Diabetic state induces lipid loading and altered expression and secretion of lipoprotein lipase in human monocyte-derived macrophages

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Abstract

Non-insulin-dependent diabetes mellitus (NIDDM) is frequently associated with macroangiopathies and coronary heart diseases. Lipoprotein lipase (LPL), an enzyme known to undergo significant functional alterations in diabetic state, is also a potential atherogenic protein. Since, to the best of our knowledge, there are no data concerning LPL secreted by macrophages of NIDDM patients we conducted a study to assess the expression and activity of LPL secreted by monocyte-derived macrophages from NIDDM patients with cardiovascular complications versus cardiovascular patients without diabetes (controls). Isolated cells from NIDDM patients, after 7 days in culture in the presence of 20% autologous serum, readily exhibit a foam cell phenotype, in contrast to the cells from controls. Macrophages were mainly loaded with triglycerides, whose cellular amount was well correlated to triglyceridemia of NIDDM subjects. Concomitantly, macrophages from NIDDM patients displayed a ~ six-fold decrease of mRNA expression and a ~ two-fold reduction of the activity of secreted LPL, as compared to control cells. These data suggest that in complicated diabetic state, macrophage loading leading to foam cell formation is accelerated, at least in part, due to a diminished expression and activity of LPL. These observations add and extend the data that may explain the occurrence of accelerated atherogenesis and of the atherosclerotic complications associated with diabetes. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Atherosclerosis; Polymerase chain reaction; Foam cell; Non-insulin-dependent diabetes mellitus; Fluorometry

1. Introduction

Lipoprotein lipase (LPL), a hydrolytic enzyme located on the capillary endothelium, is a rate limiting factor for the catabolism of plasma triglyceride-rich lipoproteins [1,2]. Data in the literature indicate that in human subjects with type 2 diabetes mellitus, the LPL activity in post-heparin plasma is decreased [3,4], and that the polymorphism of LPL gene is associated with a high risk of coronary heart disease [5]. Besides the main location of the enzyme over the capillary beds, it was also detected within the arterial wall [6], especially in the regions affected by atherosclerotic plaque [7].

The atherogenicity of LPL of the arterial wall is rather controversial [8]. However, there are several reports that emphasise the potential atherogenic effects of LPL such as, enhanced LDL subendothelial retention via interaction with proteoglycans [9] increased lipid uptake by VLDL and LDL receptor on macrophages [10,11] as well as cytotoxic effects of their reaction products on cells of the arterial wall [12]. Studies conducted in vitro have demonstrated LPL secretion by the major cell types of the plaque, i.e. macrophages [13,14] and smooth muscle cells [15]. Consistent with the in vitro observations, O'Brien et al., using immunocytochemistry and in situ hybridisation performed on human coronary atherosclerotic plaques, found that macrophage-derived foam cells are the major source of LPL [16]. It is also stated that both in murine peri-
tional macrophages [17] and in human macrophages isolated from atherosclerotic plaques [18] lipid loading is accompanied by a marked decrease in expression and activity of lipoprotein lipase. Accelerated atherosclerosis is the main complication of diabetes. Also, diabetic state is known to induce metabolic activation in monocyte/macrophages [19] as well as enhanced phagocytic [20] and chemotactic [21] properties and increased cytokine production [19]. To the best of our knowledge there are no reported data either on LPL expression and secretion by monocyte-derived macrophages obtained from patients with non-insulin-dependent diabetes mellitus or about the loading of these cells with lipids. Therefore, in the present study, we tested (a) the level of LPL mRNA expression and LPL activity secreted by monocyte/macrophages from patients with NIDDM versus non-diabetic subjects; and (b) the possible correlation between LPL expression and activity and the lipid loading of these macrophages in culture.

2. Materials and methods

2.1. Patients

The subjects chosen for the study fall into two categories, (1) non-insulin-dependent diabetics (NIDDM), diagnosed for more than 1 year, with associated ischemic heart diseases (stable angina pectoris and history of myocardial infarction) and; (2) patients suffering from similar ischemia but without diabetes mellitus (controls). A number of 30 NIDDM patients and 24 non-diabetics (controls) ageing 40-70 years were selected from 'D. Bagdasar' emergency clinic in Bucharest, with the personal patient consent. Patients were hospitalised for their coronary heart disease and not for diabetes. However, diabetic patients were diagnosed for at least 1 year and their diabetes was generally well controlled. For each patient the following parameters were assayed: serum cholesterol, HDL cholesterol, triglycerides, glycemia, blood pressure and body mass index. Coronary atherosclerosis in both groups of patients was documented by electrocardiograms, exercise tolerance tests, and, in some cases, by coronary angiography. The patients in the two groups were age and sex matched. Data are expressed as mean values ± S.D. and statistically analysed using the one-way ANOVA test for comparison between diabetic and non-diabetic patients.

