Lipoprotein Lipase (LPL) Strongly Links Native and Oxidized Low Density Lipoprotein Particles to Decorin-coated Collagen

ROLES FOR BOTH DIMERIC AND MONOMERIC FORMS OF LPL*

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Low density lipoprotein (LDL) and oxidized LDL are associated with collagen in the arterial intima, where the collagen is coated by the small proteoglycan decorin. When incubated in physiological ionic conditions, decorin-coated collagen bound only small amounts of native and oxidized LDLs, the interaction being weak. When decorin-coated collagen was first allowed to bind lipoprotein lipase (LPL), binding of native and oxidized LDL increased dramatically (23- and 7-fold, respectively). This increase depended on strong interactions between LPL that was bound to the glycosaminoglycan chains of the collagen-bound decorin and native and oxidized LDL (kDa 12 and 5.9 nM, respectively). To distinguish between binding to monomeric (inactive) and dimeric (catalytically active) forms of LDL, affinity chromatography on heparin columns was conducted, which showed that native LDL bound to the monomeric LPL, whereas oxidized LDL, irrespective of the type of modification (Cu²⁺, 2,2'-azobis(2-amidinopropane)hydrochloride, hypochlorite, or soybean 15-lipoxygenase), bound preferably to dimeric LPL. However, catalytic activity of LPL was not required for binding to oxidized LDL. Finally, immunohistochemistry of atherosclerotic lesions of human coronary arteries revealed specific areas in which LDL, LPL, decorin, and collagen type I were present. The results suggest that LPL can retain LDL in atherosclerotic lesions along decorin-coated collagen fibers.

The role of collagen in retention of low density lipoprotein (LDL)† particles was recently highlighted, when incubation of LDL with rabbit cardiac leaflets in vitro resulted in preferential accumulation of LDL along collagen fibers in the subendothelial extracellular matrix (1). Ultrastructural analysis of this association revealed that the LDL actually interacted with small filaments extending perpendicularly from the collagen fibers (1), and electron microscopic analyses of glycosaminoglycans (GAG) in the arterial intima have shown that these filaments are the GAG of collagen-binding dermatan sulfate-rich proteoglycans (PG) (2). We have recently shown that decorin, a small collagen-binding dermatan sulfate-rich PG, can link native LDL to decorin-coated collagen immobilized to microtiter wells (3), which depends on the relatively weak interaction between apoB-100 of LDL and the GAG of decorin.

A fraction of the LDL particles that have entered the arterial intima become modified, e.g. oxidized. The presence of oxidized LDL (oxLDL) in the arterial intima has been demonstrated by immunohistochemistry (4, 5), and, moreover, LDL isolated from the arterial wall shows characteristics with LDL oxidized in vitro (6). A recent study of cholesterol-fed miniature pigs has even suggested that virtually all of the LDL in the arterial intima is oxidized (7). OxLDL is thought to be taken up rapidly by cells via the scavenger receptor(s), but the ability of oxLDL to generate foam cells, at least in vitro, has been questioned (8). Some of the epitopes for oxLDL in the arterial intima are found in acellular areas rich in connective tissue and appear to be associated with the matrix (5).

LPL, in addition to its catalytic role as the key enzyme involved in the intravascular metabolism of triglyceride-rich lipoproteins, has been shown to have a noncatalytic function. Thus, LPL has been shown to link lipoproteins to the LDL receptor-related protein/a₂-macroglobulin receptor, to the very low density lipoprotein (VLDL) receptor, and to PG, as reviewed (9). LPL is synthesized in catalytically active homodimeric form, but, in the absence of stabilizing compounds, it rapidly dissociates into inactive monomers in vitro (10). Inactive LPL is also present in vivo. Indeed, a significant proportion of LPL in tissues is present as inactive monomers (11). It is of interest that both dimeric and monomeric LPL are present in plasma, the former being primarily associated with triglyceride-rich lipoproteins (12) and the latter with cholesterol-rich lipoproteins (13). Although linking of triglyceride-rich lipoproteins (VLDL) and cholesterol-rich lipoproteins (LDL) to the extracellular matrix and to the cell surface by LPL in vitro has been studied extensively (14–20), the studies have not evaluated the possibility that the monomeric and dimeric forms of LPL might be different in their ability to bind lipoproteins.

