review

Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis

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Abstract Although it has been known for over 50 years that lipoprotein lipase (LPL) hydrolyzes triglyceride in chylomicrons, during the past half decade there has been a reinterest in the physiologic and pathophysiologic actions of this enzyme. In part, this has coincided with clinical studies implicating increased postprandial lipemia as a risk factor for atherosclerosis development. In addition, the recent creation of genetically altered mice with hypertriglyceridemia has focused the interest of geneticists and physiologists on the pathophysiology of triglyceride metabolism. As reviewed in this article, it is apparent that the lipolysis reaction is only partially understood. Several factors other than LPL are critical modulators of this process, in part, because the reaction requires the lipoproteins to interact with the arterial or capillary wall. Among the factors that affect this are the apolipoprotein composition of the particles, the size of the lipoproteins, and how LPL is displayed along the endothelial luminal surface. Zilversmit's observation that LPL activity is found in greater amounts in atherosclerotic than normal arteries has led to a large number of experiments linking LPL with atherogenesis. In medium and large arteries LPL is found on the luminal endothelial surface and in macrophage-rich areas within the plaque. LPL actions in both of these locations probably have major effects on the biology of the blood vessel. Possible atherogenic actions for this LPL based on in vitro experiments are reviewed.–**Goldberg, I. J.** Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J. Lipid Res.* 1996. **37:** 693–707.

Supplementary key words atherosclerosis • lipoproteins • triglyceride • cholesterol • heparin • proteoglycans • endothelial cells • apolipoprotein • fatty acids

INTRODUCTION

Lipoprotein lipase (LPL) is the major enzyme responsible for hydrolysis of triglyceride (TG) molecules present in circulating lipoproteins. LPL is associated with the luminal side of capillaries and arteries where it hydrolyzes TG to produce free fatty acids. These free fatty acids are assimilated by muscle and adipose tissue. Within the plasma compartment, LPL converts chylomicrons to remnants and begins the cascade required for conversion of VLDL to LDL. Both changes in these circulating lipoproteins and the creation of lipolytic products could modulate a number of processes that affect the biology of the vessel wall.

The initial portion of this review will concentrate on LPL actions within the capillary beds, actions that modulate the concentrations of circulating lipoproteins. Regulation of LPL expression in tissues and structure function aspects of LPL biochemistry have been reviewed elsewhere (1–3). Factors that determine how endothelial-bound LPL is able to interact with circulating lipoproteins will be considered. Aside from its relationship to circulating lipoproteins, LPL hydrolysis of lipoproteins along the arterial wall to produce lipolytic products and remnant lipoproteins may be an atherogenic process. In addition, intra-arterial LPL has been hypothesized to affect lipid accumulation within the artery. A number of other effects of arterial LPL on the biology of macrophages and other cells in the artery will be considered. Finally, the relationship of LPL activity to the generation of atherogenesis in humans will be reviewed.

LPL CONTROL OF ATHEROGENIC AND ANTIATHEROGENIC LIPOPROTEINS

LPL hydrolysis of chylomicrons

Endothelial cell-associated LPL is a central enzyme in the metabolism of all classes of lipoproteins. Its initial discovery occurred when Hahn (4) noted that injection of heparin into dogs led to a decrease in postprandial

Abbreviations: LPL, lipoprotein lipase; TG, triglyceride; HSPG, heparan sulfate proteoglycans; CS, chondriotin sulfate; DS, dermatin sulfate; GAG, glycosaminoglycans; GPI, glycosylphosphotidylinositol; PIPLC, phosphoinositol specific phospholipase C; LRP, LDL receptor-related protein; HTGL, hepatic triglyceride lipase; CAD, coronary artery disease; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein.

lipemia. The subsequent demonstration that postheparin plasma from humans with a severe hyperchylomicronemia (type 1 hyperlipoproteinemia) did not hydrolyze chylomicrons in vitro (5) clearly linked LPL with this disorder and showed an essential role for LPL in initiating chylomicron catabolism. Similarly, in vitro data showing that apoC-II was the activator of LPL (6) were correlated with a human disorder, genetic apoC-II deficiency (7) that also caused severe hyperchylomicronemia.

