Transendothelial exchange of low-density lipoprotein is unaffected by the presence of severe atherosclerosis

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Received 29 March 2004; received in revised form 16 June 2004; accepted 28 June 2004

Available online 29 July 2004

Time for primary review 27 days

Abstract

Objective: We tested the hypothesis that transendothelial exchange of low-density lipoprotein (LDL) is influenced by the presence of severe atherosclerosis; we previously found this exchange elevated in diabetes patients.

Methods: By an in vivo isotope method, we compared transendothelial LDL exchange in 24 patients with angiographically verified coronary atherosclerosis, 11 patients with angiographically verified peripheral atherosclerosis, 60 patients with diabetes, and in 42 controls. Autologous 131-iodinated LDL (131I-LDL) and 125-iodinated albumin (125I-albumin) were injected intravenously (i.v.), and the 1-h fractional escape rates (FER_{LDL} and FER_{albumin}) were taken as indices of transendothelial exchange.

Results: Patients with coronary or peripheral atherosclerosis had FER_{LDL} similar to that of controls [4.3 (3.5–5.1) and 3.2 (2.3–4.1) versus 4.2 (3.7–4.7)%/h; P>0.05], even after adjustment for LDL distribution volume (DV_{LDL}). In contrast, diabetes patients had significantly higher FER_{LDL} than controls [5.2 (4.6–5.7) versus 4.2 (3.7–4.7)%/h; P<0.05]. This difference was even more pronounced after correction for the distribution volume of LDL. Compared with controls, FER_{albumin} was not elevated in patients with coronary atherosclerosis, possibly elevated in patients with peripheral atherosclerosis, but was elevated in diabetes patients. There was a tight positive correlation between FER_{LDL} and FER_{albumin} in all groups of patients and controls.

Conclusion: Transendothelial exchange of low-density lipoprotein is not elevated in patients having severe atherosclerosis. This suggests that the observed vascular leakiness in diabetes precedes and possibly contributes to accelerated atherosclerosis in diabetes.

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Keywords: Capillary permeability; Diabetes; Lipid metabolism; Lipoproteins

1. Introduction

Although hyperlipidemia is a well-established cause of atherosclerosis, its prognostic specificity and sensitivity for the development of cardiovascular disease are far from 100% [1]. We therefore hypothesized that transendothelial exchange of lipoproteins from blood to the vessel wall, in addition to lipoprotein levels in plasma, determines the degree of atherosclerosis [2]. According to this, the atherogenic effect of lipoproteins may be augmented if transendothelial exchange is high, and vice versa if transendothelial exchange is low.

We have developed a noninvasive in vivo isotope technique for estimating transendothelial exchange of low-density lipoprotein (LDL) in humans: we measure fractional...
escape rate of LDL from the intravascular compartment during 1-h after intravenous (i.v.) injection of radiolabeled autologous LDL (fractional escape rate of LDL, \( \text{FER}_{\text{LDL}} \)) [3]. Using this method, we previously found that type 1 and type 2 diabetic patients have higher \( \text{FER}_{\text{LDL}} \) than healthy controls [3,4], suggesting that the accelerated development of atherosclerosis in diabetes could partly be due to the increased vascular leakiness of lipoproteins that precedes atherosclerosis. This is analogous with previous observations of increased fractional escape rate of albumin (FER\(_{\text{alb}}\)) in patients with diabetes mellitus versus controls [5,6]. However, atherosclerosis with its many endothelial lesions could in itself be the cause of increased \( \text{FER}_{\text{LDL}} \), rather than increased \( \text{FER}_{\text{LDL}} \) leading to atherosclerosis: arterial lesions increase arterial LDL influx [7]. In accordance with this hypothesis, we have previously observed increased \( \text{FER}_{\text{alb}} \) in patients with severe atherosclerosis versus controls [2]. It is, however, unknown whether \( \text{FER}_{\text{LDL}} \) is also higher in atherosclerotic patients compared with healthy individuals.

