Diabetic LDL Triggers Apoptosis in Vascular Endothelial Cells

Michaela Artwohl,1 Wolfgang F. Graier,2 Michael Roden,1 Martin Bischof,1 Angelika Freudenthaler,1 Werner Waldhäusl,1 and Sabina M. Baumgartner-Parzer1

This study compares the effects of LDL glycated either in vitro (LDLiv) or in vivo in diabetic patients (LDLd) on apoptosis, proliferation, and associated protein expression in cultured human umbilical vein endothelial cells. At 100 mg/l, both LDL species considerably increase apoptosis (LDLiv 63%, LDLd 40%; P < 0.05) compared with intraindividual nonglycated LDL subfractions. Considering its lower degree of glycation (LDLiv 5–10%, LDLd 42%), LDLd’s relative proapoptotic activity is 2.7-fold greater than that of LDLiv. Glycated LDL-induced apoptosis is associated with increased expression of apoptosis promoters (LDLiv: bak 88%, CPP-32 49%; LDLd: bak 18%, CPP-32 11%; P < 0.05) and is attenuated by caspase inhibitors. Glycated LDL’s antiproliferative activity (LDLiv −34%, LDLd −9%; P < 0.01) relates to reduction (P < 0.05) of cyclin D3 (LDLiv −27%, LDLd −24%) and of hyp- (LDLiv −22%, LDLd −19%) and hyperphosphorylated (LDLiv −53%, LDLd −22%) retinoblastoma protein and is paralleled by reduced expression of endothelial nitric oxide synthase (LDLiv −30%, LDLd −23%). In response to lipoprotein lipase, LDLd more markedly triggers endothelial apoptosis (27.1-fold) compared with LDLiv, suggesting that LDLd owns a higher potential for endothelial cell damage than LDLiv. The observed behavior of LDLd versus LDLiv could be of clinical importance and well relate to differences in structure and cellular uptake of LDLd compared with LDLiv. Diabetes 52:1240–1247, 2003

Nonenzymatic glycation of LDL naturally occurs in all individuals due to condensation of reducing sugars with the apoB moiety of LDL particles. LDL glycation is increased in diabetic patients because of their elevated plasma glucose concentrations (1–6). The toxicity of glycated LDL (7) and its role in the pathogenesis of atherosclerosis appears to relate to its prolonged presence in the circulation (8) due to its impaired cellular uptake (9,10). Glycated LDL increases adhesion molecule expression (11) and modulates the fibrinolytic potential of vascular endothelial cells (9). Furthermore, both in vitro– and in vivo–glycated (diabetic) LDL alter endothelial vasoactive response by attenuating shear stress–induced l-arginine uptake and nitric oxide (NO) synthesis (12).

Endothelial dysfunction plays a key role in the development of diabetic angiopathy (13,14), which is characterized by the increased turnover and exaggerated proliferation (neoangiogenesis) of the vascular endothelium and by procoagulant as well as proadhesive activity of apoptotic endothelial cells (15–18).

In contrast to other diabetes associated “proatherogenic” risk factors, such as hyperglycemia (13), hyperhomocysteinemia, and increased plasma free fatty acid concentrations, which trigger apoptosis in vascular endothelial cells (19–22), no proapoptotic potential has been described for glycated LDL.

Consequently, this study was designed 1) to evaluate the effects of glycated LDL on apoptosis and proliferation in vascular endothelial cells in the absence and presence of lipoprotein lipase (LPL), which, by binding to the luminal surface of endothelial cells, mediates the cellular uptake of glycated LDL (10), and 2) to compare the action of LDL glycated in vitro (LDLiv) with that of diabetic LDL (LDLd; glycated in vivo), the latter being assumed to possess a more pronounced atherogenic potential (23) than LDLiv.