The initiation of chylomicron catabolism by LPL is indisputable; however, several steps in the LPL-mediated chylomicron catabolic process are uncertain. Chylomicrons are formed in the intestinal epithelium, where cells that do not express large amounts of apolipoproteins from the apoC-I–E–C-II gene family. Chylomicrons from lymph, which are presumably nascent particles, are relatively deficient in these apolipoproteins (8). Therefore, much of the association of apoCs and apoE with the chylomicron occurs after it has left the lymph and entered the bloodstream. How do apoE and apoC-II transfer to the chylomicron? As the amount of chylomicron apoCs and apoE increases, the apoA-I and A-IV content of the chylomicrons is reduced. Presumably, there is an exchange of HDL Cs and E for apoA-I and apoA-IV on chylomicrons (9–13). A relative deficiency in HDL apoC-II would then be expected to cause a lipolytic defect, and perhaps this is the reason for the hypertriglyceridemia occasionally seen with some human hypoalphalipoproteinemias (14, 15). In addition, not all apoC-II is equivalent (16). Only some HDL apoC-II is readily exchangeable (17) and the affinity of the apoC-II for the HDL appears to be a function of the lipid composition of the particle.

Chylomicron association with the capillary lumen. How do lipoproteins interact with LPL on the vessel wall? A recent article by Olivecrona and Olivecrona (18) reviewed the data that show that lipolysis is limited by factors other than LPL itself. For this reason lipolysis in the plasma that occurs after LPL release from the endothelial cells by heparin, i.e., in postheparin plasma, is thought to be a more vigorous reaction although presumably with the same amount of enzyme. Similarly, LPL in solution is a more effective lipolytic enzyme than is LPL on the surface of cultured endothelial cells (19). Some data, however, suggest that much of the apparent in vivo postheparin lipolysis is actually due to LPL actions that occur in vitro, after the plasma samples are obtained (20). Moreover, some LPL probably dissociates from the endothelial cell during lipolysis and continues to lipolyze lipoprotein TG in the bloodstream. Thus, it is unknown how much lipolysis occurs by endothelial cell anchored versus plasma LPL.

Regardless of whether LPL actions in postheparin plasma and the capillary lumen are similar, most lipolysis is initiated when lipoproteins interact with endothelial cell-associated LPL. Two theories will be presented that describe how lipoproteins contact the capillary wall. One is an apolipoprotein-mediated mechanism. The second is a purely geometric view of LPL interaction with TG-rich lipoproteins. Both hypotheses illustrate some of the added complexity that occurs in vivo but does not occur when LPL activity is assayed in vitro using TG-containing emulsions.

Apolipoprotein regulation of lipolysis in vivo. As chylomicrons traverse the capillary, they come into contact with endothelial cell-associated LPL and the apoC-II associated with the chylomicron activates the enzyme. It has been postulated that apoE, a strong heparin-binding protein, anchors TG-rich lipoproteins to the endothelial cells. In vitro data from Clark and Quarfordt (21) demonstrated that addition of apoE increased the hydrolysis of TG-containing particles that were passed through LPL-containing heparin-affinity gel. Other investigators have shown that apoE allows lipoproteins to associate with cell surface heparan sulfate proteoglycan (HSPG) (22, 23). The exposure of the apoE on the lipoprotein surface may be a function of *a)* size of the particle (24, 25); *b)* how the apoE arrived there (i.e., whether it exchanged from HDL or was included in the assembly of the nascent lipoprotein); and *c)* whether the particle is already partially lipolyzed (26–28) and therefore differs in composition and from its precursor (29). Two additional factors in this scheme are the relative heparin binding affinity of the apoE isoforms and the accessibility and, perhaps, composition of the HSPG.

