Relationship between classic risk factors, plasma antioxidants and indicators of oxidant stress in angina pectoris (AP) in Tehran

S. Meraji a,*, P.M. Abuja b, M. Hayn b, G.M. Kostner c, R. Morris d, S. Oraii a, F. Tatzber b, W. Wonisch b, R. Zechner b, K.F. Gey e

a Shahid Rajaii Hospital, Cardiovascular Research Centre, Tehran University of Medical Sciences, Tehran, Iran
b Institute of Biochemistry, University of Graz, Graz, Austria
c Institute of Medical Biochemistry, University of Graz, Graz, Austria
d Primary Care and Population Sciences, Royal Free and University College Medical School, London, UK
e Institute of Biochemistry and Molecular Biology, University of Berne, Berne, Switzerland

Received 9 March 1999; received in revised form 2 September 1999; accepted 15 September 1999

Abstract

Cardiovascular disease (CVD) in general seems to be the leading cause of death in the Eastern Mediterranean Region (EMR) including Iran. This may be due to classic risk factors such as high triglyceride (TG), high total cholesterol (TC), and low levels of high density lipoprotein cholesterol (HDL-C). The impact of antioxidants as potentially protective risk factors against early coronary heart disease (CHD) is unknown in Iran. Therefore, relationships between angina and plasma antioxidants and indicators of lipid peroxidation were investigated in a case-control study. In this study, 82 cases of previously undiagnosed angina pectoris (AP), identified by a modified WHO Rose chest pain questionnaire and verified by electrocardiography during treadmill exercise testing, were compared with 146 controls selected from the same population of over 4000 male civil servants aged 40–60 years. Subjects with AP declared significantly less physical activity and had higher serum TG [means (S.E.M.) 2.32 (0.18) versus 1.61 (0.07) mmol/l] but lower HDL-C [1.01 (0.04) versus 1.18 (0.03) mmol/l] than age-matched controls. Levels of total serum cholesterol, low-density lipoprotein cholesterol (LDL-C) and lipoprotein(a) [Lp(a)] were not significantly different between the two groups, while the ratio of LDL-C/HDL-C was significantly higher [4.51 (0.23) versus 3.54 (0.11)] for subjects with AP than for the controls. There was no significant difference in plasma levels of α-tocopherol, vitamin C, α- and β-carotene. However, retinol [1.90 (0.06) versus 2.09 (0.05)] and β-cryptoxanthin [0.398 (0.04) versus 0.467 (0.03)] were significantly lower in AP. Furthermore, angina cases exhibited a higher index of lipid peroxidation than controls (e.g. malondialdehyde, MDA; 0.376 (0.010) versus 0.337 (0.009) mmol/l). On multiple logistic regression analysis, retinol with odds ratio (OR) of 0.644 [95% confidence interval (CI; 0.425–0.978)], β-cryptoxanthin, with an OR of 0.675 (CI; 0.487–0.940), oxidation indices, MDA with OR of 1.612 (95% CI; 1.119–2.322) and LDL-C/HDL-C ratio with OR of 2.006 (95% CI; 1.416–2.849) showed the most significant independent associations with AP in this group of Iranians. In conclusion, the state of lipid peroxidation as well as the status of special antioxidants may be co-determinants of AP in Iran, in parallel with the influence of classical risk factors for cardiovascular disease. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Antioxidants; Lipid peroxidation; Malondialdehyde; Autoantibodies; Lipoproteins; Coronary heart disease

1. Introduction

Cardiovascular diseases (CVD) are the leading cause of death in the Eastern Mediterranean Region including The Islamic Republic of Iran [1]. Despite the lack of accurate mortality data and modern medical care, there is enough evidence that CVD is increasing in Iran. The proportion of deaths due to CVD reached around 38% in 1989 (1983–1989) [2]. The national survey data have suggested that elevated plasma levels of classic risk factors such as a high total cholesterol (TC), triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) might be important underlying causes of this
increased mortality [3,4]. A recent community-based programme showed that 64% of a healthy normal population in Tehran were suffering from some kind of dyslipidaemia [5].

In industrialized countries, where classical risk factors for coronary heart disease (CHD) such as blood pressure, plasma cholesterol and smoking have been known for some time, corresponding public education and other interventions have led to a decline in the mortality rate in these countries in the last three decades [6]. However, in the present decade it has been suggested that other factors such as plasma antioxidant vitamins and peroxide concentrations play an important role in the etiology of CHD including angina pectoris (AP). Oxidative modification of low density lipoprotein (LDL) has been inferred to be important in the development of atherosclerosis, since modified LDL is readily taken up by macrophages, and as a consequence, leads to foam cell and plaque formation [7,8]. High levels of lipid proxidation were also reported to be associated with angina pectoris [9,10] and progressing coronary artery disease [7,8,11].

