Lipoprotein Lipase Mediates the Uptake of Glycated LDL in Fibroblasts, Endothelial Cells, and Macrophages

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The nonenzymatic glycation of LDL is a naturally occurring chemical modification of apolipoprotein (apo)-B lysine residues by glucose. Once glycated, LDL is only poorly recognized by lipoprotein receptors including the LDL receptor (LDL-R), the LDL-R–related protein (LRP), and scavenger receptors. Glycated LDL (gLDL) is a preferred target for oxidative modifications. Additionally, its presence initiates different processes that can be considered "proatherogenic." Thus, LDL glycation might contribute to the increased atherosclerotic risk of patients with diabetes and familial hypercholesterolemia. Here we investigate whether lipoprotein lipase (LPL) can mediate the cellular uptake of gLDL. The addition of exogenous LPL to the culture medium of human skin fibroblasts, porcine aortic endothelial cells, and mouse peritoneal macrophages enhanced the binding, uptake, and degradation of gLDL markedly, and the relative effect of LPL on lipoprotein uptake increased with the degree of apoB glycation. The efficient uptake of gLDL by LDL-R–deficient fibroblasts and LRP-deficient Chinese hamster ovary cells in the presence of LPL suggested a mechanism that was independent of the LDL-R and LRP. In macrophages, the uptake of gLDL was also correlated with their ability to produce LPL endogenously. Mouse peritoneal macrophages from genetically modified mice, which lacked LPL, exhibited a 75% reduction of gLDL uptake compared with normal macrophages. The LPL-mediated effect required the association of the enzyme with cell surface glycosaminoglycans but was independent of its enzymatic activity. The uptake of gLDL in different cell types by an LPL-mediated process might have important implications for the cellular response after gLDL exposure as well as the removal of gLDL from the circulation. *Diabetes* 50: 1643–1653, 2001

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acLDL, acetylated LDL; AGE, advanced glycation end product; apo, apolipoprotein; CHO, Chinese hamster ovary; cLDL, control LDL; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; DMEM, Dulbecco's minimal essential medium; FCS, fetal calf serum; FH-HSF, LDL receptor–deficient fibroblasts; gHDL, glycated HDL; gLDL, glycated LDL; gVLDL, glycated VLDL; mgLDL, moderately glycated LDL; HSF, human skin fibroblasts; ko-macrophages, macrophages from LPL-knockout mice; LDL-R, LDL receptor; LPDS, lipoprotein-deficient serum; LPL, lipoprotein lipase; LRP, LDL-R–related protein; PAEC, porcine aortic endothelial cell; PBS, phosphate-buffered saline; TCA, trichloroacetic acid; TC, total cholesterol; THL, tetrahydrolipstatin; TNBS, trinitrobenzenesulfonic acid; wt-macrophages, macrophages from wild-type mice.

Protein glycation is a nonenzymatic reaction of glucose with susceptible amino groups that occurs at a rate linearly related to the plasma glucose concentration. During glycation, glucose reacts with lysine residues of target proteins to form a labile Schiff's base. This product may further react into a more stable fructosamine that is characteristic of glycated proteins (1). Chemical modification of lysine residues of apolipoprotein (apo)-B_{100} for example by acetylation is known to decrease the recognition of LDL by the LDL receptor (LDL-R) (2). Similarly, in vitro glycated LDL (gLDL) is bound and internalized less efficiently than normal LDL in different cell types including fibroblasts (3,4) and endothelial cells (5). Depending on the in vitro glycation protocol, different degrees of LDL glycation and different types of lysine modifications can be achieved (6). The decreased recognition of gLDL particles by the LDL-R correlates directly with the degree of apoB modification. In contrast to LDL acetylation, glycation of LDL does not result in the highly efficient uptake of the modified lipoprotein by scavenger receptors (4,7). Decreased LDL-R affinity of gLDL increases the relative circulation time of the lipoprotein (8), which may result in increased particle oxidation (9,10), the formation of advanced glycation end products (AGEs) (11), and the activation of alternative uptake mechanisms by non–LDL-R–mediated pathways. Because these processes are considered "proatherogenic," it has been proposed that the nonenzymatic glycation of apoB could contribute to the increased susceptibility of diabetic patients to atherosclerosis and coronary heart disease.

Besides the LDL-R, members of a whole gene family of receptors have been shown to participate in lipoprotein metabolism, including the LDL-R–related protein (LRP) and the VLDL receptor. Importantly, lipoprotein lipase (LPL), in addition to its function as the major enzymatic component for triglyceride catabolism, can facilitate the cellular uptake of lipoproteins. Through nonenzymatic mechanisms, LPL mediates the binding of lipoproteins to receptors by direct interaction with receptors (ligand function) (12,13) or by forming a molecular bridge between lipoproteins and proteoglycans (bridging function) (14–16). LPL has been shown to increase the binding and internalization of chylomicrons, chylomicron remnants, VLDL, LDL, and modified lipoproteins to a variety of cells (17). In the present study, we investigated the role of LPL in the cellular uptake of gLDL. In addition to human skin fibroblasts (HSF), we investigated cell types of the vascu-

lar system that produce LPL (macrophages) or bind large amounts of the enzyme (endothelial cells). We found that LPL markedly increased the capacity of cells to bind and internalize gLDL independently of the classic LDL-R pathway and LRP.

RESEARCH DESIGN AND METHODS

Lipoprotein preparation. Lipoproteins were prepared from plasma of fasted normolipidemic subjects by sequential ultracentrifugation using standard techniques (18) in the presence of EDTA (0.1%) and NaN_3 (0.1%). The pooled plasma was stored in the presence of 0.6% sucrose and 0.1% EDTA at −70°C. Protein concentrations of lipoproteins were determined by the method of Lowry et al. (19) using bovine serum albumin as standard. Total cholesterol (TC) was determined using an enzymatic procedure (CHOD-PAP; Boehringer Mannheim).

Lipoprotein glycation and LDL acetylation. In vitro glycation of lipoproteins was performed in the absence and presence of $NaBH_3CN$ as described previously (8). LDL at a concentration of 1 mg TC/ml was incubated in 10 mmol/l phosphate-buffered saline (PBS) (pH 7.4) containing 0.1% EDTA, 25 μmol/l butylhydroxytoluene, 50 μmol/l diethylentriaminepenta-acetic acid, 12 mg/ml $NaBH_3CN$, and different concentrations of D-glucose (2.5, 5, 10, 20, 40, and 80 mmol/l). Incubation of control LDL (cLDL) was performed without glucose. The mixture was filter-sterilized (0.22-μm filters), N_2-purged, and incubated at 37°C for 6 days in the dark. HDL, VLDL, and albumin were glycated under identical conditions at a protein concentration of 0.625 mg/ml and in the presence of 40 mmol/l glucose. Glycation of LDL in the absence of $NaBH_3CN$ was performed with 80 mmol/l glucose for 14 days at 37°C.

The amount of glycation was estimated by the trinitrobenzenesulfonic acid (TNBS) assay (Pierce). TNBS reacts specifically with free lysines and NH_2-terminal amino acid residues to form trinitrophenyl derivatives. The relative reduction of absorbance of gLDL compared with cLDL is a linear function of the concentration of trinitrophenyl derivatives (20). LDL (200 μl) (1 mg TC/ml) was mixed with 200 μl of 4% $NaHCO_3$, pH 8.4, and 200 μl of 0.1% TNBS. The mixture was incubated for 2 h at 37°C. Optical density was measured at 340 nmol/l. The degree of glycation was calculated from the reduction of absorbance compared with cLDL. Acetylation of LDL was performed as described by Basu et al. (21).

