsu². Dept. of Internal Diseases, Military Hospital, Olszyn; 2Clinic of Cardiology, Central Military Clinical Hospital, Warsaw, Poland

The "CORO" Program is primary prevention program realized among professional soldiers of Polish Army.

The aim of the present study was to evaluate the prevalence of cigarette smoking – the major risk of cardiovascular diseases (CVD), in this population and to estimate the relationship of this risk factor with some metabolic (lipid) risk factors of CVD.

In 1477 men (mean age 43.0 ± 5.0; range 35–65 yrs) we evaluated clinical (history of smoking) and biochemical measurements (total cholesterol-TG, LDL-cholesterol by Friedwald’s equation, HDL-cholesterol, triglycerides-TG and TC/HDL-chol index).

The prevalence of cigarette smoking among studied group was 48.4%. Among smokers we found unfavourable shifts in lipid metabolism parameters: higher concentrations of TC (p < 0.05); LDL-cholesterol (ns) and TG (ns); and lower concentrations of HDL-cholesterol (ns). Among this group we observed significantly (p < 0.01) higher values of TC/HDL-chol index.

Generally, cigarette smoking is not only a major risk for CVD but also probably plays important role in increasing levels of the serum lipids.

ThT4:W31

Hypertriglyceridaemia among professional soldiers of North East Poland: "CORO" program

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The "CORO" Program is prophylactic program realized among professional soldiers of Polish Army. The purpose of this program is first of all primary prevention and early diagnosis and treatment of coronary heart disease (CHD).

Many data suggest an important role of high concentrations of triglycerides (TG) in developing of CHD.

The aim of the present study was to examine the incidence of hypertriglyceridaemia and association between overweight (and obesity), impaired glucose metabolism and plasma triglycerides (TG) concentrations.

In 1477 men (mean age 43.0 ± 5.0; range 35–65 yrs) we evaluated anthropometric (Body Mass Index-BMI) and biochemical parameters (fasting plasma glucose).

Mean TG concentration in the studied group was 145.6 mg/dL with SD 85.5 mg/dL. TG levels below 200 mg/dL (desirable) were found in 82.6% of this population; levels between 200 and 500 mg/dL were found in 16% and levels above 500 mg/dL were found in 1.4% of studied group. A statistically significant (p < 0.01) correlation between serum concentrations of TG and BMI (r = 0.23) and serum glc concentrations (r = 0.25) were observed.

In studied group we found strong positive correlation between BMI, glc and
values of TG. It suggests a common pathophysiologic basis of these clinical abnormalities and need of active preventive strategies in Polish Army oriented to shift the distribution of CHD risk factors to a more acceptable levels.

### ThTh:W31

**Evolutionary data of early-onset coronary disease**

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**Purpose:** To determine the clinical evolution in males with coronary heart disease under 50 years of age.

**Methods:** Consecutively, 229 male patients before 50 years of age (mean age 43 ± 5 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease, were prospectively studied. A mean follow-up of 32 ± 13 months was made with special attention to mortality and the cause of death, appearance of angina, myocardial infarction, heart failure and the need for coronary revascularisation.

**Results:**

<table>
<thead>
<tr>
<th>Event</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>11 (5%)</td>
</tr>
<tr>
<td>Angina</td>
<td>82 (36%)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>10 (4%)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>11 (5%)</td>
</tr>
<tr>
<td>Revascularisation</td>
<td>21 (9%)</td>
</tr>
</tbody>
</table>

Two patients died in the acute phase: one due to sigmoid adenocarcinoma with severe carcinomatosis and the other due to sudden death. During follow-up the causes of mortality were as follows: sudden death 8 (73%), heart failure 1 (9%) and neoplasia 2 (18%).

**Conclusions:** Medium term prognosis of young male patients with coronary disease shows a mortality of around 5% being sudden death the principal etiology. The appearance of an episode of angina is frequent in the follow-up of these patients (36%). The number of patients presenting heart failure or myocardial infarction is small. Almost 10% of these patients require coronary revascularisation during a follow-up of 3 years.

### ThTh:W31

**Identification of markers of different severity of lesion in the proximal left main coronary artery**

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**Purpose:** To identify clinical and invasive markers which distinguish stenosis of the left main coronary artery (LMCA), between 50–70% and greater than 50%.

**Methods:** Out of 4000 catheterisms made in our Hospital, we studied 73 patients (pts) with significant stenosis in the LMCA. Two groups were established: Group A made up of those pts with a stenosis in the LMCA between 50–70%, and Group B consisted of those with a stenosis in the LMCA greater than 70%. We analysed 25 clinical and haemodynamics variables in order to finding significant differences between both groups.

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 21)</th>
<th>p</th>
<th>Group B (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>66%</td>
<td>&lt;0.05</td>
<td>60%</td>
</tr>
<tr>
<td>Distal LMCA</td>
<td>61%</td>
<td>&lt;0.05</td>
<td>80%</td>
</tr>
<tr>
<td>Complexity</td>
<td>53%</td>
<td>&lt;0.05</td>
<td>53%</td>
</tr>
<tr>
<td>Collaterals</td>
<td>62%</td>
<td>&lt;0.05</td>
<td>27%</td>
</tr>
<tr>
<td>Diffuse disease</td>
<td>62%</td>
<td>&lt;0.05</td>
<td>42%</td>
</tr>
<tr>
<td>Number vessels</td>
<td>2.4 ± 0.9</td>
<td>&lt;0.01</td>
<td>1.6 ± 0.9</td>
</tr>
</tbody>
</table>

**Conclusions:** Patients with stenosis in LMCA between 50–70% presented a greater frequency of hypertension, multi-arterial disease, presence of collateral circulation and diffuse coronary artery disease. The severe lesion (>70%) of LMCA was found predominantly at distal level and presented complexity in its angiographic characteristics and less number of affected vessels, which suggests a more acute coronary disease.

### ThTh:W31

**Effects of policosanol and pravastatin on lipid profile, platelet aggregation and endothelium in older hypercholesterolemic patients**

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This randomized, double-blind study was undertaken to compare the effects of policosanol and pravastatin administered at 10 mg/d on lipid profile, platelet aggregation and endothelium in older patients with type II hypercholesterolemia and high coronary risk. After 6 weeks on lipid-lowering diet, patients were randomized to receive, under double-blind conditions, policosanol or pravastatin 10 mg tablets that were taken for 8 weeks. Policosanol significantly (p < 0.00001) lowered LDL-C (19.3%), TC (13.9%) and the ratios of LDL-C/HDL-C (28.3%) and TC/HDL-C (24.4%). Pravastatin significantly (p < 0.00001) lowered LDL-C (15.6%), TC (11.8%) and the ratios (p < 0.0001) of LDL-C/HDL-C (18.9%) and TC/HDL-C (15.7%). Policosanol, but not pravastatin, significantly increased (p < 0.001) levels of HDL-C (18.4%) and reduced (p < 0.01) triglycerides (14.1%). Policosanol was more effective (p < 0.05) than pravastatin to inhibit platelet aggregation induced by arachidonic acid (AA) at 1.5 and 3 mmol/L, by 42.2% and 65.9%, respectively; by collagen 0.5 μg/mL (p < 0.05) (16.6%) and by ADP 1 μmol/L (p < 0.01) (20.3%), meanwhile pravastatin reduced significantly (p < 0.001) (27%) platelet aggregation induced by AA 3 mmol/L. Both drugs significantly decreased (p < 0.0001) endothelium levels, but final values were significantly lower (p < 0.001) in policosanol than in pravastatin group. Both treatments were safe and well tolerated. Two pravastatin patients discontinued the study because of adverse experiences (jouissance and myocardial infarction). It is concluded that effects of policosanol (10 mg/d) shows more favorable effects on lipid profile, platelet aggregation and endothelium than similar doses of pravastatin.

### ThTh:W31

**Angiographic findings in non-Q wave myocardial infarction: Differences in relation with CK peak**

A. Batalla, J. Mayordomo, G.I. Cubero, J.J.R. Reguero, S. Hevia, J.C. Sanmartí, J. Gutiérrez, T. Raviña, Hospital de Cabueñas (Gijón); Hospital Central de Asturias (Oviedo), Spain

**Purpose:** To determine the angiographic findings in non-Q wave myocardial infarction according to CK peak.

**Methods:** From a cohort of 48 patients who were consecutively admitted to our hospital with a diagnosis (14.5%) non-Q wave myocardial infarction and underwent a coronarographic study a group of 23 patients with a CK peak less than 700 (Group A) and a group of 25 patients with a CK peak higher than 700 (Group B) were made. We examined differences between both groups.

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 23)</th>
<th>Group B (n = 25)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart failure</td>
<td>9%</td>
<td>16%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Three vessels disease</td>
<td>24%</td>
<td>44%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Collateral flow</td>
<td>82%</td>
<td>56%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Coronary calcification</td>
<td>21%</td>
<td>48%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Proximal disease</td>
<td>39%</td>
<td>60%</td>
<td>m</td>
</tr>
</tbody>
</table>

**Conclusions:** Patients with non-Q wave infarction and CK peak less than 700 have a significantly more coronary collateral perfusion as compared with those patients with non-Q wave infarction and CK peak higher than 700, which however have more frequently heart failure, coronary calcification and three vessels disease.