In the present study, we tested the hypothesis that transendothelial exchange of low-density lipoprotein is influenced by the presence of severe atherosclerosis: we compared \( \text{FER}_{\text{LDL}} \) and \( \text{FER}_{\text{alb}} \) in patients with severe coronary or peripheral atherosclerosis with values in diabetic patients as well as in healthy controls. We also measured LDL diameter and distribution volumes of both LDL and albumin (DV\(_{\text{LDL}}\) and DV\(_{\text{alb}}\)), as this could affect FER values.

2. Methods

2.1. Subjects

We studied four groups of subjects with similar age and sex distributions: 24 nondiabetic patients with angiographically verified coronary atherosclerosis, i.e., coronary heart disease (CHD); 11 nondiabetic patients with angiographically verified peripheral atherosclerosis, i.e., peripheral arterial disease (PAD); 42 clinically healthy subjects recruited from the Copenhagen City Heart Study, a major epidemiological general population study of cardiovascular disease and risk factors [8,9]; and 60 patients with diabetes mellitus (27 with type 1 diabetes and 33 with type 2 diabetes). Among the diabetes patients, 21 (35%) had clinical symptoms of CHD or PAD, but the diagnosis was only verified by angiography in 5 (8%). Insulin was instituted in 48 (80%) of the diabetes patients, whereas the rest were on oral antidiabetic agents; 7 (12%) received beta adrenoceptor blockers; 11 (18%) calcium channel blockers; 16 (27%) angiotensin-converting enzyme inhibitors or angiotensin II-receptor blockers; and 8 (13%) statins. In patients with CHD, 10 (42%) received beta adrenoceptor blockers; 7 (29%) calcium channel blockers; 18 (75%) angiotensin-converting enzyme inhibitors or angiotensin II-receptor blockers; and 18 (75%) statins. In patients with PAD, 2 (18%) received calcium channel blockers and 2 (18%) angiotensin-converting enzyme inhibitors or angiotensin II-receptor blockers; whereas none were on beta adrenoceptor blockers or statins. All participants gave written informed consent. The study was in accordance with the Declaration of Helsinki and was approved by the Copenhagen and Frederiksberg Ethics Committee (file no. 01-302/97). The study was surveyed by the Danish National Department of Isotope Pharmacy who permitted investigation of maximally two subjects per week.

2.2. Procedures

Fractional escape rates of low-density lipoprotein (FER\(_{\text{LDL}}\)) and albumin (FER\(_{\text{alb}}\)) were measured by means of plasma decay curves 1 h after intravenous injection of autologous 131-iodinated LDL (\( ^{131}\text{I}-\text{LDL} \)) and commercially available 125-iodinated human serum albumin (\( ^{125}\text{I}\)-albumin), respectively.

LDL was isolated from 100 ml of blood by sequential ultracentrifugation at 4 °C in solvent densities of 1.019 and 1.050 g/ml, respectively, at 50,000 rpm for 20 h in a Beckman 50.4 Ti rotor. 131-Iodination of LDL was done with 18.5 MBq 131-iodine using iodine monochloride [10]. The iodination efficiency was 25±5% (\( n=54 \)) which corresponds to 52±13 cpm/ng LDL protein. In the labeled preparations, 98.6±0.4% of the \( ^{131}\text{I} \) radioactivity was precipitable with 15 vol./vol.% trichloroacetic acid (TCA), and 5.6±0.5% of the \( ^{131}\text{I} \) radioactivity was lipid-soluble, i.e., extractable into chloroform–methanol (1:1, vol/vol). In fixed-density ultracentrifugation analysis of labeled LDL in the presence of added carrier plasma, ≥96% of the total radioactivity was in the LDL density range of 1.019–1.063 g/ml. No evidence of fragmentation of the labeled LDL was detected using a 3% to 8% Tris-Acetate Gradient Gel followed by autoradiography. In all labeled preparations, test for sterility was negative, and <5 pg of pyrogenes was detectable per milliliter sample.