Light scattering analysis. Dynamic light scattering experiments were performed to investigate potential aggregation of LDL during the glycation procedure. Experiments were performed on a laboratory-built goniometer equipped with an $Argon^+$ laser (Spectra Physics; model 2060-55, $P_{max} = 5$ W, $\lambda = 514$ nm), single-mode fiber detection, and an ALV-5000 correlator (ALV, Germany). Measurements were performed at 20°C at a scattering angle of 90° and a laser power of 400 mW, which was reduced by a beam splitter to ~200 mW. Any aggregation of LDL particles led to an increase of the apparent hydrodynamic radius (R_H) and an increase in the integrated intensity as described previously (22).

Lipoprotein labeling procedures. Lipoproteins were labeled with the fluorescent dye 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) (Lambda Probes) by the method previously described (23). LDL was diluted to a concentration of 1 mg/ml (TC) in PBS containing 0.01% EDTA, and 3 ml lipoprotein-deficient serum (LPDS) was added to 1 ml LDL solution. The mixture was filter-sterilized (using 0.22-μm filters), and 75 μl DiI was added from a stock solution (3 mg/ml in DMSO) under gentle agitation. Incubation was performed under nitrogen at 37°C for 15 h in the dark. The labeled lipoprotein was reisolated by ultracentrifugation and extensively dialyzed against PBS, 0.01% EDTA.

Iodination of lipoproteins was performed as described by Sinn et al. (24) using N-Br-succinimide as the coupling agent. $Na^{125}I$ (1 mCi) (Amersham) was used to label 5 mg protein. The labeled lipoprotein was desalted using a Sephadex column (PD-10) and dialyzed against PBS, 0.01% EDTA.

Isolation of LPL. Bovine LPL was isolated as described previously (25). Briefly, fresh bovine milk was centrifuged to separate cream, and NaCl was added to the remaining skim milk for a final concentration of 350 mmol/l. After filtration, 40 ml heparin-Sepharose (Amersham Pharmacia) was added to the skim milk, and the mixture was incubated overnight with gentle agitation at 4°C. LPL was further purified using affinity chromatography on heparin-Sepharose and hydrophobic interaction chromatography on phenyl-Sepharose as described for human LPL (26).

Cells, cell lines, and culture conditions. Cells were cultured under standard conditions (37°C, 5% CO_2, 95% humidity). HSF, LDL-R–deficient fibroblasts (FH-HSF), Chinese hamster ovary (CHO)-K1 cells, and LRP-deficient CHO cells (designated 13-5-1) (27) were grown in 75-cm^2 flasks in Dulbecco's minimal essential medium (DMEM) containing 10% fetal calf serum (FCS). Subsequently, cells were trypsinized and seeded into 24-well trays. When cells were confluent, the medium was refreshed or switched to DMEM containing 10% LPDS 24 h before the experiment.

Endothelial cells were isolated from porcine aortas, as described by Graier et al. (28). Briefly, fresh porcine aortas were incubated at 37°C in DMEM containing 200 U/ml collagenase (type II), 2% DMEM essential amino acids (Life Technologies), 1% DMEM nonessential amino acids (Life Technologies), 1% DMEM vitamins (Life Technologies), and trypsin inhibitor (soybean type I, 1 mg/ml). Isolated cells were cultured in 10-cm Petri dishes in Opti-DMEM containing 3% FCS. For experiments, cells of passage 1 were trypsinized and seeded into 24-well trays. When cells were confluent, the medium was refreshed 24 h before the experiments.

Isolation of peritoneal mouse macrophages from mice was performed as described previously (29). Briefly, peritoneal macrophages from wild-type mice (wt-macrophages) were elicited by the intraperitoneal injection of 2 ml thioglycollate medium (3% in H_2O; Life Technologies) and harvested 3 days after injection. Cells were plated on 24-well plates for 4 h. Subsequently, macrophages were washed three times with PBS (10 mmol/l, pH 7.4, 0.15 mol/l NaCl) and cultured overnight in DMEM containing 10% FCS. For the isolation of LPL-deficient peritoneal macrophages (ko-macrophages), the identical protocol was used starting with genetically modified mice that expressed the enzyme exclusively in muscle. Peritoneal macrophages from these mice have been shown previously to completely lack LPL expression (30).

Lipoprotein binding, uptake, and degradation studies. For determination of binding of lipoproteins, cells were placed on ice and incubated with the labeled lipoproteins for 4 h at 4°C under constant shaking. For determination of binding and internalization of lipoproteins at 37°C, cells were incubated with the labeled lipoproteins for 6 h under standard conditions. Cells were then placed on ice and washed twice with PBS containing 0.5% BSA and twice with PBS without BSA. Surface-bound lipoproteins were removed by incubating the cells with DMEM containing heparin (100 U/ml) under constant shaking for 1 h at 4°C.

Degradation of ^{125}I-labeled apolipoproteins was estimated by measuring the non–trichloroacetic acid (TCA) precipitable radioactivity in the medium after precipitation of free iodine with $AgNO_3$ (31). Medium (0.25 ml) was mixed with 50 μl BSA (30 mg/ml) and 0.5 ml ice-cold TCA (0.7 mol/l) and incubated for 30 min at 4°C. Subsequently, 125 μl $AgNO_3$ (0.7 mol/l) was added. The mixture was vortexed and centrifuged for 15 min at 8,000 rpm at 4°C. The radioactivity in 0.5 ml of the supernatant was quantitated in a gamma counter. Cell-associated (nondegraded) radioactivity was measured after lysis of cells in 0.1 N NaOH, 0.1% SDS. Control experiments without cells were performed in parallel to determine the amounts of degradation not attributable to cellular effects.

The internalization of the DiI-labeled lipoproteins was measured after lysis of cells in 0.1 N NaOH, 0.1% SDS, for 4 h at room temperature as described by Teupser et al. (32). Fluorescence was measured on microtiter plates in a microplate reader (1420 Multilabel Counter; Wallac), with excitation and emission wavelengths set at 520 and 572 nm, respectively. The fluorescence intensity of DiI-labeled LDL was linear throughout the range monitored (from 20 ng TC/ml to 1 μg TC/ml, $r = 0.999$). All fluorimetric data were corrected for the autofluorescence of cells incubated with medium alone. The concentration of protein in the cell lysate was measured by the method of Lowry et al. (19).

RESULTS

To study the effect of glycation on the LDL-R–mediated uptake of LDL in HSF, it was necessary to establish a reproducible glycation protocol and to determine the degree of LDL glycation. Incubation of LDL with D-glucose resulted in a dose-dependent glycation of the susceptible amino groups of apoB (Fig. 1*A*). In the presence of $NaBH_3CN$ and at a glucose concentration of 40 mmol/l, 40% of all susceptible lysine residues in apoB were modified. This preparation is expected to contain both glucitol-lysine and fructoselysine modifications (6) and was termed glycated LDL. Additionally, a glycation protocol without the use of $NaBH_3CN$ was utilized that resulted in lower glycation efficiency (12% modification). This moderately glycated LDL (mgLDL) is expected to predominantly contain fructoselysine modifications. The glycation procedures did not cause the aggregation of LDL, as demonstrated by the lack of changes in the dynamic and static light scattering behavior of the particles (Fig. 1*B*). Even at