Here, we studied the interaction between oxLDL and decorin-coated collagen in the absence and presence of LPL. We found that LPL, by binding to decorin, could anchor both oxidized and native LDL to decorin-coated collagen with high affinity. The interactions of native and oxLDL with LPL were studied further, using affinity chromatography on heparin columns loaded with LPL, a method that enabled us to study the binding of various apoB-100-containing lipoproteins separately to dimeric and to monomeric LPL. Finally, to assess the relevance of the present in vitro observations to human atherosclerosis, we studied the ultrastructural localization of LPL with regard to LDL, oxLDL, decorin, and collagen in human atherosclerotic lesions.

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† The abbreviations used are: LDL, low density lipoprotein(s); AAPH, 2,2'-azobis(2-amidinopropane)hydrochloride; BSA, bovine serum albumin; CL, cholesteryl linoleate; GAG, glycosaminoglycan(s); IDL, intermediate density lipoprotein(s); LPL, lipoprotein lipase; MDA, malondialdehyde; oxLDL, oxidized LDL; PG, proteoglycan(s); VLDL, very low density lipoprotein(s).
EXPERIMENTAL PROCEDURES

Isolation, Characterization, and Modification of Decorin—PG was isolated from bovine fetal skin exactly as described (3). The GAG composition consisted of 63% dermatan sulfate, 12% chondroitin 6-sulfate, and 25% chondroitin 4-sulfate. NH₂-terminal sequencing showed that this PG preparation contained more than 90% of decorin. 35S-Labeled decorin was prepared by labeling the core protein of decorin with 35S-potassium 35S-labeling reagent (Amersham Pharmacia Biotech) by the Bolton-Hunter procedure (21). Chondroitinase ABC-treated decorin was prepared by incubating 30 μg of 35S-decorin with 250 million units of chondroitinase ABC (Seikagaku, Tokyo, Japan) in 100 μl of Dulbecco’s phosphate-buffered saline (Life Technologies, Inc., Paisley, Scotland) containing 100 μg/ml bovine serum albumin (BSA; Sigma) for 18 h at 37 °C. Degradation of the GAG chain of decorin was confirmed by loss of electrophoretic mobility of decorin on cellulose acetate (Helena Biosciences, Sunderland, Tyne & Wear, U.K.). The amounts and concentrations of decorin are expressed in terms of protein.

Isolation and Modification of Lipoproteins—Human VLDL, IDL, and LDL were isolated from plasma of healthy volunteers by sequential ultracentrifugation (22) and dialyzed extensively against buffer A (150 mM NaCl, 1 mM EDTA, pH 7.4). Apolipoproteins of these lipoproteins were tritiated by the Bolton-Hunter procedure (21) with N-succinimidyl-1,2,3,3H)propionate (Amersham Pharmacia Biotech) to yield [3H]VLDL, [3H]IDL, and [3H]LDL and iodinated by the iodine monochloride method as described by McFarlane (23) and Bilheimer et al. (24). LDL was also labeled with [3H]cholesterol linolate (Amersham Pharmacia Biotech), as described (25), to yield [3H]IDL-LDL.

LDL was oxidized in four different ways. 0.5 mg/ml [3H]IDL-LDL was incubated with copper sulfate-phosphate-buffered saline at 37 °C for 18 h or the indicated period of time; (ii) with 25 mM 2,2′-azobis(2-aminopropane)hydrochloride (AAPH; Polysciences, Warrington, PA) in buffer A for 18 h at 37 °C, after which AAPH was inactivated LPL (100 μg/ml bovine serum albumin (BSA); Sigma) for 18 h at 37 °C for 6 h. The proteolyzed native-sized LDL was reisolated by size exclusion chromatography of apoB-100 was analyzed by SDS-polyacrylamide gel electrophoresis in the presence of b-mercaptoethanol (27). The amounts and concentrations of lipoproteins are expressed in terms of protein. Protein concentrations of decorin are expressed in terms of protein.