The most abundant diet-derived antioxidants available in plasma to protect against reactive oxygen species are \( \alpha \)-tocopherol (vitamin E) and ascorbic acid (vitamin C) [12]. \( \alpha \)-Tocopherol is the major lipophilic antioxidant present in the outer lipid monolayer of LDL, which may protect the polyunsaturated fatty acids against oxidation by accepting electrons to form \( \alpha \)-tocopheryl radicals [13,14], whereas carotenoids [14] and retinol (vitamin A) [15] are minor lipophilic antioxidants. Vitamin C, in the aqueous phase of the plasma, permits regeneration of \( \alpha \)-tocopherol [16]. Cross cultural studies in Europe [17,18], Scotland and Finland [for review see Ref. [19]] have revealed an inverse relationship between the plasma vitamins C and E and CHD [17,18]. Other studies of large cohorts have shown that correction of an inadequately low dietary intake of vitamins E [20] and C [for review see Ref. [19]] reduces CHD expression.

Although the mortality rate of CHD seems to be increasing in urban areas of Iran, there has not been any systematic study on associations of risk factors with angina pectoris and CHD. A study of previously undiagnosed AP may provide most valuable information on an early stage of CHD with mild or transient clinical symptoms, which is not yet biased by recent changes of life style or any other treatment. In Edinburgh, AP of middle-aged males was associated with low plasma levels of vitamins A, C, E and carotene [21]. A small pilot study on young students in Tehran, found a higher level of plasma malondialdehyde (MDA) as an indicator of lipid peroxidation, but similar amounts of \( \alpha \)-tocopherol and \( \beta \)-carotene and even higher levels of some other carotenoids (lycopene, canthaxanthin, lutein + zeaxanthin) as compared with a matched group of Australians [22]. Results of a household food security survey in Tehran [23] indicated that 96% of the population of Tehran consume vitamin C in excess of and 32% consume retinol below the recommended intake [23], of which only 18% is from meat and dairy products.

Generally, the dietary habits of Iranians are different from those prevailing in industrialized countries and their life style has been influenced to some extent by war in recent decades. A recent nationwide survey found that the main sources of carbohydrates are rice (at least once a day) and bread (66% of energy comes from carbohydrates): oil or hard margarine as the main sources of fat. Twenty two percent of energy was derived from fat in which saturated fatty acids exceed the recommended level: the intake of animal protein was low [24]. Leafy vegetables such as parsley, coriander, dill weed, onions, spinach, horse radish, mint, watercress and other herbs are traditionally eaten every day, but other vegetable are consumed to a lesser extent and all mostly in pickled form. However, tomato puree, lemon juice and puree from pomegranate and plums are also used for enhancing the taste, but often requiring prolonged cooking times for stews. Green salad, yoghourt or pickled vegetables are served with every meal.

The aim of this study was to identify subjects with AP among middle-aged males in Tehran and to measure both classic risk factors, as well as plasma antioxidant concentrations and indicators related to lipoprotein oxidation as potentially independent predictor risk factors for AP.

2. Material and methods

2.1. Experimental design

The design of the investigation was based on a case-control study. The selected population consisted of men aged 40–60 years who were not taking any medication for treatment of CHD, hyperlipidaemia, diabetes or hypertension. They were also not taking vitamin supplements and had not undertaken recent dietary changes that might affect their plasma antioxidant status. Male employees of eleven different Governmental Organizations in Tehran were screened for previously undiagnosed AP. More than 4000 questionnaires were returned. Three hundred reported coronary chest pain according to a modified Rose chest pain questionnaire [25] and were invited for exercise test with a 97% response rate. The ECG revealed abnormalities after exercise loading in 104 subjects, but only 82 subjects with horizontal or downslope ST depression of equal or more than 1 mm in two or more leads, were admitted to the study. These 82 verified AP cases were matched with 146 male controls of similar age drawn from the
remaining population (3700) who had a negative response to the Rose chest pain questionnaire (reconfirmed by telephone), no history of CHD and a negative electrocardiography exercise test. All subjects completed documentation with informed consent.