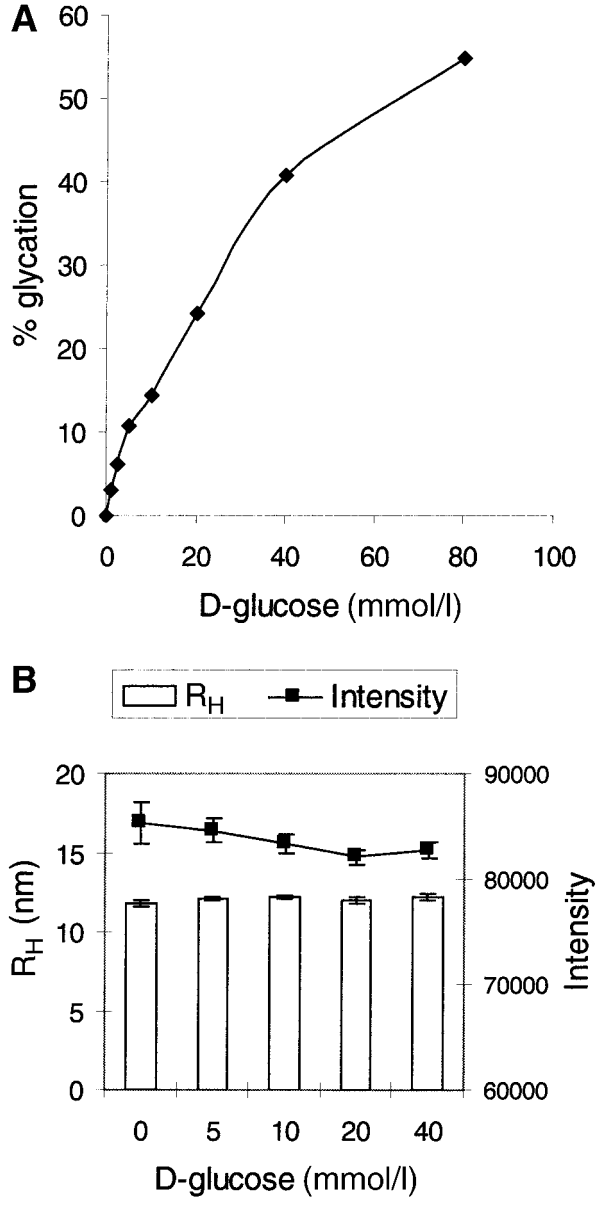

FIG. 1. *A*: Glycation of LDL. LDL (1 mg TC/ml) was incubated with the indicated concentrations of D-glucose for 6 days at 37°C. The degree of glycation was estimated using the TNBS assay, as described in RESEARCH DESIGN AND METHODS. Data represent the means of duplicate determinations of one typical experiment. *B*: Static and dynamic light scattering analysis of cLDL and gLDL. LDL was glycated with the indicated concentrations of D-glucose. Experiments were performed on a laboratory-built goniometer at a LDL concentration of 1 mg TC/ml. The hydrodynamic radius (R_H) of cLDL and gLDL was obtained from the dynamic scattering experiments. The intensities were obtained from static scattering. Data represent the mean of 10 determinations for ± SD.

the highest degree of LDL glycation, no increase in particle diameter was observed. As shown previously (33), the in vitro glycation procedures did not lead to oxidative modifications of LDL.

The inhibitory effect of glycation on LDL-R–mediated uptake was analyzed in HSF cell cultures (Fig. 2*A*). Compared with nonglycated cLDL, a decrease of DiI-LDL uptake was observed when gLDL was used in cell uptake experiments. Decreased uptake correlated directly with the extent of LDL glycation. When the LDL-R was upregulated by preincubation of HSF with medium containing LPDS, a similar result was obtained. Although uptake of

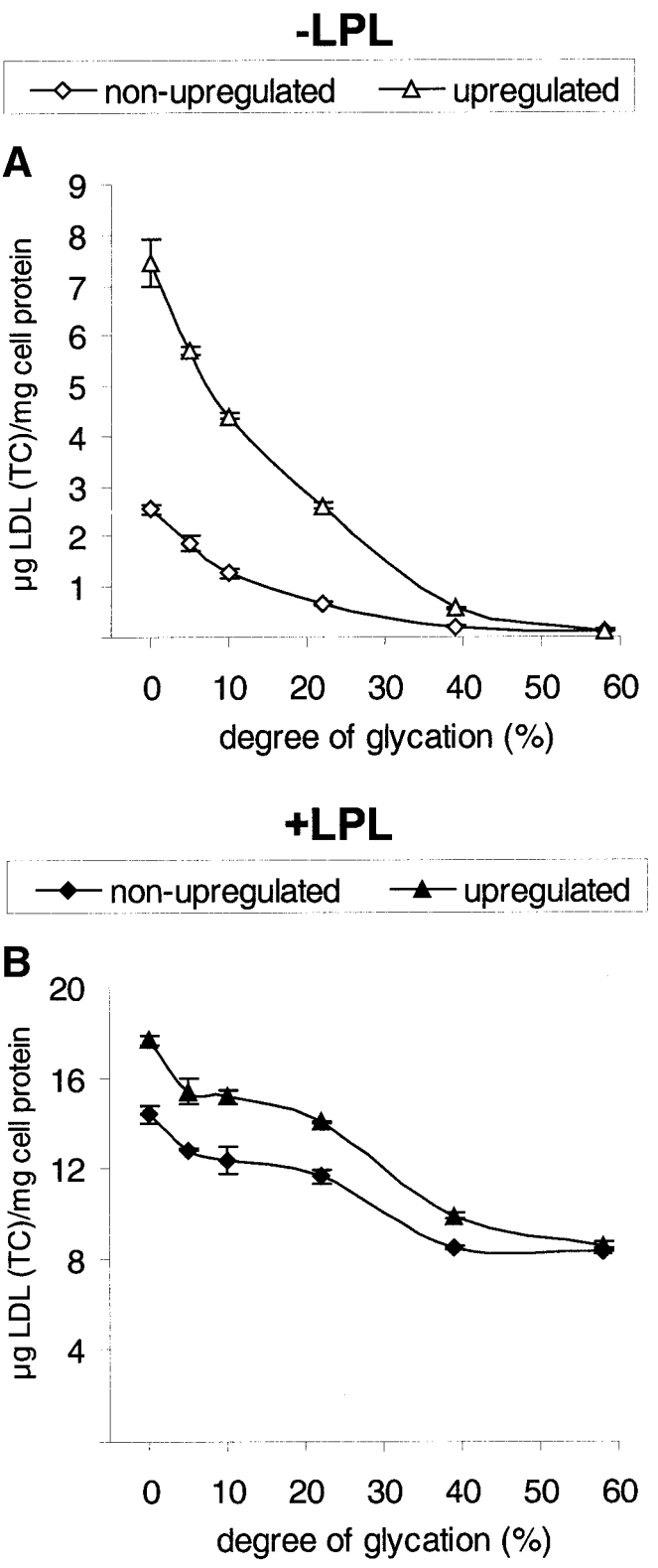

FIG. 2. Effect of glycation on LDL-R–mediated and LPL-mediated uptake of LDL in HSF. HSF were grown to confluency and incubated in DMEM containing either 10% LPDS (upregulated) or 10% FCS (nonupregulated) for 24 h. After this period, cells were incubated with DiI-labeled LDL (20 µg TC/ml) with different degrees of glycation for 6 h at 37°C in the absence (*A*) or in the presence (*B*) of 5 µg/ml LPL. Subsequently, the cells were washed and incubated with 100 U/ml heparin for 1 h at 4°C under constant shaking to remove cell-bound lipoproteins. Uptake was determined after lysis of cells in 0.1N NaOH/0.1% SDS. Data represent means ± SD from triplicate wells.

