Lipoprotein Lipase in the Arterial Wall
Linking LDL to the Arterial Extracellular Matrix and Much More

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Abstract—For low density lipoprotein (LDL) particles to be atherogenic, increasing evidence indicates that their residence time in the arterial intima must be sufficient to allow their modification into forms capable of triggering extracellular and intracellular lipid accumulation. Recent reports have confirmed the longstanding hypothesis that the major determinant(s) of initial LDL retention in the preatherosclerotic arterial intima is the proteoglycans. However, once the initial atherosclerotic lesions have formed, a shift to retention facilitated by macrophage-derived lipoprotein lipase (LPL) appears, leading to the progression of the lesions. Here, we review recent findings on the mechanisms enabling LPL to promote LDL retention and extracellular lipid accumulation in the arterial intima, and we describe the structures in the extracellular matrix that are held to be important in this process. Finally, the potentially harmful consequences of LDL linking by LPL and of other LPL actions in the arterial intima are briefly reviewed. (Arterioscler Thromb Vasc Biol. 2002;22:211-217.)

Key Words: lipoprotein lipase ■ extracellular matrix ■ lipoproteins ■ retention

The major lipolytic enzyme involved in the intravascular metabolism of postprandial triglyceride-rich lipoproteins is lipoprotein lipase (LPL).1 In adipose tissue and in muscle, this enzyme is synthesized and secreted in catalytically active form by adipocytes and myocytes, respectively, and is then transported to the capillary endothelial surface. Its physiological function is to hydrolyze the triglycerides of chylomicrons and VLDL and IDL particles on the luminal side of the capillary endothelium, with release of free fatty acids, which are stored as triglycerides (adipose tissue) or are oxidized for production of energy (muscle). The majority of the so-formed triglyceride-poor and cholesterol-enriched lipoprotein remnant particles are cleared by the liver. However, experiments with transgenic mice expressing catalytically inactive LPL show that the enzyme is dissociated into monomers, its activity is lost.

LPL belongs to the mammalian triacylglycerol lipase gene family, which also contains pancreatic lipase, hepatic lipase, and endothelial lipase. According to homology modeling3,4 based on the crystal structure of human pancreatic lipase,5 LPL has a larger N-terminal folding domain (amino acids 1 to 312) and a smaller C-terminal domain (amino acids 313 to 418). The catalytic site, consisting of Ser132, Asp156, and His241, is localized in the N-terminal folding domain and is covered by a lipid-binding lid. Interestingly, interaction of the C-terminal folding domain with lipoprotein substrates has been proposed to result in a conformational change in LPL that leads to the opening of the lid, thus allowing the enzyme to conduct its catalytic function.6 Other functionally important parts of LPL include heparin-binding sites located in the N-terminal and the C-terminal folding domains, a binding site for the cofactor apoC-II in the N-terminal folding domain, and a binding site for LDL receptor–related protein in the C-terminal folding domain.

The catalytically active LPL is a noncovalent 96-kDa homodimer that is likely to be oriented in a head-to-tail conformation.7 The dimeric form of the enzyme is stabilized by its interaction with the heparan sulfate glycosaminoglycans (GAGs) present on the endothelial surface. As illustrated by experiments with mice expressing heparin-binding defective LPL, binding of LPL to proteoglycans is important not only for the stability of the enzyme but also for anchoring the enzyme to sites at which its activity is required.8 Once the enzyme is dissociated into monomers, its activity is lost. Interestingly, monomers of LPL are present in significant amounts in the circulation and also in extrahepatic tissues, such as adipose tissue and the heart.9,10 In the circulation, LPL monomers associate with the lipoprotein remnant particles and, in this way, may enhance their clearance by the liver.10,11 The vast majority of the total LPL in the body is located in the capillary endothelium. However, LPL is also found on the arterial endothelium. This LPL, albeit a tiny fraction of the total body LPL, has been suggested to have a role in atherogenesis by creating, locally on the arterial endothelium, remnant particles that can be deposited in the arterial intima. As in the capillary bed, such remnants are generated from triglyceride-rich lipoproteins of intestinal origin. Accordingly, this action of arterial LPL has been suggested to be a postprandial phenomenon and so may link the fat-containing...
Western diet with the development of atherosclerosis. A second pool of arterial LPL is located subendothelially, ie, within the inner layer of the artery, the arterial intima, where lipids accumulate during atherogenesis. The present review deals with this fraction of LPL and its potential relation to intimal lipid accumulation and to some other processes that are important in the development of atherosclerotic lesions.