Blood pressure was measured in the right arm after resting sitting for 10 min, by mercury sphygmomanometer. Two measurements of blood pressure were recorded at 5 min intervals, by a trained person. Height and weight were measured, and questionnaire data was collected on food frequency, years of education, numbers of cigarettes smoked per day, exercise habits, walking, past history of reported high BP, diabetes and dyslipidaemia, in the subjects and their first degree relatives. Four of the AP cases underwent coronary artery bypass surgery immediately after angiography.

Exercise testing and all blood sampling was performed during the period September 1997 to mid-March 1998, before the beginning of spring when stored fruit supplies gradually decline. In particular, it was important to finish sample collection before the celebrations of the Iranian New Year which starts on 21 March and lasts for 2 weeks, when people eat more nuts, sweets and fruits. The frozen samples were transferred on dry ice to the University of Graz, Austria, in early May 1998. The biochemical analyses were completed by the end of June 1998.

2.2. Materials

All solvents and further specified chemicals were analytical grade, supplied by Merck (Darmstadt, Germany) or Prochem (Wesel, Germany) or Sigma (St. Louis, MO). The kit to measure autoantibodies to oxidized LDL (oLab) was provided by Dr Franz Tatzber, KEG, Vienna, Austria.

2.3. Methods

2.3.1. Storage of plasma

Blood samples (30 ml) were taken by venipuncture of the antecubital vein, with brief use of a tourniquet, in the morning after 12 h fasting. Serum was obtained for lipid profile. For parameters other than vitamin C, blood samples containing 1 mg/ml EDTA, pH 7.4, were centrifuged at 2000 × g for 10 min. The aliquots of plasma were stored at −80°C for 3–8 months. A separate aliquot of 0.5 ml of plasma EDTA was mixed with 0.5 ml of 10% metaphosphoric acid, within 30 min of venipuncture and stored at −80°C for assay of vitamin C.

2.3.2. Determination of lipid profile in serum

Cholesterol was measured by the CHOD-PAP method. TG by the GPO-PAP method, high density lipoprotein cholesterol (HDL-C) was determined after separation with phosphotungstic acid and magnesium chloride, all using established kit methods from Boehringer, Mannheim, Germany. LDL-C concentration was calculated [26]. The methods and results were validated against the UK-NEQAS scheme and Boehringer QC standards.

2.3.3. Determination of lipid soluble antioxidants

Antioxidants were determined as described previously [27]. Briefly, 0.4 ml of plasma with 0.4 ml of water were mixed with an equal volume (0.8 ml) of ice-cold ethanol (containing 0.5 mg/ml butylated hydroxy-toluene (BHT)). A total of 2.0 ml of n-hexane (stored over bidistilled water containing 20 µM EDTA) was added and after centrifugation (3000 rpm, 5 min), a volume of 1.2 ml of hexane phase was transferred into a brown crimp vial and dried in a speed-vac for 10 min, at room temperature. The residue was dissolved in 0.3 ml mixture of ethanol:ethylacetate (10:1 v/v, containing 1 mg/l BHT). A sample (20 µl) was injected into an HPLC system (Lichrosphere 100 RP18 column, 5 µm, 125 × 4 mm: Merck, Germany). Separation was performed in isocratic mode with a mixture of acetonitrile/methanol/ethanol/water (60:50:20:2 v/v), containing 0.01% ammonium acetate, flow rate 1.2 ml/min. The effluent was monitored with two detectors in series, a UV-VIS detector (L4250, Merck, Hitachi) set to 450 nm for detection of carotenoids and a fluorescence detector (Jasco 821 FP) set initially to 325/500 nm for detection of retinol and after 3.9 min to 292/335 nm for detection of tocopherols. The intra assay coefficient of variation was 4–7% for all antioxidants.

2.3.4. Determination of vitamin C with HPLC

Vitamin C was measured according to the method of Bui et al. [28]. The frozen plasma-EDTA, in metaphosphoric acid, was thawed, mixed for 5 s and centrifuged on an Eppendorf centrifuge (Hamburg, Germany, Mod.5415C) at 10 000 × g for 4 min. A volume of 0.1 ml of supernatant was mixed with 0.4 ml of HPLC-eluent, centrifuged and injected by the autosampler into the HPLC System (Lichrosphere 100 RP18, 5 mm, 250 × 4 mm). The HPLC-eluent was prepared by adding 4.3 ml of 70% perchloric acid and 100 mg EDTA to 1 l of bidistilled water. Flow rate was 1 ml/min. The effluent was monitored with an electrochemical detector set to +0.6 V against an Ag/AgCl reference electrode filled with 3 M LiCl. Peak quantification was performed with at least two standard mixtures of ascorbic acid. The time between thawing and HPLC separation did not exceed 3 h.