RESEARCH DESIGN AND METHODS

Materials. M199, delipidated calf serum, butylated hydroxytoluene, diethylenetriamin-pentaacetic acid, sodium cyanoborohydride, trinitr-benzenesulfonic acid (TNBSA), LPL, Triton X-100, Nonidet P-40, NaVO3, β-glycerophosphate, phenylmethanesulfonyl fluoride, aprotinin, leupeptin, and collagenase were purchased from Sigma Chemical (St. Louis, MO); PBS, L-glutamine, pen/strep, fungizone, and FCS were from Hyclone (Logan, UT). Optimem 1 was from Gibco (Paisley, UK) and glucose solution for infusion (20%) from Mayrhofer Pharmaceuticals (Linz, Austria). BtE, EDTA, NH4COOH, NaCl, Na2SO4, MgCl2, NaOH, NaHCO3, Tris(hydroxymethyl)-aminomethan (Tris), and HEPES were purchased from Merck (Darmstadt, Germany). Glyco Gel II and HEPES were purchased from MicroQuest (Santa Cruz, CA; p53). Transduction Laboratories (Lexington, KY; p21WAF-1/Cip1, p27Kip, Cyclin D3, CPP-32, and endothelial NO synthase [eNOS]), and Calbiochem (Cambridge, MA; retinoblastoma protein [pRb], bcl-2, and bak). The caspase inhibitory peptides Z-VA-D-CHO were from Calbiochem (Cambridge, MA) and Bachem (Bubendorf, Switzerland), respectively.

Preparation of LDL. LDL was prepared from fasting EDTA plasma of healthy normolipidemic subjects (n = 17) and diabetic patients (13 men and 4 women, 1 type 1 diabetic patient, 16 type 2 diabetic patients, mean age 60 ± 9 years).
EDTA was present throughout the entire isolation procedure. In diabetic patients, mean values were 10.3 ± 1.5% (reference range 4.8–5.2%) for HbA1c, 229 ± 50 mg/dl (150–200) for cholesterol, 43 ± 10 mg/dl (45) for HDL, 142 ± 44 mg/dl (<130) for LDL, and 225 ± 153 mg/dl (172) for triglycerides. LDL was isolated by sequential ultracentrifugation in the presence of EDTA using standard techniques (10,12,24). In brief, after density adjustment of plasma to 1.063 g/ml with solid KBr and a centrifugation step (100,000g at 15°C for 18 h, 70,170 rpm) fixed angle rotor; Beckman Instruments, Fullerton, CA), floating lipoproteins (adjusted to ρ = 1.2 g/ml with KBr) were separated by centrifugation (230,000g at 15°C for 26 h, SW-40Ti swinging bucket rotor; Beckman Instruments) in a linear density gradient (ρ = 1.2–1.01 g/ml). The obtained LDL fraction (ρ = 1.019–1.063 g/ml) was covered with nitrogen to prevent oxidation and dialysed for 3 days at 4°C in the dark against 2 l PBS containing 0.01% EDTA, with daily changes of dialysis solution. Total cholesterol, representing the concentration of isolated LDL, was measured by a standard procedure (CHOD-PAP method).

In vitro glycation of LDL. LDL isolated from the plasma of healthy volunteers was separated into glycated and nonglycated LDL subfractions by Glyco Gel II boronate affinity chromatography.

The nonglycated LDL fraction (diluted to 1 mg/ml total cholesterol) was then incubated (1 week at 37°C in the dark) in PBS containing 2 mg/ml EDTA, 25 mMol/l butylated hydroxytoluene, and 50 mMol/l diethylenetriamin-penta-acetic acid with either various concentrations of ρ-glucose (range 30–350 mMol/l; LDLα) or without ρ-glucose added (LDLβ) under nitrogen (10,12). Nonglycated control cultures (LDLα) were dialysed with N2 and incubated at 37°C for 24 or 48 h against 2 l PBS containing 0.01% EDTA, with daily changes of dialysis solution, stored under N2 at 4°C in the dark, and used in 1 week.