**Retention of LDL in the Arterial Intima**

The initial step in the development of atherosclerotic lesions is suggested to be the retention of LDL. Kinetic studies have provided evidence for sequestration of LDL in the normal arterial wall in a pool that exchanges slowly with plasma LDL. In atherosclerosis-prone areas, ie, in branch points of normal arteries and also in injured or atherosclerotic arteries, the size of this sequestered pool is increased. The observed differences in LDL sequestration are correlated with changes in the proteoglycan composition in arterial branch sites and in early atherosclerotic lesions, which is consistent with the idea that sequestration results, at least partly, from the binding of LDL to proteoglycans in the arterial intima. Direct experimental support for this idea has come from experiments by Borén et al, who showed that if the principal proteoglycan-binding site of apoB-100 is mutated, LDL retention in the normal arterial intima of mice is virtually abolished. Importantly, Borén et al found that in mice expressing proteoglycan-binding-defective human apoB-100 compared with mice expressing wild-type human apoB-100, the development of atherosclerotic lesions was retarded. Although this experiment provides direct evidence that LDL-proteoglycan interaction has a role in the initiation and early development of atherosclerosis, it also reveals that lipid-containing atherosclerotic lesions may develop even when LDL-proteoglycan interaction is prevented.

Then, the same group made an important observation: Retention of the proteoglycan-binding-defective LDL to preformed atherosclerotic lesions was found to be similar to that of wild-type LDL. This finding must be interpreted to mean that later during the process of atherogenesis, factors other than direct binding to proteoglycans are involved in the retention of LDL to the arterial intima. Because LPL can bind to GAGs (proteoglycans) and to LDL, LPL could be the factor mediating the retention of the proteoglycan-binding-deficient LDL particles in the proteoglycan-rich areas of atherosclerotic lesions.

Several experiments with genetically engineered mice have attempted to elucidate the role of LPL in atherogenesis. In the LPL receptor−/− and the apoE−/− mouse models of atherosclerosis with pronounced remnant hyperlipidemia, overexpression of LPL resulted in less extensive atherosclerotic lesions than those found in their nontransgenic littermates. In contrast to mice with enhanced LPL activity, apoE−/− mice with heterozygous LPL deficiency, although having enhanced hyperlipidemia, show a similar degree of atherosclerosis or less atherosclerosis than do apoE−/− mice with full LPL activity. The paradoxically lacking atherogenic effect of remnant hyperlipidemia in these animals could have been due to low LPL activity locally on the arterial endothelium and in the arterial wall. However, in all of the above-described experiments, the total body LPL activity was either increased or decreased, making an unequivocal decision about the roles of arterial LPL in atherogenesis difficult.

With the advent of elegant novel techniques for producing chimeric mice, it has become possible to assess the impact of arterial wall LPL on atherosclerosis. Thus, by transplanting either LPL−/− or LPL−/− fetal liver cells as the source of hematopoietic cells (and, ultimately, of monocytes/macrophages infiltrating the arterial wall), Babaev et al were able to show that cholesterol-fed C57Bl/6 mice with normal expression of macrophage LPL developed more extensive atherosclerosis than did mice with no expression of macrophage LPL. Using the same strain of mice but using bone marrow transplantation, Van Eck et al showed that deficiency of macrophage LPL production during cholesterol feeding led to a 50% decrease in serum cholesterol and apoE and to a 52% decrease in the area of aortic fatty streak lesions. Finally, Babaev et al, by transplanting either LPL−/− or LPL−/− fetal liver cells into LDL receptor−/− mice, showed that macrophage LPL production enhanced the formation of fatty streak lesions. In their experiment, development of advanced atherosclerotic lesions was not affected by macrophage LPL production, which is consistent with the observed scarcity of macrophages in this type of lesion. Collectively, the above experiments showed that macrophage-derived LPL exerts a proatherogenic effect by inducing aortic foam cell formation, ie, formation of the earliest type of atherogenic lesions, the fatty streaks.