The participants met at 0800 h after an 8-h fast and tobacco abstinence. A 17-G Teflon cannula was inserted in an antecubital vein in both arms, one for blood sampling and one for injection. After 30-min rest at recumbency, the preparation containing \( ^{131}\text{I}-\text{LDL} \) (700 kBq) and \( ^{125}\text{I} \)-albumin (500 kBq; code IFE-IT.23S or IFE-IT.20S, Isopharma, Kjeller, Norway (free \( ^{125}\text{I} \) radioactivity <1.5±0.2%) was injected intravenously. Venous blood samples of 10 ml were drawn without stasis in heparinized tubes before and at 10, 20, 30, 40, 50, and 60 min after injection. Proteins in plasma (3 ml) and doses (0.1 ml with 2.9 ml unlabeled plasma added) were precipitated at 4 °C with TCA at a final concentration of 15 vol.%/vol.%. Following mixing and centrifugation, total radioactivity as well as radioactivity in the supernatant was counted for 20 min in a double-channel gamma counter (1282 Compugamma, LKB, Wallac, Turku, Finland). For both tracers, the TCA-precipitable radioactivity at each time point was plotted versus time after injection.

We also measured LDL diameter and distribution volumes of both LDL and albumin (DV\(_{\text{LDL}}\) and DV\(_{\text{alb}}\)), as this could affect FER values.

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logarithmic transformation. FER_{LDL} and FER_{abl} (in %/h) were then calculated on the basis of the slopes ($\beta$) of the best linear curves fitted by the least squares method using the formula $(1-60e^{-\beta \times 100\%})$, thus assuming that radioactivities declined monoexponentially with time (1-compartment system). Distribution volumes of LDL (DV_{LDL}) and albumin (DV_{abl}=plasma volume) were calculated from the amounts of injected radioactivities divided by the plasma radioactivities at time zero, as derived from the intercepts of the fitted lines for the two tracers, respectively. The obtained DV values were corrected for body surface area ($m^2$) by the formula $0.007184 \times weight^{0.425} \times height^{0.725}$. Because there could be an inflection point of the decay curves after only 30 min, curve fits of both 60 and 30 min after reinjection were used for calculations. Finally, the absolute net efflux of LDL cholesterol, $J_{DL}$ in mmol/(m$^2 \times h$) was calculated by the formula $FER_{LDL} \times DV_{LDL} \times$ plasma LDL cholesterol.

The contribution of receptor mediated elimination of LDL from the intravascular compartment to $FER_{DL}$ during the 1-h blood sampling period was elucidated by comparing $FER_{DL}$ with $FER_{gly-LDL}$ in three humans without diabetes mellitus [3]. Glycosylated LDL is not recognized by LDL receptors [11,12], and thus the difference between $FER_{DL}$ and $FER_{gly-LDL}$ represents receptor elimination. Glycosylation of LDL was performed as previously described by others [11,13,14]. Autologous $^{131}$I-LDL (700 kBq) and autologous $^{125}$I-Gly-LDL (500 kBq) was reinjected under similar conditions as described above. Venous blood samples of 10 ml were drawn without stasis in heparinized tubes before and at 10, 20, 30, 40, 50, and 60 min after reinjection. Eleven additional blood samples were obtained during the subsequent 6 days. Plasma was precipitated with TCA and counted for radioactivity as described above. For both tracers, the logarithmically transformed TCA-precipitable radioactivity was plotted versus time, and FER_{DL} and FER_{gly-LDL} were calculated as previously described. Mean $FER_{DL}$ was about 1%/h higher than mean $FER_{gly-LDL}$ (3.6±1.1%/h vs. 2.6±1.1%/h). Moreover, fractional catabolic rates, $FCR_{DL}$ and $FCR_{gly-LDL}$ (in %/h), were calculated according to Matthews [15] using the formula $(C_1/\beta_1+C_2/\beta_2)^{-1}$, where $\beta_1$ and $\beta_2$ are slopes and $C_1$ and $C_2$ intercepts of the late and initial linear curve fits, respectively. Mean $FCR_{DL}$ was about two times higher than mean $FCR_{gly-LDL}$ ($P<0.01$), documenting that Gly-LDL was indeed glycosylated.