Binding of LDL to Decorated Collagen—LPL was isolated from bovine fetal skin exactly as described (3). Oxidation of LDL by copper acetate (32). The catalytic activity of LPL was inhibited by the addition of tetrahydrolipstatin (Orlistat®, Hoffman-La Roche, Basel, Switzerland) over LPL in buffer containing 20 mM NaCl, 2 mM CaCl₂, 2 mM MgCl₂, 10 mM HEPES, pH 7.4, and 30 mg/ml BSA.

Microtiter Well Binding Assay—Microtiter well assays were performed by incubating the compound to be tested in 50 μl of buffer A at 4 °C (BSA-coated wells) overnight and the BSA-coated and decorin-coated wells for 1 h at room temperature for nonspecific binding. In some experiments, the wells were preincubated sequentially with the indicated concentrations of decorin and LDL for 1 h at room temperature and washed twice with 250 μl of buffer B between incubations. Unbound compounds were removed by aspirating the incubation medium, and the wells were then rinsed three times with 250 μl of buffer B. The wells were detached, and their radioactivity was determined by liquid scintillation counting. The data points shown in Figs. 1, 2, and 5 indicate ligand specifically bound to the compound(s) tested, which was calculated by subtracting the amounts of ligand bound to the BSA-coated wells from the amounts of ligand bound to the wells coated with the compound(s) tested. The former values were <10% of the latter ones.

Affinity Chromatography of Hepatocyte Columns—Binding of VLDL, IDL, and native and modified LDL, and microemulsions to 1-mL heparin-Sepharose columns (Amersham Pharmacia Biotech) was studied in the absence and presence of native and inactivated LDL. Native or inactivated LDL (100 μg/ml diluted 1:5 with 20 mM Tris-HCl, pH 7.4, 30% glycerol) was first injected into the column equilibrated with 20 mM Tris-HCl, pH 7.4. In some experiments, inactive forms of LDL were removed from the column by washing it with buffer containing 1 mM CaCl₂, 20 mM Tris-HCl, pH 7.4. After re-equilibration to 20 mM Tris-HCl, pH 7.4, tritiated lipoproteins (10 μg) were injected into the column and eluted with a linear gradient of NaCl (0—2 M) in 20 mM Tris-HCl in 10 min. 500-μl fractions were collected, and aliquots of the fractions were analyzed for radioactivity by scintillation counting and for triacylglycerol content by the method of Bolin and Havel (28). The amount of lipoprotein in each fraction was determined by measuring the radioactivity and the triacylglycerol content. Nonspecific binding was blocked by an equimolar concentration of NaCl in the standard and the gradient elution buffer.

Immunohistochromistry—Samples of coronary arteries were obtained from four human hearts explanted at cardiac transplantation and snap frozen in OCT compound (Miles, Elkhart, IN); serial frozen sections were cut at 5 μm and dried at room temperature. Sections were then incubated with 3% glutaraldehyde-modified lysine residues (1:100; produced in the Immunolab, Witztum), 5D2 for LPL (1:100; a kind gift of Dr. J. Brunzell, University of Washington, Seattle), MAB1340 for collagen type I (1:10, Chemicon International, Sunnyvale, CA). Acetone-fixed sections were stained with standard protocols using the indirect immunoperoxidase method with horseradish peroxidase-conjugated goat anti-mouse or biotinylated goat anti-rabbit and horseradish peroxidase-conjugated streptavidin detecting antibodies (DAKO, Glostrup, Denmark) for mouse monoclonal and rabbit polyclonal antibodies, respectively. 3-Amino-9-ethylcarbazole (Sigma) was used as chromogen.