2.3.5. Determination of MDA in plasma

MDA was determined using a slight modification of the HPLC method of Rabl et al. [29]. The plasma
samples were thawed immediately before the assay and 100 μl was mixed with 100 μl water, 300 μl of 0.15 mol phosphoric acid/l, 10 μl of BHT, 4% methanolic solution and 100 μl 0.6% thiobarbituric acid (TBA) and incubated at 95°C for 60 min. The chromogen was extracted with 1.25 ml butanol-1 and fractionated by HPLC with fluorometric detection (excitation wavelength: 525 nm, emission wavelength 550 nm). MDA–TBA adduct was calibrated with tetramethoxypropane standard solution.

2.3.6. Measurement of autoantibodies to oLab

Determination of oLab (EliTec, Bisamberg, Austria) was performed according to the method of Tatzber and Esterbauer, 1995 [30]. Briefly, human LDL (50 μg protein/ml) in PBS, (pH 7.4), was oxidized with CuCl2 at a final concentration of 1.66 μM for 24 h. Microtitration plates were coated using a concentration of LDL at 5 μg protein/ml (250 μl/well, 1.25 μg protein) in phosphate carbonate buffer (pH 9.6) overnight. Unspecific binding sites were blocked with 1% bovine serum albumin (BSA) in PBS (pH 7.4) for 2 h at room temperature. The sera of the subjects were diluted 1:21 in PBS and incubated for 2 h at 37°C in the wells coated with oLDL. After washing, 100 μl of antihuman IgG horse radish peroxidase conjugate was added to each well and incubated for 45 min at room temperature. Tetramethylbenzidine was used as a chromogenic substrate and the absorbance was read at 450 nm. The absorbance of samples collected before supplementation was taken as the 100% value for each subject, the measurements of oLab obtained after supplementation were calculated as a percent of this value.

2.3.7. Determination of lipoprotein (a) [Lp(a)] and apolipoprotein (a) [apo(a)]

Quantitation was performed by a sandwich assay on the DELFIA system (LKB-Pharmacia) [31]. In brief, a polyclonal rabbit antibody was purified by immunofinity by passing over a column loaded with plasminogen, and coated onto 96-well Costar plates. The purified antibody was free of any detectable cross reactivity against plasminogen, or other plasma constituents as tested by Western bolt analysis using Glu1-plasminogen. Non-specific bindings sites were blocked by exposure to 250 μl of 0.5% (w/v) BSA for 30 min. Two hundred μl aliquot of the samples were added to the wells and incubated for 2 h at 20°C. After three successive washing steps with 50 mM Tris–HCl, pH 7.7, the polyclonal antibody against apo(a), labelled with Europium (Eu) was added to the wells and incubated additionally for 2 h at 20°C. Fluorescence was determined in a DELFIA reader after 15 min. Plasma samples were diluted 1500–3000-fold.

2.4. Statistical analysis

Statistical comparisons between angina cases and the controls were carried out using unpaired $t$-tests. The non-parametric Wilcoxon test was used for comparing non-normally distributed data. For comparison of the percentage of smokers and of genetic history in first degree relatives, chi-squared tests were used. Multiple logistic regression with a forwards stepwise approach was used to determine the independent predictors of AP in the total sample. The following variables were not normally distributed and were log transformed before analysis: α, β-carotene, β-cryptoxanthin, γ-tocopherol, oLab, YadjMDA and triglycerides. Variables entered into the logistic regression included lipid profile, LDL-C and HDL-C (entered as LDL-C/HDL-C ratio, not individually, as this term can give more power in risk assessment [32]), and all the water soluble and lipid soluble antioxidants and indices of lipid peroxidation, whether they were statistically significant or not. Correlations between variables were calculated with Pearson (denoted $r$) or Spearman’s rank correlations (denoted $r_s$), according to whether variables followed a normal distribution. There was a strong positive correlation between cholesterol ($r = 0.498, P = 0.0001$) and triglyceride ($r_s = 0.440, P = 0.0001$) and α-tocopherol, therefore α-tocopherol was adjusted for these two parameters according to the procedure of Jordan et al. [33], before entering into the logistic regression. There was a weak correlation between the other antioxidants (e.g. β-cryptoxanthin) and TC ($r_s = 0.279, P = 0.0001$) and TG ($r_s = 0.014, P = 0.841$), therefore they were entered without adjustments. Although, the correlation between MDA and TC ($r_s = 0.223, P = 0.002$) and TG ($r_s = 0.221, P = 0.002$), were not strong, MDA was adjusted for both TC and TG according to the procedure of Jordan et al. [33] in view of the high TG in AP cases. However, the relationship between MDA and angina was similar whether or not such an adjustment was made. The protective and the risk factors for angina pectoris were evaluated by logistic multiple regression with adjustment for other variables entering the statistical model. OR and their 95% confidence limits are presented as the relative odds associated with a standard deviation increase in that particular variable. A $P$ value less than 0.05 was considered significant. All values shown in the table are mean (S.E.M.). All statistical analyses were performed by using the computer software SPSS Rel.6.1.2.