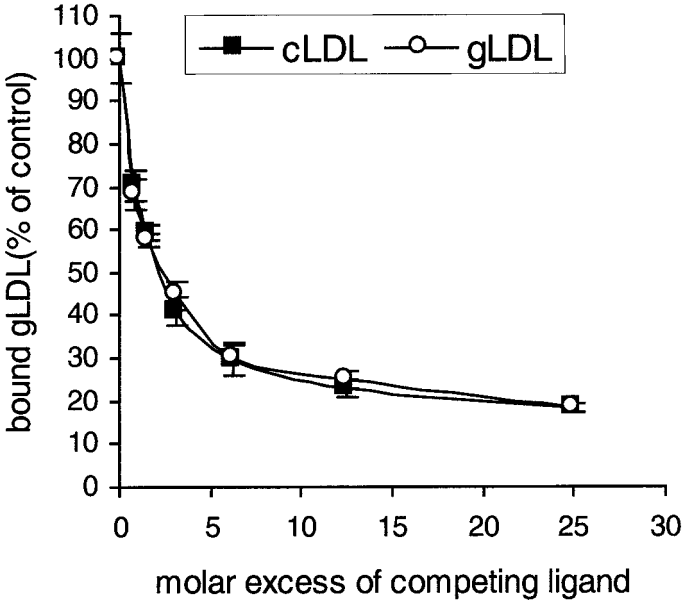

FIG. 3. Displacement curves of ^{125}I-labeled gLDL by cLDL or gLDL. Confluent layers of HSF were incubated in DMEM containing 10% LPDS and bovine LPL (100 ng/well) for 1 h at 4°C. Subsequently, the medium was removed and cells were incubated for another 4 h in DMEM 10% LPDS containing ^{125}I-labeled gLDL (1 μg/ml) and the indicated concentrations of nonlabeled LDL or gLDL. Thereafter, cells were washed, and the LPL-bound lipoproteins were displaced from cells with 100 U/ml heparin for 5 min at 4°C under constant shaking. Data represent means ± SD from triplicate wells.

cLDL was increased threefold in upregulated cells compared with nonupregulated cells, this difference was gradually abolished with increasing glycation. At ~50% glycation of apoB, virtually no LDL uptake was observed in both upregulated and nonupregulated HSF.

To test whether LPL can facilitate the binding and uptake of gLDL, bovine LPL was added at a concentration of 5 μg/ml to the incubation medium. As shown in Fig. 2*B*, in the presence of LPL, uptake of LDL at different degrees of glycation was markedly increased. However, similar to the situation in which LPL was absent, a linear decrease of lipoprotein uptake was found depending on the level of LDL glycation. Interestingly, even at the highest degree of glycation, LDL was still internalized efficiently by HSF, independently of whether the LDL-R was upregulated. These findings strongly imply that there is an LDL-R–independent pathway for the LPL-mediated uptake of gLDL.

Protein-protein interaction of LPL with the NH_2-terminus of apoB is known to promote the binding of LPL to LDL (34). To investigate whether glycation affects the interaction of LPL with LDL, we performed binding experiments with ^{125}I-labeled gLDL to fibroblasts at 4°C. In the absence of LPL, the binding of gLDL to cells was not detectable. When cells were preincubated with LPL (100 ng/well) for 1 h at 4°C, 204 ± 12 ng/mg cell protein of gLDL bound to fibroblasts. Competition of gLDL binding with nonlabeled cLDL and gLDL is shown in Fig. 3. Both cLDL and gLDL were capable of competing for the lipase-specific binding in a concentration-dependent manner. Identical displacement suggested that glycation of LDL did not affect the binding of LDL to LPL.

Binding, uptake, and degradation of ^{125}I-labeled apolipoproteins of cLDL and gLDL by HSF in the presence and absence of LPL are shown in Fig. 4. At a concentration of 10 μg LDL (TC)/ml, the addition of LPL (5 μg/ml) increased the binding of cLDL and gLDL to approximately the same maximum levels, which represents a 41- and 283-fold increase, respectively (Fig. 4*A* and *B*). The same conditions resulted in a 38% increase in the uptake of cLDL and a 15-fold increase in gLDL uptake (Fig. 4*C* and *D*). Degradation of cLDL (Fig. 4*E*) was only marginally increased (7%). Although degradation of gLDL was enhanced ninefold (Fig. 4*F*), it did not reach the levels observed for LDL-R–mediated degradation of cLDL.

To test the role of LDL-R and LRP in LPL-mediated uptake of gLDL, we performed uptake experiments with mutant cell lines. The participation of the LDL-R was examined by investigating the effect of LPL on the uptake of ^{125}I-labeled gLDL in FH-HSF and HSF (Fig. 5*A*). In the absence of LPL, uptake of gLDL was very low in both cell lines. Addition of LPL caused a more than 10-fold increase in the uptake of gLDL in both HSF and FH-HSF. LRP involvement in LPL-mediated uptake of gLDL was studied in LRP-deficient CHO cells and CHO-K1 wild-type cells. As shown in Fig. 5*B*, very low amounts of gLDL were internalized in the absence of LPL in both cell types. The addition of LPL enhanced the uptake of gLDL in CHO-K1 and in LRP-null CHO cells to a similar extent. These results provided direct evidence that the LPL-mediated uptake of gLDL does not depend on the expression of LDL-R and LRP.

To extend our studies to a cell system exhibiting more physiologically relevant features, lipoprotein binding, uptake, and degradation were determined in HSF, porcine aortic endothelial cells (PAECs), and mouse peritoneal macrophages. Endothelial cells are a particularly suitable model because, first, they represent the cell type to which LPL is bound in vivo and, second, they directly encounter increased levels of gLDL in diabetic individuals. Macrophages have been shown to produce endogenously high levels of LPL (35). Additionally, in these experiments, we compared the effect of LPL on the binding, uptake, and degradation of gLDL versus mgLDL. mgLDL was produced by a glycation protocol without the use of $NaBH_3CN$, which resulted in a lower glycation efficiency and the presumed absence of glucitollysine formation. In a dose-response experiment (Fig. 6), the addition of increasing amounts of LPL to the incubation medium led to a pronounced dose-dependent increase of both gLDL and mgLDL binding in all investigated cell types (Fig. 6*A*). Uptake and degradation of gLDL and mgLDL are shown in Fig. 6*B* and *C*, respectively. The addition of LPL caused a marked increase of both gLDL and mgLDL uptake and degradation, which was saturable between 1 and 2 μg LPL/ml in all cell types. The pronounced increase in the uptake and degradation of both gLDL and mgLDL indicated that the nature of lysine modification did not affect LPL action. The consistently higher uptake and degradation rates of mgLDL in the absence and presence of LPL can be explained by the more moderate degree of glycation that did not completely abolish its binding to the LDL receptor.

To investigate the role of endogenously synthesized macrophage LPL in the uptake of gLDL, we used peritoneal macrophages from induced mutant mice that lack LPL (ko-macrophages) and compared them with macrophages from wild-type mice (wt-macrophages). Figure 7 shows the uptake of DiI-labeled gLDL and acetylated LDL