Separation of nonglycated (LDLα) fractions from LDLβ. LDL isolated from the plasma of diabetic patients (LDLNa) was divided into two portions, of which one was applied to Glyco Gel II boronate affinity chromatography column (using the monoclonal antibody mabOB/04 raised against copper binding sites (27,28)) for separation of nonglycated LDL (LDLα). Both LDLα and the nonglycated “control” fraction (LDLc), were covered with N2, dialysed, and stored as described above.

Determination of LDL glycation. The degree of glycation was determined using TNBSA, which couples with primary amines and peptides in aqueous solution to give trinitrophenyl derivates (25). In brief, 50 μl LDLα and 50 μl LDLβ, both containing 50 μg total cholesterol, were mixed with 50 μl TNBSA (0.1%) and 1 ml NaHCO3 (4%, pH 8.5). Solutions were covered with nitrogen and incubated at 37°C for 2 h in the dark. Samples were read at 340 nm against a mixture of LDL, NaHCO3, and distilled H2O (instead of TNBSA) on a UV/VIS Liquid Scintillation Analyser; Canberra Packard, Meriden, CT). Results are expressed as percentage of the respective intraindividual control (set to 100%). Samples were tested in quadruplicates.

In situ apoptosis staining. Human umbilical vein endothelial cells (HUVECs) were isolated in vitro–glycated (LDLβ) or diabetic LDL (LDLNa) in Optimem 1 supplemented with 5% delipidated bovine serum and antibiotics. For control experiments, each HUVEC culture was exposed to 100 μg/ml diethylenetriamine-penta-acetic acid with either 30, 100, and 350 mg/dl glucose, respectively (Fig. IA).

Results

Preparation of LDL glycated LDL. Mean concentrations of isolated LDL were 5.1 ± 1.1 g/l cholesterol for healthy donors (n = 17) and 3.4 ± 1.3 g/l for diabetic patients (n = 17). Oxidation products were not detectable in any of the LDL preparations (data not shown). Mean glycation of LDL isolated from healthy donors and subsequently glycated in vitro were 0, 5 ± 1, 19 ± 1.6, and 42 ± 3% upon exposure to 0, 30, 100, and 350 mMol/l glucose, respectively (Fig. 1A).

Apoptosis. Depending on the degree of glycation, in vitro glycated LDL (LDLβ) increased apoptosis in HUVECs (n = 4) up to 163% compared with LDLα (set to 100%; P < 0.05), as determined by DNA fragmentation assays (data not shown) and in situ staining (Fig. 1B–D).

LPL (100 units/ml) per se did not affect basal apoptosis of HUVECs (91 ± 6% of control without LPL added, set to 100%), but reduced that triggered by LDLβ from 163 to 113 ± 9% of control (LDLα + LPL set to 100%; n = 4, P < 0.01; data not shown in figures). Diabetic LDL (LDLNa) increased apoptosis in HUVECs after 48 h up to 140% of LDLα (set to 100%, P < 0.01) (Fig. 2A and B) in the absence of LPL, as documented by both [3H]thymidine assays (Fig. 2A) and in situ staining (Fig. 2B). By adding LPL, LDLNa triggered apoptosis was further increased to 184% of...
control (LDLN + LPL set to 100%, P < 0.001) (Fig. 2A and B) in a time-dependent fashion (Fig. 2A). LDLN-induced apoptosis was reduced close to baseline (110 ± 9%/n = 5, P < 0.05) by the caspase inhibitors Ac-DEVD-CHO (100 μmol/l) and Z-VAD-FMK (40 μmol/l).

In pilot experiments (n = 2; data not shown in figures), exposure (24 h) of individual HUVEC cultures to high LDLN concentrations (500 mg/l) increased rates of apoptosis to 125% (99% at 100 mg/l) (compare Fig. 2A) in the absence of LPL and to 155% (140% at 100 mg/l) (compare Fig. 2A) in the presence of LPL, each compared with 500 mg/l LDLN, suggesting some concentration dependency of LDLN action.