Note: The reason the number of analyses is not consistent is due either to insufficient plasma for all analyses for some subjects, or lack of time, as only 2 months were available to complete all the biochemical analyses. Regarding blood pressure and body mass index (BMI), their entry did not affect the outcome of the logistic regression, therefore they were omitted.
Also, the physical activity value was self-declared and unmeasurable, of interest but considered less reliable than the biochemical measurements and it was also omitted from the logistic analysis.

3. Results

3.1. Anthropometric measurements, smoking and BP

In the present case-control study the apparently healthy controls were compared with subjects with previously undiagnosed AP regarding sex, age (40–60), profession (civil servants of the Governmental staff) and a medium-low socioeconomic status with similar mean years of education and a similar BMI around 25. Also, they declared that they did not drink alcohol.

There was no significant difference in the BP between these two groups (Table 1) and levels were within the reference range according to European and National Cholesterol Educational Program guidelines [34,35]. The percentages of cigarette smokers were similar in the two groups, however, the percentages of those with a family history of CHD in first degree relatives, before age of 60, were significantly higher (Table 1) in the angina group than in the controls.

3.2. Lipid profile

There were no significant differences in TC and LDL between the two groups (Table 2). TG (44%) and ratio of LDL-C/HDL-C were significantly higher (27%) in the cases than controls (Table 2) and HDL-C was significantly lower (15%) in the cases than controls. There was a significant inverse correlation between HDL and plasma TG ($r_s = -0.433, P = 0.0001$). It has been suggested that HDL-C $< 1.0$ mmol/l and/or fasting triglycerides $> 2.0$ mmol/l and TC $> 5.0$ mmol/l are markers of increased coronary risk [34,35]. Seventy five percent of either angina subjects or controls had TC $> 5.0$ mmol/l; 46% of the angina and 24% of controls had TG $> 2.0$ mmol/l; and 53% of angina cases and 26% of the controls had HDL-C $< 1.0$ mmol/l. Thus, these classical risk factors occurred at least twice as often in the angina cases as the controls, in combination with a lower declared physical activity and an increase in the family background of premature CHD ($P = 0.0055$ and $P = 0.0063$, respectively).

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Control</th>
<th>n</th>
<th>Angina</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>146</td>
<td>48.4 (0.38)</td>
<td>82</td>
<td>48.7 (0.54)</td>
<td>0.586</td>
</tr>
<tr>
<td>EDU</td>
<td>146</td>
<td>14.3 (0.27)</td>
<td>82</td>
<td>13.6 (0.37)</td>
<td>0.068*</td>
</tr>
<tr>
<td>BMI</td>
<td>146</td>
<td>24.6 (0.24)</td>
<td>82</td>
<td>25.1 (0.34)</td>
<td>0.207</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>146</td>
<td>123.5 (1.1)</td>
<td>82</td>
<td>126.6 (1.3)</td>
<td>0.124*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>146</td>
<td>77.10 (0.7)</td>
<td>82</td>
<td>78.7 (1.0)</td>
<td>0.269*</td>
</tr>
<tr>
<td>Activity (min)</td>
<td>146</td>
<td>270 (20)</td>
<td>82</td>
<td>181 (20)</td>
<td>0.0055*</td>
</tr>
<tr>
<td>Smoking $&gt;5$/day</td>
<td>146</td>
<td>13.0%</td>
<td>82</td>
<td>15.9%</td>
<td>0.553**</td>
</tr>
<tr>
<td>History of CHD in the 1st relative $&lt;60$</td>
<td>146</td>
<td>17.1%</td>
<td>82</td>
<td>32.9%</td>
<td>0.0063**</td>
</tr>
</tbody>
</table>

* Data are presented as mean (S.E.M.). Education by years EDU; weight (kg)/height (m)$^2$, BMI; blood pressure, BP; walking + exercise/week. Activity (min); smoking more than 5 cigarettes/day considered as smoker. Data were compared with the unpaired t-test.
** Data were compared by $\chi^2$ test.