### ThTh:W31

**Risk factors for cardiovascular disease awareness in patients referred for hypertensive crisis: Preliminary data**

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**Objective:** The control of risk factors reduces the incidence of cardiovascular disease (CVD). Indeed, in the strategy against CVD, the awareness of risk factors represents a key point for management of patients. The purpose of our study was to assess the degree of awareness of risk factors for CVD in patients referred for hypertensive crisis. We reported preliminary data of the first 20 patients.

**Methods:** We enrolled patients referred for hypertensive crisis undergoing oral antihypertensive treatment in Emergency Room. They were subsequently
examined for hyperlipidemia, diabetes mellitus and hypertension in our Internal Medicine Unit. The subjects enrolled filled in a questionnaire concerning personal and familial CVD, smoking habits and drug consumption and received also ABPM, ECG, echocardiogram and echoDoppler of carotid arteries.

**Results:** In all patients, but not one, the hypertension was confirmed by ABPM. No patients showed secondary hypertension. Among hypertensives, 14 were aware of their condition but only 3 were treated. Thirteen patients were found to have hyperlipidemia and 2 to have diabetes mellitus. Only 5 of patients with hyperlipidemia were aware of their condition, no one was treated. The 2 diabetic patients were aware of their condition, they were treated and they had HbA1C levels > 7%.

**Conclusions:** Although we reported preliminary data, our findings show that, in these selected patients, the awareness and the control of risk factors for CVD is not negligible. Indeed, an approach by primary and secondary prevention program might be of help to improve the awareness of the risk factors for CVD.

**ThT10W31 Cardiovascular risk factors analysis in patients with sudden death after acute myocardial infarction**

Gabriela Ciojboratu, D. Burghina, Deliana Vassiliuta, R. Popa, Minodora Mihalas, Anca Isac, Oana Tuna, C. Domide, I.A. Rivas. *1st Internal Medicine Clinic – City Hospital Timisoara, Romania*

The present work aimed to correlate the risk factors in acute myocardial infarction (AMI) patients and their role in appearance and control of sudden death.

**Material and Method:** We studied 174 patients with AMI, which presented sudden death, hospitalized between 1995–1999. There was analyzed: the existence of the risk factors with predictive value for the sudden death, the heart hypertrophy – dilatation, EKG aspects.

**Results:** 31.5% from the patients were successfully resuscitated after ventricular fibrillation (20 patients) and after ventricular tachycardia (4 patients). The location of AMI in surviving patients was 50% inferior, 37.5% anterior, 12.5% non-Q. The average age was 54.5 in surviving patients and 66.1% in deceased. The risk factors for the unsuccessfully resuscitation were: male (Odds Ratio: OR = 1.12, and Relative Risk: RR = 1.03); age over 60 years (OR = 10.4 and RR = 3.92); the anterior location of AMI as compared to inferior location (OR = 36, RR = 4.88); the presence of hypertrophied-dilated heart (OR = 2.24, RR = 1.29). Patients invasive treated (PTCA, stent or CABG) and their rate of survival at 1 year was 75% as compared to 41.6% in patients medically treated. The rate of survival was less than 6 month in patients with dilated heart.

**Conclusions:** Risk factors synergy increases the sudden death risk in young persons, males, aged over 60, anterior location of AMI. Cardiac dilatation reduced postresuscitation survival less than 3 month. Ventricular fibrillation was the main mechanism of sudden death in resuscitated patients. Myocardial revascularization techniques increase postresuscitation survival duration more than 1 year.

**ThT11W31 Nutrition and hypertension**

M. Bozbiram, M. Rafiei, A. Jalali, N. Sarrafzadegan, F.A. Sayed-Tabatabaei. *Isfahan Cardiovascular Research Center, Isfahan, Iran*

**Objective:** Hypertension considered as a major risk factor for coronary artery disease (CAD). As the CAD is the a major cause of death in Isfahan (Iran), and also Isfahan Salt Study couldn’t show any relationship between blood pressure and salt, we decided to investigate the relation between dietary habit and blood pressure.

**Methods:** The analyses conducted on the data of the 2nd ICVDRF Survey. The sample size for this study was 1200 men and women selected from Isfahan population. Dietary intakes were estimated using 24-hr record-assisted recall for 3 days by FCP-HC software. Also for each subject, inquired information was obtained by a standard questionnaire. A fasting blood specimen for the analyses of serum lipids was taken from each one. Blood pressures of all subjects were measured according to WHO criteria from right arm in sitting position after at least 5 minutes of rest. All people who are on a diet were excluded from the analysis.

**Results:** The table presents the difference between the mean values of some nutrients between hypertensive and normotensive subjects.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Hypertensives Mean ± SD</th>
<th>Normotensives Mean ± SD</th>
<th>95% CI P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (Kcal)</td>
<td>2251.6 ± 923.3</td>
<td>2522.6 ± 1271.5</td>
<td>117.2, 424.8</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>76.9 ± 29.2</td>
<td>82.3 ± 33.3</td>
<td>128.6, 9.6</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>327.5 ± 123.0</td>
<td>362.3 ± 225.7</td>
<td>90.2, 60.6</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>252.8 ± 156.2</td>
<td>273.1 ± 190.4</td>
<td>25.4, 69.3</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>71.9 ± 65.9</td>
<td>83.6 ± 45.8</td>
<td>4.5, 10.6</td>
</tr>
<tr>
<td>SFA (g)</td>
<td>32.6 ± 38.4</td>
<td>37.3 ± 21.9</td>
<td>1.07, 8.36</td>
</tr>
<tr>
<td>MUFA (g)</td>
<td>23.2 ± 20.5</td>
<td>28.8 ± 15.0</td>
<td>143.5, 82</td>
</tr>
<tr>
<td>PUFA (g)</td>
<td>12.34 ± 10.2</td>
<td>15.2 ± 10.3</td>
<td>1.42, 4.4</td>
</tr>
</tbody>
</table>

In hypertensives than normotensives but only cholesterol intake can be a contributor dietary factors for hypertension in Iranian population.

**ThT12W31 Effects of cardiac rehabilitation and exercise training on exercise capacity and body mass index in women**

K. Rohjie, J. Najafian. *Isfahan Cardiovascular Research Center, Isfahan, Iran*

**Objective:** The benefits of cardiac rehabilitation programs on improving exercise capacity and body mass index were reported previously. This study assesses the relative benefits of cardiac rehabilitation program in women compared to men.

**Methods:** We compared the results of cardiac rehabilitation in 27 women (age 54 ± 8.21 years) with 99 men (age 51.84 ± 9.58 years). All patients completed a rehabilitation program of 24 sessions, each 60 minutes (three sessions weekly).

**Results:** Following the cardiac rehabilitation and exercise training program, women had significant improvements in exercise capacity (42%, P < 0.000) and body mass index (~3.6%, P < 0.001). For parameters, improvements following the cardiac rehabilitation program were statistically similar in women and men.

**Conclusions:** We believe these data further support the idea that women should be routinely referred for cardiac rehabilitation programs following major CAD events.

**ThT13W31 Comparing the efficacy of statins in various patient subgroups in the atorvastatin comparative cholesterol efficacy and safety study (ACCESS)**

V.M. Campese1, J.B. Kotnis2, C.L. Shear3. *For the ACCESS Investigators; 1LACUSC Medical Center, Los Angeles, CA; 2UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ; 3Pfizer Central Research, Groton, CT, USA*

**Background:** Data consistently show that many patients do not reach National Cholesterol Education Program (NCEP) LDL-C goals. ACCESS study subanalyses compared statin efficacy in various patient populations including women, patients ≥ 70 years and African-Americans.

**Methods:** Patients (n = 3169) meeting NCEP drug treatment criteria were randomized to atorvastatin (10–80 mg), simvastatin (10–40 mg), pravastatin (10–40 mg), fluvastatin (20–80 mg) or lovastatin (20–80 mg). Patients were randomized in a 4 to 1 ratio (atorv, n = 1598; simv, n = 482; prav, n = 481; fluv, n = 497; lov, n = 498) and received initial doses for 6 weeks. Thereafter, patients were titrated if they did not reach NCEP LDL-C goals and were followed to 54 weeks.

**Results:** At Week 6, reductions from baseline in LDL-C, TC and TG were significantly greater with atorvastatin 10 mg compared with other statins at their initial doses for women and patients ≥ 70 years (all p < 0.05). This was also true for African-Americans (with the exception of TG levels vs pravastatin). Proportions of patients reaching NCEP goals are given below:

<table>
<thead>
<tr>
<th>Patient subgroup</th>
<th>% of patients reaching NCEP goal at Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>(p* &lt; 0.05 vs atorv)</td>
<td></td>
</tr>
<tr>
<td>Atorv</td>
<td>Simv</td>
</tr>
<tr>
<td>Women</td>
<td>57.2</td>
</tr>
<tr>
<td>Patients ≥ 70 years</td>
<td>54.8</td>
</tr>
<tr>
<td>African-Americans</td>
<td>41.9</td>
</tr>
</tbody>
</table>

All statins were welltolerated and produced comparable adverse event rates.

**Conclusion:** In the patient subgroups studied, a significantly greater proportion of atorvastatin-treated patients achieved NCEP goals at starting dose compared with patients treated with other statins.