**Interaction of LPL With Different Components of the Arterial Intima**

Theoretically, LPL in the arterial intima could originate from circulating LPL and/or from local synthesis by the various cells present in the arterial intima. Because LPL has been shown to be produced in the intima by macrophages and smooth muscle cells and because virtually no LPL is present in the intimas of mice deficient in macrophage LPL production, it appears that most of the LPL in the arterial intima is derived from local synthesis, notably by the monocyte-derived macrophages. Most interestingly, immunohistochemical staining for LPL in the arterial intima has shown that LPL is not only associated with intimal cells but is also abundantly present within the extracellular matrix. Thus, the intima clearly differs from other tissues in which the subendothelially synthesized LPL is not stored within the stromal tissue but is effectively transported to the luminal surface of the capillary endothelium. One reason for this difference may be the lack of capillaries in the arterial intima.

In vitro experiments have shown that LPL can bind to heparan sulfate and dermatan sulfate GAGs but not to collagen, fibronectin, vitronectin, or, surprisingly, chondroitin sulfate GAGs. However, differentiated macrophages have been shown to synthesize oversulfated chondroitin sulfate–rich proteoglycans that can bind to LPL. Moreover, LPL has been shown to bind to chondroitin sulfate and especially to dermatan sulfate–rich proteoglycans isolated from the human aorta. In a recent study, we did not observe any direct binding of LPL to collagen but found that the collagen-binding small proteoglycan decorin was able to efficiently link LPL to GAGs.
has been suggested to be mediated by 3 continuous clusters in the N-terminal domain and a noncontinuous cluster in the C-terminal domain of positively charged amino acids that are brought together by folding of the enzyme. The dimeric structure of LPL causes an increase in the affinity for heparin, presumably because sites in both monomers can participate in the binding of 1 heparin molecule. The dimeric C-terminal domain of LPL binds much more strongly to heparin molecules than does the corresponding monomeric domain. This presumably because sites in both monomers can participate in the binding of 1 heparin molecule.

Although LDL can also bind to various types of proteoglycans with relatively similar affinities in vitro, a recent study showed that the pattern of LDL retention closely followed that of biglycan, a small proteoglycan. To elucidate the type of proteoglycan to which LPL binds in the extracellular matrix of the arterial intima, we compared the distribution of LPL with that of various proteoglycans in sections of human atherosclerotic lesions. Figure 1 shows an atherosclerotic lesion (atheroma or American Heart Association type IV lesion) characterized by an almost acellular lipid-rich core, numerous inflammatory cells in the shoulder region, and a relatively thin fibrous cap. In this lesion, LPL is found diffusely throughout the superficial proteoglycan-rich layer and is clearly absent from the deeper musculoelastic layer of the intima. Similarly, versican and biglycan are present throughout the upper layer of the intima. Decorin, in turn, is present only in the core region of the plaque. Collagen type I, again, is present in the core region and also in the superficial part of the lesion, forming a thin fibrous cap. Finally, LDL (apoB-100) is extensively deposited in the core region of the plaque and also in the fibrous cap, colocalizing with collagen I. Notably, apoB-100 is completely absent from the highly cellular area between the core and the collagenous cap, ie, the shoulder area, a finding compatible with the idea that macrophages ingest and degrade the LDL particles.