RESULTS

The interaction between LDL and decorin was first studied in microtiter wells coated with decorin. Under physiological ionic conditions and in the absence of LDL, binding of native LDL was low and not saturable (Fig. 1A). But when the immobilized decorin was first preincubated with purified LDL from bovine milk at 10 μg/ml and the unbound LDL removed, the remaining LDL/LDL decorin interaction increased. Surface plasmon analysis of this interaction revealed an association constant of 11.7 ± 2.9 10⁻³ pm (mean ± S.D., n = 3). Oxidation of LDL by copper for 18 h, which resulted in an electrophoretic mobility of 1.7 ± 0.2 (n = 7) relative to native LDL on cellulose acetate (which corresponds to an electrophoretic mobility of 3.5 relative to LDL on agarose electrophoresis), effectively inhibited the interaction between LDL and decorin.
Interaction between LDL and decorin (Fig. 1B). However, preincubation of decorin with LPL at 10 μg/ml dramatically enhanced the interaction between oxLDL and decorin, yielding an association constant of 5.9 ± 1.0 nM (n = 4). When LPL was directly immobilized to microtiter wells, both native and oxLDL bound strongly to the LPL (K_D 12.3 nM and 5.3 nM, respectively), revealing that the presence of decorin did not markedly modulate the interactions between LPL and the two lipoproteins. When radiolabeled decorin was incubated with LPL immobilized to microtiter wells, the decorin bound to LPL with high affinity (K_D 56 nM). Digestion of the GAG chain of decorin by chondroitinase ABC inhibited this binding by more than 90%, revealing that decorin interacts with LPL via its GAG chain.

Fig. 2 shows binding of both native and oxLDL to collagen under conditions of physiological ionic strength. Under these conditions, only minimal binding of LDL or oxLDL to collagen or decorin-coated collagen was observed. However, when LPL was first allowed to bind to the decorin-coated collagen, both native and oxLDL showed high affinity and saturable binding to the pretreated collagen. In three independent experiments, LPL increased the amount of LDL bound to decorin-coated collagen by 9-, 29-, and 64-fold and that of oxLDL by 4-, 6-, and 12-fold. Thus, it appears that LPL can associate with collagen only in the presence of decorin and can efficiently link both native and oxLDL to decorin-coated collagen.

To gain further insight into the mechanisms of the interactions between native and oxLDL, LPL, and decorin, we performed affinity chromatography on a heparin column in the absence and presence of LPL. As shown in Fig. 3A, in the absence of LPL, most of native LDL, IDL, and VLDL bound to the immobilized heparin, their elution peaking at 215, 180, and 120 mM NaCl, respectively. Consistent with previous results (35), oxidation of LDL with copper for 18 h resulted in decreased affinity of LDL for heparin and formation of a fraction not binding to heparin. Next, we loaded the heparin column with LPL and compared the elution of the above series of lipoproteins. As shown in Fig. 3B, elution of native LDL was markedly retarded, although it still eluted earlier than LPL activity. In contrast, IDL, VLDL, and oxLDL all bound tightly to the column and eluted at roughly the same...
time as the LPL activity. Similar results were obtained with decorin columns, although all of the studied components eluted at lower NaCl concentrations. Accordingly, in the absence of LPL, native LDL eluted at 50 mM NaCl, and oxLDL failed to bind to the column, whereas in the presence of LPL, native and oxLDL eluted at 600 and 800 mM NaCl, respectively. LPL activity eluted at 800 mM NaCl.

To study the effect of different degrees of LDL oxidation on binding to LPL, we incubated LDL (0.5 mg/ml) with 5 μM copper sulfate for various times and studied its effect on the elution profiles from heparin columns loaded with LPL. As shown in Fig. 3C, inset, oxidation for 2 h resulted in ~2-fold electrophoretic mobility on agarose gel relative to that of native LDL and degradation of apoB-100 into a range of large fragments when analyzed by SDS-polyacrylamide gel electrophoresis (not shown). This degree of oxidation markedly increased the binding of LDL to LPL (Fig. 3C) but, in contrast, had only a minor effect on the binding to heparin in the absence of LPL (not shown). As oxidation progressed, the affinity of oxLDL to LPL continued to increase until it partly coeluted with the active LPL. Progressive oxidation of LDL particles also progressively decreased their affinity for heparin in the absence of LPL (not shown; the effect of 18-h oxidation is shown in Fig. 3A). We also studied the effects of oxidation with AAPH, hypochlorite, and soybean 15-lipoxygenase on the binding of LDL to LPL. Moreover, the oxidative modification of LDL lysines was mimicked by acetylation of LDL. As shown in Fig. 3D, all of these modifications caused a significant increase in the affinity of LDL for LPL.