XIIth International Symposium on Atherosclerosis, Stockholm, Sweden, June 25-29, 2000
Correlations between risk factors in coronary heart disease

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Objective: To study correlation between coronary risk factors and the occurrence of the ST segment abnormalities.

Methods: The data describe occurrence of ST segment abnormalities in 300 patients with CHD who were divided into five groups according to symptoms and objective diagnostic cardiovascular parameters. The data describe patients who entered our Institute during the period of a few months. The descriptor set used includes anamnestic data (10 items), laboratory test results (6), the resting ECG data (5), the exercise test ECG data (6), echocardiography results (2), and long term continuous ECG recording data (3). It makes all together 34 data items. The classification of patients was performed by the cardiologist using generally accepted medical knowledge.

Results: Complex statistical analysis of the data set (1020 items) shows that some of the descriptors are more significant than the others (like body mass index, diabetes mellitus, arterial hypertension in anamnestic data set, or high density lipoproteins, acid uric and fibrinogen in laboratory tests). Analysis is performed by calculating the pair and higher order correlation functions between different descriptors.

Conclusion: The performed analysis generally verifies the existing medical practice in non-invasive diagnosis of CHD and has been used for development of an useful machine learning algorithm.

The MEMPED-FH program in Australia

I.R. Hamilton-Craig, M. Platts. For the MEMPED-FH (Australia) Steering Committee, Adelaide, South Australia

Objective: To present the current status of the MEMPED-FH (Make Early Diagnosis, Prevent Early Deaths in Familial Hypercholesterolemia) program in Australia as part of the international program to improve the management of patients with FH.

Methods: Patients with FH were registered with the Adelaide coordinating centre after notification by participating physicians. Patients and physicians completed questionnaires based on the US international coordinating centre. Follow-up of relatives was performed through telephone and mail contact.

Results: Clinical criteria only were used to diagnose FH, as LDL receptor DNA analyses were not available at the time of data collection. Since the program began in 1993, 572 patients have been registered, and 123 of their relatives contacted to inform them of the need for lipid testing and further family studies if indicated. At present 38 specialist physicians and general practitioners participate in the program. This compares with an estimated FH population of about 35,000 for a total population of 17 million, with about 17000 general practitioners. At present a multinational multicentre trial of a new statin is being conducted in some patients registered in the MEMPED-FH program.

Conclusions: Only a small proportion of FH patients have been registered in the MEMPED-FH program, through the participation of a small proportion of participating physicians, in spite of several years of continued effort. Current research collaboration is encouraging. In order to achieve its goals of greater public awareness of FH, the MEMPED program needs to be oriented to the general population rather than to medical practitioners, and requires greater funding to achieve success.

Estrogen replacement therapy (ERT): Prevalence in a cardiology ambulatory

A.A. Faludo1, M.C. Bertolo1, A.T. Paes1, J.M. Alighi2. 1 Instituto Dante Pozzobonese de Cardiologia; 2 Faculdade de Saude Publica da Universidade de Sao Paulo, Sao Paulo, Brazil

Objective: To determine the prevalence of ERT among postmenopausal women who searched a cardiology ambulatory for primary prevention of coronary heart disease (CHD) by controlling risk factors (RF).

Methods: An observational study where all postmenopausal women more than 50 years old, that were present for a consultation in a cardiology ambulatory during 1995, answered a questionnaire about the use of ERT, physical activity and smoking and were submitted to an investigation of hypertension, diabetes mellitus, hyperlipidemia and obesity.

Results: Five hundred seventy six women were included, ages varying from 50 to 92 years old (average = 64.5 ± 8.73). From the total of women, 70.8% were hypertensive, 21.7% diabetics, 84.2% hyperlipidemic and 29.3% obese. ERT was being used by 18.1% of the participants. Among the remaining groups, the causes of not using ERT were: no gynecological consultation (29%), no indication by the gynecologist (24%), adverse effects (10.5%), suspension by the gynecologist (5.4%), no interest in the use (4.9%), belief of prejudicial effects (4.2%) and contra-indication by the gynecologist (3.8%). ERT was used by only 15.9% of the hypertensive women (p = 0.052), 5.6% of diabetics (p < 0.001), 15.9% of hyperlipidemic (p = 0.003) and 8.9% of obese (p < 0.04).

Conclusion: The prevalence of ERT in the studied population was low. It was significantly lower among women who presented diabetes, hyperlipidemia or obesity, with a tendency to lesser use by hypertensive women.

Risk factors for coronary heart disease in children: A study in Lodz, Poland


Coronary heart disease becomes a major cause of mortality and morbidity in Poland. It is mainly adult disease but atherosclerosis begins in childhood and preventive program should be started in childhood.

The aim of our study was to estimate the risk factors in children. Our studies demonstrate that the most frequent risk factors are obesity, hypertension arterials, smoking habits, low physical activity, family history of coronary heart disease and lipid disorders. The study comprised 496 children aged 7–15 from primary school. Among of them we have selected 55 children with risk factors.

Results: We have shown that the most frequent risk factors of atherosclerosis were lipid disorders and obesity. Most investigated children had more then one risk factor (60%). Among lipid disorders our studies demonstrate that the most frequent are: increase level of cholesterol and triglycerides, decrease of HDL-cholesterol. We determined the total plasma lipid enzymatic methods.

Conclusion: High frequency of risk factors of atherosclerosis in investigated group demonstrates the necessity of using such investigations as a common activity in public health.

Prevention of atherosclerosis in hyperuricemia and in gout

J. Figuerinhas1, L. Luisa Faleiro2, J. Vaz Patto1, M. Cara Martins3, M. Odete Rodrigues4,1 Reumatologia; 2 Cardiologia-Instituto Português de Reumatologia; 3 Instituto Nacional de Saúde. Lisboa, Portugal

Objective: Most common causes of the death in the industrialized countries, is the cardio and cerebrovascular disease. The precocious detection of risk factors in the populations should be imposed. At this time, we are doing the approach of the prevention of the atherosclerosis in the rheumatic chronic diseases, just conming the referring results of gout and hyperuricemia, both situations very frequent in Portugal.

Patients and Methods: We observed 166 patients with mean age 59.4 years, being 161 male (97.0%). The laboratory methods for lipid parameters were: CHOD-PAP goes total cholesterol and HDL-cholesterol after precipitation of LDL + VLDL with magnesium and tungstate ions, the totally enzymatic method was used goes triglyceride determinations and the LDL cholesterol quantified using the Friedwalt it formulates. It goes glycerina and uric acid were used enzymatic methods.

Results and Discussion: The hyperuricemia was present in 162 patients (97.6%), the hypercholesterolemia, in 81 (48.8%) the hypertriglyceridemia in 101 (60.8%), the hyperproproteinemia type IV in 81 (48.8%) and the reduction of the HDL cholesterol in 23 (13.9%). Other risk factors are the obesity verified in 88 (53.0%), diabetes in 47 (28.3%) and the hypertension in 104 (62.7%). There are frequently associated with tabagia and alcoholic habits and in the family history it appears the gout in 44 (26.5%), the obesity in 66 (39.1%), the diabetes in 39 (23.5%) and the arterial hypertension in about 50%.

Conclusion: The results suggest the need of the atherosclerosis prevention in this chronic rheumatic disease, in order to prevent the modifiable risk factors.
**TH19:W31**  Five years experience with preventive activities in hypercholesterolemic children

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¹Dept. Clin. Biochem., Metab. Unit, Hospital, "Na Homolce", Prague, Czech Republic.

**Objective:** To present our five years experience with preventive activities of "Parents's Club for Children Suffering from Familiar Hypercholesterolemia".

**Methods and Results:** 100 children (4-18 yrs) suffering from familiar hypercholesterolemia (FH), born from parents with high cardiovascular risk (at least one parent or grandparent after operation of aortoconary or peripheral artery bypasses) attend the metabolic surgery for dietetic or medical treatment of FH. Parents of these children are organized in the "Club" in order to support the preventive activities in risk families and to increase the production and quality control of low-cholesterol products on the market. The prominent activities and efforts are as follows:

- Education: lectures and courses of invited specialists dealing with genetic, metabolic and environmental risks for these children; dietetic rules for preparation and cooking of low-cholesterol foods and dishes; warning against not recommended "pseudolowcholesterol" products (burners, supervitamins etc.).

- Organization: rehabilitation of children in swimmingpool, summer holiday camps in mountains for whole families; weekends-trips.

- Quality control: controlling of recommended low-cholesterol products labelled in Delvalta supermarkets with the logo "Program For Health". These low-cholesterol products are selected and recommended only after laboratory confirmation of low-cholesterol content in the State Veterinary Institute.

**Conclusions:** We have good experience with the managing of this Club. It can help us to recognize the nutritional and psychosocial behaviour of the family and to increase the compliance for dietetic treatment of FH. We can detect the anxious families where children could be damaged by strong hypercholesterolemia diet.

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**TH20:W31**  Estrogen replacement therapy (ERT) and LDL-cholesterol targets

A.A. Faludi¹, M.C. Bertolam², A.T. Paes¹, J.M. Aldrighi², ¹Instituto Dante Pozzanese de Cardiologia; ²Faculdade de Saúde Pública da Universidade de São Paulo, São Paulo, Brazil.