2.2. Isolation and culture of monocyte-derived macrophages

Mononuclear cells were isolated from whole blood, collected on 3.8% Na₂-citrate using Hystopaque 1077, according to [22]. Monocytes were subsequently purified by centrifugation using a Percoll gradient [23]. After two washes with Hank’s Balanced Salt Solution, the cells were seeded (10⁶ cells per well) in 24-well culture dishes in RPMI 1640 medium supplemented with 20% autologous serum, 100 U/ml penicillin and 100 µg/ml streptomycin. After 2 h incubation, the non-adherent cells were washed and the remaining cells were cultured for 6 days, in 5% CO₂, humidified atmosphere, at 37°C, replacing the medium every 2 days. All the assays were performed on the 7th day, after a 20 h incubation of cells with fresh medium. The cell viability was evaluated using a LIVE/DEAD Eukolight Viability/Cytotoxicity Kit (Molecular Probes, Inc., Eugene, OR). Briefly, after washing with warm medium, and fixation using 2% p-formaldehyde, cells were incubated with both calcein (stain for living cells) and ethidium bromide homodimer (stain for death cells), according to the protocol indicated by the manufacturer. Finally, cells were counted under a Nikon fluorescence microscope.

2.3. Lipoprotein lipase activity assays

One milliliter medium (collected from four wells) was concentrated to a final volume of 100 µl using CentriPrep concentration tubes (cut-off 10 000 Da, Millipore Intertech, Marlborough, MA). In some experiments, the cells were previously treated with heparin (4 U/ml), for 20 min, in order to release the LPL associated with the cell surface. The LPL activity was assayed by a non-radioactive method adapted from Nilsson–Ehle and Schotz [24]. Briefly, 100 µl of concentrated medium was incubated with 150 µl of a substrate mixture, yielding a final concentration of 1.7 mM glycerol trioleate (corrected according to each individual plasma triglyceride values) and 0.5 mM lecithin, 1% BSA in 80 mM Tris–HCl, NaCl 150 mM, pH 8.2 and 50 µl heat inactivated human serum, in 500 µl total volume. In all cases, the triglyceride hydrolysis does not exceed 5–8% of the total amount of substrate in the mixture. Free fatty acids released in the reaction were measured using the method of Itaya [25]. A control consisting from RPMI-1640 culture medium with 20% autologous serum was treated similarly to the corresponding probe and the activity value obtained was subtracted from the latter. All measurements of LPL activity were performed within 2 h from medium collection.

2.4. Quantitative RT-PCR for LPL mRNA

Total RNA was extracted from 10⁴ to 10⁶ macrophages after 7 days in culture. Cells were scraped from the plates using a rubber policeman, than washed twice with serum-free medium and the total RNA was extracted using a GlassMax RNA Microisolation Spin
centrifugation at 5000 min, cells were scraped from the plates and pelleted by saturated solution of ferrous citrate on ice [29]. After 40 minutes in 1.1 M cacodylate buffer (pH 7.4) and paraformaldehyde, 5% glutaraldehyde, 2% osmium tetroxide, the pelleted cells were dehydrated with increasing concentrations of ethanol and embedded in Epon. Thin sections stained with uranyl acetate and lead citrate were examined with a Phillips 400 Electron Microscope. Quantitative measurements of lipid droplet number were made directly on electron micrographs of macrophages, in which the plane of the section was through the body of the cell. A number of 20 cells for each experimental condition were randomly selected (n = 5 per group).

2.6. Lipid analysis

To assess the intracellular lipid content, the cells plated on four culture wells were washed three times with PBS and scraped with a rubber policeman in 1 ml total volume of water. After a 30 s sonication of the cell suspension, lipids were extracted in a mixture of chloroform:methanol:water (2:2:1). The organic phase containing lipids was dried under nitrogen and the pellet was solubilised in a chloroform:methanol:water (1:1:1) mixture. The amount of triglyceride and cholesterol (free and esterified) was measured using enzymatic kits provided by Sigma (St. Louis, MO). Blanks and standard curves for each lipid class were run in parallel. Intracellular lipids were expressed as µg lipid per mg cell protein.

