Transvascular Low-Density Lipoprotein Transport in Patients With Diabetes Mellitus (Type 2)

A Noninvasive In Vivo Isotope Technique

Karen Kornerup, Børge Grønne Nordestgaard, Bo Feldt-Rasmussen, Knut Borch-Johnsen, Kurt Svarre Jensen, Jan Skov Jensen

Objective—The increased risk of atherosclerosis associated with diabetes cannot be explained by conventional cardiovascular risk factors alone. We hypothesized that transvascular lipoprotein transport may be increased in patients with diabetes, possibly explaining increased intimal lipoprotein accumulation and, thus, atherosclerosis.

Methods and Results—We developed an in vivo method for measurement of transvascular transport of low density lipoprotein (LDL) and applied it in 16 patients with maturity-onset diabetes (type 2) and 29 healthy control subjects. Autologous $^{131}$I-labeled LDL was reinjected intravenously in addition to $^{125}$I-labeled albumin, and the 1-hour fractional escape rates were taken as indices of transvascular transport. Both parameters were normally distributed, and they were tightly correlated ($R^2=0.69, P<0.0001$). Transvascular LDL transport was $5.4\pm2.9$%/h and $4.1\pm1.5$%/h in patients with diabetes and control subjects, respectively ($P<0.05$); equivalent values for albumin were $6.5\pm2.5$%/h and $5.3\pm1.6$%/h ($P<0.05$). This difference most likely was not caused by altered hepatic LDL receptor expression, glycosylation of LDL, small LDL size, nephropathy, statin use, or different plasma insulin levels in diabetic patients.

Conclusions—Transvascular LDL transport may be increased in patients with type 2 diabetes. This suggests that lipoprotein flux into the arterial wall is increased in people with diabetes, possibly explaining the accelerated development of atherosclerosis. (Arterioscler Thromb Vasc Biol. 2002;22:1168-1174.)

Key Words: low density lipoprotein ■ transvascular transport ■ lipoprotein size ■ diabetes mellitus

Diabetes mellitus is the most serious individual risk factor of atherosclerotic vascular disease, leading to coronary heart disease, stroke, and peripheral arterial insufficiency. This strong association is probably not solely explained by diabetes-related atherosclerotic risk factors, such as hyperlipidemia, hypertension, obesity, hyperglycemia, and hyperinsulinemia. An additional explanation may come from increased transvascular sieving of macromolecules such as albumin and lipoproteins in the diabetic state, as suggested from the increased urinary albumin excretion and increased transcapillary escape rate (TER) of albumin (TERalb) observed in individuals with diabetes versus control subjects. This could augment the atherogenic effect of lipoproteins by increased intimal deposition.

The transvascular transport of lipoproteins has predominantly been studied in animal models, in which the accumulation of lipoproteins is measured in excised arterial tissue. Alternatively, TERalb is a well-established in vivo method to assess the transvascular (mainly transcapillary) transport of albumin in humans by its fractional disappearance from the intravascular compartment during 1 hour after intravenous injection. We have previously shown that TERalb is elevated in patients with diabetes and in patients with severe atherosclerosis. However, it is unknown whether LDL also has a higher escape rate in diabetic and atherosclerotic patients compared with healthy individuals. Whereas the initial disappearance of albumin from the intravascular compartment takes place by transvascular transport exclusively, the initial disappearance of LDL perhaps is due to transvascular transport and hepatic receptor–mediated elimination via LDL receptors. Consequently, in the present study, we designate the variable under study as fractional escape rate (FER) instead of TER.