**Objective:** To evaluate, in postmenopausal women, the importance of ERT in the modification of LDL-c targets from <130 mg/dL to <160 mg/dL, considering that ERT dismisses menopause as a risk factor (RF) for coronary heart disease.

**Methods:** We analyzed postmenopausal women, followed in a Cardiology Ambulatory for primary prevention of coronary heart disease. The association of RF (hypertension, obesity, smoking status and familiar antecedents) was investigated

**Results:** Among 227 included women, ages varying from 50 to 92 years old (average 64.5), 66 (28.2%) were ERT users. Considering that ERT modifies LDL-c as a RF, the table shows the percentages of women with and without ERT, and after of amenopause of a RF, that already were in the target LDL-c zones <130 and 160 mg/dL. Thirty six women from a total of 45 (80%) who were on ERT and presented at least two RF and accordingly had LDL-c target <130 mg/dL, changed the therapeutic target to <160 mg/dL.

<table>
<thead>
<tr>
<th>LDL-c</th>
<th>No ERT</th>
<th>With ERT</th>
<th>With ERT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;130</td>
<td>138/4 (125)</td>
<td>40% (18/45)</td>
<td>22% (2/9)</td>
</tr>
<tr>
<td>&gt;160</td>
<td>66 (25/36)</td>
<td>52% (10/19)</td>
<td>70% (15/21)**</td>
</tr>
</tbody>
</table>

*p and numbers of women who presented LDL-c target with and without ERT. Total numbers of each target group appear between brackets. *after amenopause of menopause as a RF due to ERT. **p = 0.04.

**Conclusion:** In this population, the percentage of ERT users that had their LDL-c therapeutic target increased from 130 to 160 was significant, justifying the discussion of the importance of ERT in this context.

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**TH21:W31**  Prevalence of blood hypertension in dyslipidemic patients

S. Bottaro, G.C. Griffin, P. Zanchi. Medical Dep., Umberto I Hospital, Venice-Mestre, Italy.

The value of blood pressure in an important parameter in order to evaluate the risk of coronary heart disease in dyslipidemic patients. The aim of this study was to measure blood pressure (BP) in a group of hyperlipidemic subjects using office conventional (OBP) and ambulatory monitoring blood pressure (AMBP) methods.

In 50 (30 F, 20 M, aged 57 ± 7 yrs) dyslipidemic patients clinical BP was taken at two separate sitting by manual device and the average was recordered. All patients were submitted to 24-hour BP monitoring by pressurometric device (Del Mar Avionic P6).

The 24-h, daytime and nighttime measures were considered. The BP values (mean ± SD) are shown in the Table:

<table>
<thead>
<tr>
<th></th>
<th>OBP 24-h</th>
<th>Daytime</th>
<th>Nighttime</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>148 ± 14</td>
<td>120 ± 12</td>
<td>112 ± 12</td>
</tr>
<tr>
<td>PAD</td>
<td>92 ± 10</td>
<td>69 ± 9</td>
<td>65 ± 9</td>
</tr>
</tbody>
</table>

With OBP values, 53% of patients have to be considered hypertensive (BP > 140/90 mm Hg) while with ABPM (Daytime > 135/85 mm Hg) only 29%.

**Conclusions:** EAS guidelines recommend to evaluate BP in all dyslipidemic patients. In our study hypertension was overestimated by OBP respect to ABPM. In patients with hyperlipidemia we suggest to perform a ABPM before to assess the global risk for CHD.
but also of total mortality) increases both with the degree of reduction of the cholesterol levels and with its prolongation in time, we come to conclusion that cardiologists should consider hypercholesterolemia as a chronic disease that must be treated and treated without interruptions. If the attitude towards the cholesterol-lowering therapy changed in the way we are suggesting, the high coronary risk primary prevention and the secondary prevention of CHD could become more efficacious.

**T:W32 INTRACELLULAR LIPOID METABOLISM**

**ThT1:W32** Tyrosine phosphorylation of macrophage by low density lipoprotein from hemodialysis patients and vitamin E

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**Objective:** We investigated whether interaction of low density lipoprotein (LDL) from hemodialysis patients (HD) and macrophages induces tyrosine phosphorylated proteins in the macrophages.

**Methods:** Human monocyte-derived macrophages were incubated with HD-LDL (100 µg/ml) with (E+) or without (E−) vitamin E (600 µg/day) for 24 hours. Lysed proteins were divided into Triton-soluble and insoluble fractions. Both fractions (soluble and insoluble) were separated by SDS-PAGE and electroblotted onto a PVDF membrane. Immunoblotting was performed using antibody against phosphorytyrosine.

**Results:** Several proteins in the range 50 KDa-100 KDa were found to be phosphorylated constitutively in he macrophages, not affected by the addition of HD-LDL. HD-LDL either from E− or E+ did not induce any tyrosine phosphorylated proteins. Macrophages pretreated with tyrosine kinase inhibitor, genestein drastically inhibited tyrosine phosphorylation of these proteins.

**Conclusion:** These data suggest that tyrosine autophosphorylated proteins may play a role in the early step of signal transduction in the macrophages.

**ThT2:W32** Liver sterol 27 hydroxylase in hamster: Modulation by steroids and diets

C. Lutton, M. Souidi, S. Dubrac, M. Parquet. Physiologie de la Nutrition, Université Paris-Sud, Orsay, France

**Objective:** To investigate, in hamster liver, the effect of various oxysterols, bile acids or a sucrose-rich versus commercial diet on sterol 27-hydroxylase (S27), an ubiquitous enzymatic activity implicated in anti-atherogenic process and bile acid synthesis.

**Methods:** A sensitive assay (Life Sciences 1999, 64, 1585–1593) was used in order to obtain linearity for the accumulation of labelled 27-hydroxycholesterol in hamster mitochondria.

**Results:** In vitro, epiprostanol and 27 hydroxycholesterol lowered S27 activity (88 and 29%) while hydroxycholesterol and 25 hydroxycholesterol were without effect. A sucrose-rich diet induced a marked decrease in S27 activity (–54%) compared with the commercial diet.

In vivo, epiprostanol and hydroxycholesterol acid lowered (–46 and 70%) as well as the lithogenic diet (–56%) but 25 and 27 hydroxycholesterol were without effect.

**Conclusion:** Hepatic sterol 27 hydroxylase activity can be modulated by steroids and diets. Researching conditions able to activate this activity might be of great interest.

**T:W33 PROLIFERATION AND DIFFERENTIATION OF SMOOTH MUSCLE CELLS**

**ThT1:W33** Diffuse intimal thickening emerges in atherosclerosis-prone arteries in childhood and the intima/media ratio increases with age

Y. Nakashima, Y.C. Chen, K. Sueishi. Pathophysiological and Experimental Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

**Objective:** Diffuse intimal thickening (DIT) is considered as an adaptive change of arterial intima and suggested to have a close relation to atherosclerosis. The aim of this study is to clarify the somatic distribution and chronic proliferative change of DIT.

**Methods:** Aorta, coronary artery and cerebral artery as well as other aortic branches and visceral arteries were systematically examined in autopsy cases of Japanese children and young adults. The age groups were 0 year, 1–10 years, 11–20 years and 21–30 years with 16 cases in each group. Histological sections were stained with Elastica van-Gieson stain, and intima to media (IM) ratio was measured on a digitalised image.

**Results:** DIT was a organized structure of elastin, proteoglycan and smooth muscle cells and devoid of lipid pool and foamy macrophages. DIT emerged in the coronary artery as early as in 1 month of age and IM ratio increased proportionately with age. The abdominal aorta, carotid artery and iliac artery showed similar trends, though DIT appeared later than the coronary artery. The cerebral artery and other visceral arteries did not develop DIT until third decades in general, and the thickening, if any, was very thin.

**Conclusion:** DIT is different from atherosclerosis, but emerges in atherosclerosis-prone arteries from childhood and thickens with age. These findings suggest an important role of DIT as a ground for atherogenesis.

**ThT2:W33** Inhibition of vascular smooth muscle cell proliferation by red wine polyphenols is associated with downregulation of cyclin A gene expression

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Red wine polyphenols have been shown to contribute to the “French paradox” phenomenon consisting of lower mortality from coronary heart disease in the French population. Although vascular smooth muscle cell (VSMC) proliferation plays an important role in the progression of atherosclerotic lesions, the effects of red wine polyphenols (RWP) on VSMC proliferation have not been elucidated.

**Methods and Results:** To examine the effect of RWP on the growth of VSMC, we extracted a total polyphenolic fraction, called RWP-PF, from red wine using column chromatography. Cultured rat smooth muscle cells (RASMC3) were incubated with RWP-PF (1–100 microgram/ml) for 72 hr. In cell count and thymidine uptake assay, treatment with RWP-PF showed a potent inhibitory effect on cell growth and DNA synthesis in a dose-dependent manner. In contrast, this polyphenolic fraction did not affect the growth of bovine carotid endothelial cells (BCEC). To elucidate the mechanism of this anti-proliferative effect of RWP-PF in RASMC, we investigated expression of cyclin A mRNA and cyclin A promoter activity. RWP-PF dramatically downregulated the cyclin A mRNA and reduced the cyclin A promoter activity in a dose-dependent manner in RASMC but not in BCEC. In addition, RWP-PF decreased the binding of nuclear proteins to the activating transcription factor (ATF) site in the cyclin A promoter, and the expression of transcription factors, cyclin A, AMP-responsive element-binding protein (CREB) and ATF-1, was also downregulated by RWP-PF.