2.7. Other assays

Serum triglycerides, cholesterol and glycemia were assayed by using Sigma enzymatic kits. HDL cholesterol was measured following precipitation with sodium phosphotungstatate/MgCl₂ as in [30]. LDL cholesterol was calculated according to Friedewald formula. Cell protein was determined according to Bradford, as described in [31].

3. Experimental results

3.1. Characterisation of the patients

Non-insulin-dependent diabetic subjects with cardiovascular complications (NIDDM) and patients with similar cardiovascular disease but without diabetes (controls) were sex- and age-matched. As shown in Table 1, NIDDM patients did not display severe obesity (BMI < 33 kg/m²) or severe hypertriglyceridemia (serum triglycerides did not exceed 200 mg/dl) and there were no significant statistical differences in serum total cholesterol, triglycerides and HDL/LDL cholesterol between the patients in the two groups (P > 0.05), except that the blood glucose was highly increased in NIDDM patients. In addition, all patients in the NIDDM group received hypoglycemic sulphonylureas, but no insulin. With respect to cardiovascular disease,
patients in both groups presented stable angina pectoris (12 patients NIDDM, 15 controls), myocardial infarction in history (six patients NIDDM, five controls) or both (12 patients NIDDM, 15 controls). Based on the clinical and biochemical data, we could assume that any significant difference in the results between NIDDM and controls may be mostly ascribed to the diabetic state.

3.2. Enzymatic activity of LPL secreted by macrophages isolated from NIDDM and controls

Monocyte-derived macrophages (MDM) from both NIDDM and control subjects were cultured in RPMI 1640 medium containing 20% autologous serum and the activity of LPL secreted by the cells was assayed in 20 h conditioned media, starting with day 1 of culture for 11 days. For cells belonging to both groups of patients, the results indicated a linear increase of the activity during the first 5 days, followed by a plateau for the next 4 days, and a slight decrease up to the 11th day, partly due to an increased cell mortality as assayed by LIVE/DEAD Cytotoxicity/Viability kit (data not shown). Also, over the 11-day period, the absolute values for LPL activity of diabetic macrophages were constantly lower than that found in control cells. Consequently, we performed all the assays for the activity of secreted LPL in the 20 h conditioned media at day 7 of culture. For each probe, LPL activity was assayed in parallel in the conditioned media and in an equal volume of RPMI with 20% autologous serum maintained without cells in the same conditions, in order to subtract the intrinsic residual serum LPL activity and free fatty acids of the autologous serum used in the culture medium. To increase the reproducibility of the results, as a source of apo CII (for activation of LPL) a unique pool of heat-inactivated human serum, stored at -70°C, was used. During the 60-min assay the reaction was linear with respect to enzyme concentration and time and in most cases the substrate hydrolysis was kept under 8%. LPL activity secreted by MDM separated from NIDDM was highly decreased (42%), as compared to controls (298.5 ± 40.7 vs. 691.1 ± 103.1 nmol FFA per mg cell protein/h, 37°C, P = 0.00035) (Fig. 1). The values obtained for LPL activity in our experimental conditions were in the same range as those reported by other groups [14,32].

3.3. Expression of LPL mRNA secretion by human monocyte-derived macrophages of NIDDM and controls

The expression of mRNA for LPL in MDM was measured after total RNA extraction as described in Section 2. Each probe was reverse transcribed together

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>NIDDM (n = 31)</th>
<th>Controls (n = 28)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.1 ± 1.46</td>
<td>58.4 ± 1.8</td>
<td>N.S.</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>68.9</td>
<td>64.2</td>
<td></td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>29.4 ± 1.1</td>
<td>26.8 ± 2.4</td>
<td>N.S.</td>
</tr>
<tr>
<td>Glycemia (mg/dl)</td>
<td>164.2 ± 12.3</td>
<td>88.4 ± 3.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>6.1 ± 0.7</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>156.8 ± 12.9</td>
<td>148.4 ± 12.7</td>
<td>N.S.</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>191.4 ± 8.13</td>
<td>199.4 ± 6.95</td>
<td>N.S.</td>
</tr>
<tr>
<td>HDL/LDL cholesterol</td>
<td>3.85 ± 0.6</td>
<td>3.31 ± 0.5</td>
<td>N.S.</td>
</tr>
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</table>