The use of a 1-compartment system for calculation of $FER_{DL}$ was validated by comparing $FER_{DL}$ with transvascular LDL permeability as described by Matthews [15], which takes into account an extravascular protein compartment, receptor-mediated metabolism, and excretion. In eight subjects without diabetes, blood samples were collected every 10 min during the first hour and subsequently once a day during the next week upon reinjection of autologous $^{131}$I-LDL. Transvascular LDL permeability using the multicompartment model was calculated by the formula $C_1 C_2 (\beta_2-\beta_1)^2/(C_1 \beta_2+C_2 \beta_1)$ [15]. There was a positive correlation between $FER_{DL}$ using 1-compartment kinetics and transvascular LDL permeability using multicompartment kinetics ($R^2=0.41; n=8$; one-sided $P<0.05$). The equation for the linear correlation was 1-compartment $FER_{DL}=0.43 \times$ multicompartment $FER_{DL}+2.8$ (all in %/h). The overestimation of $FER_{DL}$ by 1-compartment kinetics was most pronounced in the lower range.

Finally, we correlated $FER_{DL}$ to $FCR_{DL}$ in 27 subjects, where $FCR_{DL}$ was calculated by multicompartmental analysis using the SAAM II software (SAAM Institute, Seattle, WA) [16]. Because there was no correlation between $FER_{DL}$ and $FCR_{DL}$ ($R<0.01; n=27; P=0.99$), it is unlikely that metabolism, i.e., receptor-mediated elimination of LDL, contributes to $FER_{DL}$ to any significant degree.

Plasma concentrations of total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides, and blood concentration of glucose were all measured by commercially available assays (Roche Diagnostics, Mannheim, Germany) using a Hitachi analyzer. Plasma concentration of insulin was measured by a fluoroimmunoassay. Fraction of glycosylated hemoglobin in blood, hemoglobinA1c, was measured by high-performance liquid chromatography. LDL particle diameter was measured by nondenaturing pore gradient gel electrophoresis as previously described [3,17]. All blood samples were drawn after an 8-h fast and tobacco abstinence. Systolic and diastolic blood pressures were measured by auscultation using a manometre and an appropriately sized cuff. Body mass index (BMI; kg/m$^2$) was calculated as weight/height$^2$.

### 2.3. Statistical analysis

Comparisons between groups were performed by Student’s $t$-test, analysis of variance (ANOVA), or the $\chi^2$ test. Factors associated with $FER_{DL}$ were analyzed by linear regression analyses. Plasma triglycerides, plasma insulin, and blood hemoglobinA1c were log-transformed prior to the analyses because of nonnormal distribution. $P$-values<0.05 were significant. We used the SPSS version 11.0 statistical software package.