**Conclusion:** The downregulation of cyclin A gene expression may contribute to the antiproliferative effect of RWP on VSMC through the inhibition of transcription factor expression.

**ThT3:W33** Magnolol induces a regression of aortic hypertrophy in spontaneously hypertensive rats

T.-C. Chou. Graduate of Medical Sciences, National Defense Medical Center, Taipei, Taiwan

**Objective:** To evaluate whether the Chinese herb, magnolol, induces regression of hypertension and possesses antihypertensive effect in spontaneously hypertensive rats (SHR).

**Methods:** After administration of magnolol (50 µg/kg, p.o.) for 6 weeks, the vascular morphological change, blood pressure, endothelium-dependent response, and the O2− formation from aorta in SHR were evaluated.

**Results:** Magnolol resulted in a significant reduction of mean blood pressure compared with that of untreated group in SHR (140.2 ± 3.7 vs 161.0 ± 7.0 mmHg, P < 0.05) and the endothelium-dependent relaxation in response to acetylcholine was also improved. In addition, the intima/media thickness and area ratio of aorta were significantly lower in magnolol treated group (Table 1). The production of O2− from aorta in SHR was also significantly attenuated by magnolol treatment.

**Conclusion:** Our results show that magnolol possesses beneficial effect of regression of vascular hypertrophy and antihypertensive effect in SHR. In
addition, the antioxidant effect of magnolol may be involved in these effects of magnolol.

**ThT4:W33** Effects of lysophosphatidylcholine on nuclear protein import

L.H. W. Stronger, M.P. Crubry, G.N. Pierce. *Div. of Stroke & Vascular Disease, St. Boniface Research Ctr., Winnipeg, MB, Canada*

**Objectives:** Lysophosphatidylcholine (LPC) stimulates growth of smooth muscle cells. Increased nuclear protein import is associated with proliferating cells, therefore we examined the effect of LPC on nuclear import.

**Methods:** Import cocktail (cytosol, protease inhibitors, an energy-generating system and a fluorescent nuclear import substrate) was treated with various [LPC] for various times. Nuclear import of the substrate in digitothin-permeabilized vascular smooth muscle cells was quantified by confocal microscopy. In one study, import cocktail was pretreated with 20 μM PD98059 for 30 min. prior to use.

**Results:** 10 μM LPC had a biphasic effect on nuclear protein import at a function of time. A steady increase in import to 190% of control was observed after 30 min. incubation with LPC, but this level stopped to 60% of control by 60 min. LPC also exhibited dose-dependent effects, causing a significant increase in import at 1 to 10 μM, but with no effect at higher concentrations.

The effect of treatment with 10 μM LPC for 30 min. could be reversed by pre-treatment with 20 μM PD98059, a MEK1 inhibitor, for 30 min.

**Conclusions:** LPC both stimulates and inhibits nuclear protein import, depending on concentration and exposure time. LPC’s effect on import is mediated by the ERK MAP kinase pathway.

Supported by the Medical Research Council of Canada.

**ThT5:W33** Role of phosphoinositides in nuclear protein import in smooth muscle

M.P. Crubry, R. Jankowski, G.N. Pierce. *Division of Stroke and Vascular Disease, St. Boniface Research Centre, Winnipeg, Manitoba, Canada*

**Objectives:** Phosphoinositides are critical second messengers in smooth muscle. This study investigates how phosphoinositides may regulate nuclear protein import, which in turn may affect smooth muscle cell proliferation in situations such as restenosis.

**Methods:** Digitonin-permeabilized rabbit aortic vascular smooth muscle cells were treated with various [IP3] for varying times. Alternatively, IP4 or L-IP5 was used. Import was then assayed using an import cocktail consisting of rat liver cytosol, an energy-generating system, protease inhibitors, and a fluorescent nuclear import substrate, and quantified by confocal microscopy.

**Results:** IP3 inhibited import in a time dependent manner, with peak inhibition (67% of control) at 30 min. incubation with 1 μM IP3. Incubation for 60 min. or more had no effect. Concentrations of IP3 from 1 to 10 μM had similar inhibitory effects on import. Treatment with 1 μM IP3 inhibited import to a lesser degree (~90% of control). L-IP3, the stereo enantiomer of IP3, had no effect on import.

**Conclusions:** IP3, and to a lesser degree IP4, are able to inhibit nuclear protein import. The effect of IP3 is likely mediated by the IP3 receptor, since L-IP3, which doesn’t bind to the IP3 receptor, had no effect on import.

Supported by the Medical Research Council of Canada.

**ThT2:W34** Tissue distribution of the very low density lipoprotein receptor variants

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We have previously reported that some cell types (e.g. vascular endothelium) only express the variant of the very low density lipoprotein (VLDL) receptor that lacks the O-linked sugar domain. The presence or absence of the O-linked sugars determine the VLDL receptor stability or release from the cell, respectively.

It is important to know which of the two VLDL receptor variants are present in the different tissues for this purpose. For this, we have identified mRNA splicing variants in bovine tissues by relative quantitative reverse transcription-polymerase chain reaction (RT-PCR) and ribonuclease protection assays. We found that the variants are preferentially expressed in some bovine tissues.

These results, together with the differences found in the receptor stability, suggest a tissue-specific role of the VLDL receptor variants.

**ThT3:W34** Hypcholesterolemia in cancer

A. Walter1, C. Reuter1, P. Fraenberger, D. Seidel, A.K. Wall. *Inst. of Clin. Chem., Grosshadern University Hospital; 1Clinic Barmherzige Brüder, Munich, Germany*

**Objective:** Hypcholesterolemia is often observed in patients with cancer. Although tumor tissue show enhanced cholesterol utilization, it is unlikely that tumors of small size can decrease circulating cholesterol levels due to increased uptake. The aim of the present study was to investigate, whether tumors are capable to release humoral factors which increase systemic choles-

**Methods:** 125I-LDL binding to membranes of cancer tissue of various origin as well as corresponding tumor free tissue was measured. In addition effects of tumor conditioned medium on the LDL uptake and proliferation in fibroblasts was investigated. We also generated conditioned medium by incubating established colon carcinoma cell line SW 480 in the presence and absence of simvastatin and cerivastatin. To identify the responsible humoral factors, the effects of conditioned medium in the presence of various neutralizing antibodies were examined.

**Results:** All tumors investigated revealed increased LDL receptor activity compared to tumor free tissue. Conditioned medium from tumors and tumor cell line SW480 stimulated LDL uptake and proliferation in fibroblasts. Neutralizing antibodies against PFG reduced the stimulation of LDL uptake induced by renal tumor conditioned medium by about 50%, whereas anti PDGF-AA inhibited the effect of SW 480 conditioned medium by about 90%. Both cerivastatin and simvastatin inhibited the release of PDGF-AA by SW480 cell line.

**Conclusion:** Although tumor tissue itself show an increased LDL-receptor activity, tumor cells also release humoral factors with the capacity to increase systemic LDL uptake. Both mechanism may contribute to the hypocholesterolemia during cancer disease. Statins abolish the tumor conditioned medium induced LDL uptake and cell proliferation. Use of statins as an adjuvant therapy in patients with cancer deserves attention.
The proteoglycan receptor for lipoprotein docking at the blood-endothelium-matrix interface

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Objective, Methods, Results: Proteoglycan sulfate can be adsorbed to a methylated silica surface in a monomolecular layer via its transmembrane hydrophobic protein core domain. Due to electrostatic repulsion, its anionic glycosaminoglycan side chains are stretched out into the blood substitute solution representing a receptor site for specific lipoprotein binding through basic amino acid-rich residues within their apolipoproteins. The binding process was studied by ellipsometric techniques showing that HDL has a high binding affinity to the receptor and a protective effect on interfacial heparan sulfate proteoglycan layers with respect to LDL and Ca2+ complexation. LDL was found to deposit strongly at the proteoglycan surface, particularly in the presence of Ca2+ thus creating the ternary complex formation 'proteoglycan–low density lipoprotein–calcium'. This heterometric complex build-up may be interpreted as arteriosclerotic microplaque formation on the molecular level responsible for the arteriosclerotic primary lesion. On the other hand, HDL bound to heparan sulfate proteoglycan protected against LDL docking and completely suppressed calcification of the proteoglycan–lipoprotein complex.

Conclusions: Although much remains unclear regarding the mechanism of lipoprotein deposition at proteoglycan-coated surfaces, it seems clear that the site of such systems offers possibilities for investigating lipoprotein deposition at a microscopic level under close to physiological conditions. In particular, Ca2+-promoted LDL deposition and the protective effect of HDL even at high Ca2+ and LDL concentrations agree well with previous clinical observations regarding risk factors and beneficial factors for early stages of atherosclerosis. Therefore, we believe that the system can be of some use in investigations, e.g., of the interaction between different lipoproteins in arteriosclerotic plaque formation, as well as in high throughput screening of candidate drugs to atherosclerosis in a biosensor application (PCT/EP 97/05212, USPTO 09/319,970).