* NIDDM, patients with non-insulin-dependent diabetes mellitus and cardiovascular complications; controls, patients with cardiovascular disease only; values represent means ± S.D.; N.S., a non-significant one-way ANOVA test.
with AW109 as an internal standard and the four dilutions of the resulting cDNAs were amplified by PCR for 35 cycles. In each case, the two amplification curves were parallel, which indicates that the amplification efficiencies were the same for both the target and the standard. This feature attests an accurate quantitative measurement of LPL mRNA expression. The reaction products from AW109 cRNA (300 bp) and the target mRNA for LPL (277 bp) were separated by 3% agarose gel electrophoresis. An example of PCR products of LPL mRNA from MDM isolated from a cardiovascular patient (control) and the internal standard in serial 1:3 dilutions of the cDNA mixture is shown in Fig. 2. The products were visualised using SYBR Green I staining and quantitated by measuring the fluorescence intensities of the resolved bands. The arbitrary units of fluorescence (AUF) for each band were plotted against the template concentration. The specificity of the reaction was validated by the lack of any detectable signal every time we included a control experiment in which the reverse transcriptase was omitted or in which mRNA was replaced by water. Monocyte-derived macrophages from both NIDDM and control subjects were assayed for LPL mRNA expression on day 7 of culture. On average, the total number of cells subjected to RNA extraction was between $5 \times 10^4$ and $5 \times 10^5$ and the reverse transcribed RNA was $\sim 200$ ng. In each experiment, $10^8$ copies of AW109 cRNA were used. The quantity of LPL mRNA obtained from the amplification curves was normalised to the number of viable cells subjected to the total RNA extraction. Results showed a 6.5-fold decrease in mRNA expression for LPL in MDM isolated from NIDDM as compared to control patients (Table 2). The data regarding the LPL mRNA expression correlate well with those concerning the activity of secreted LPL in NIDDM and controls. The difference between the decrease in LPL mRNA expression (85%) and in secreted

![Figure 2](image)

**Fig. 2.** Quantitative analysis of LPL mRNA levels in monocyte-derived macrophages of a patient with cardiovascular disease only (control); SYBR Green I staining of PCR products separated in 3% agarose gel. Lanes 1–5 indicate LPL mRNA PCR products from 1:3 dilutions of a sample containing 250 ng total RNA from macrophages and $10^5$ molecules of AW109 cRNA after amplification for 35 cycles. Lane 6 is the 0.5 Kb molecular weight marker.

<table>
<thead>
<tr>
<th>Patient</th>
<th>LPL mRNA (molecules per cell)</th>
</tr>
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<tbody>
<tr>
<td>NIDDM</td>
<td>$15.5 \pm 5.42$</td>
</tr>
<tr>
<td>Controls</td>
<td>$101.7 \pm 40.5$</td>
</tr>
</tbody>
</table>

*Total RNA was isolated from monocyte-derived macrophages of NIDDM or controls and cultured for 7 days in RPMI 1640 supplemented with 20% autologous serum. LPL mRNA molecules per cell is calculated considering only viable cells counted after fluorescent staining with LIVE/DEAD Cytotoxicity kit. Data represent mean value $\pm$ S.D. of four experiments. The means are statistically different as judged by $P = 0.05$, calculated using one-way ANOVA test. NIDDM, non-insulin-dependent diabetic patients with associated cardiovascular disease; controls, patients with cardiovascular disease only.

LPL activity (58%) in NIDDM versus control patients could be due, at least in part, to a different intracellular pool of LPL in the macrophages of the two groups.