**Proliferation.** In vitro-glycated LDL (LDLgv) reduced proliferation of HUVECs in both the absence (−34%, P < 0.01) and presence (−37%, P < 0.001) of LPL (100 units/l) in a glycation-dependent fashion, when compared with LDL0 (Fig. 3A). LPL (100 units/l) per se did not affect HUVEC proliferation (98 ± 7% of control without LPL added, set to 100%).

Diabetic LDL (LDLd) slightly reduced proliferation of HUVECs in the absence (−9%, P < 0.01), but not the presence, of LPL (Fig. 3B) when compared with LDLN action.

**Apoptosis-related protein expression.** In vitro-glycated LDL (LDLgv) increased protein expression of the apoptosis promotor bak (Fig. 4A and C) and CPP-32 (Fig. 4B and D) in a glycation-dependent fashion to 188% and 149%, respectively (P < 0.05 compared with LDL0, set to 100%). These effects were blunted by LPL (data not shown), as was apoptosis. In contrast to bak and CPP-32, cellular expression of bcl-2, a prominent inhibitor of apoptosis, remained unaffected by LDLgv (111 ± 17% of LDL0; data not shown within figures).

Diabetic LDL (LDLD) likewise upregulated (P < 0.05) bak (18%) (Fig. 5A) and CPP-32 (11%) (Fig. 5B), which were somewhat further increased upon addition of LPL (bcl-2 and CPP-32 22%, P < 0.05 compared with control [= LDLN + LPL]). However, LDLD, like LDLgv, did not affect bcl-2 expression in HUVECs (110 ± 7% of LDL0; data not shown within figures).

**Cell cycle-related protein expression.** HUVECs exposed to in vitro glycated LDL (LDLgv) reduced (P < 0.05) cyclin D3 expression (Fig. 6A) in both the absence (−27%) and presence of LPL (−34%) in a glycation-dependent manner. In parallel, hypo- (−LPL: −22 ± 6%; +LPL: −27 ± 8%; data not shown within figures) and hyperphosphorylated (−LPL: −53%; +LPL: −61%) (Fig. 6C) forms of the
pRb were reduced by glycated LDL ($P < 0.05$), while the tumor suppressor p53 and the inhibitors of cyclin-dependent kinases p21$^{WAF-1/Cip1}$ and p27$^{kip}$ remained unaffected (data not shown).

Diabetic LDL (LDL$_D$) reduced ($P < 0.05$) expression of cyclin D3 ($-24\%$, Fig. 6B) and of both hypo- ($-19 \pm 3\%$; data not shown within figures) and hyperphosphorylated ($-22\%$) (Fig. 6D) forms of pRb in the absence of LPL, but not in its presence (Fig. 6B and D; data of hypophosphorylated pRb are not shown within figures). Expression of the cell-cycle checkpoint molecules p53, p21$^{WAF-1/Cip1}$, and p27$^{kip}$ was not affected by LDL$_D$ (data not shown).

**Vasoactive proteins.** eNOS expression was reduced in HUVECs by both LDL$_{iv}$ ($-30\%$, $P < 0.05$) (Fig. 7A) and LDL$_{iv}$ ($-23\%$, $P < 0.001$) (Fig. 7B) compared with the respective control cultures exposed to LDL$_0$ or LDL$_N$ (set to 100%), respectively. This effect was augmented by addition of LPL for LDL$_{iv}$ ($-51\%$, $P < 0.001$ vs. LDL$_0$ + LPL) (Fig. 7A) but not for LDL$_D$ ($-23\%$, $P < 0.05$ vs. LDL$_N$ + LPL) (Fig. 7B).