Moreover, although the electrophoretic mobility of LDL differed among the different types of oxidation (Cu²⁺, hypochlorite, AAPH, soybean 15-lipoxygenase), all types of oxidation dose-dependently increased the electrophoretic mobility of LDL and dose-dependently increased their affinity for LPL. Additional experiments showed that oxidation of LDL by reagent peroxynitrite and modification of LDL by MDA increased the affinity of LDL for LPL, and this effect depended on the degree of modification (not shown).

Previous studies have shown that inactive forms of LPL have a weaker affinity for heparin than has the catalytically active dimeric LPL (36). The observed elution of native LDL from the heparin column loaded with LPL (both active and inactive) by increasing NaCl concentration before the elution of catalytically active LPL may have reflected release of the LDL from either the inactive form or the catalytically active form of LPL (43). To distinguish between these two alternatives, we compared the binding of native and of copper-oxidized LDL to heparin columns containing one or the other form of LPL. Fig. 4 shows the elution profiles of native and copper-oxidized LDL in the absence (panel A) and presence (panel B) of LPL. Elution of LDL was monitored by absorbance at 280 nm and was found to be roughly similar both in the absence and presence of the added lipoproteins (not shown). LDL was found to elute in three separate peaks, the last of which was the active form of the enzyme (panel B). Incubation of LPL either with 1 M guanidinium HCl for 1 h at room temperature (panel C) or as a dilute solution (10 μg/ml LPL protein) for 1 h at 37 °C (not shown) resulted in total loss of LPL activity and disappearance of the peak corresponding to active LPL in heparin affinity chromatography (panel C). Loading of the heparin column with this inactivated enzyme retarded the elution of native LDL in a manner similar to the effect found with the LPL preparation containing both the inactive and the active forms of the enzyme (C versus B). In sharp contrast, the elution profile of oxLDL was dramatically changed: the peak eluting near the position of active LPL disappeared completely, the elution profile resembling that found when no LPL was present in the column, except for a small peak coeluting with the inactive forms of LPL (C versus A and B). Finally, we studied the ability of native and oxLDL to bind to dimeric LPL. An affinity column containing only the dimeric form of the enzyme was obtained by removing inactive forms of LPL by washing the LPL-loaded heparin column with 1 M NaCl. As shown in panel D, this treatment had no effect on the elution of oxLDL (D versus B) but dramatically changed the elution profile of native LDL, which now resembled that found in the absence of LPL (panel A). To study...
whether binding of oxLDL to catalytically active LPL depends on the dimeric state or the catalytic activity of the enzyme, heparin-bound LPL, before application of oxLDL to the column, was treated with tetrahydrolipstatin, an inhibitor of the catalytic site of LPL. We found that tetrahydrolipstatin did not inhibit binding of oxLDL to LPL, although it inhibited the LPL activity by 86%. This result demonstrates that binding of oxLDL to dimeric LPL does not depend on the catalytic activity of the enzyme and agrees with the results of Zambon et al. (12), who showed that catalytically inhibited LPL was associated with triglyceride-rich lipoproteins in blood plasma. To confirm the results in an independent system, we compared the abilities of monomeric and dimeric LPL to link native and oxLDL to decorin-coated microtiter wells. We found that mild dissociation of LPL into monomers by guanidinium HCl decreased LPL-mediated linking of native LDL to decorin by only 30%, whereas LPL-mediated linking of oxLDL to decorin was decreased by >90%. Taken together, the present results show that native LDL binds to monomeric LPL, whereas oxLDL binds preferentially to dimeric LPL.