### 3.4. Morphology and lipid accumulation in MDM from NIDDM and control patients

Freshly isolated human monocytes from both subject groups contained no detectable lipid inclusions and displayed similar morphological appearance (Fig. 3a and d). As early as day 4 in culture, MDM isolated from the NIDDM patients started to display numerous lipid droplets in the cytoplasm ($30 \pm 12$), which increased steadily in number and by the 7th day, practically all cells exhibited a typical foam cell morphology (with $90 \pm 18$ lipid droplets in the cytoplasm), with few organelles and a small, sometimes eccentric, nucleus (Fig. 3e and f). The MDM originating from control patients showed few, if any, intracellular lipid droplets after 4 days in culture ($8 \pm 4$) and on the 7th day, in some cells, a variable number of lipid inclusions were found ($38 \pm 17$), a figure significantly lower when compared to NIDDM macrophages at the same time point ($P < 0.05$). Extreme examples of control MDM are shown in Fig. 3b and c. After 11 days in culture the differences in lipid loading of cells from the two groups, were similar to those observed on day 7. However, though unlikely, we cannot rule out the possibility that eventually, after 2–3 weeks in culture, the macrophages isolated from controls would also exhibit a foam cell-like phenotype. To assess the lipid composition, the MDM were subjected to chloroform:methanol extraction and the triglycerides and total cholesterol quantified. The results showed a significant triglyceride enrichment in NIDDM ($837 \pm 110$ μg per mg cell protein) versus controls ($500 \pm 130$ μg per mg cell protein) (Table 3). For total cholesterol, although the difference between the MDM of the two groups did not reach a statistical significance ($P > 0.05$), the mean was...
32% lower in NIDDM group (Table 3). In addition, results indicated that for both group, freshly isolated monocytes has lower values for the total cholesterol (114 ± 27 μg per mg cell protein) and triglycerides (270 ± 54 μg per mg cell protein), respectively. As observed from the above data, the main intracellular lipid pool is represented by triglycerides (especially in MDM from NIDDM group), which is in accordance with the results reported by others for MDM of healthy human subjects [33]. To test the influence of autologous serum on lipid loading of MDM, cross-experiments were performed, in which the serum from controls was used to culture cells of NIDDM and vice versa. Data about the intracellular cholesterol and triglycerides mass in macrophages from NIDDM and control subjects cultivated in autologous serum are set out in Table 3. When the serum was switched between the cells isolated from the two groups, the results showed that MDM from NIDDM patients were still loaded with lipids in day 7 of culture (735 ± 97 μg triglyceride per mg cell protein and 237.5 ± 64 μg cholesterol per mg cell protein), while the MDM from controls displayed only a moderate lipid loading when grown in NIDDM serum-containing medium (385 ± 66 μg triglyceride per mg cell protein and 360 ± 37 μg cholesterol per mg cell protein). These data may suggest a different mechanism of lipid uptake and/or metabolism in macrophages of NIDDM subjects as compared to controls. The potential correlations between each of the total cholesterol and triglycerides concentrations in serum and their respective intracellular mass for each individual within the two subject groups were further investigated (Fig. 4). For the control group, there is a lack of correlation between the serum cholesterol and triglycerides and their intracellular counterparts \( P = 0.97, R = 0.01 \) and \( P = 0.65, R = 0.13 \), respectively (Fig. 4A and B). MDM of NIDDM patients also exhibit a weak correlation with respect to serum versus intracellular cholesterol \( P = 0.2, R = 0.35 \) (Fig. 4D). In contradistinction, serum and intracellular triglycerides are very well correlated in macrophages of NIDDM group \( P = 0.0001, R = 0.83 \) (Fig. 4C). These data bring further support for possible alteration in triglyceride metabolism of diabetic MDM. The prominent lipid loading of cells from diabetic patients is accompanied by a decrease in the expression and activity of LPL as described above. Fig. 5 shows a good correlation between the triglyceride loading of macrophages and the LPL activity in the cell medium. Both in control group (Fig. 5A) and in NIDDM group (Fig. 5B) higher LPL activity values corresponded to lower intracellular triglyceride amounts, leading to a progressive decrease of LPL.

Fig. 3. Electron micrographs of monocytes and monocyte-derived macrophages from non-insulin diabetic patients with cardiovascular disease (NIDDM) and patients with cardiovascular disease only (controls). After isolation, monocytes from either controls (a) or NIDDM (d), were allowed to adhere for 2 h. on 24-well culture plates in RPMI 1640 with 20% autologous serum and after extensive washings with serum free medium, cells were fixed for 20 min, scraped from the plates and processed for electron microscopy. Alternatively, monocyte-derived macrophages were cultured for 7 days and then processed (as above). In the cells isolated from controls various numbers of cytoplasmic lipid droplets (LD) are present (38 ± 17); two extreme cases are shown in (b and c). The 7 day cultured macrophages from two NIDDM patients, (e) and (f), have the typical morphological aspect of foam cells, i.e. the cell cytoplasm is filled with droplets. Bar, 1 μm.
activity while cells became loaded with lipids. For both groups the values were well corrected \((r = 0.657\) for controls and \(r = 0.763\) for NIDDM) and \(P\) is less than 0.03 as determined by ANOVA analysis.