**DISCUSSION**

This study demonstrates that both in vitro (LDL$_{iv}$) and in vivo glycated (LDL$_D$ [diabetic]) LDLs reduce proliferation and trigger apoptosis in HUVECs. Glycated LDL’s proapoptotic activity already occurs at a concentration (100 mg/l) corresponding to $\sim 10\%$ of that seen in normal human plasma and further increases with higher concentrations, as shown for 500 mg/l (half of the normal plasma concentration). From this, it appears that glycated LDL could contribute to endothelial dysfunction in vivo and thus could be critically involved in the acceleration and pathogenesis of diabetic angiopathies (13,14,23), as reported for other inducers of endothelial apoptosis, such as...
high glucose (13,19), oxidized LDL (30,31), free fatty acids (22), and homocysteine (21). Endothelial cells undergoing apoptosis impair the intact endothelial monolayer and barrier function, trigger plaque erosion as well as rupture, and can lead to coronary thrombosis due to provision of proadhesive and procoagulatory activity (14,18,32).

The present study was performed in HUVECs that are readily available and represent an established in vitro model for endothelial cells. Although preliminary data obtained at our laboratory suggest LDL<sub>rv</sub>'s antiproliferative and LDL<sub>rv</sub>'s proapoptotic activity to also relate to other types of human endothelial cells (adult venous and microvascular endothelial cells; M.A., unpublished observations), it cannot be excluded that endothelial cells originating from other vascular beds might show a different response.

FIG. 5. Protein expression of bak (A) and CPP-32 (B) in HUVECs (n = 6) exposed to diabetic LDL (100 mg/l, 48 h) compared with control cultures incubated with the respective nonglycated LDL subfractions (LDL<sub>n</sub>), set to 100%. Experiments were carried out in the presence and absence of LPL (100 units/l). *P < 0.05 vs. LDL<sub>n</sub>.

FIG. 6. Protein expression of cyclin D3 (A and B) and hyperphosphorylated pRb (C and D) in HUVECs (n = 6) after their exposure (48 h) to 100 mg/l in–vitro glycated (A and C) and diabetic (B and D) LDL compared with control cultures incubated with the respective nonglycated LDL subfractions (LDL<sub>n</sub> or LDL<sub>m</sub>), set to 100%. Experiments were carried out in the presence and absence of LPL (100 units/l). *P < 0.05, **P < 0.01, ***P < 0.001 vs. LDL<sub>n</sub> (A and C) or LDL<sub>m</sub> (B and D).
Since oxidation products were not detectable in any of the LDL preparations, the observed action of glycated LDL is not attributable to LDL oxidation. Notably, although diabetes-associated oxidative stress is assumed to account for oxidation of lipoproteins, recent data suggest that neither diabetic nor in vitro–glycated LDL per se show any significant degree of oxidation (12,33,34). These observations could relate to the presence of 1) defense systems as antioxidants and metal ion binding proteins present in plasma and 2) the antioxidants EDTA and butylated hydroxytoluene throughout the isolation and in vitro glycation procedures. In vivo, LDL’s oxidative modification appears to predominantly occur in the arterial intima, being in line with the presence of oxidized LDL in atherosclerotic lesions (35). Since LDL\textsubscript{\textregistered} and LDL\textsubscript{\textsuperscript{iv}} differ in their action against the endothelium, one could speculate that such differences relate to their different oxidizability in vascular cells.

Compared with LDL\textsubscript{\textsuperscript{iv}} preparations, which were glycated up to 42%, glycation of LDL in diabetic patients, previously shown to correlate with HbAl\textsubscript{c} (1,4–6), is lower and ranges from 5 to 10% only (1,5,6). Thus, in relation to its degree of glycation, LDL\textsubscript{\textregistered} exhibits a 2.7-fold higher proapoptotic activity in endothelial cells than LDL\textsubscript{\textsuperscript{iv}}.

Apoptosis induced by LDL\textsubscript{\textsuperscript{iv}}, as well as by LDL\textsubscript{\textsuperscript{iv}} in the presence of LPL, is associated with increased expression of the apoptosis promotors bak and CPP-32 (synonym for caspase-3) (Table 1). These proteolytically activated cystein proteases are responsible for the destruction of the cellular architecture during apoptosis and for detachment and subsequent clearance of apoptotic cells from the surrounding tissue (36,37). The major importance of caspasas in glycated LDL-evoked endothelial cell death is confirmed by the finding that HUVECs are rescued from LDL\textsubscript{\textsuperscript{iv}}-induced apoptosis by caspase inhibitors such as Ac-DEVD-CHO (inhibiting caspase-3, -6, -7, -8, and -10) and Z-VAD-FMK (inhibiting caspase-1, -3, -4, and -7) in the presence and absence of LPL.