Table 2

<table>
<thead>
<tr>
<th>Parameter (mmol/l)</th>
<th>n</th>
<th>Control</th>
<th>n</th>
<th>Angina</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>146</td>
<td>5.78 (0.09)</td>
<td>82</td>
<td>6.13 (0.15)</td>
<td>0.053</td>
</tr>
<tr>
<td>LDL-C</td>
<td>146</td>
<td>3.87 (0.09)</td>
<td>82</td>
<td>4.09 (0.14)</td>
<td>0.215</td>
</tr>
<tr>
<td>HDL-C</td>
<td>146</td>
<td>1.18 (0.03)</td>
<td>82</td>
<td>1.91 (0.04)</td>
<td>$&lt;0.0001$*</td>
</tr>
<tr>
<td>LDL-C/HDL-C</td>
<td>146</td>
<td>3.54 (0.11)</td>
<td>82</td>
<td>4.51 (0.23)</td>
<td>$&lt;0.0001$*</td>
</tr>
<tr>
<td>TG</td>
<td>146</td>
<td>1.61 (0.07)</td>
<td>82</td>
<td>2.32 (0.18)</td>
<td>$&lt;0.0001$*</td>
</tr>
<tr>
<td>Lp(a) (mg/dl)</td>
<td>140</td>
<td>21.5 (1.7)</td>
<td>79</td>
<td>29.6 (3.5)</td>
<td>0.131*</td>
</tr>
</tbody>
</table>

* Values are presented as mean (S.E.M.). Data were compared with the unpaired t-test. Total cholesterol, TC; high density lipoprotein cholesterol, HDL-C; low density lipoprotein cholesterol, LDL-C; triglyceride, TG; lipoprotein (a), Lp(a).
** Data were compared by non-parametric Wilcoxon two sample test.
3.3. Lp(a)

The mean level of Lp(a) was (37%) higher ($P = 0.131$; ns) in the cases than the controls (Table 2). Plasma level of Lp(a) above 30 mg/dl was found in 21% of controls and 33% of angina cases.

3.4. Oxidation indices

Indices of lipid peroxidation such as MDA were significantly higher (12%) in the cases than controls ($P = 0.0001$, Table 3). Also, when MDA was standardized for lipid [YadjMDA, which incorporated cholesterol and triglycerides [33]] it was significantly higher in the cases than controls ($P = 0.0021$). Circulating autoantibodies against oLab were also 27% higher in the cases than controls, but at marginal significance only if outlying values were excluded ($P = 0.049$, Table 3). There was no correlation between MDA and oLab ($r_s = -0.051$, $P = 0.505$).

3.5. Vitamin C

Plasma vitamin C level was close to the optimal level recommended to protect against CHD [19,20,34,36], with no significant differences between the cases and controls (Table 3). Plasma vitamin C was positively correlated with the number of oranges consumed per day ($r = 0.271$, $P = 0.0001$). Vitamin C was significantly correlated with β-cryptoxanthin ($r_s = 0.372$, $P = 0.0001$).

3.6. Lipophilic antioxidants

Plasma retinol was significantly (9%) lower in the cases than in controls. β-Cryptoxanthin exhibited noticeable significant differences between the two groups. The control group had significantly higher β-cryptoxanthin (15%) than in the cases (Table 3). β-Cryptoxanthin was also weakly correlated with the number of oranges consumed/day ($r_s = 0.217$, $P = 0.002$). The plasma carotene (α-β-carotene) seemed to be similar to the levels recorded for Austrians [22] and other European males [21], yet β-carotene did not reach desirable recommended levels [36]. The absolute amount of α-tocopherol was 9.6% higher in the cases than controls, the difference was not statistically significant. When α-tocopherol was standardized for concurrent cholesterol-rich carriers, the α-tocopherol status within lipids (α-tocopherol/TC) as well as lipid standardized α-tocopherol (YadjE, which incorporates cholesterol and TG [33]) the values were clearly below the recommended desirable levels [36], but without any differences between cases and controls (Table 3). Although β-carotene did not exhibit any differences between the two groups, levels were lower than recommended for primary prevention for both controls and cases [36].

3.7. Multiple regression analyses

Considering all the variables assessed for AP and controls, plasma levels of retinol, β-cryptoxanthin, LDL-C/HDL-C and MDA were independently associ-
ated with AP in this Iranian population (Table 4). For one standard deviation (S.D.) increase in retinol and log_2 (β-cryptoxanthin), there was a 0.644 and 0.675-fold decrease in the odds of angina. However, for an increase of one S.D. in log_2 (YadjMDA) and LDL-C/HDL-C, there was a 1.612 and 2.006-fold increase for risk of angina, respectively.