4. Discussion

In patients with NIDDM, macrovascular complications such as coronary artery and peripheral vascular disorders are the major health problems; in Caucasian patients with NIDDM their contribution to mortality overrides the impact of microvascular complications [34]. In order to emphasise the role of long lasting diabetic state on lipid metabolism of macrophages, we selected for this study one group of patients with non-insulin-dependent diabetes mellitus and ischemic heart diseases and one control group represented by patients with the same type of ischemia, but without diabetes. Our results indicated that the activity of LPL secreted by macrophages of NIDDM patients is 42% decreased as compared to controls. A similar diminution in LPL activity is also reported for murine macrophages in streptozotocin-induced diabetes [35]. There are several possible reasons, which could account for the observed change in LPL activity. First, the high blood glucose concentration in NIDDM may serve as an alternative to free fatty acids as source of energy for the cell, leading to decreased synthesis and activity of LPL. This is also supported by the fact that, among all serum biochemical parameters that we have tested, fasting glycaemia was the only statistically different one. Furthermore, our current experiments on murine peritoneal macrophages cultured in RPMI supplemented with 0.1 and 0.2% glucose revealed a two-fold decrease in LPL activity in the latter condition. Another possible explanation is the activated state of monocytes in diabetic state on lipid metabolism of macrophages [36], which induces the secretion of several inflammatory cytokines such as IL-1, TNF or IFN\(_gamma\), that in turn, were shown to suppress secreted LPL activity in human MDM [37] or mouse peritoneal macrophages [38]. Suppression of LPL activity in murine peritoneal macrophages loaded with triglyceride [17] or in macrophage-derived foam cells isolated from the arterial wall of human subjects [18] were also reported. These data are in accordance to our results, in which MDM from NIDDM patients exhibit a decreased secreted LPL activity concomitantly with a foam cell phenotype (after 7 days in culture) as compared to MDM from controls, which are only moderately loaded with triglycerides. Thus, a massive triglyceride loading may be partly responsible for the decreased LPL activity observed in MDM of NIDDM patients.

Our results also showed that LPL mRNA synthesis is ~ six-fold reduced in MDM from NIDDM versus controls, while the activity is only ~ two-fold decreased. A similar result is reported for LPL in adipose tissue of guinea pig during fasting [39]. The possible explanations may be the existence of a pool of inactive LPL stored in a high-mannose form within the endoplasmic reticulum [40] of control MDM or reduced stability of LPL mRNA in macrophages of NIDDM patients. A similar discrepancy was reported for J744 macrophages grown in high versus normal glucose medium, and the authors suggest that post-translational modifications may account for the difference observed [41]. Finally, we may speculate that due to a higher efficiency of the diabetic LPL than that of the normal enzyme in degrading the substrate, the specific activity of the LPL from diabetics is actually more than two-fold decreased.

The results presented here indicate that the NIDDM macrophages are prone to become loaded with lipids, mainly as triglycerides. Recent data [33] show that human monocyte derived macrophages from healthy subjects cultured in RPMI with 10% human serum, but not foetal calf serum, turn into triglyceride-rich foam cells, with no change in LPL activity. Also there is one report about an increase, rather than a decrease of LPL activity in macrophages isolated from diabetic patients compared to healthy controls [42]. In contrast to these results, we found a selective lipid loading of MDM accompanied by a decrease in LPL activity and expression. This behaviour of macrophages obtained from NIDDM patients is in accordance with previous observations [17,18] that indicated that macrophage derived-foam cells express lower LPL mRNA and activity. On the other hand, Mattsson et al. [18] showed that only the CD14 positive cells from human atheroma exhibit a decreased LPL activity and lipid loading; these cells are

### Table 3

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>NIDDM (µg per mg cell protein)</th>
<th>Controls (µg per mg cell protein)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>837 ± 110</td>
<td>500 ± 130</td>
<td>(P = 0.05)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>287.5 ± 55</td>
<td>420 ± 107</td>
<td>(P = 0.28)</td>
</tr>
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</table>