### 3. Results

Characteristics of the four study groups are given in Table 1. Patients with peripheral arterial disease (PAD) had higher systolic blood pressure, plasma triglycerides, plasma C-reactive protein, and proportion of smokers than controls. Patients with coronary heart disease (CHD) had higher body mass index, plasma triglycerides, plasma insulin, and plasma C-reactive protein and lower plasma LDL cholesterol and HDL cholesterol than healthy controls. Diabetes patients had higher systolic blood pressure, body mass index, plasma
Table 1
Characteristics of patients with diabetes mellitus, coronary heart disease (CHD), peripheral arterial disease (PAD), and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=42)</th>
<th>CHD (n=24)</th>
<th>PAD (n=11)</th>
<th>Diabetes (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (%)</td>
<td>76 (63–89)</td>
<td>88 (75–100)</td>
<td>82 (59–100)</td>
<td>77 (66–88)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52 (46–55)</td>
<td>55 (52–58)</td>
<td>60 (55–65)</td>
<td>53 (49–56)</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>33 (19–47)</td>
<td>50 (30–70)</td>
<td>73 (47–99)</td>
<td>33 (21–44)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>119 (114–124)</td>
<td>121 (115–127)</td>
<td>140 (132–149)*</td>
<td>134 (128–139)*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75 (71–78)</td>
<td>72 (67–76)</td>
<td>81 (72–93)</td>
<td>76 (73–79)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.5 (23.5–25.5)</td>
<td>27.0 (25.6–28.4)</td>
<td>27.0 (26.0–28.0)</td>
<td>27.0 (26.0–28.0)</td>
</tr>
<tr>
<td>Plasma total cholesterol (mmol/l)</td>
<td>5.2 (5.0–5.4)</td>
<td>4.8 (4.3–5.3)</td>
<td>5.3 (4.8–5.8)</td>
<td>5.2 (4.8–5.6)</td>
</tr>
<tr>
<td>Plasma LDL cholesterol (mmol/l)</td>
<td>3.4 (3.2–3.6)</td>
<td>2.9 (2.5–3.3)</td>
<td>3.3 (2.9–3.7)</td>
<td>3.0 (2.8–3.2)*</td>
</tr>
<tr>
<td>Plasma LDL cholesterol (mmol/l)</td>
<td>3.16 (1.22–1.50)</td>
<td>1.06 (0.94–1.18)</td>
<td>1.18 (0.98–1.38)</td>
<td>1.35 (1.23–1.47)</td>
</tr>
<tr>
<td>Plasma triglycerides (mmol/l)</td>
<td>0.8 (0.7–0.9)</td>
<td>1.5 (1.1–1.9)*</td>
<td>1.6 (1.1–2.2)*</td>
<td>1.0 (0.9–1.3)</td>
</tr>
<tr>
<td>Blood hemoglobin A1c (%)</td>
<td>5.7 (5.6–5.8)</td>
<td>5.9 (5.8–6.0)</td>
<td>6.2 (5.8–6.6)</td>
<td>8.6 (8.2–8.9)*</td>
</tr>
</tbody>
</table>

Continuous variables are shown by means or geometric means (data not shown). To increase the statistical power for triglycerides than controls (Tables 1 and 2).

The mean plasma decay curves for 131I-LDL in patients with CHD+PAD, diabetes, and healthy controls are given in Fig. 1: evaluation of these raw data suggests that iodinated LDL disappears faster from plasma in diabetes patients versus controls, but not in patients with CHD+PAD versus controls.

Patients with CHD or PAD had similar FERLDL as healthy controls, irrespective of adjustment for differences in DVLDL (Table 2 and Fig. 2). Due to variations in DVLDL between groups, this difference was even more pronounced after correction for DVLDL (Table 2 and Fig. 2), and it remained statistical significance after adjustment for systolic blood pressure, body mass index, plasma insulin, hemoglobinA1c, C-reactive protein, plasma LDL cholesterol, LDL diameter, and plasma triglycerides (data not shown). To increase the statistical power for comparison with controls, CHD and PAD patients were combined because both groups are thought to have universal atherosclerosis: FERLDL in CHD+PAD patients did not differ from that in controls, unadjusted or adjusted for DVLDL (Fig. 2).

Diabetes patients had significantly higher FERLDL than controls (Table 2 and Fig. 2). Due to variations in DVLDL between groups, this difference was even more pronounced after correction for DVLDL (Table 2 and Fig. 2), and it remained statistical significance after adjustment for systolic blood pressure, body mass index, plasma insulin, hemoglobinA1c, C-reactive protein, plasma LDL cholesterol, LDL diameter, and plasma triglycerides (data not shown). To increase the statistical power for comparison with controls, CHD and PAD patients were combined because both groups are thought to have universal atherosclerosis: FERLDL in CHD+PAD patients did not differ from that in controls, unadjusted or adjusted for DVLDL (Fig. 2).