4. Discussion

According to the guidelines of the European Atherosclerosis Society [34] and the National Cholesterol Education Program [35], the AP cases had approximately twice the target levels of lipid-related cardiovascular risk, and the position of the control population was also not ideal.

Thus levels of HDL were below the guidelines (53% of AP and 26% of controls had HDL < 1.0 mmol/l), TGs were higher (46% of AP and 24% of controls had TG > 2.0) but levels of TC were not significantly different between two groups (75% of AP or controls had TC > 5.0 mmol/l). Some 75% of the population of Tehran suffer from some kind of dyslipidaemia [5]. The levels of HDL were lower, but TG and LDL-C/HDL-C ratio were significantly higher in the cases than controls (Table 2). It is well established that high HDL is considered to be antiatherogenic and associated with lower risk for CHD [37,38] and may mitigate the toxic effect of LDL [39]. On the other hand, an increase in total triglycerides and a decrease in HDL are reported to be associated with progression of coronary atherosclerosis [40]. As generally reported, and in the present study, TG level was not an independent predictor of AP in the logistic regression, but the ratio of LDL-C/HDL-C was an independent predictor (OR 2.006: CI 1.416–2.849). This was mainly due to low levels of HDL which might respond to increases in physical activity and changes of the dietary habits such as less reliance on hard saturated margarine [24].

No significant differences in vitamin C levels were observed between the cases and the controls. The concentration of plasma vitamin C was apparently sufficient since it was above the critical threshold of 50 µmol/l suggested for antioxidant protection of LDL against CHD [36]. It was even 45% higher than levels reported for an AP group studied in Edinburgh [21]. Indeed, the consumption of vitamin C in Iran is rather high compared to other vitamins [23] and exceeds the intake recommended from a household survey [23]. Although it has previously been reported that α-tocopherol status (< 20–25 µmol/l with α-tocopherol/TC ratios < 4.25) can play a key role in AP [21] and CHD [18–20], in this study there was no significant difference between levels for angina and controls. In this study, level of plasma α-tocopherol was 14.5% and the ratio of α-tocopherol/TC was 19.9% higher than those reported in the Edinburgh AP study in which vitamin E inadequacy was the most prominent risk factor [21]. Therefore, it was unlikely that any vitamin E inadequacy specifically contributed to AP in Tehran. Also, there was no significant difference in levels of α- and β-carotene between the two groups although both were below recommended levels for protection against CHD [36].

In contrast to vitamin C, E, and carotene, in the present study the levels of retinol and β-cryptoxanthin were significantly lower in the AP than controls (Table 3) and were predictors of AP with OR 0.644 (P = 0.0392) and 0.675 (P = 0.0205) for retinol and β-cryptoxanthin respectively (Table 4). In this study, the mean levels of retinol in AP (1.9 µmol/l) were 20% below that found for European population with AP [21] and 10% below the general recommended levels for protection against CHD [19]. According to a cross cultural study, this plasma level of retinol was associated with an increased mortality rate for CHD [17,18]. The low level of retinol in this population in Tehran may not only be due to inadequacy of preformed retinol in the diet [23] but could also reflect reduced retinol formation from carotenoids with potential pro-vitamin A activity such as α- and β-carotene and β-cryptoxanthin. As there was no significant difference for the carotenoes between the AP and controls, plasma β-cryptoxanthin might be

<table>
<thead>
<tr>
<th>Variables</th>
<th>Regression coefficient (B)</th>
<th>S.E. of (B)</th>
<th>S.D. of controls</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol</td>
<td>−0.745</td>
<td>0.361</td>
<td>0.59</td>
<td>0.644</td>
<td>0.425–0.978</td>
<td>0.0392</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>−0.485</td>
<td>0.209</td>
<td>0.81</td>
<td>0.675</td>
<td>0.487–0.940</td>
<td>0.0205</td>
</tr>
<tr>
<td>YadjMDA</td>
<td>1.910</td>
<td>0.745</td>
<td>0.25</td>
<td>1.612</td>
<td>1.119–2.322</td>
<td>0.0103</td>
</tr>
<tr>
<td>LDL-C/HDL-C</td>
<td>0.512</td>
<td>0.132</td>
<td>1.36</td>
<td>2.006</td>
<td>1.416–2.849</td>
<td>0.0001</td>
</tr>
<tr>
<td>Constant</td>
<td>0.553</td>
<td>1.239</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Odds ratios (OR) were calculated for an increase in each variable equivalent to one standard deviation (S.D.) (estimated from control group data) of the variable. Thus odds ratio was calculated as \( \exp(B \times \text{S.D.}) \). The 95% confidence interval (CI) was calculated as \( \exp(B \pm 1.96 \times \text{S.E. of } B) \). Each OR given was adjusted for all other variables in data. β-cryptoxanthin and YadjMDA were log transformed before entering multiple logistic regression, because this produced a more normally distributed variable. LDL-C/HDL-C; low/high density lipoprotein cholesterol.
causally related to the lower level of plasma retinol. However, although low levels of β-cryptoxanthin were a weak but significant independent predictor of AP, this does not necessarily imply any exclusive causal relationship between AP and carotenoids such as β-cryptoxanthin. Since β-cryptoxanthin is a relatively specific marker of consumption of oranges and tangerines [41], the latter will provide other antioxidants with presumed CHD-protective properties such as bioflavonoids and polyphenols [19]. Vitamin A deficiency has been reported in Iran [23] and this study population may require a higher intake of performed vitamin A such as oily fish and certain dairy products and its precursors found in varieties of coloured vegetables.