The present study of patients with maturity-onset diabetes mellitus (type 2) and healthy control subjects had the following purposes: (1) development and validation of a method to measure FER of LDL (FERLDL), (2) assessment of the contribution of receptor elimination to FERLDL, (3) description of the distribution of FERLDL and Fer of albumin (FERalb), (5)
TABLE 1. Characteristics of Patients With Type 2 Diabetes and of Controls

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Diabetes Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/Women</td>
<td>25/4</td>
<td>14/2</td>
</tr>
<tr>
<td>Age, § y</td>
<td>57 (42–65)</td>
<td>58 (48–67)</td>
</tr>
<tr>
<td>Smokers/Nonsmokers</td>
<td>10/19</td>
<td>4/12</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>118±15</td>
<td>142±15*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>75±11</td>
<td>81±10†</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.1±2.7</td>
<td>28.8±4.4*</td>
</tr>
<tr>
<td>Plasma total cholesterol, mmol/L</td>
<td>5.3±0.6</td>
<td>5.2±1.0</td>
</tr>
<tr>
<td>Plasma LDL cholesterol, mmol/L</td>
<td>3.5±0.6</td>
<td>3.2±0.8</td>
</tr>
<tr>
<td>Plasma triglycerides,</td>
<td></td>
<td>mmol/L</td>
</tr>
<tr>
<td>Plasma insulin,</td>
<td></td>
<td>pmol/L</td>
</tr>
<tr>
<td>Blood glucose, mmol/L</td>
<td>4.9±0.1</td>
<td>9.2±0.7*</td>
</tr>
<tr>
<td>Blood hemoglobinA₁₀₀, %</td>
<td>5.7 (5.5–5.9)</td>
<td>8.5 (8.0–9.5)*</td>
</tr>
</tbody>
</table>

Data are mean±SD except for § which is median (range) and † which are medians (quartiles).

*P<0.001; †P<0.005; ‡P<0.05 (Student unpaired t test).

This table does not include the 3 people reported on in Table 2 or the 8 people reported on in Figure 1.

comparison of FER_LDL between patients with diabetes and healthy control subjects, and (6) determination of variables associated with FER_LDL, including the particle size of LDL.

Methods

Subjects

We studied 16 patients with type 2 diabetes mellitus (14 men and 2 women, age range 48 to 67 years); all were recruited from the Steno Diabetes Center in Copenhagen. Type 2 diabetes was defined according to World Health Organization criteria as onset after the age of 40 years. Any chronic disease (with possible influence on transvascular transport of macromolecules, such as found in inflammation, infection, or malignancy) other than diabetes or its related complications constituted exclusion criteria. Among these 16 patients, 3 had coronary heart disease, 2 had nephropathy, 7 had retinopathy, and 8 had peripheral arterial insufficiency. Twelve were on insulin therapy, 8 received peroral antidiabetic agents, 4 received diuretics.

In addition, we studied 29 clinically healthy individuals with age and sex distributions similar to those of the patients (25 men and 4 women, age range 42 to 65 years). Healthy individuals were recruited from the Copenhagen City Heart Study, a major epidemiological population study of cardiovascular disease and risk factors. None of the 29 control subjects received any medication or had any familial predisposition to atherosclerosis. All participants gave written informed consent. Characteristics of patients and control subjects are given in Table 1. The present 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 National Department of Isotope Pharmacy, which permitted the investigation of maximally 2 subjects per week.

FER_LDL and FER_alb

FER_LDL and FER_alb were measured by means of plasma decay curves during 1 hour after intravenous injection of autologous iodinated LDL (¹²⁵I-LDL) and commercially available iodinated human serum albumin (¹²⁵I-albumin), respectively.

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Intravenous Injection of ¹²⁵I-LDL and ¹²⁵I-Albumin

Participants met at 8:00 AM after an 8-hour fast and tobacco abstinence. A 17-gauge polytetrafluoroethylene (Teflon) cannula was inserted in an antecubital vein in both arms, one for blood sampling and one for injection. After 30 minutes of rest at recumbency, the preparation containing ¹²⁵I-LDL (700 kBq) and ¹²⁵I-albumin (500 kBq, code IFE-IT.235 or IFE-IT.20S, Isopharma AS) was injected intravenously. Venous blood samples of 10 mL were drawn without stasis into heparinized tubes before and at 10, 20, 30, 40, 50, and 60 minutes 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 to a final concentration of 15% (vol/vol). After the mixing and centrifugation, total radioactivity, as well as radioactivity in the supernatant, was counted for 20 minutes in a double-channel γ-counter (1282 Compugamma, LKB, Wallac).