Table 2
Fractional escape rates (FER), distribution volumes (DV), and absolute net efflux (J) of LDL and albumin, and LDL size in patients with diabetes mellitus, coronary heart disease (CHD), peripheral arterial disease (PAD), and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=42)</th>
<th>CHD (n=24)</th>
<th>PAD (n=11)</th>
<th>Diabetes (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FERLDL60 (%/h)</td>
<td>4.2 (3.7–4.7)</td>
<td>4.3 (3.5–5.1)</td>
<td>3.2 (2.3–4.1)</td>
<td>5.2 (4.6–5.7)*</td>
</tr>
<tr>
<td>FERLDL60 (%/h)</td>
<td>6.1 (5.0–7.2)</td>
<td>7.3 (5.5–9.1)</td>
<td>6.4 (4.9–7.9)</td>
<td>8.3 (7.2–9.4)</td>
</tr>
<tr>
<td>DV1LDL60 (l/m²)</td>
<td>2.39 (2.30–2.48)</td>
<td>2.55 (2.42–2.69)*</td>
<td>1.85 (1.65–2.05)*</td>
<td>2.19 (2.13–2.26)*</td>
</tr>
<tr>
<td>DV2LDL60 (l/m²)</td>
<td>2.37 (2.28–2.46)</td>
<td>2.48 (2.34–2.62)</td>
<td>1.78 (1.51–2.05)*</td>
<td>2.20 (2.13–2.27)*</td>
</tr>
<tr>
<td>LDL diameter (nm)</td>
<td>24.2 (24.1–24.3)</td>
<td>24.1 (23.9–24.2)</td>
<td>24.1 (23.9–24.3)</td>
<td>24.0 (23.9–24.1)*</td>
</tr>
<tr>
<td>FERab30 (%/h)</td>
<td>5.6 (5.0–6.2)</td>
<td>5.6 (5.0–6.2)</td>
<td>5.6 (4.8–6.4)</td>
<td>6.4 (5.8–6.9)</td>
</tr>
<tr>
<td>FERab30 (%/h)</td>
<td>6.6 (5.5–7.7)</td>
<td>7.2 (5.7–8.7)</td>
<td>9.0 (8.0–10.0)*</td>
<td>9.1 (8.0–10.2)*</td>
</tr>
<tr>
<td>DVab30 (l/m²)</td>
<td>2.36 (2.27–2.45)</td>
<td>2.49 (2.36–2.62)</td>
<td>1.83 (1.63–2.03)*</td>
<td>2.15 (2.08–2.21)*</td>
</tr>
<tr>
<td>DVab30 (l/m²)</td>
<td>2.35 (2.26–2.44)</td>
<td>2.44 (2.30–2.58)</td>
<td>1.75 (1.48–2.02)*</td>
<td>2.16 (2.09–2.23)*</td>
</tr>
<tr>
<td>Jab30 [mmol/m²*h]</td>
<td>2.4 (2.1–2.7)</td>
<td>2.3 (2.0–2.6)</td>
<td>3.2 (2.5–3.9)*</td>
<td>3.0 (2.7–3.3)*</td>
</tr>
<tr>
<td>Jab30 [mmol/m²*h]</td>
<td>2.9 (2.4–3.4)</td>
<td>2.9 (2.4–3.4)</td>
<td>5.4 (4.1–6.7)*</td>
<td>4.3 (3.8–4.8)*</td>
</tr>
</tbody>
</table>

FER, DV, and J are calculated both 30 and 60 min after injection of the tracers. Data are means with 95% confidence intervals in parentheses.

* P<0.001 compared with controls.
§ P<0.05 compared with controls.
¶ P<0.005 compared with controls.
‖ P<0.01 compared with controls.
FERLDL was similar in diabetes patients with or without clinical symptoms of CHD or PAD (data not shown). After correction for DVab, FERab was also elevated in diabetes patients compared with controls (Table 2 and Fig. 3). This was also the case in patients with PAD, whereas patients with CHD had FERab similar to healthy controls (Table 2). However, CHD+PAD patients had FERab similar to that of controls (Fig. 3).

FERLDL and FERab were generally higher when only the initial 30 min of the decay curves were used, which is in support of a possible inflection point at about 30 min after reinjection. However, the relative differences between groups were of similar magnitude as the corresponding 60-min values (Table 2). Net efflux of LDL cholesterol from the plasma compartment, JLDL, was due to a lower DVLDL not elevated in diabetes patients versus controls, but significantly reduced in patients with PAD (Table 2).