Since LDL\textsubscript{\textsuperscript{iv}}’s proapoptotic activity exceeds that of LDL\textsubscript{\textsuperscript{iv}}, by 2.7-fold (in the absence of LPL) when corrected for the degree of glycation, LDL\textsubscript{\textsuperscript{iv}}-induced apoptosis is associated with relatively lower bak and CPP-32 expression (Table 1) when compared with LDL\textsubscript{\textsuperscript{iv}}. LDL\textsubscript{\textsuperscript{iv}}-triggered apoptosis could thus involve additional, yet unidentified, mechanisms. However, neither LDL\textsubscript{\textsuperscript{iv}} nor LDL\textsubscript{\textsuperscript{iv}} affected the expression of bcl-2, a prominent inhibitor of apoptosis (28). The resulting increased ratio of bak/bcl-2 favors a proapoptotic state and is thus in line with the observed phenomena.

LDL\textsubscript{\textsuperscript{iv}}-induced apoptosis (163% of control) is reduced (113% of control) by addition of LPL, as is the associated expression of bak and CPP32. However, in the presence of LPL, LDL\textsubscript{\textsuperscript{iv}}’s proapoptotic activity (352.8% when corrected for the degree of glycation) clearly exceeds (27.1-fold) that of LDL\textsubscript{\textsuperscript{iv}}.

While diabetic LDL has higher proapoptotic potential compared with in vitro glycated LDL, the antiproliferative activities of LDL\textsubscript{\textsuperscript{iv}} and LDL\textsubscript{\textsuperscript{iv}} are comparable considering the latter’s lower degree of glycation (Table 1). Since diabetic LDL exhibits its antiproliferative activity in the absence but not the presence of LPL, it remains elusive whether its antiproliferative activity has a role in the development of vascular endothelial dysfunction in vivo, where LPL is bound to the endothelium.

Reduction of cyclin D3 and hypo- and hyperphosphorylated pRb is exclusively seen in association with the antiproliferative activity exerted by LDL\textsubscript{\textsuperscript{iv}} (with or without LPL) and LDL\textsubscript{\textsuperscript{iv}} (without LPL) in HUVECs. Such findings suggest that the antiproliferative activity of both glycated LDLs is not mediated by increased LPL levels.