Identification of families with inherited predisposition to familial hypercholesterolaemia

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Objectives: Familial Hypercholesterolaemia (FH) is characterised by autosomal co-dominant inheritance with elevated plasma-cholesterol, the presence of xanthomas and premature atherosclerosis. The majority of cases can be accounted for by either mutations in the Low density-lipoprotein receptor (LDLR) or the Apo B-100 gene. As cholesterol lowering is possible, the decision of treatment should be based on a risk assessment. Considering that at least thirty percent of individuals with coronary artery disease die prior to receiving medical attention primary prevention is important.

Methods: We have established an efficient DGGE screening procedure for the detection of mutations in the LDLR or Apo B-100 gene. A network corporation with clinical doctors on Medical departments throughout Denmark has been established. Individuals typed as positive for a pathogenic mutation receives genetic counselling and functions as contacts to undiagnosed relatives.

Results: Within the present project we have received samples from 92 patients. 75 individuals has been typed. In six of these no disease causing mutations has been identified. A number of previously unpublished mutations have been identified. Three patients represented unaffected relatives with no identified mutations.

Conclusion: The positive impact of tracing relatives to the proband has been demonstrated in a number of families characterised by a particular severe clinical outcome. In these families the clinical manifestation additionally necessitated genotyping of a number of children.

Sepsis-induced hypocholesterolaemia

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Objective: Severe inflammatory diseases such as sepsis and septic shock are associated with a significant reduction of circulating cholesterol. However the underlying mechanisms are unclear. The aim of the present work was to investigate the diagnostic and pathophysiological relevance of hypocholesterolaemia during sepsis.

Methods: In patients with sepsis, septic shock and/or multi-organ failure circulating cholesterol, triglycerides, apo A1 and apo B levels were measured. In addition inflammatory mediators such as IL-6, TNF, and soluble TNF receptors were also measured. Effects of TNF-α and IL-6 on LDL-cholesterol activity in cultured fibroblasts, endothelial cells and HepG-2 cells was investigated in order to evaluate a possible effect of these cytokines on cellular lipoprotein metabolism. We also examined effects of isolated lipoproteins on endotoxin induced release of TNF and IL-6 in PMA-activated monocytes.

Results: An inverse correlation between total cholesterol and mortality in patients with sepsis was noted. Cholesterol levels below 30 mg/dl were associated with a mortality > 50%. Serum levels of inflammatory cytokines show an inverse correlation with total cholesterol. TNF increased the uptake of 125I-LDL in all cultured cell lines used in this study. IL-1 and IL-6 also increased LDL uptake in HepG-2 cells. Incubation of monocytes with LDL, HDL or VLDL led to a concentration dependent reduction of endotoxin induced release of inflammatory cytokines.

Conclusion: The drastic reduction of cholesterol in patients with sepsis may be in part explained by the release of cytokines with subsequent increase of lipoprotein uptake. The prognostic relevance of low cholesterol level in these patients may be due to a protective and antiinflammatory effect of lipoproteins.

Upptake by macrophages of triacylglycerol-rich artificial emulsion particles co-incubated with native or modified LDL is a lipoprotein lipase-dependent process

M.D.T. Carvalho, L.M. Harada, E.C.R. Quintão. Lipids Lab. University of São Paulo Medical School, SP/SP. Brazil

Aim: investigate the cell uptake of the triacylglycerol (TG)-rich artificial emulsion particles (EM) co-incubated with LDL and lipopolysaccharide (LPS), an inhibitor of lipoprotein lipase.

Methods: EM simulating natural chylomicrons (except for lack of apoLp) containing 69% TG, 23% lecithin, 6% cholesterol oleate and 2% cholesterol were co-incubated with 1x, 2x(n) 1H-cholesterol oleoyl ether [1H-CO-EM]. EM was co-incubated (37°C, 4 hours in DMEM), with mouse peritoneal macrophages (5 x 10⁶) with LDL: native (na), aggregated (ag), acetylated (ac) or oxidized (ox), with or without LPS. Cell-associated radioactivity median values shown below were expressed as an uptake index of 1H-CO-EM: percent uptake of 1H-CO-EM with LDL without LDL (n = 8): na ag ag + LPS ac ac + LPS ox ox + LPS

Results: LPS inhibits the uptake of 1H-CO-EM when co-incubated with naLDL, agLDL or acLDL, but not with oxLDL.

Conclusion: the uptake of 1H-CO-EM co-incubated with native or modified LDL depends on the lipoprotein lipase enzyme.

Macrophages take up triacylglycerol-rich artificial emulsion particles co-incubated with native or modified LDL by an actin-dependent mechanism

M.D.T. Carvalho, L.M. Harada, E.C.R. Quintão. Lipids Lab. University of São Paulo Medical School, SP/SP. Brazil

Aim: investigate the cell uptake of the triacylglycerol (TG)-rich artificial emulsion particles (EM) co-incubated with LDL and cytochalasin B (cy), an inhibitor of the actin-dependent process.

Methods: EM simulating natural chylomicrons (except for lack of apoLp) containing 69% TG, 23% lecithin, 6% cholesterol oleate and 2% cholesterol were labeled with [1x, 2x(n)] 1H-cholesterol oleoyl ether [1H-CO-EM]. EM was co-incubated with 37°C, 4 hours in DMEM), with mouse peritoneal macrophages (5 x 10⁶) with LDL: native (na), aggregated (ag), acetylated (ac) or oxidized (ox), with or without cy. Cell-associated radioactivity median values shown below were expressed as an uptake index of 1H-CO-EM: percent uptake of 1H-CO-EM with LDL without LDL (n = 8): na ag ag + cy ac ac + cy ox ox + cy

Results: LPS inhibits the uptake of 1H-CO-EM when co-incubated with naLDL, agLDL or acLDL, but not with oxLDL.

Conclusion: the uptake of 1H-CO-EM co-incubated with native or modified LDL depends on the lipoprotein lipase enzyme.
Results: cytochalasin inhibits the uptake of $^{3}H$-CO-EM when co-incubated with nanLDL, agLDL or acLDL, but not with oxLDL.

Conclusion: acLDL is involved in the macrophage uptake of $^{3}H$-CO-EM co-incubating with nanLDL or with ag and ac LDLS.

ThT9; W34
Uptake by macrophages of triacylglycerol-rich artificial emulsion particles co-incubated with native or with modified LDL is scavenger receptor-dependent

M.D.T. Carvalho, L.M. Harada, E.C.R. Quintão, Lipids Lab, University of São Paulo Medical School, SP/SP, Brazil

Aim: investigate the cell uptake of the triacylglycerol (TG)-rich artificial emulsion particles (EM) co-incubated with LDL and fucoidan (fuc), a scavenger receptor (SRA-I) inhibitor.

Methods: EM simulating natural chylomicrons (except for lack of apoLP) containing 69% TG, 23% lecithin, 6% cholesteryl oleate and 2% cholesterol were labeled with $[1^\alpha, 2^\alpha]$-$^{3}H$-cholesterol oleoyl ether ($^{3}H$-CO-EM). EM was co-incubated (37°C, 4 hours in DMEM), with mouse peritoneal macrophages ($5 \times 10^6$) with LDL: native (na), acetylated (acx), or without fucoidan. Cell-associated radioactivity median values shown below were expressed as an $^{3}H$-CO-EM uptake index: percent uptake of $^{3}H$-CO-EM with LDL$^{3}H$-CO-EM without LDL (n = 8):

<table>
<thead>
<tr>
<th>na</th>
<th>na + fuc</th>
<th>ac</th>
<th>ac + fuc</th>
<th>ox</th>
<th>ox + fuc</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.30*</td>
<td>0.00</td>
<td>0.18*</td>
<td>-0.10</td>
<td>-0.27</td>
<td>-0.27</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test: p < 0.05. *EM + LDL + vEM + LDL + fuc.

Results: fucoidan inhibits the uptake of $^{3}H$-CO-EM when co-incubated with LDL or acLDL, but not with oxLDL.

Conclusions: in macrophages the SRA-I receptor is involved in the uptake of $^{3}H$-CO-EM co-incubated with LDL and acLDL.

ThT10; W34
Mild oxidation of LDL by macrophages increases the uptake of triacylglycerol-rich artificial emulsion particles co-incubated with native LDL

M.D.T. Carvalho, L.M. Harada, E.C.R. Quintão, Lipids Lab, University of São Paulo Medical School, SP/SP, Brazil

Aim: the influence of a mild oxidation of native LDL on the uptake of the triacylglycerol (TG)-rich artificial emulsion particles (EM).

Methods: EM simulating natural chylomicrons (except for lack of apoLP) containing 69% TG, 23% lecithin, 6% cholesteryl oleate and 2% cholesterol were labeled with $[1^\alpha, 2^\alpha]^{-}{3}H$-cholesterol oleoyl ether ($^{3}H$-CO-EM).