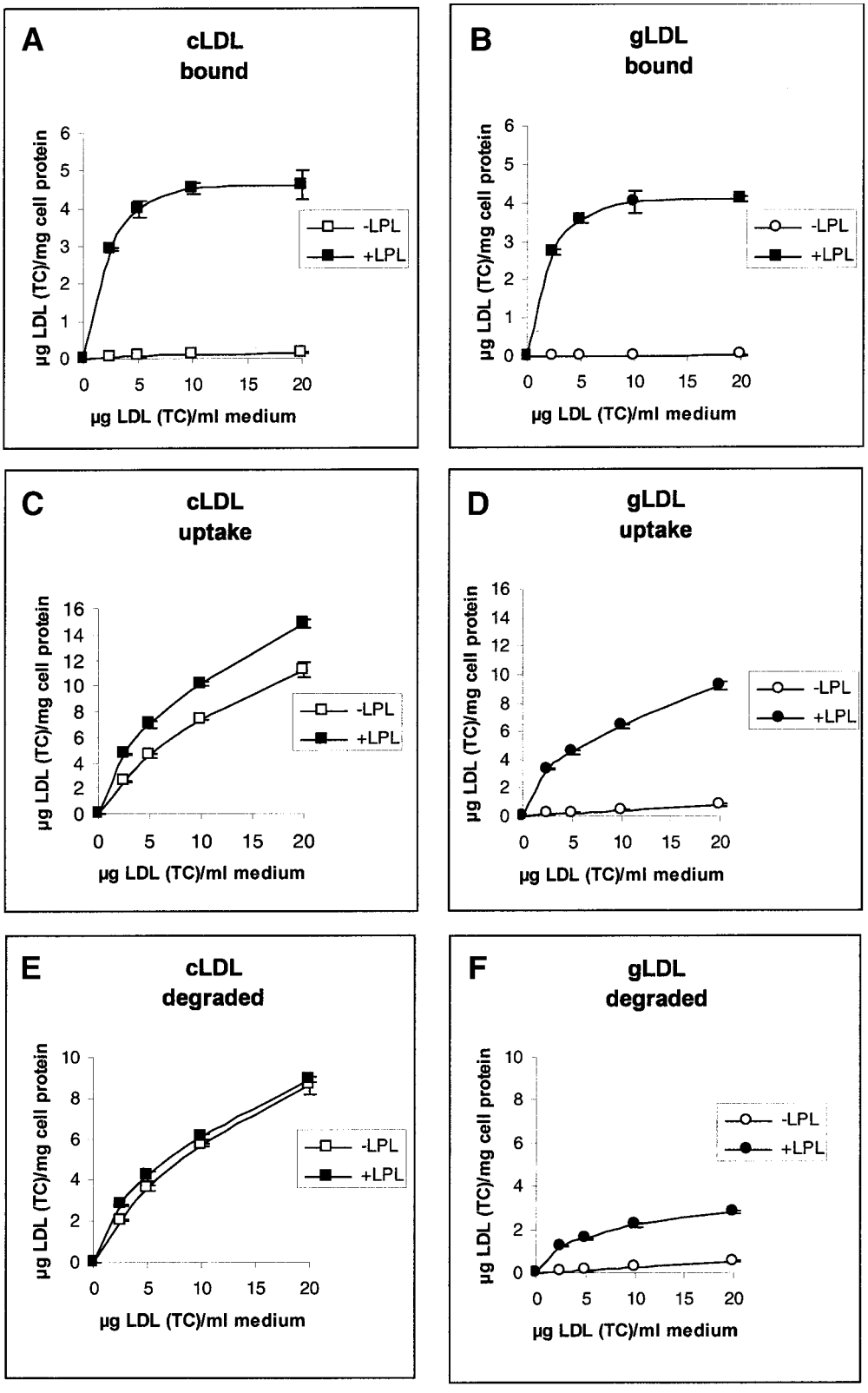

FIG. 4. Effect of LPL on binding, uptake, and degradation of ^{125}I-labeled cLDL and gLDL in HSF. HSF were grown to confluence and incubated in DMEM containing 10% LPDS for 24 h before treatment. Subsequently, the cells were incubated in DMEM containing 10% LPDS and the indicated concentrations of ^{125}I-labeled cLDL and gLDL for 6 h at 37°C. After washing, heparin releasable binding was determined by incubating cells with 100 U/ml heparin (*A* and *B*). Uptake represents cell-associated (nondegraded) LDL plus degraded LDL (*C* and *D*). Degradation of lipoproteins was measured as non-TCA precipitable radioactivity in the medium (*E* and *F*). Data represent means ± SD from triplicate wells.

(acLDL) in wt- and ko-macrophages. ko-Macrophages exhibited a marked decrease in their capacity to internalize gLDL. At a gLDL concentration of 40 µg/ml LDL (TC)/ml medium, gLDL uptake was 2.6-fold higher in macrophages that expressed LPL compared with ko-macrophages (Fig. 7*A*). In contrast, the uptake of acLDL, which is mediated

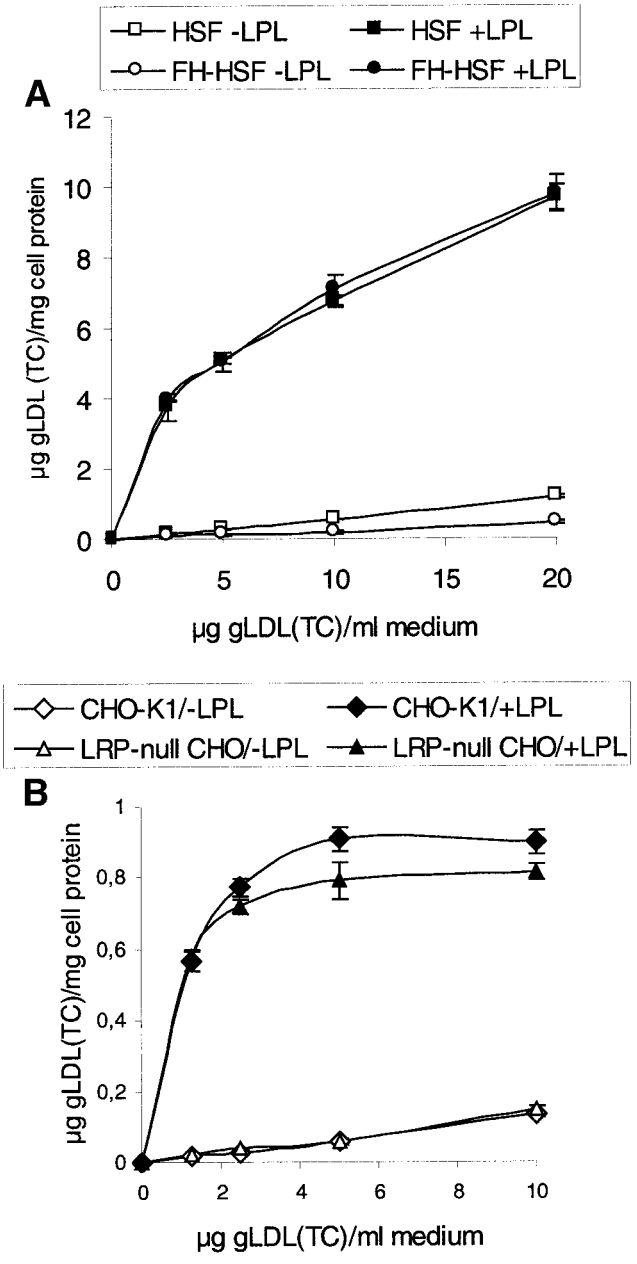

FIG. 5. Effect of LPL on the uptake of ^{125}I-labeled gLDL in LDL-R–deficent fibroblasts (FH-HSF, *A*) and LRP-deficient CHO cells (*B*). *A*: Confluent layers of HSF and FH-HSF were incubated with the indicated concentrations of gLDL for 6 h at 37°C in the absence or presence of 5 µg/ml LPL. *B*: Confluent layers of CHO-K1 and LRP-deficient CHO cells were incubated with the indicated concentrations of gLDL for 6 h at 37°C in the absence or presence of 1 µg/ml LPL. After incubation with gLDL, the cells were washed and incubated with 100 U/ml heparin for 1 h at 4°C under constant shaking to remove cell-bound lipoproteins. Uptake represents cell-associated (nondegraded) LDL plus degraded LDL. Data represent means ± SD from triplicate wells.

by scavenger receptors, was not affected by the presence or absence of LPL (Fig. 7*B*). Additionally, the amount of acLDL internalized by macrophages was more than 10-fold higher than the amount of gLDL taken up, confirming that LDL glycation did not transform the lipoprotein into a particle readily internalized by macrophages. Increased uptake also affected the lipoprotein degradation (Fig. 8). Degradation of ^{125}I-labeled cLDL was increased by 28% in wt-macrophages compared with ko-macrophages, whereas degradation of labeled gLDL was increased sixfold. In the additional presence of exogenously added bovine LPL (1 µg/ml), lipoprotein degradation was further increased, and the difference between wt- and ko-macrophages was abolished.