**TABLE 1**

<table>
<thead>
<tr>
<th>Degree of glycation</th>
<th>LDL\textsubscript{\textsuperscript{iv}}</th>
<th>LDL\textsubscript{\textsuperscript{iv}} in relation to LDL\textsubscript{\textsuperscript{iv}}</th>
<th>Efficacy of LDL\textsubscript{\textsuperscript{iv}} in relation to LDL\textsubscript{\textsuperscript{iv}}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>42%</td>
<td>to 10%</td>
<td></td>
</tr>
<tr>
<td>Apoptosis</td>
<td>63%</td>
<td>40%</td>
<td>2.7-fold</td>
</tr>
<tr>
<td>bak expression</td>
<td>88%</td>
<td>18%</td>
<td>0.86-fold</td>
</tr>
<tr>
<td>CPP-32 expression</td>
<td>40%</td>
<td>11%</td>
<td>0.94-fold</td>
</tr>
<tr>
<td>Proliferation</td>
<td>–34%</td>
<td>–9%</td>
<td>1.1-fold</td>
</tr>
<tr>
<td>cyclin D3 expression</td>
<td>–27%</td>
<td>–24%</td>
<td>3.7-fold</td>
</tr>
<tr>
<td>hypophosphorylated pRb expression</td>
<td>–22%</td>
<td>–19%</td>
<td>3.6-fold</td>
</tr>
<tr>
<td>hyperphosphorylated pRb expression</td>
<td>–53%</td>
<td>–22%</td>
<td>1.7-fold</td>
</tr>
<tr>
<td>eNOS expression</td>
<td>–30%</td>
<td>–23%</td>
<td>3.2-fold</td>
</tr>
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Degree of glycation: LDL\textsubscript{\textsuperscript{iv}} up to 10% vs. LDL\textsubscript{\textsuperscript{iv}}, 42%, i.e., *correction factor = 4.2.
LDL species relates to reduced expression of the growth factor sensor cyclin D3 and of pRb, particularly of its hyperphosphorylated form. It remains, however, unknown why the LDL_{D}\textsuperscript{a}–associated reduction of cyclin D3 and hyperphosphorylated pRb exceeds that induced by LDL_{v}, (3.7- and 3.6-fold, respectively) (Table 1) when corrected for the degree of glycation. Reduction of cyclin D3 is rate limiting for S-phase entry (38), and underphosphorylation of pRb results in diminished liberation and availability of E2F (39), a transcription factor that triggers cell progression through the cell cycle by induction of target genes (e.g. cyclins E and A and several DNA replication enzymes) (40,41). In contrast to other models of apoptosis and associated growth arrest (42), in subconfluent HUVECs, the tumor suppressor p53 and the inhibitors of cyclin-dependent kinases p21^{WAF-1,Cip1} and p27^{Kip} are affected by neither LDL_{v}, nor LDL_{D}. eNOS has been shown to be a prerequisite of vascular endothelial growth factor–induced endothelial proliferation (43) and to be reduced in association with endothelial apoptosis and growth arrest (27,44,45). The present study shows that although reduced by both LDL species, LDL_{D}'s induced reduction of eNOS is more pronounced in both the absence (3.2-fold) and presence (1.9-fold) of LPL when corrected for the degree of glycation.

Abrogation by LPL of LDL_{v}'s proapoptotic and LDL_{D}'s antiproliferative activity, but not of the respective reduction in eNOS expression (Table 2), however, excludes a regulatory role of eNOS in glyicated LDL's proapoptotic or antiproliferative responses of endothelial cells. Since reduction of eNOS expression could diminish NO bioavailability (12), LDL_{D} could well account for the impaired endothelial vasodilation seen in hyperglycemic states (46;47).

The effects elicited in the vascular endothelium by LDL_{D} are more pronounced than those induced by LDL_{v}, with respect to apoptosis (−LPL: 2.7-fold; +LPL: 2.1-fold) and reduction of eNOS expression (−LPL: 3.2-fold; +LPL: 1.9-fold). In addition, the proapoptotic and antiproliferative activity of LDL_{v} and LDL_{D} differs completely in response to LPL (Table 2). These findings suggest that the effects of LDL_{v} and LDL_{D} in vascular endothelial cells are caused by the molecules' different structure or their different cellular uptake, which is currently poorly characterized. In this context, it is worth noting that nonglycated LDL and LDL_{v} are taken up by either the LDL receptor–dependent pathway (nonglycated LDL) or nonclassical pathways that are independent of LDL receptor and LDL receptor–related protein (10). Such nonclassical pathways could involve LPL (10), surface glycosaminoglycans (10,48), or yet uncharacterized interactions of glycated LDL with the respective cell surface in the absence of LPL. The observation that binding, uptake, and degradation of moderately compared with highly in vitro–glycated LDL preparations are different (10) suggests that such differences might also exist for binding, oxidizability, and catabolism of in vivo–glycated diabetic LDL.

In conclusion, the present study shows that in the presence of LPL, the activity of LDL_{v} (isolated from the plasma of diabetic patients) clearly exceeds that of in vitro glycated LDL_{v}, with respect to apoptosis (27.1-fold) when corrected for the degree of glycation. From this it is apparent that in vitro–glycated LDL cannot generally be used as a model substance for diabetic LDL, which, due to its more pronounced activity, carries a higher potential for endothelial cell damage than in vitro–glycated LDL preparations.

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