Calculations

For both tracers, the TCA-precipitable radioactivity at each time point was plotted versus time after logarithmic transformation. FER_LDL and FER_alb (as percentage per hour) were then calculated on the basis of the slopes (b) of the best linear curves fitted by the least squares method with the formula 1–60×β×100%, because the radioactivities declined monoexponentially with time, suggestive of a 1-compartment system during this initial phase. Distribution volumes (DV) of LDL (DV_LDL) and albumin (DV_ab [plasma volume]) were calculated from the amounts of injected radioactivities divided by the plasma radioactivities at time 0, as derived from the intercepts of the fitted lines for the 2 tracers, respectively. The obtained DV values were corrected for body surface area (m²) by the formula 0.007184×weight[0.668×height[0.40]⁻¹].

LDL Particle Size

To determine whether FER_LDL measurements are influenced by LDL particle size, particle diameters of LDL in all participants were measured as described by Nordestgaard and Zilversmit with minor modifications: 100 mL of blood was drawn into tubes containing Na₂-EDTA (final plasma concentration 1.2 mg/mL), benzamidine (10 mg/mL), a-amino-n-caproic acid (1.3 mg/mL), and aprotinin (10 kallikrein units/mL); all were from Sigma Chemical Co. Sequential ultracentrifugation at 4°C was performed in solvent densities of 1.019 g/mL and then 1.050 g/mL at 50 000 rpm for 20 hours in a Beckman 50.4 Ti rotor; fractions were collected by tube puncture. LDLs [density 1.019 to 1.050 g/mL to exclude Lp(a)] were equilibrated to PBS containing 0.1 mg/mL Na₂-EDTA by prepacked PD-10 columns (Amersham Pharmacia Biotech) and were passed through a 0.22-μm filter (Millipore SGLV R25 LS). The protein concentration in isolated LDL was estimated from the absorbance at 220 nm, with serum albumin used as a standard.

Iodination of LDL (5 mg protein) was performed with 18.5 MBq (Amersham Pharmacia Biotech and New England Nuklear) with the use of iodine monochloride. Unbound iodine was removed with a PD-10 column equilibrated with PBS containing 0.1 mg/mL Na₂-EDTA. The preparation of labeled LDL was immediately added to 3 mL of a 200-g/L human albumin solution (Statens Seruminstitut) and passed through a 0.22-μm filter. The iodination efficiency was 25.7% (n=45), which corresponds to 52±20 cpm/μg LDL protein. In the labeled preparations, 98.6±0.4% of the ¹²⁵I radioactivity was precipitable with 15% (vol/vol) trichloroacetic acid (TCA), and 5.6±0.5% of the ¹²⁵I radioactivity was lipid soluble, ie, 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 to 1.063 g/mL. No evidence of fragmentation of the labeled LDL was detected with the use of a 3% to 8% Tris-Acetate Gradient Gel (NuPage catalogue No. EA0375, Novex), followed by autoradiography. In all labeled preparations, tests for sterility were negative, and <5 μg pyrogen was detectable per milliliter sample (Coaest-Endotoxin, Chromogenix).
determined on commercially available 3% to 8% Tris-Acetate Gradient Gels (NuPage catalogue No. EA0375, Novex) as described by Krauss and Burke.23 with minor modifications. Standards of known diameter (apoferritin and thyroglobulin, HMW Electrophoresis Calibration Kit, Amershams Pharmacia Biotech) and an isolated LDL standard were included on each gel. The particle diameter of the LDL standard was determined by negative staining electron microscopy.26 Apoferritin, thyroglobulin, and LDL standards, a frozen LDL quality control, and isolated autologous LDL were diluted 1:2 in native sample buffer (No. LC2672, Novex), and 10 μL was applied to the gel. Electrophoresis was conducted in native running buffer (No. LC2672, Novex) for 5.5 hours at 150 constant voltage in an Xcell II apparatus (Novex) and stained by Bio-Safe Coomassie (Bio-Rad Laboratories). Gels were scanned, and LDL peak sizes were estimated against a standard curve created from the standards of known diameter: the dominant peak in the LDL standard, 23.5 nm; thyroglobulin, 17.0 nm, and apoferritin 12.2 nm. For unknown LDL samples having a multiple-peak profile, the mean diameter was calculated by using the migration distance of each peak multiplied by its respective area. The LDL quality control sample (stored in aliquots at −20°C) had a coefficient of variation on size measurement throughout the entire study of 1.0%, which corresponds to a mean ± SD of 23.75 ± 0.24 nm (n = 26 gels).