There was a tight positive correlation between FERLDL and FERab in patients with CHD+PAD, diabetes patients, as well as in healthy controls (Fig. 4). This was true for unadjusted values, values adjusted for differences in distribution volumes of LDL and albumin, respectively, as well as values adjusted for other covariates as listed above (latter not shown).

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Apart from blood glucose lowering agents, lesser medication was instituted in diabetes patients than in
patients with CHD or PAD. The use of insulin or oral antidiabetic agents was not associated with FER.

4. Discussion

The present study has shown that patients with presence of clinical atherosclerosis in either coronary or lower extremity arteries have transendothelial exchange of low-density lipoprotein (measured as FER_{LDL}) similar to that of healthy controls, while patients with diabetes mellitus have higher FER_{LDL} values than controls. It is therefore not likely that increased transendothelial LDL exchange in diabetes is due to atherosclerosis per se but rather is due to some other factors in the diabetes state. This could be generalized vascular leakiness of macromolecules, i.e., increased permeability, which is not seen in nondiabetic subjects. Our observations are particularly important because they, for the first time, support that vascular LDL leakiness could precede development of atherosclerosis in diabetes and therefore may help explain the accelerated atherogenesis seen in diabetes. The reason why FER_{LDL} is not increased in nondiabetic patients with atherosclerosis could be that such patients have only localized arterial lesions in contrast to diabetes patients who may have microvascular as well as macrovascular leakiness.

It is well recognized that diabetes patients have more atherosclerosis than controls [18]. Generally, patients with type 1 diabetes are at an increased coronary heart disease risk only if they have renal disease [19], although FER_{LDL} was not correlated to diabetes duration, microalbuminuria, or renal disease [4]. This is different from type 2 diabetes where insulin resistance predominates and the risk of atherosclerosis starts very early [20]. Because atherosclerotic lesions per se increase arterial LDL influx to intima [7], it is important to exclude that the larger FER_{LDL} in diabetes patients versus controls [3,4] is not simply caused by atherosclerosis, rather than the opposite. As patients with PAD or CHD likely have more atherosclerosis than diabetes patients in the present study, our demonstration of elevated FER_{LDL} in diabetics, but not in those with PAD or CHD who have severe atherosclerosis, excludes that increased transendothelial LDL exchange in diabetes is caused by severe atherosclerosis. Therefore, vascular LDL leakiness in diabetes likely may lead to accelerated atherogenesis, as increased arterial permeability to LDL is associated with later increased development of atherosclerosis after exposure to hyperlipidemia [21].
An alternative explanation of elevated FER\textsubscript{LDL} in diabetes patients may be that FER\textsubscript{LDL} reflects metabolism of LDL rather than transendothelial exchange. However, at least four points are against this possibility:

1. The difference in FER\textsubscript{LDL} between diabetes patients and controls remained unaffected after adjustment for plasma LDL levels. If metabolism was operating, the number of hepatic LDL receptors and consequently uptake of labeled LDL would depend on the concentration of unlabeled LDL in plasma \[22\];
2. the distribution volume of LDL was smaller in diabetes patients than in controls, excluding that uptake of LDL by LDL receptors should be larger in diabetes patients versus controls;
3. not only FER\textsubscript{LDL} but also FER\textsubscript{ab} was elevated in diabetes patients, and FER\textsubscript{ab} cannot be influenced by LDL metabolism; and
4. there was no relationship between FER\textsubscript{LDL} and FCR\textsubscript{LDL}.