To study whether the protein (apoB-100) component of LDL is important in binding to either form of LPL, we tested the effect of proteolyzing apoB-100. Proteolysis of LDL with α-chymotrypsin for 6 h led to formation of ~20% trichloroacetic acid-soluble material, i.e. this amount of apoB-100 peptides had been released from the LDL particles (25). SDS-polyacrylamide gel electrophoresis (Fig. 5A) showed that no intact apoB-
100 but only fragments of apoB-100 below 100 kDa had remained associated with the particles. Interestingly, a fraction coeluted with LPL activity, demonstrating binding to dimeric LPL (Fig. 5A). Next, we treated LDL with plasmin, which leads to fragmentation of apoB-100 (Fig. 5B) but does not to lead to any loss of apoB-100 from the particles (25). Such fragmentation of apoB-100 had no effect on the interaction of LDL with LPL (Fig. 5B). To assess whether the lipids of LDL can bind to dimeric LPL, we isolated the lipids from LDL and reconstructed lipid particles (microemulsions) from the LDL lipids by sonication in aqueous buffer. As expected, these microemulsions did not bind to heparin in the absence of LPL (Fig. 5C) but bound to dimeric LPL with high affinity. This interaction appeared to be of ionic type because increasing concentration of NaCl efficiently decreased the binding of the microemulsions to LPL adsorbed on microtiter wells (Fig. 5D). To study whether binding of the microemulsions to the dimeric LPL depends on particle size, the large microemulsions were compared with LDL-sized phosphatidylcholine vesicles for their ability to bind LPL. No differences in binding to LPL were observed (not shown). Finally, to study whether changes in the lipid surface of LDL can lead to binding of LDL to dimeric LPL, LDL was enriched with free fatty acids or treated with bee venom phospholipase A2 either in the absence or presence of albumin. However, the lipid surface-modified LDL bound preferentially to monomeric LPL (not shown). Thus, it appears that dimeric LPL has the potential to bind to LDL lipids, but this interaction is hindered by intact apoB-100 in LDL.

Finally, we were interested in studying the spatial relationship of these components in the arterial intima. For this purpose we obtained samples of coronary arteries from four explanted human hearts and stained frozen sections immunohistochemically for apoB-100, MDA-lysines, LPL, decorin, and collagen type I. The samples were graded according to the AHA guidelines (37, 38), and we found areas of type I lesion in all four samples, type II lesion in two samples, type III lesion in one sample, and type V lesion on two samples. ApoB-100, MDA-lysines, LPL, decorin, and collagen were present in distinct, characteristic, partially overlapping areas of the intima. Thus, apoB-100 was present in all of the early lesions (types I–III) in both the superficial PG-rich and the deep muscular layer of the intima and concentrated in distinct sectors of the arterial circumference. In advanced lesions (type V), staining for apoB-100 was most intense around the lesion core and its shoulder areas. Staining for oxidized epitopes (MDA-lysines) was weak or absent in the grossly normal intima but was clear in type I–III lesions in the PG-rich layer, being associated mainly with intracellular lipid deposits. In advanced lesions (type V), oxidized epitopes were concentrated, like apoB-100, around the core and shoulder areas of the lesion. LPL was present in early lesions (types I–III) subendothelial and in the PG-rich layer but not at all. In advanced lesions LPL was found in foam cell-rich areas of the shoulders and sometimes also deeper around the core. Decorin was present in the PG-rich layer in early lesions (types I–III) and around core regions in advanced lesions (type V). Staining for collagen type I was weak in areas of thin, grossly normal intima, but in thick intima was clearly present in the PG-rich layer (lesion types I–III). In advanced lesions, staining for collagen type I was concentrated around the lesion core. Interestingly, we were able to find an early atherosclerotic lesion, characterized by extracellular Oil Red O-positive material, in which there was colocalization of all of the studied components (Fig. 6).

**FIG. 6. Immunohistochemistry of human coronary arteries.** Frozen sections of a human coronary artery were stained with Oil Red O (panel A) or by indirect immunoperoxidase using antibodies against apoB-100 (panel B), MDA-lysines (panel C), LPL (panel D), decorin (panel E), and collagen type I (panel F). Panel G shows a control section where primary antibody was omitted. L, lumen; I, intima; IEL, internal elastic lamina.