**Contribution of Receptor Elimination: Normal LDL (FER L DL ) Versus Glycosylated LDL (FER G L DL )**

To assess the contribution of receptor-mediated elimination of LDL from the intravascular compartment to FER L DL during the 1-hour blood sampling period, we compared FER L DL with FER of glycosylated LDL (FER G L DL ) in 5 rabbits and 3 humans free of diabetes mellitus. Glycosylated LDL (Gly-LDL) is not recognized by LDL receptors25; thus, the difference between FER L DL and FER G L DL represents receptor elimination.

Glycosylation of LDL was performed as described by Sasaki and Cottam.27 Bitheimer et al.28 and Witztum et al.30 with minor modifications. Sterile LDL (final concentration 2.5 to 5.0 mg/mL of LDL protein) was incubated under sterile conditions for 5 days at 37°C in PBS, pH 7.4, containing Na2-EDTA (0.1 mg/mL), 200 mmol/L glucose, and 30 mmol/L sodium cyanoborohydride (all from Sigma). Unbound reactants were removed, and the Gly-LDL preparation was equilibrated to PBS containing Na2-EDTA by prepacked PD-10 columns. A second aliquot of the original native LDL was incubated without the addition of glucose but otherwise identical conditions. Gly-LDL was labeled with 125I and normal LDL was labeled with 131I as described above. After iodination, <2 ng cyanide was detectable per milliliter sample. Tests for sterility were negative, and <5 pg pyrogen was detectable per milliliter sample. In the labeled preparations, >96% of the radioactivity in both lipoproteins was precipitable with TCA, and 6.8 ± 0.1% and 5.6 ± 0.5% of the radioactivity in Gly-LDL and LDL, respectively, was lipid soluble, ie, extractable into chloroform/methanol (1:1 vol/vol). In fixed-density ultracentrifugation analysis of labeled LDL in the presence of added carrier plasma, >93% of the total radioactivity was found in the LDL density range (from 1.019 to 1.063 g/mL). An aliquot of native plasma, native LDL, 131I-LDL, and 125I-Gly-LDL from the same individual was applied to a 0.8% agarose gel; electrophoresis was conducted in a barbitral buffer (0.05 mol/L, pH 8.6) for 2 hours at 125 constant voltage; and the gel was stained by a saturated solution of Sudan black. There were no significant differences in gel mobility between the β-lipoprotein band of plasma, native LDL, and 131I-LDL. The mobility of 125I-Gly-LDL was increased, as previously reported by Witztum et al.30

**Intravenous Injection of 131I-LDL and 125I-Gly-LDL**

A preparation containing human 131I-LDL (700 kBq) and 125I-Gly-LDL (500 kBq) was injected into an ear vein in 5 rabbits of the Danish Country strain (Statens Seruminstitut, Copenhagen, Denmark; weight 3.4 ± 0.4 kg). From the opposite ear, blood samples were drawn before and at 10, 20, 30, 40, 50, and 60 minutes after injection. Ten additional blood samples were obtained during the next 96 hours.