**Interaction of LPL With Native and Modified LDL**

The role of apolipoproteins in the interaction between LPL and triglyceride-rich lipoproteins during hydrolysis of triglycerides has been studied extensively. Efficient hydrolysis of triglyceride-rich lipoproteins depends on 1 of the several apolipoproteins present on the surface of these particles, namely, apoC-II, an activator of LPL. However, LPL has been shown to also bind to artificial lipid emulsions lacking apolipoproteins. Thus, it appears that LPL can interact directly with phospholipid molecules in the absence of apolipoproteins. It has been proposed that the lipid-binding region in LPL is constituted of the lid region containing amphipathic α-helices of the N-terminal folding domain and of tryptophan residues in an exposed loop between the β strands of the C-terminal folding domain (involving amino acids 390 to 394). Interestingly, dimeric LPL lacking the C-terminal domains binds to phospholipid vesicles as efficiently as native dimeric LPL but is totally unable to interact with chylomicrons. On the other hand, although full-length LPL binds to chylomicrons, VLDL, and LDL with similar affinity, the C-terminal domain binds much more strongly to chylomicrons and VLDL than to LDL. Thus, it appears that the C-terminal domain of LPL is important for binding to chylomicrons and VLDL, whereas the N-terminal folding domain of LPL mediates the binding of LPL to phospholipid vesicles and possibly also to LDL particles.

Results regarding the role of apoB-100 in the interaction of LPL with LDL, which is not a physiological substrate for LPL (unlike the triglyceride-rich lipoproteins), have been conflicting. Goldberg’s group (Choi, Pang, and colleagues) published data indicating that LPL interacts with the amino-terminal part of apoB-100. However, we found no evidence to support an interaction of LPL with apoB-100 and could even demonstrate enhanced binding of LPL to LDL that had been treated with proteolytic enzymes to obtain degradation of apoB-100 and subsequent loss of apoB-100 peptides. Moreover, in a very recent study, Borén et al. studied this issue extensively and found binding of LPL to LDL lipids and, moreover, suggested that the binding of LPL to the recombinant amino-terminal part of apoB-100 (apoB-17) can be explained by the presence of lipids bound to this apolipoprotein. Finally, since 1 LDL particle contains only 1 apoB-100 polypeptide, the stoichiometry of the LPL-LDL binding was shown to be 1 apoB-100/LPL.
interaction (binding of up to 15 dimers per LDL particle) speaks against a specific interaction between LPL and apoB-100 on the interaction of LPL with LDL.

The interaction of LPL with LDL has been shown to involve electrostatic and hydrophobic interactions. Using “sandwich” affinity chromatography, we made the unexpected observation that although the catalytically active dimeric form of LPL efficiently interacts with triglyceride-rich lipoproteins, the monomeric form of LPL preferentially interacts with LDL. These results are in line with those of Hendriks et al., who found that LPL in its native dimeric form was unable to link LDL to J774 macrophages.

LDL has been suggested to undergo several types of modification in the arterial intima, including oxidation, and hydrolysis of apoB-100 and of lipids. Interestingly, although oxidation decreases the direct binding of LDL to GAGs, even mild oxidation significantly enhances the interaction between LDL and LPL. The mechanism(s) responsible for this increased binding is not known but may involve the shift of LDL to a more hydrophobic particle after partial hydrolysis of the surface phospholipids or of apoB-100. Moreover, as noted above, in addition to oxidation of LDL, proteolytic degradation of apoB-100 alone could enhance the interaction between LDL and LPL. Thus, it is

Figure 2. Diagram showing the various effects of LPL in the arterial intima. TG indicates triglyceride; FFA, free fatty acid.
possible that any modification that disturbs the well-organized surface structure of LDL may generate particles with increased affinity for LPL.