In the present study, the low levels of retinol and β-cryptoxanthin in AP were associated at least with a significantly higher susceptibility towards lipid peroxidation in the AP cases than controls as indicated by an increase in plasma MDA (Table 3), as reported by previous studies [9–11]. As we measured MDA in the presence of BHT, a phenolic antioxidant, MDA precursors or even preformed MDA may at least in part have occurred in vivo. Increased plasma MDA was an independent predictor of AP (OR 1.612; \( P = 0.0103 \); Table 4). However, in a study of AP in Aberdeen [9], high levels of plasma lipid peroxidation products were accompanied by differences in plasma levels of vitamin E but not of vitamins C or A. In the present study, high levels of lipid peroxidation was associated with a lower plasma level of retinol and β-cryptoxanthin but not with lower levels of vitamins C and E. In other studies [19–21], lower levels of vitamins C, E and carotene were associated with angina and this may be related to the special dietary habits in different countries. A high level of lipid peroxidation in the patient in Tehran could well accelerate the process of atherosclerotic plaque formation [7,8], particularly in the presence of low retinol and accelerate AP. It has been shown that supplementation with vitamin E [42,43], β-carotene and retinol retards the process of oxidation [15,22,44]. The underlying cause of lipid peroxidation is not known, but it could partly be due to lack of antioxidants, or to oxidised fat accumulated during processing, storage and/or cooking. Overall this study supports the hypothesis that lipid peroxidation is involved in AP and CHD respectively and that cardiovascular health requires the concurrent adequacy of various antioxidants [9,11,19–21].

5. Conclusion

A high ratio of LDL/HDL and increased tendency towards lipid peroxidation, together with low levels of retinol and β-cryptoxanthin in conjunction with some genetic disposition of the patients and a low declared physical activity, may contribute to the promotion of AP in Tehran. In full accordance with findings from other authors it is therefore advisable that the Iranian people should modify their life style by consuming a greater variety of fruits and more vegetables and, in particular, those rich in retinol precursors such as oranges, tangerines, carrots as well as sources of performed retinol such as milk and its products and oily fish. For individuals who have a very low level of retinol perhaps short term retinol supplementation may be advisable: long term supplementation can be associated with toxic effects. Furthermore, it is recommended that Iranians, and in particular this selected population, should replace saturated margarine [24] with a moderate intake of less saturated fats such as sunflower-oil and the more stable olive oil.

Acknowledgements

This study is supported by international Nutrition Foundation for Promotion of Nutrition Education (ISFE), in particular Professor Dr P Walter, Switzerland. Ministry of Health and Medical Education, Deputy for Research Affairs, Iran. Also, Austrian Science Foundation, Project No. SFB 709. We are grateful to these organizations. Also, we would like to thank for their invaluable support, the organization and the subjects who took part in this study. Also, the director Dr F. Noohi, the research director, Dr M. Maleki, in Rajaii Heart Hospital, Tehran for allowing the study to be conducted in this center and Dr F. Safarpour for his constant help with exercise test. Also, we are grateful to Dr M. Heydari in Cardiovascular Research Center Tehran University of Medical Sciences for lipid analyses and Mr M. Shekarloo of Austrian Airline for his support in transporting the samples without charge to Austria. Further thanks go to Dr Peter Whincup for his advice on chest pain questionnaire, Professor K.R. Bruckdorfer and Professor A. Winder of The Royal Free and University College Medical School, London for their advice and support.

References