A preparation containing autologous 131I-LDL (700 kBq) and autologous 125I-Gly-LDL (500 kBq) was reinjected into 3 humans under conditions similar to those 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 minutes 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.

**Calculations**

For both tracers, the logarithmically transformed TCA-precipitable radioactivity was plotted versus time, and FER L DL and FER G L DL were calculated as previously described. Moreover, fractional catabolic rates (FCRs) of LDL and Gly-LDL (FCR L DL and FCR G L DL, respectively; as percentage per hour) were calculated according to Matthews31 by using the formula: ((C1/b1 + C2/b2)− 1)/(C1/b1 + C2/b2).11

**Validation of 1-Compartment Model**

To validate the assumption of the 1-compartment model for measurement of FER L DL, we compared measures of FER L DL as described above by measures of transvascular LDL permeability as described by Matthews,31 which takes into account an extravascular protein compartment and a urine and feces compartment and, thus, includes receptor-mediated metabolism. In 8 subjects without diabetes, blood samples were collected every 10th minute during the first hour and subsequently once a day during the next week on reinjection of autologous 131I-LDL. Transvascular LDL permeability with the use of the multicompartment model was calculated by the following formula: C1(C2/b2 − b1)/b1(C1/b1 + C2/b2).31

**Other Measurements**

Plasma concentrations of total cholesterol, LDL cholesterol, and triglycerides and blood concentration of glucose were all measured by commercially available assays (Roche Diagnostics, GmbH) with the use of a Hitachi analyzer. Plasma concentration of insulin was measured by a fluoroinmunossay. The fraction of glycosylated hemoglobin in blood, hemoglobin A1c, was measured by high-performance liquid chromatography. All blood samples were drawn after an 8-hour fast and tobacco abstinence. Systolic and diastolic blood pressures were measured by auscultation with the use of a manometer and an appropriately sized cuff. Body mass index (kg/m2) was calculated as weight/height2.

**Statistical Analysis**

Comparisons between groups were performed by Student t test, ANOVA, or the χ2 test. Factors associated with TER L DL were analyzed by linear regression analyses, allowing for the presence of diabetes. Plasma triglycerides, plasma insulin, and hemoglobin A1c were logarithmically transformed before the analyses, because of non-normal distribution. Values of P < 0.05 were considered significant and were always 2-sided, unless otherwise stated.

**Results**

**Contribution of Receptor Elimination: FER G L DL and FCR G L DL Compared With FER L DL and FCR L DL**

The results of experiments in nondoniabetic rabbits (n = 5) and humans (n = 3, all males, aged 23, 28, and 51 years) are shown in Table 2. In rabbits, mean FER L DL was >5%/h higher than mean FER G L DL (P < 0.001). In humans, however, mean FER L DL was only 1%/h higher than mean FER G L DL, and this difference in humans was not statistically significant. Mean
FCR LDL was \( \approx 2 \) times higher than mean FCR Gly-LDL in rabbits and humans \((P<0.01)\), documenting that Gly-LDL was indeed glycosylated.

Validation of 1-Compartment Model for Measurement of FER LDL

There was a positive correlation between FER LDL with the use of 1-compartment kinetics and transvascular LDL permeability with the use of multicompartment kinetics \((R^2=0.41, n=8, 1\text{-sided } P<0.05; \text{Figure 1})\). The equation for the linear correlation was as follows: 1-compartment FER LDL = 0.43 \times \text{multicompartment FER LDL} + 2.8 (all given in \%/h). The overestimation of FER LDL by 1-compartment kinetics was most pronounced in the lower range.

Distributions of FER LDL and FER alb

Frequency histograms of FER LDL and FER alb show normal distribution of both parameters in controls \((n=29; \text{Figure 2})\). The distributions in diabetic patients alone did not reject the assumption of normal distribution (data not shown).