EM was co-incubated (37°C, 4 hours in DMEM), with mouse peritoneal macrophages ($5 \times 10^6$) with native (na) LDL or pretreated with probucol (naLDLp), an inhibitor of LDL oxidation, with or without fucoidan (fuc), a scavenger receptor inhibitor. Cell-associated radioactivity median percent $^{3}H$-CO-EM uptake/mg of cell protein is shown below:

<table>
<thead>
<tr>
<th>(n = 8)</th>
<th>without fuc</th>
<th>with fuc</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM + naLDL</td>
<td>3.97*</td>
<td>2.86</td>
</tr>
<tr>
<td>EM + naLDLp</td>
<td>2.32</td>
<td>2.34</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test: p < 0.05. *EM + naLDL + vEM + naLDL + fuc; or EM + naLDLp or EM + naLDLp + fuc.

Results: the percent uptake of $^{3}H$-CO-EM co-incubated with native LDL diminished with fucoidan probucol, or both together.

Conclusions: a mild degree of LDL in vitro oxidation increased the $^{3}H$-CO-EM uptake by macrophages by the SRA-I receptor.

ThT11; W34
Uptake by macrophages of triacylglycerol-rich artificial emulsion particles co-incubated with native LDL is a clathrin-dependent process

M.D.T. Carvalho, L.M. Harada, E.C.R. Quintão, Lipids Lab, University of São Paulo Medical School, SP/SP, Brazil

Aim: investigate the cell uptake of the triacylglycerol (TG)-rich artificial emulsion particles (EM) co-incubated with native LDL and EDTA, an uptake inhibitor by clathrin-dependent mechanisms involving the LDL receptor and pinocytosis.

Methods: EM simulating natural chylomicrons (except for lack of apoLP) containing 69% TG, 23% lecithin, 6% cholesteryl oleate and 2% cholesterol were labeled with $[1^\alpha, 2^\alpha]-^{3}H$-cholesterol oleoyl ether ($^{3}H$-CO-EM). EM was co-incubated (37°C, 4 hours in DMEM), with mouse peritoneal macrophages ($5 \times 10^6$) and inhibitors of endocytosis that work by different mechanisms [between square brackets]: EDTA [LDL receptor]; fucoidan (fuc) [scavenger receptor = SR-A]; cytchalasin B (cy) [phagocytosis], and LPS [lipoprotein lipase enzyme = LPL]. Cell-associated radioactivity median percent $^{3}H$-CO-EM uptake/mg of cell protein is shown below (n = 8):

<table>
<thead>
<tr>
<th>(n = 8)</th>
<th>EM + EDTA</th>
<th>EM + cy</th>
<th>EM + fuc</th>
<th>EM + LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9</td>
<td>6.1</td>
<td>5.9</td>
<td>4.7</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test: p < 0.05. *EM × EM + EDTA.

Results: EDTA alone, but not the other inhibitors, diminishes the percent uptake of $^{3}H$-CO-EM.

Conclusions: the cell LDL receptor, but neither the SR-A receptor, nor phagocytosis and LPL are involved in the uptake of EM alone.
T:W35 MONOCENIC HYPERLIPIDEMIA

ThT1:W35
Familial defective apolipoprotein B-100 in hypercholesterolemic patients in Poland
M. Bednarska-Makaruk1, M. Bisko2, M.F. Putwalska2, D. Hoffmann-Zacharska1, M. Rodo1, M. Rosaszykno1, A. Solik-Tomassini2, G. Broda3, M. Polakowska2, A. Pytlak2, H. Wohl1, 1 Institute of Psychiatry and Neurology; 2 National Institute of Cardiology, Warsaw, Poland

Defects of a ligand domain of apolipoprotein B-100 (apo B) for the low density lipoprotein (LDL) receptor results in hypercholesterolemia and premature atherosclerosis. The most common mutation is an arginine to glutamine substitution in position 3500. The aim of the study was to evaluate the prevalence of the familial defective apolipoprotein B-100 (FDB) Arg3500Gln mutation in hypercholesterolemic Polish subjects. A group of 525 unrelated patients with moderate and severe hypercholesterolemia (LDL cholesterol level ≥ 160 mg/dl) without (group IA; n = 351) or with (group IB; n = 174) mild hypertriglyceridemia was screened for the presence of FDB mutation using SSCP analysis according to Chaves (1996). Presence of the Arg3500Gln mutation was confirmed using a mismatch MspI PCR strategy according to Defesche (1993). FDB mutation was detected in 13 unrelated subjects and in 23 of 67 their relatives. All the probands belonged to the IA group. Plasma lipid levels and clinical characteristics of 36 carriers of the Arg3500Gln mutation were analysed and compared with their 44 nonaffected relatives. In the affected individuals a variable expression of lipid concentrations and atherosclerosis symptoms were observed. In one hypercholesteremic subject with early CAD symptoms a non-hiiberto described mutation in apo B gene was identified using DNA sequencing. The C → T transition in codon 3402 (ACT → ATT) which is producing a change from threonine to isoleucine in the encoded amino acid sequence was found. In summary, our study showed that the prevalence of the Arg3500Gln mutation in Polish hypercholesterolemic patients was 3.7% which was similar to several other European countries.

T:W37 OTHER TOPICS

ThT1:W37
The importance of the ejection fraction in the prognostics of early-onset coronary disease
A. Batalla1, G.I. Cubero2, J.J.R. Reguero3, S. Hevia2, E. Merino2, J.C. Sanmartin2, A. Cortina2, Department of Cardiology; 1Hospital de Cabueñas (Gijon); 2Hospital Central de Asturias (Oviedo), Spain

Purpose: To determine whether the ejection fraction is a prognosis predictor in early onset coronary disease.

Methods: Consecutively, 230 male patients before 50 years of age (mean age 43 ± 5 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease, were prospectively studied. In the acute phase by means of a structured questionnaire the presence of smoking habits, hypertension, diabetes and dyslipidemia were determined. A physical examen and fasting analysis were also made. Due to clinical instability or persistent myocardial ischemia 140 patients underwent a cardiac catheterization. According to left ventricular ejection fraction (LVEF) was greater to or minor than 60%, patients were divided into 2 groups. A mean follow-up of 32 ± 13 months was made. For statistical analysis, the Chi-Square test with Fisher's test was applied.

Results:

<table>
<thead>
<tr>
<th></th>
<th>LVEF &lt; 60% (n = 50)</th>
<th>LVEF ≥ 60% (n = 50)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Coronarography</td>
<td>12 (24%)</td>
<td>38 (76%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Mortality</td>
<td>7 (14%)</td>
<td>2 (2%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Heart failure</td>
<td>6 (12%)</td>
<td>0 (0%)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

No differences were found in the prevalence of hypertension, smoking habits, diabetes and dyslipidemia. Nor were there any difference in the appearance of angina, myocardial infarction and the need for coronary revascularisation in the follow-up.

Conclusions: Patients with early-onset ischemic heart disease and LVEF ≥ 60% present a greater prevalence of normal angiograms, less episodes of heart failure and minor mortality in the follow-up.

ThT2:W37
Differences between first episode and recurrent coronary disease
A. Batalla1, G.I. Cubero2, J.J.R. Reguero3, S. Hevia2, E. Merino2, J.C. Sanmartin2, A. Cortina2, Department of Cardiology; 1Hospital de Cabueñas (Gijon); 2Hospital Central de Asturias (Oviedo), Spain

Purpose: To determine differences exist in the morbi-mortality of patients under 50 years of age with coronary disease in the first episode or recurrent coronary disease.

Methods: Consecutively, 227 male patients before 50 years of age (mean age 43 ± 5 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease, were prospectively studied. A mean follow-up of 32 ± 13 months was made with special attention to the presence of coronary events. Two groups were established depending on whether it was (Group A) their first episode of coronary disease or not (Group B). For statistical analysis, the Chi-Square test was employed for the comparison of percentages.

Results:

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 153)</th>
<th>Group B (n = 74)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events</td>
<td>48 (31%)</td>
<td>42 (57%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Angina</td>
<td>45 (29%)</td>
<td>61 (82%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Revascularisation</td>
<td>37 (24%)</td>
<td>39 (53%)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

No differences were found in relation to heart failure, myocardial infarction or mortality.

Conclusions: Patients with a first clinical episode of coronary disease before 50 years of age present during follow-up fewer episodes of angina and less need for coronary revascularisation than these with various previous clinical episodes.

ThT3:W37
Control of risk factors in early-onset coronary disease
A. Batalla1, G.I. Cubero2, J.J.R. Reguero3, S. Hevia2, E. Merino2, J.C. Sanmartin2, A. Cortina2, Department of Cardiology; 1Hospital de Cabueñas (Gijon); 2Hospital Central de Asturias (Oviedo), Spain

Purpose: To determine the level of control of the principal risk factors in patients with early onset coronary disease.

Methods: Consecutively, 230 male patients before 50 years of age (mean age 43 ± 5 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease (166 myocardial infarction, 64 unstable angina), were prospectively studied. Smoking habits, arterial hypertension, diabetes and dyslipidemia were determined by means of a questionnaire, physical examen and fasting analysis during the acute phase and during a mean follow-up of 32 ± 13 months.