To reveal whether the observed effects of LPL required an active enzyme that is bound to the cell surface by glycosaminoglycans, the degradation of gLDL was measured in the presence of inactivated LPL and in the presence of heparin. LPL inhibition was achieved by the reversible active site inhibitor tetrahydrolipstatin (THL) (Orlistat). At a concentration of 20 µg/ml, this serine hydrolase inhibitor completely abolished the activity of LPL in conventional LPL activity assays (not shown). As shown in Fig. 9, the addition of THL to the incubation medium did not significantly affect the degradation of gLDL. Enzymatically active LPL (1 µg/ml) increased the degradation 16-fold, and THL-inactivated LPL led to a 13-fold increase in gLDL degradation. In contrast, heparin treatment completely abolished the effect of LPL. These experiments indicated that an enzymatically inactive LPL is sufficient to enhance the uptake of gLDL, but the effect critically depends on the interaction of LPL with cell surface proteoglycans.

Finally, we examined the effect of LPL on the degradation of ^{125}I-labeled glycated VLDL (gVLDL) and ^{125}I-labeled glycated HDL (gHDL). In the absence of LPL, glycation of VLDL and HDL decreased the degradation of the particles by 44 and 72%, respectively, compared with nonglycated lipoproteins (Fig. 10). The addition of LPL enhanced the degradation of both control VLDL (sixfold) and gVLDL (ninefold) (Fig. 10*A*). The degradation of control HDL and gHDL was enhanced 1.4- and 3.5-fold, respectively (Fig. 10*B*). LPL had no measurable effect on the degradation of albumin or glycated albumin (not shown). These results indicated that the effect of LPL can also be observed in other lipoprotein classes.

DISCUSSION

ApoB glycation is a naturally occurring modification of LDL that might produce a particle of high atherogenic potential. Increased concentrations of gLDL have been measured in diabetic patients as well as individuals suffering from hypercholesterolemia (36). Once glycated, LDL becomes a preferential target for different oxidative modifications (11). Additionally, gLDL can enhance chemotaxis and the production of superoxide anions in macrophages (37). In endothelial cells, it prevents shear stress-mediated L-arginine uptake and nitric oxide formation (33) and causes increased production of plasminogen-activator inhibitor 1 (38) and prostaglandins (39), while inhibiting the expression of tissue plasminogen activator (38). All these processes can contribute to the development of atherosclerosis. Although little is known about how gLDL initiates these processes, it is reasonable to assume that gLDL must interact with the cell surface of target cells—namely endothelial cells and macrophages.

In contrast to native LDL, which is efficiently removed from plasma by the LDL-R, gLDL is only poorly recognized by lipoprotein receptors. Neither the LDL-R nor LRP or macrophage scavenger receptors bind and internalize gLDL with high affinity. In search of a potential binding partner and uptake mechanism for gLDL, we investigated the ability of LPL to facilitate this process. LPL appeared to be

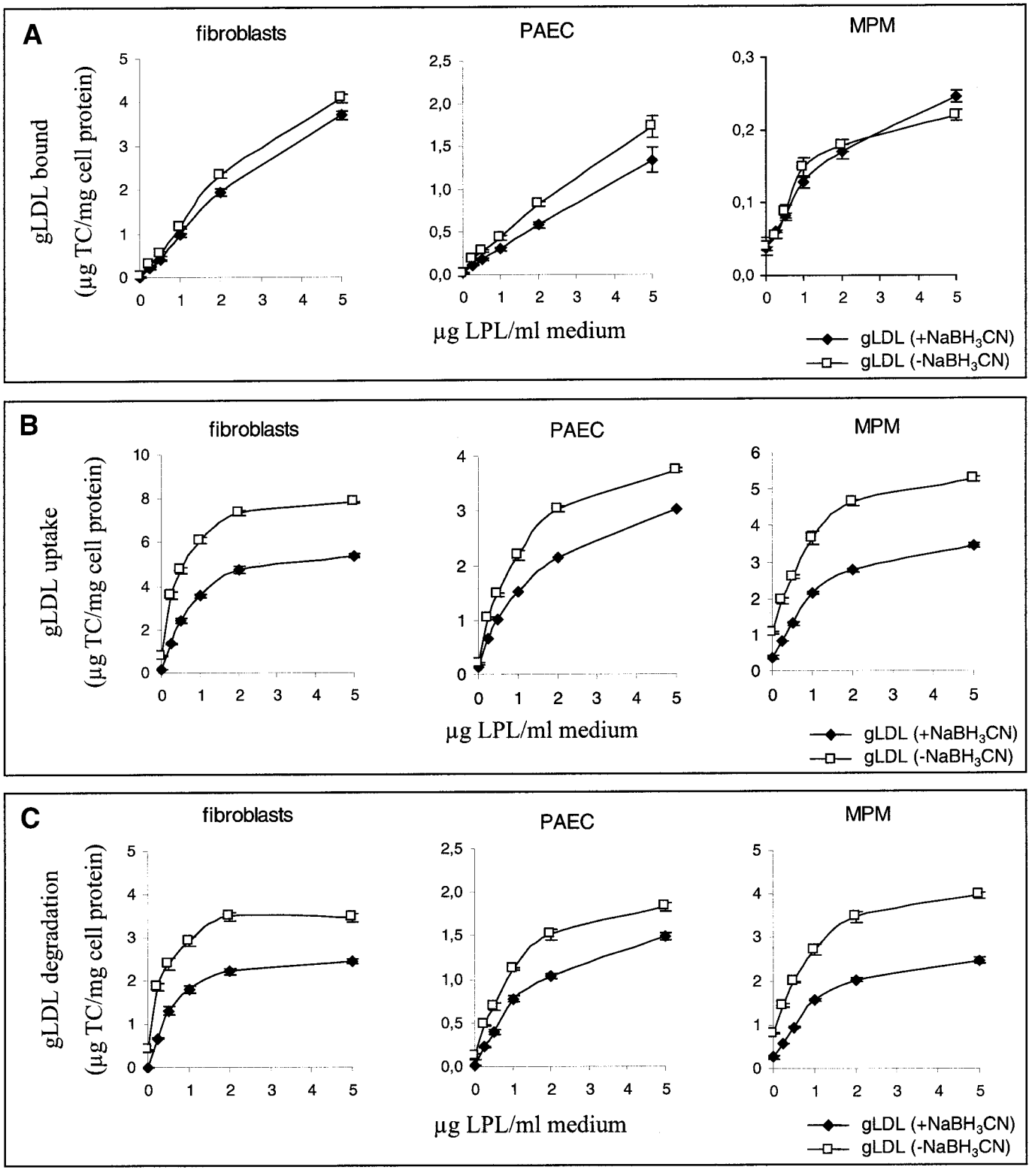

FIG. 6. Binding, uptake, and degradation of ^{125}I-labeled gLDL and mgLDL in HSF, PAEC, and mouse macrophages. Cells were incubated with LDL (10 μg TC/ml) glycated in the absence (12% glycation, mgLDL) or in the presence (40% glycation, gLDL) of $NaBH_3CN$ and with increasing concentrations of LPL for 6 h at 37°C. Subsequently, the cells were washed and the heparin-releasable binding was determined after incubating the cells with 100 U/ml heparin for 1 h at 4°C under constant shaking (*A*). Uptake represents cell-associated (nondegraded) LDL plus degraded LDL (*B*). Degradation of lipoproteins was measured as non-TCA precipitable radioactivity in the medium (*C*). Data represent means ± SD from triplicate wells.

an attractive candidate because, first, it is expressed in or located on the surface of cells that exhibit the most pronounced biological response to gLDL (macrophages and endothelial cells) and, second, LPL has been shown to interact with native and modified LDL mediating their cellular uptake (40,41). Several basic mechanisms have