Comparison and Association Between FER LDL and FER alb

There was a tight positive correlation between FER LDL and FER alb \((R^2=0.70, 1\text{-sided } P<0.0001, \text{adjusted for the presence of diabetes by inclusion of diabetes as a dummy variable; Figure 3})\). This correlation was independent of the presence of diabetic nephropathy, statin use, plasma insulin levels, or any of the variables listed in Table 1. The equation for the linear correlation was as follows: FER LDL = 0.88 \times \text{FER alb} - 0.53 (all given in \%/h). FER LDL was lower than FER alb; the mean difference was 1.2 \pm 1.2\%/h \((P<0.001)\). This was the case in diabetic patients and in healthy control subjects (Figure 3).

Comparison of FER LDL and FER alb Between Diabetic Patients and Control Subjects

Compared with control subjects, diabetic patients exhibited significantly higher values of FER LDL and FER alb (Table 3 and Figure 4). These differences remained statistically significant after adjustment for the presence of diabetic nephropathy, use of statins, or plasma insulin levels. In contrast, DV LDL and DV alb were both larger in control subjects than in diabetic patients. DV LDL was slightly larger than DV alb; the mean difference was 0.028 \pm 0.003 L/1.73 m² \((P<0.001)\). The LDL diameter was smaller by 0.5 nm in diabetic patients versus control subjects (Table 3).

Association of FER LDL With Other Variables

FER LDL was tested for correlations with the variables listed in Table 1: the only significant correlation found was a negative correlation between FER LDL and plasma LDL cholesterol \((R^2=0.20, P<0.001, \text{adjusted for presence of diabetes, by the inclusion of diabetes as an independent dummy variable in a linear regression analysis})\). There was no correlation between FER LDL and LDL size or between FER LDL and DVs of LDL

| TABLE 2. FERs and FCRs of Native LDL and Glycosylated LDL (Gly-LDL) in Nondiabetic Rabbits and Humans |
|---------------------------------------------------|---------|---------|---------|---------|
| Rabbits \((n=5)\)                                 | 16.2 \pm 7.6 | 10.8 \pm 7.1* | 6.6 \pm 2.1 | 2.8 \pm 0.5† |
| Humans \((n=3)\)                                  | 3.6 \pm 1.1  | 2.6 \pm 1.9  | 1.9 \pm 0.2  | 0.9 \pm 0.3‡ |

Data are mean \pm SD.

*\(P<0.001\); †\(P<0.01\); ‡\(P<0.05\) (Student paired \(t\) test).

Figure 1. Scatter plot of the relationship between FER LDL assuming a 1-compartment model and transvascular LDL permeability assuming a multicompartment model in 8 healthy subjects. \(R^2=0.41; \text{one-sided } P<0.05; \text{1-compartment FER LDL} = 0.43 \times \text{multicompartment FER LDL} + 2.8 \) (all in \%/h).

Figure 2. Distributions of FER LDL (upper histogram) and FER alb (lower histogram) in healthy subjects \((n=29)\). Normal distributions are superimposed for comparison.

Figure 3. Scatter plot of the relationship between FER LDL and FER alb in type 2 diabetic patients \((n=16, \text{closed circles})\) and healthy controls \((n=29, \text{open circles})\). \(R^2=0.69; \text{one-sided } P<0.0001\) (adjusted for presence of diabetes); FER LDL = 0.88 \times \text{FER alb} - 0.53 (all in \%/h).
and diabetes. Indeed, the relationship between FERLDL and diabetes was accentuated if variations in the DVs of LDL or albumin. Indeed, the relationship between FERLDL and diabetes was accentuated if variations in the DVs of LDL or albumin. Finally, among diabetic patients, FERLDL was not correlated with diabetic hemoglobin A1c or with plasma insulin levels. Finally, among diabetic patients, FERLDL was not correlated with diabetic hemoglobin A1c or with plasma insulin levels.