Results:

<table>
<thead>
<tr>
<th></th>
<th>Acute phase</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td>169 (73%)</td>
<td>45 (20%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>84 (36%)</td>
<td>76 (34%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>21 (9%)</td>
<td>20 (8%)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>181 (77%)</td>
<td>172 (75%)</td>
</tr>
</tbody>
</table>

Conclusions: In early onset coronary disease smoking is the risk factor which is better controlled during the follow-up. However, an improvement in the lipid profile of these patients is not seen.

coronary events a mean follow-up of 32 ± 13 months was carried out. For statistical analysis the Chi-Square test with Fisher’s test was applied.

Results:

<table>
<thead>
<tr>
<th>Tchol/HDL</th>
<th>Hypertension</th>
<th>No Hypertension</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tchol/Tchol/HDL &lt; 5 (n=39)</td>
<td>7 (18%)</td>
<td>77 (40%)</td>
<td>0.007</td>
</tr>
<tr>
<td>Tchol/Tchol/HDL &gt; 5 (n=100)</td>
<td>7 (18%)</td>
<td>13 (59.5%)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

No differences were found in the prevalence of diabetes or smoking habits in either group. No differences were seen during follow-up in the presence of angina, myocardial infarction, heart failure or need for revascularisation.

Conclusions: Our group of patients younger than 50 years old with ischaemic heart disease and showing a Tchol/HDL less than or equal to 5, present less prevalence of hypertension and show more frequently absence of significant corona lesions in comparison with those patients with an altered lipid profile.

ThT5:W37 Early-onset single vessel coronary disease

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Purpose: To determine common characteristics of patients under 50 years of age with single vessel coronary disease.

Methods: Consecutively, 119 male patients before 50 years of age (mean age 42 ± 4 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease and at least a coronary lesion greater than 70% in the coronaryangiography, were prospectively studied. Smoking habits, arterial hypertension, diabetes and dyslipidaemia were determined during the acute phase by means of a questionnaire, physical examen and fasting analysis. The follow cutoff points were established: Total cholesterol (Tchol) 200 mg/dl; HDL cholesterol (HDL) 35 mg/dl; LDL cholesterol (LDL) 130 mg/dl; Triglycerides (TG) 200 mg/dl and Lp(a) 30 mg/dl. In order to determine new coronary events, a mean follow-up of 32 ± 13 months was carried out. For statistical analysis the Student’s t test was employed for the comparison of percentages of independent groups

Results:

<table>
<thead>
<tr>
<th>mg/dl</th>
<th>Pts (%)</th>
<th>Events (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT ≥ 200</td>
<td>144 (63%)</td>
<td>64 (45%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CT ≥ 200</td>
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<td>LDL &lt; 130</td>
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<td>TG &lt; 200</td>
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<td>38 (52%)</td>
<td>0.007</td>
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No differences were found according to the cutoff points for HDL and Lp(a).

Conclusions: Differences exist in the appearance of coronary events during follow-up of patients with early onset coronary disease according to the levels of Tchol, LDL and TG in the acute phase being the number of events significantly less in patients with Tchol levels less than 200 mg/dl, LDL levels less than 130 mg/dl and TG levels less than 200 mg/dl.

ThT8:W37 The relationship of dyslipidaemia with other coronary risk factors in males with early coronary disease

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Purpose: To determine a possible association between dyslipidaemia and other coronary risk factors in early coronary disease.

Methods: Consecutively, 229 male patients before 50 years of age (mean age 42 ± 4 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease were prospectively studied. Smoking habits, arterial hypertension, diabetes and dyslipidaemia were determined during the acute phase by means of a questionnaire, physical examen and fasting analysis. An attempt was made to determine a possible association between dyslipidaemia and other risk factors. We considered dyslipidaemia a Total cholesterol−HDL cholesterol ratio (Tchol/HDL) greater than 5. For statistical analysis the Chi-Square test was applied.

Results: No differences were found in the relation to smoking habits and diabetes.

Conclusions: In our study of males with early coronary disease an association was found between dyslipidaemia and arterial hypertension that the prevalence of arterial hypertension is significantly greater in the group of patients with a Tchol/HDL greater than 5.

ThT7:W37 Lipid levels and coronary events

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Purpose: To determine whether differences exist in the appearance of coronary events in patients with coronary disease before 50 years of age according to the levels of different lipoprotein fractions.

Methods: Consecutively, 227 male patients (pts) before 50 years of age (mean age 42 ± 4 years), who were admitted to our Coronary Care Unit as result of an episode of coronary disease were prospectively studied. Levels of Total cholesterol (Tchol), HDL cholesterol (HDL), triglycerides (TG) and Lp(a) were analysed after patients had fasted for more than 12 hours. LDL cholesterol (LDL) was calculated using Friedwald’s formula, when the TG did not exceed 300 mg/dl. The following cutoff points were established in order to divide patients into 2 groups: Tchol 200 mg/dl, LDL 35 mg/dl, LDL 130 mg/dl, TG 200 mg/dl and Lp(a) 30 mg/dl. In order to determine new coronary events, a mean follow-up of 32 ± 13 months was carried out. For statistical analysis the Student’s t test was employed for the comparison of percentages of independent groups.

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ThT7:W37 Is rehabilitation in women as beneficial as men?

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Rationale: Cardiac rehabilitation is an essential component of the long term comprehensive management of coronary patients.

Objective: Determining the effect of rehabilitation on blood lipid profile in men and women.

Methods: The study sample was 40 women with the mean age = 54.2 ± 8.21 and 100 men with the mean age = 51.8 ± 9.58 who were selected randomly from all patients with myocardial infarction referred to the rehabilitation unit in Isfahan cardiovascular research center.

Eight-week rehabilitation program was started. Each session included a 20-minute stretching and warming up, a 20-minute conditioning period and a 15-minute cooling down. This program was performed as 3 sessions per week. Serum lipoproteins of all patients were measured before and after rehabilitation.

Results: There was no significant difference between age in the men and women. The obtained results show that rehabilitation can decreased TG, Tchol and LDL-cholesterol and the HDL-cholesterol level to be increased significantly (P < 0.05) in both sexes. Also, it was determined by statistical unpaired t-test that there were no significant differences between two sexes regarding the effect of rehabilitation on these factors.

Conclusions: As determined that increasing blood lipids is a major risk factor for cardiovascular disease and also rehabilitation improves lipid profiles in both men and women. It means that both men and women benefit from rehabilitation.
Homocysteine levels in young myocardial infarction survivors

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Homocysteine has been demonstrated to have a role in thrombotic and atherogenic events and hyperhomocysteinemia has been implicated as a risk factor for premature coronary vascular disease.

Objective: to study the plasma total homocysteine in young acute myocardial infarction survivors and compare the levels with a control group of healthy subjects.

Methods: population: 23 patients, mean age 35.2 ± 4.9 years (22-40), male: 20 patients, admitted for acute myocardial infarction since January 1995 until June 1998. Risk factors: tobacco smoking 22/23, systemic arterial hypertension 4/23, hypercholesterolemia 17/23, positive family history for coronary heart disease 5 patients, previous cardiac history 4 patients, none of these patients had diabetes mellitus. The location of the infarct was anterior in 12 patients, inferior in 10 patients and non-Q wave in one patients. Controls: 20 subjects, mean age 35.5 ± 5.8 years (23-45), male: 17 subjects. Blood samples were drawn after overnight fasting and the plasma total homocysteine was determined by fluorescence polarized immuno-assay.

Results: there was no significant difference between the homocysteine levels of patients (mean 10.9 ± 2.9 mmol/L) and controls (mean 11.2 ± 2.31 mmol/L) although the lipid profile was significantly altered in the patients as compared to controls: total cholesterol 240 ± 53.8 mg/dL vs 193.6 ± 34.3 (p < 0.01); LDL 184.3 ± 36.0 mg/dL vs 94.3 ± 28.4 mg/dL (p < 0.001); triglycerides 169.1 ± 103.3 mg/dL vs 126.5 ± 50.9 mg/dL (p < 0.01) and HDL 37 ± 5.4 mg/dL vs 51.5 ± 10.8 mg/dL (p < 0.001).

Conclusions: in this group of patients the plasma total homocysteine levels didn’t differ significantly from controls. The authors consider that the most important risk factors were probably the lipid levels and smoking habits.

Cholesterol and triglycerides in high density lipoproteins sub-classes

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Objective: To determine cholesterol and triglycerides HDL reference ranges in a Brazilian population.

Methods: Ninety-nine healthy volunteers, aged 18 to 86 years, had their lipids, lipoproteins and apolipoproteins measured by enzymatic, calculated or direct, and nephelometric methods, respectively. HDL₂ and HDL₃ were separated by preparative microultracentrifugation.

Results: The only parameter that presented gaussian distribution was HDL₂ triglycerides (TG) (Shapiro-Wilk test). HDL₂ cholesterol (CHOL) did present it after a log transformation and HDL₂ TG and HDL₃ CHOL did not show it. Sex differences were found only for HDL₃ CHOL and no differences were found between sexes and age groups (> and ≤50 years) for all the other parameters (ANOVA and Mann-Whitney tests).

Conclusions: The reference ranges for this population were determined as (mg/dL, ± 2SD): HDL₂ CHOL = 11 ± 2 (n = 82); HDL₂ TG = 15 ± 5 (n = 52); HDL₃ CHOL, men = 39 ± 9 (n = 47), women = 43 ± 9 (n = 52) and HDL₃ TG = 14 ± 6 (n = 52).