**DISCUSSION**

The present findings show that LPL dramatically changed the characteristics of decorin-coated collagen with regard to interaction with lipoproteins; in the absence of LPL, decorin-coated collagen bound small amounts of native LDL relatively weakly and no oxLDL at all, whereas in the presence of LPL, large amounts of both native and oxLDL were bound with high affinity. In this system, LPL binds to the GAG chain of collagen-bound decorin, and native or oxLDL then binds to the immobilized LPL. Native LDL, unlike oxLDL, can bind directly to decorin but is readily dissociated from decorin at physiological ionic strength because the affinity of this interaction is weak. On the other hand, the affinities of both LDL and decorin for LPL are a magnitude higher, and thus LPL acts as a strong link between native LDL and decorin. Such strong links are likely to be required if LDL is to be retained in tissues in vivo, where many other molecules are also competing for binding.

LDL is known to bind to GAG by the positively charged lysine and arginine residues of apoB-100 (39, 40), and oxidation of the lysines of apoB-100 has been shown to abolish this interaction (35). The mechanism of binding of native and oxLDL to LPL is less well understood. Because LPL is known to be notoriously labile to dissociate into monomers (10), LPL immobilized directly on microtiter wells or on preparations of extracellular matrix is likely to be a mixture of mono-
meric and dimeric forms of LPL. Careful preparation of dimeric LPL by washing off monomeric LPL and leaving dimeric LPL bound to heparin yielded the unexpected results that dimeric LPL did not bind to native LDL but bound strongly to oxLDL. In addition, we found that VLDL and IDL also bound to dimeric LPL (not shown). These experimental in vitro results are supported by findings that, in human plasma, dimeric LPL is associated with postprandial lipoproteins larger than LDL (12, 41), whereas inactive LPL is associated with cholesterol-rich lipoproteins (13). These findings may be of importance in understanding lipoprotein metabolism in that LPL is allowed to perform its enzymatic function, i.e., to hydrolyze triglycerides of triglyceride-rich particles on capillary surfaces without competition from a high concentration of nonsubstrate lipoproteins (such as LDL). Similarly, it is important that the LDL particles can be transported without hindrance across the capillary endothelium into the extracellular fluids to be able to provide cells with cholesterol (42).

We found that oxidative modification of LDL, regardless of the oxidation method used, increased the affinity of LDL for dimeric LPL dose-dependently. This is consistent with the results of Auerbach et al. (18) and Makoveichuk et al. (20). In contrast, Hendriks et al. found that binding of LDL to J774 cells in the presence of LPL was decreased by oxidation and by acetylation (44), a finding likely reflecting the use of heat-inactivated, i.e., monomeric/aggregated LPL. Interestingly, we found that, in addition to other types of oxidative modification of LDL, even a small degree of oxidation by soybean 15-lipoxygenase allowed binding of LDL to dimeric LPL. In line with this observation, Makoveichuk et al. demonstrated recently that copper oxidation of triglyceride emulsion particles (Intralipid®) leads to their increased affinity for LPL (20).

Dimeric LPL binds lipid particles devoid of apoB-100 similarly to oxLDL, IDL, and VLDL, which strongly suggests that dimeric LPL interacts with the phospholipid surface of these lipoproteins. Proteolytic degradation of apoB-100 and charge modification of apoB-100 by oxidation, but not modification of the surface phospholipids of LDL by addition of free fatty acids or by treatment with phospholipase A_2 (not shown), allowed binding of LDL to dimeric LPL. This strongly suggests that apoB-100 in LDL inhibits binding of LDL to dimeric LPL, possibly by steric hindrance via its positively charged lysine and arginine residues. Interestingly, in addition to binding to the lipids in LDL, LPL has been found to bind to the NH_2-terminal part of apoB-100 of LDL (45). However, in the present experiments, no evidence of binding of LPL to apoB-100 was found.