* Lipids were extracted from monocyte-derived macrophages after 7 days in culture, and quantitated using enzymatic kits. Values are given as mean \(±\) S.D. \((n = 13)\). The ‘\(P\)’ value is calculated using one-way ANOVA test. NIDDM, non-insulin-dependent diabetic patients with cardiovascular disease; controls, patients with cardiovascular disease and without diabetes.
capable of phagocytosis. Although scarce, there are some data in the literature to document an increased phagocytic capacity of human monocytes in diabetes [36]. Thus, we cannot rule out the possibility of a different number of CD14 positive cells among MDM population of NIDDM versus control patients. Moreover, Behr et al. [43] have shown that a high degree of activation of monocytes/macrophages leads to a decreased LPL activity.

Another observation concerns the strong correlation that exists between serum and intracellular triglyceride in MDM from NIDDM, but not in control patients. This suggests that NIDDM macrophages take up triglyceride-rich lipoproteins via a non-down regulated pathway. One of these pathways could involve the VLDL receptor [44], recently demonstrated by in situ hybridisation and immunohistochemistry on macrophages and endothelium of human arteries [45]. The decreased LPL activity in NIDDM–MDM versus controls, together with a lower LPL affinity for diabetic VLDL, as reported in [46] may lead to a higher concentration of intact VLDL in the medium, and thus an increased number of ligands available for the VLDL receptor, that could eventually lead to the foam cell formation by different mechanisms. Furthermore, diabetic patients have been noted to have elevations of

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Fig. 4. Correlation between intracellular and serum triglycerides and total cholesterol in the two subject groups. After 7 days of culture with RPMI 1640 supplemented with 20% autologous serum, monocyte-derived macrophages were washed in warm medium, than scraped from the plates and total cholesterol and triglycerides determined after lipid extraction using enzymatic kits; serum lipids were quantitated similarly. A and B, patients with cardiovascular disease (controls, ■); C and D, non-insulin-dependent diabetic patients with cardiovascular complications (NIDDM, □).
abnormal triglyceride-rich lipoproteins that are enriched in apoE [47] and are more avidly taken up by macrophages [48]. On the other hand, we may speculate that, due to an increased activation reported for diabetic monocytes [37], the cells isolated from NIDDM patients had an increased capacity of oxidising lipoproteins in serum-containing culture media that, in turn, are recognised by the macrophage scavenger receptors, leading to fast lipid loading of the cells [49]. In support of this hypothesis, and of our data there is a recent report showing that inhibition of LPL expression in human monocyte-derived macrophages is dependent on LDL oxidation [50]. Another possible explanation for triglyceride loading of macrophages isolated from NIDDM patients is related to the apoE secreted by the cells [51]. A recent study demonstrated that apoE enhances lipid uptake by macrophages in LPL deficiency [52].

On the other hand, Evans et al. [53] showed that extracellular lipolysis of VLDL subfractions by LPL is necessary for lipid loading of J744A.1 macrophages via a LDL receptor-mediated mechanism. Our data showing a decreased LPL secretion in diabetic macrophages may suggest though, that lipoproteins could be taken up with decreased efficiency by the cells via the LDL receptor. However, as a result of this decreased uptake, the local residence time of the lipoproteins would be prolonged, making modification (lipid peroxidation) and uptake by scavenger receptors more likely. Collectively, these data may provide a plausible explanation as to why a reduced LPL activity in NIDDM derived macrophages may be able to potentiate triglycerides and, to a lesser extent, cholesterol loading of the cells.

In conclusion, the results reported here indicate that, (i) monocyte-derived macrophages from NIDDM patients became foam cells after 7 days in culture, whereas in the same conditions MDM from control patients are only moderately loaded with lipids; (ii) in diabetic patients the accumulation of triglycerides in macrophages is well correlated with triglyceridemia; and (iii) macrophage-derived foam cell formation coincides with a decreased LPL mRNA expression and enzymatic activity in these cells.

We can postulate that in diabetic state, the decreased synthesis of LPL may cause accumulation of triglycerides in macrophages that ultimately accelerates their transformation into foam cells thus contributing to the rapid formation of atheroma and the accelerated atherosclerosis characteristic for diabetic condition.

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References