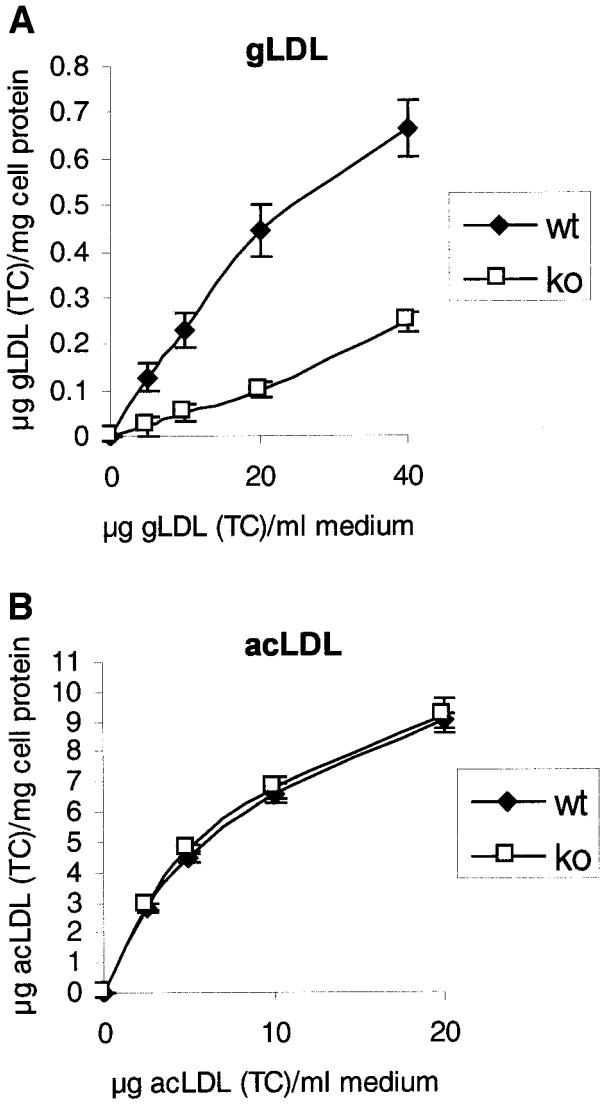

FIG. 7. Uptake of DiI-labeled gLDL (*A*) and acLDL (*B*) in wt- and ko-macrophages. Cells were cultured in DMEM containing 10% FCS for 24 h. Subsequently, the cells were incubated with the indicated concentrations of DiI-labeled gLDL or acLDL for 6 h at 37°C. Uptake was determined after lysis of cells in 0.1N NaOH/0.1% SDS. Data represent means ± SD of duplicate wells from macrophages of two completely independent experiments starting from different wild-type (wt) and knockout (ko) mice.

been shown to contribute to LPL-mediated lipoprotein uptake. Beisiegel et al. (12) showed in cross-linking experiments that LPL can directly bind to LRP (ligand function). Other authors (15,42) suggested that the increased binding of LDL to cell surface heparan sulfate proteoglycans in the presence of LPL facilitates LDL-R–mediated LDL uptake (bridging function), whereas Rumsey et al. (16) concluded from their experiments that LPL increases lipoprotein uptake by a non–LDL-R pathway. Finally, Fuki et al. (43) showed that in the presence of LPL, syndecan proteoglycans can mediate the uptake of lipoproteins, a process with characteristics distinct from classic receptor pathways.

The present study demonstrates that LPL can markedly enhance the binding, uptake, and degradation of gLDL by HSF, PAEC, and mouse peritoneal macrophages. LPL promoted the uptake of both "moderately glycated" mgLDL and "heavily glycated" gLDL. This suggested that the type of lysine modification (glucositollysine vs. fructoselysine) introduced by the presence or absence of $NaBH_3CN$ in the glycation procedure did not affect the LPL-mediated process. A number of experimental observations indicated that the LPL-mediated binding and uptake of gLDL is independent of the LDL-R and LRP. First, and in accordance with previous studies (4), gLDL was not recognized by cells in the absence of LPL. Second, changes in LDL-R activity in HSF in response to LPDS stimulation did not affect gLDL binding or uptake. Third, LPL can mediate the

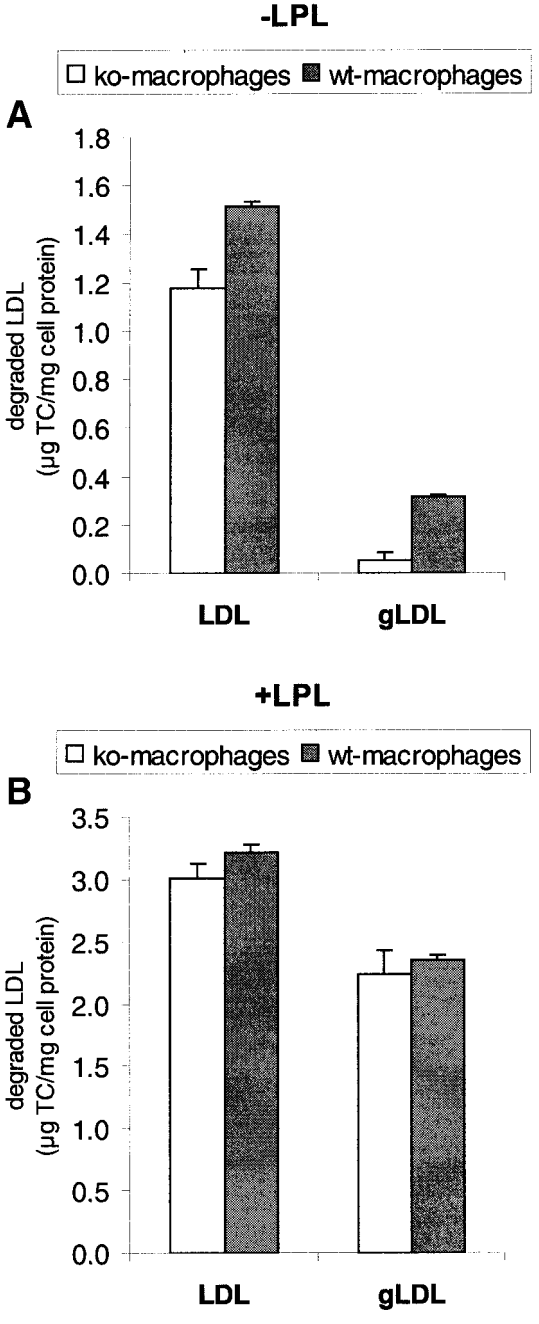

FIG. 8. Effect of endogenous (*A*) and exogenous (*B*) LPL on the degradation of ^{125}I-labeled cLDL and gLDL in wt- and ko-macrophages. Before the experiment, cells were cultured in DMEM containing 10% FCS for 24 h. After this period, cells were incubated with cLDL and gLDL (20 µg TC/ml) for 6 h at 37°C. LPL was added at a concentration of 1 µg/ml. Subsequently, degradation of lipoproteins was measured as non-TCA precipitable radioactivity in the medium. Data represent means ± SD from triplicate wells.

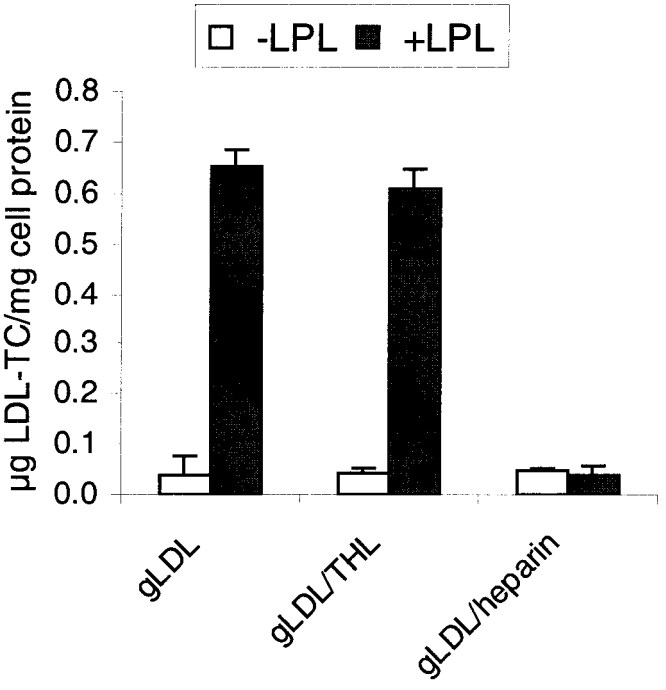

FIG. 9. Effect of THL and heparin on the degradation of ^{125}I-labeled gLDL in PAEC. Confluent layers of PAEC were incubated with gLDL (10 µg TC/ml, 40% glycation) for 6 h at 37°C in the absence or presence of 1 µg/ml LPL. THL and heparin were added at a concentration of 20 µg/ml and 1 U/ml, respectively. Subsequently, the cells were washed and incubated with 100 U/ml heparin for 1 h at 4°C under constant shaking to remove cell-bound lipoproteins. Degradation of lipoproteins was measured as non-TCA precipitable radioactivity in the medium. Data represent means ± SD from triplicate wells.