The role of LPL in atherosclerosis has been difficult to assess because the enzyme has antiatherogenic effects when present on the capillary endothelium and acting on the circulating triglyceride-rich lipoproteins, but may have proatherogenic effects when present in the arterial intima (46). Thus, decreased levels of LPL caused by mutations in the LPL gene (47–49) have been shown to have accelerated atherosclerosis, most likely the result of lipid abnormalities in the plasma. Moreover, overexpression of LPL has been shown to retard the development of atherosclerosis in apoE−/− and LDLr−/− mice (50, 51), most likely because of enhanced metabolism of remnant lipoproteins. However, the proatherogenic role of LPL in the vascular wall was demonstrated recently in mice lacking macrophage LPL expression, which had decreased diet-induced atherosclerosis compared with wild-type mice (52).

LPL activity has been detected in the endothelial layer of the arterial wall (53). However, in the arterial wall, as in other tissues, LPL is synthesized and secreted by the subendothelial cells rather than by the endothelial cells themselves (54). In deed, arterial wall cells of two types, smooth muscle cells and macrophages, have been shown to synthesize and secrete catalytically active dimeric LPL in vitro and in vivo (55) providing a cellular source in the arterial intima for an extracellularly located pool of catalytically active LPL molecules (56). Because LPL binds strongly to heparan sulfate and dermatan sulfate GAG, a fraction of this extracellular LPL pool is likely bound to the extracellular matrix of the intima. The presence of the monomeric form of LPL in the arterial intima is also possible; at least in the tissues studied so far (the adipose tissue, heart, lung, and skeletal muscle), a significant proportion of the LPL is in this inactive form (11). LPL protein has been shown to be present in the arterial intima, and the staining pattern reveals that some of the LPL is also localized within the extracellular matrix (54, 57–59). In the present study, we extended these findings and demonstrated not only localization of LPL in the arterial intima in collagen-rich areas, but also the presence of decorin, LDL, and oxLDL in these areas, i.e., providing an in vivo basis for the present in vitro observations. However, on the basis of the small number of samples studied and the small number of components stained, the present immunohistochemical results should be regarded as merely suggestive and should be followed by a larger study involving large numbers of samples and comparing the distribution of LPL with different types of GAG and with different apolipoproteins (60).

In atherosclerosis, the retention of LDL in the extracellular matrix of the arterial wall has an evident role, whereas the roles of the individual components of the extracellular matrix in the retention are less known. Retention of LDL appears to involve both direct binding to GAG, and indirect binding to GAG facilitated by molecules such as LPL and apoE, the former having a very large capacity but low affinity, and the latter having a limited capacity but high affinity to bind LDL. In view of the findings of Borén et al. (61) that elimination of PG binding of LDL appears to delay the onset of atherosclerosis, of Tozer and Carew (62) that the retention of LDL is most extensive after formation of macrophage-rich fatty streak lesions, and of Babaev et al. (52) that macrophages appear to be major sources of LDL in the arterial wall, it appears evident that the retention of LDL is initially governed by direct binding of LDL to GAG but then shifts to facilitated binding when macrophages infiltrate the intima and secrete LPL.

The feature that makes collagen a particularly interesting component of the extracellular matrix in the arterial intima, apart from its great quantity, is the demonstration of preferential accumulation of LDL along collagen fibers when incubated with cardiac leaflets of the rabbit (1). In light of the present findings we propose that retention of both native and oxLDL along collagen fibers is initiated, not by the collagen itself, but by the collagen-bound decorin and LPL. The present findings demonstrate that monomeric LPL binds native LDL, and dimeric LPL binds oxLDL. Because both of these lipoprotein classes are present in the collagen-rich areas of human coronary atheromas, the possibility exists that both dimeric and monomeric forms of LPL play a role in lipoprotein retention in the arterial intima during atherogenesis.

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Lipoprotein Lipase (LPL) Strongly Links Native and Oxidized Low Density Lipoprotein Particles to Decorin-coated Collagen: ROLES FOR BOTH DIMERIC AND MONOMERIC FORMS OF LPL
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