Glycosylation of LDL in patients with diabetes could also influence LDL receptor elimination in diabetes patients versus controls. Thus, glycosylated LDL is not recognized by LDL receptors \[11,12\], as confirmed by the significantly lower FCR\textsubscript{Gly-LDL} than FCR\textsubscript{LDL} in our validation experiments. We therefore studied the contribution of receptor elimination to FER\textsubscript{LDL} by measuring FER\textsubscript{Gly-LDL} and FER\textsubscript{LDL} simultaneously. FER\textsubscript{Gly-LDL} was about 1%/h lower than FER\textsubscript{LDL} in nondiabetic humans, indicating that receptor elimination contributes to FER\textsubscript{LDL} only by approximately 1%/h (corresponding to about one-third of FER\textsubscript{LDL}). This is in accordance with the overestimation of FER\textsubscript{LDL} by 1–3%/h when our 1-compartment model is used compared with a multicompartment model which subtracts the contribution of metabolism \[15\]. It is also in accordance with the slightly bigger distribution volume of LDL than albumin. Importantly, because glycosylation of LDL particles may be increased—although far below 100%—in diabetes patients due to the hyperglycemic milieu, LDL glycosylation does not explain the difference in FER\textsubscript{LDL} between diabetic patients and healthy subjects observed in this study, but rather the difference may be underestimated.

Yet another possibility is that the smaller LDL diameter in diabetes patients may explain the difference in FER\textsubscript{LDL} between diabetic patients and controls. Small LDL enters faster into the vessel wall than large LDL \[10\], and small LDL is associated with increased risk of coronary heart...
disease [23]. However, some arguments do not support this possibility:

1. LDL diameter was not (inversely) correlated with \( F_{\text{ER,LDL}} \);
2. not only \( F_{\text{ER,LDL}} \) but also \( F_{\text{ER,alb}} \) was elevated in diabetes patients versus controls, and \( F_{\text{ER,alb}} \) cannot be influenced by LDL diameter; and
3. the difference in \( F_{\text{ER,LDL}} \) between diabetes patients and controls remained unaffected after adjustment for LDL diameter.

Because \( F_{\text{ER,LDL}} \) was also independent of systemic arterial blood pressure and endothelial surface area as reflected by the plasma volume, we suggest the elevated \( F_{\text{ER,LDL}} \) in diabetes patients to result mainly from increased transvascular permeability. This could be a consequence of endothelial cell death or damage [24–26] due to, e.g., hyperinsulinemia [27–29], or to circulating advanced glycation end products (AGE) inducing transvascular hyperpermeability [30–33]. However, other authors have observed increased intimal LDL accumulation before the formation of AGE and suggest a direct effect of hyperglycemia on the vessel wall [34].

The main proportion of transendothelial LDL exchange probably takes place in the capillaries. However, there exists indirect evidence for similar exchange of albumin and lipoproteins in capillaries and arteries [35]. Furthermore, the tight positive correlation between \( F_{\text{ER,LDL}} \) and \( F_{\text{ER,alb}} \) observed in this study is analogous with the correlation between the transport of LDL and albumin across the arterial wall in rabbits [36]. The significantly lower \( F_{\text{ER,LDL}} \) than \( F_{\text{ER,alb}} \) is in accordance with the three-times-larger diameter of LDL particles than of albumin.

In conclusion, this human in vivo study has shown that transendothelial exchange of LDL particles from the intravascular compartment is unaffected by presence of severe atherosclerosis. In contrast, among patients with diabetes mellitus, this exchange was elevated. We believe that this difference is likely due to increased vascular leakiness of LDL in diabetes patients, potentially leading to increased LDL accumulation in the vessel wall and thus progressed atherosclerosis. Our study could not identify this mechanism in nondiabetic individuals with symptomatic and angiographically verified atherosclerosis, suggesting that this phenomenon is unique for diabetes patients.

Acknowledgements

The study was funded by the Danish Heart Foundation, the Danish Diabetes Association, the Novo Nordisk Foundation, the Copenhagen University Hospitals (H:S) Research Foundation, the Danish Medical Association Research Fund, the A. P. Møller Foundation for the Advancement of Medical Science, Bayer, the Eli Lilly Diabetological Research Foundation, the Danish Foundation of Fight Against Circulatory Diseases, the Boserup Foundation, the Aage and Johanne Louis-Hansen Foundation, the Karl G. Andersen Foundation, the Kathrine and Vigo Skovgaard Foundation, the Jacob Madsen Foundation, the Lauritz Peter Christensen Foundation, the P. A. Messerschmidt Foundation, the König-Petersen Foundation, and the Bjørnow Foundation.

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