uptake of gLDL in FH-HSF and LRP-deficient CHO cells. From these results we conclude that cLDL and gLDL are taken up by different mechanisms. Whereas cLDL once bound to the cell surface via LPL is mainly internalized and degraded by the LDL-R pathway, gLDL is internalized by other LDL-R– and LRP-independent uptake mechanisms.

Fibroblasts are a commonly studied model in lipid metabolism. However, these cells do not produce LPL, and it is not likely that fibroblasts are exposed to high concentrations of LPL in vivo. The functional site of LPL action is the microvascular endothelium. Although endothelial cells themselves do not produce LPL, the enzyme is delivered from the surrounding LPL-producing parenchymal tissues and subsequently tightly bound to the luminal surface of endothelial cells (44). Similarly to the situation observed with fibroblasts, LPL markedly increased the binding, uptake, and degradation gLDL in endothelial cells in a dose-dependent manner. Because the vascular endothelium in muscle and adipose tissue contains large amounts of LPL on its luminal surface that come in contact with plasma lipoproteins, it is likely that LPL mediates the effects of gLDL on endothelial cell physiology. Additionally, endothelial cells might participate in the removal of gLDL from the circulation through an LPL-mediated mechanism.

LPL is highly expressed in macrophages (45,35). Obunike et al. (14) showed that the LDL-R–independent uptake of LDL via LPL is much more rapid in THP-1 macrophages than in fibroblasts. Our studies corroborate and extend these observations by demonstrating that LPL can drastically enhance the binding, uptake, and degradation of gLDL in macrophages. Additionally, using genetically modified macrophages, we were able to show that the amount of endogenously produced LPL from macrophages also affects the cellular uptake of gLDL. When mouse peritoneal macrophages do not express LPL, lipoprotein uptake is diminished. The LPL-mediated uptake of gLDL in macrophages might have important pathophysiological implications because the level of LPL activity in macrophages is directly correlated with the susceptibility of inbred mouse lines to develop atherosclerotic lesions (46). Direct evidence for such a proatherogenic role of LPL in macrophages was provided in experiments with transgenic mice demonstrating a reduced atherosclerosis susceptibility in animals that lack LPL in macrophages (47,48). In atherosclerotic lesions, LPL was found on the surface of macrophages and intimal smooth muscle cells and on extracellular matrix components (49). LPL bound to proteoglycans in the subendothelial matrix might cause

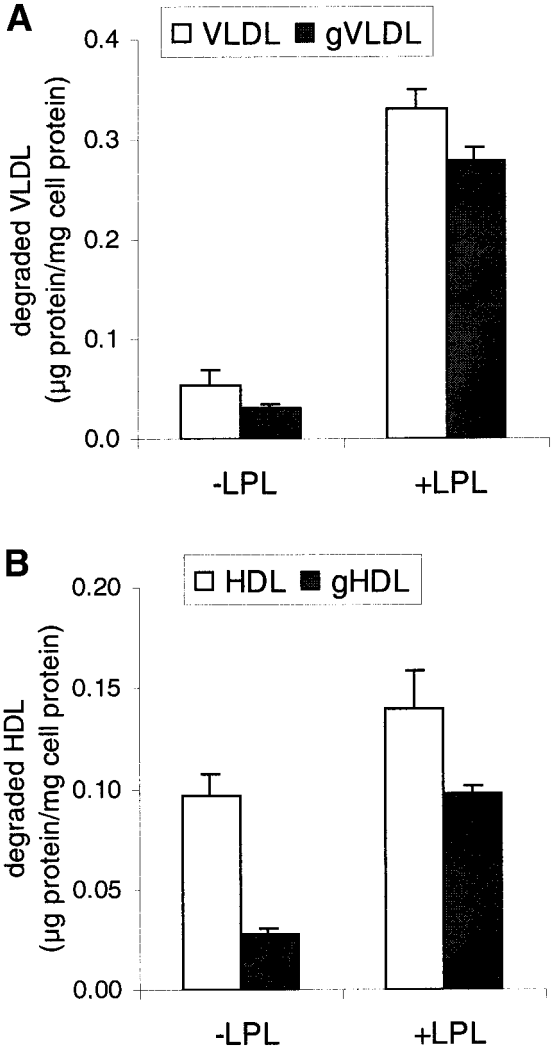

FIG. 10. Effect of LPL on the degradation of ^{125}I-labeled glycated and nonglycated VLDL and HDL in PAEC. Confluent layers of PAEC were incubated with (*A*) glycated and nonglycated VLDL (1 µg protein/ml) or (*B*) glycated and nonglycated HDL (20 µg protein/ml). Incubations were performed for 6 h at 37°C in the absence or presence of 1 µg/ml LPL. Subsequently, the cells were washed and incubated with 100 U/ml heparin for 1 h at 4°C under constant shaking to remove cell-bound lipoproteins. Degradation of lipoproteins was measured as non-TCA precipitable radioactivity in the medium. Data represent means ± SD from triplicate wells.

the trapping of gLDL and prolong its residence time in the circulation similarly to the situation demonstrated for LDL (50,51). This process possibly permits more extensive oxidation termed glucoxidation (11) and could ultimately result in the formation of AGEs, such as pentosidine and vesperlysine (52,53). AGE-modified proteins and lipoproteins are avidly internalized by macrophages via specific AGE receptors and might contribute to lipid accumulation in macrophages, foam cell formation, and the development of atherosclerotic lesions (54).

The high affinity binding between LPL and lipoproteins is thought to involve multiple ionic and hydrophobic interactions (55) that allow the enzyme to interact not only with LDL but also chylomicrons, VLDL, and HDL (15). In this investigation, we show that LPL also binds to gLDL and that glycation does not affect the interaction of LPL with LDL. LPL binding to lipoproteins facilitated the cellular uptake and degradation of gLDL, gVLDL, and gHDL. LPL enzyme activity was not required for the enhancement of gLDL uptake. In contrast, binding of LPL to cell surface glycosaminoglycans was a prerequisite for LPL-mediated gLDL uptake because heparin treatment completely abolished this effect. Similar results were obtained when the LPL-mediated uptake of native LDL was studied (14,56). Although LPL increased the binding of gLDL to cells dramatically, the amount of internalization and degradation did not reach the levels observed for nonglycated LDL. Therefore, we assume that a "slow, high-capacity" pathway, as originally described by Obunike et al. (14), for LPL-mediated uptake of LDL might be responsible for gLDL uptake. This hypothesis is in accordance with observations by Lopes-Virella et al. (57), who found that gLDL is internalized by a LDL-R–independent mechanism of high capacity in human macrophages.

In summary, we have shown that LPL can mediate the uptake of gLDL in different cell types even though the modified particle is not recognized by the LDL, LRP, or scavenger receptors. We speculate that LPL might play a role in the removal of gLDL from the circulation by LPL-containing cells. Additionally, the process of cellular binding of gLDL mediated by LPL might initiate the signal transduction pathway resulting in the cellular response of endothelial cells and macrophages after gLDL exposure.

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