**Proatherogenic Effects of LPL in the Arterial Intima**

As discussed above, LPL that is bound to the components of extracellular matrix of the arterial intima can lead to retention of LDL in these structures by acting as a molecular bridge. Retention increases the residence time of LDL in the intimal matrix, thus allowing the particles to be modified more extensively than they would otherwise have been. During such modification, biologically active lipids, such as oxidized phospholipids, oxysterols, free fatty acids, and ceramide, are formed in the LDL particles. Free fatty acids and lysophosphatidylcholine are released from the modified particles to some extent and, when bound to albumin, may be transported to the intimal cells or, when the modified LDL particles are ingested by the cells, may be taken up by them with other modified lipids (Figure 2). The biologically active lipids may have several effects on the intimal endothelial cells, macrophages, and smooth muscle cells: they are able to trigger inflammatory reactions, and at high concentrations, they may even be cytotoxic. Finally, LPL also bridges native and modified lipoproteins to cell surface heparan sulfate proteoglycans and to various lipoprotein receptors on the cell surfaces and thus facilitates the uptake of lipoproteins by the intimal cells (see review). According to a very recent finding, LPL appears to cause selective uptake of cholesterol from LDL, a process that requires cell surface proteoglycans but is independent of lipoprotein receptors and LPL activity.

During lipolysis of chylomicrons and VLDL by LPL, the formed free fatty acids have been shown to release the particles with LPL from the endothelial surface, allowing the remnant particles to be transported to the liver or to extrahepatic tissues. In addition to LDL, IDL particles, small VLDL particles, and chylomicron remnants have also been shown to enter the arterial wall, where they have even been suggested to be preferentially retained. In an elegant study, Rutledge et al. showed that LPL increased the retention of VLDL in perfused arteries and that when both the surface and core of VLDL were followed, LPL appeared to generate surface remnants of VLDL that accumulated in arteries as "lakes." Interestingly, particles that closely resemble remnants of triglyceride-rich lipoproteins hydrolyzed by LPL in vitro have been isolated from the human arterial intima. This important finding provides strong supportive evidence for the view that LPL plays a role in the retention of remnants of triglyceride-rich lipoproteins in the arterial intima. Hydrolysis of VLDL by LPL generates free fatty acids, which are able to increase the permeability of the arteries to LDL, so tending to further promote the entry and retention of LDL. Finally, fatty acids resulting from lipolysis of VLDL by LPL have been shown to fuel phagocytosis by macrophages in the presence of low concentrations of glucose, to enhance the production of tumor necrosis factor-α by monocyte/macrophages, to cause proatherogenic changes in the production of proteoglycans by smooth muscle cells, and to induce synthesis of LPL by monocytes (Figure 2).

Finally, LPL may have some actions potentially relevant to the development of atherosclerosis that are more or less independent of direct interaction with lipoproteins (Figure 2). Thus, LPL can (1) enhance the adhesion of monocytes to the endothelium, presumably as a result of the ability of the dimeric LPL to bind heparan sulfate proteoglycans on endothelial and monocyte surfaces, (2) enhance the production of proteoglycans by macrophages, and (3) have a proliferative effect on vascular smooth muscle cells that is independent of the presence of lipoproteins.

**Conclusions and Perspectives**

Although the relative importance of the various effects of LPL in the arterial intima is not known, all of the effects studied so far appear to be proatherogenic, by interfering either directly or indirectly with intimal lipoprotein metabolism and, ultimately, by enhancing the accumulation of intracellular and extracellular lipids in the intima. Similarly, the role of arterial endothelial LPL, through local production of lipoprotein remnants capable of entering the intima, has been suggested to be proatherogenic. In contrast to the arterial pool of LPL, whether present on the endothelium or deeper in the intima, the capillary endothelial LPL is clearly antiatherogenic through its anti-inflammatory actions. Because of the contrasting actions of the 2 pools of LPL (arterial versus capillary), attempts to decrease the mass or activity in the body should be restricted to LPL in the arterial intima. A first hint that this should be a therapeutic goal comes from studies showing that targeted inhibition of macrophage LPL production is protective against atherosclerosis in mice. Targeted LPL therapy may become feasible some day in humans also. Then, in selected dyslipidemic subjects with low LPL activity in the capillaries, attempts should be made to prevent atherosclerosis by stimulating LPL activity in the capillary bed while depressing it in the arterial wall.

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**References**


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