**Discussion**

The present clinical study has shown evidence of an increased efflux of FERLDL from the intravascular compartment in type 2 diabetic patients (n=16) compared with healthy control subjects. This may contribute to the high degree of atherosclerosis and coronary heart disease in diabetes mellitus, a risk that is independent of plasma lipoprotein concentrations and other conventional atherosclerotic risk factors.1 The mechanism explaining our findings could be that elevated FERLDL reflects increased intimal influx and deposition of lipoproteins in arteries.11,12

An alternative possibility is that FERLDL reflects the metabolism of LDL rather than transvascular transport, in spite of the fact that FERLDL was measured for only 1 hour after injection of labeled LDL. The inverse relationship between plasma LDL concentration and FERLDL in diabetic patients and healthy subjects suggests that receptor elimination of LDL may operate even within the first hour, because the number of hepatic LDL receptors and, consequently, the uptake of labeled LDL would be downregulated by high concentrations of unlabeled LDL in plasma.20 However, because diabetic patients and control subjects had similar plasma LDL levels, increased downregulation of LDL receptors in control subjects versus diabetic patients seems to be an unlikely explanation for the observed difference in FERLDL between the 2 groups. Furthermore, the difference in FERLDL between diabetic patients and control subjects was independent of plasma insulin levels and the use of statins, both of which have been reported to increase LDL metabolism.29,32,33

Glycosylation of LDL in patients with diabetes could also influence LDL receptor elimination in diabetic patients versus control subjects. Thus, glycosylated LDL is not recognized by LDL receptors,27,28 as confirmed by the significantly lower FCRGly-LDL than FCRLDL in rabbits and humans in our experiments. Therefore, we studied the contribution of receptor elimination to FERLDL by measuring FCRGly-LDL and FERLDL simultaneously. In nondiabetic rabbits, in which LDL receptors are highly upregulated,34 FERGly-LDL was ~5%/h lower than FERLDL, whereas this difference was only ~1%/h in nondiabetic humans, indicating that receptor elimination contributes to FERLDL by ~1%/h only (corresponding to approximately one third of FERGly-LDL). This is in accordance with the overestimation of FERLDL by 1%/h to 3%/h when our 1-compartment model is used compared with a multicompartment model, which subtracts the contribution of metabolism.35 It is also in accordance with the only slightly bigger DV of LDL than albumin. Importantly, however, because glycosylation of LDL particles may be increased in diabetic patients as a result of the hyperglycemic milieu, LDL glycosylation will not explain the difference in FERLDL between diabetic patients and healthy subjects observed in the present study; rather, the difference may be underestimated.

Yet another possibility is that differences in LDL size may explain the difference in FERLDL between diabetic patients and control subjects. Compared with large LDL, small LDL enters faster into the vessel wall,24 and small LDL is associated with an increased risk of coronary heart disease.35 In support of this possibility is the fact that LDL size was smaller in diabetic patients than in control subjects, as also observed by others.36 However, some arguments do not support this possibility: (1) LDL size was not (inversely) correlated with FERLDL; (2) not only FERLDL but also FERalb was elevated in diabetic patients versus control subjects, and FERalb cannot be influenced by LDL size; and (3) the difference in FERLDL between diabetic patients and control subjects remained unaffected after adjustment for LDL size (data not shown).

Because FERLDL was also independent of systemic arterial blood pressure and endothelial surface area, as reflected by the plasma volume, we suggest that the elevated FERLDL in type 2 diabetic patients may result mainly from increased transvascular permeability. This could be a consequence of endothelial cell death,37–42 or of circulating advanced glycation end products inducing transvascular hyperpermeability.43–46 However, other authors have observed increased intimal LDL accumulation before the formation of advanced glycation end products, and they suggest a direct effect of hyperglycemia on the
positive correlation between FER LDL and FER albumin observed in arteries. Furthermore, the tight determination of LDL standard particle size.

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