If the theory relating apoE to lipolysis is correct then a defect in apoE-HSPG interaction would decrease lipolysis. Although this might appear to be relatively simple to test, defective apoE-HSPG association affects a number of other lipoprotein metabolic processes. These include lipoprotein sequestration in the liver and interaction with the LDL receptor-related protein (LRP), a pathway that requires lipoproteins to first bind to proteoglycans (23, 30, 31). Dysbetalipoproteinemia, associated with apoE2, sometimes presents as a severe hypertriglyceridemia. Presumably, in this situation the defect in remnant lipoproteins leads to a "back-up" of the lipolytic cascade. It is equally plausible that an underlying defect in LPL actions is being uncovered. Some apoE mutations are defective in their HSPG binding and lead to dominant forms of dysbetalipoproteinemia (32). However, humans with genetic apoE deficiency (33, 34) and the apoE null mice (35, 36) have more severe hypercholesterolemia than the apoE mutants and are relatively free of hypertriglyceridemia. Thus, either the requirement for apoE in TG metabolism is not absolute, or apoE has other actions required for the assembly of

TG into lipoproteins. Additional metabolic information in the apoE null mouse and its tissues should improve our understanding of the relationship of apoE to TG metabolism.

A second possible illustration of the importance of apoE-HSPG interaction results from abnormalities in C apolipoproteins. Increased production of apoC-I (37), C-II (38), or C-III (39, 40) causes hypertriglyceridemia in transgenic mice. In humans, apoC-III deficiency leads to low TG and rapid turnover of VLDL (41). ApoCs will displace apoE from lipoproteins (42) and increased concentration of apoCs will decrease liver uptake of TG-containing lipoproteins (43, 44). However, a defect in lipolysis in this situation might also occur. Investigations of the mechanisms of the hypertriglyceridemia in apoC-III transgenic mice included an assessment of postheparin lipolytic activity in these animals and in vitro lipolysis of the circulating lipoproteins with purified LPL (39, 40). The kinetics of LPL–lipoprotein interaction in solution, or even after the release of LPL into the bloodstream, might fail to assess some factors regulating LPL actions in normal physiology. Assessment of abnormalities in the lipoprotein–endothelial interaction requires assays that also test the docking of the lipoprotein to the endothelial surface.

Evaluation of the hypertriglyceridemia with apoC-II overexpression in mice has included additional studies of possible defects in LPL actions. ApoC-II mice have an increase in large circulating VLDL, but they have normal amounts of postheparin LPL and their TG-rich lipoproteins are hydrolyzed normally in an in vitro solution type assay. These particles are, however, apoE-deficient and have a defect in their interactions with endothelial-bound LPL that may be etiologic in their hypertriglyceridemia (38). Similar abnormalities may be found in some human VLDL (45). Whether a defect in endothelial attachment due to overproduction of apoCs is responsible for some human hypertriglyceridemias is unknown.

ApoCs, especially apoC-III, could inhibit lipolysis exclusive of its effects on cell surface apoE and lipoprotein proteoglycan interaction. Optimal activation of LPL by apoC-II is inhibited by addition of apoC-III (46). In addition, association of LPL with lipid particles is decreased by addition of apoC-III and apoC-II (47). Presumably this is because LPL binding lipids are obscured by the apoCs coating the surface.

Geometric factors regulating lipolysis. A fuller understanding of the lipolysis reaction not only requires consideration of endothelial-bound LPL, but also requires an assessment of the geometry of the lipolysis site. Although LPL appears in vitro to hydrolyze larger, more TG-rich lipoproteins better that smaller particles, the affinity of the enzyme in solution may not be any greater for these particles (48). Rather, the more rapid clearance of chylomicrons than VLDL may be a function of the capillary size. A theoretical chylomicron of 500 nm diameter fills 0.25/64 of the cross-sectional area of the typical 8 micron capillary. In contrast, a smaller lipoprotein of 100 nM (e.g., a VLDL) only occupies 0.01/64 of the cross-sectional area of the capillary. The relationship of the particles to the cross-sectional lumen of the capillary is shown in **Fig. 1**; the relative size of the two particles is appropriate although their diameters are increased 10-fold for illustrative purposes. If one assumes that proximity to LPL at the edge of the lumen is a function of the area occupied by the lipoproteins, the chylomicrons have a 25-fold greater chance of interacting with each LPL. Thus, while the half-life of the

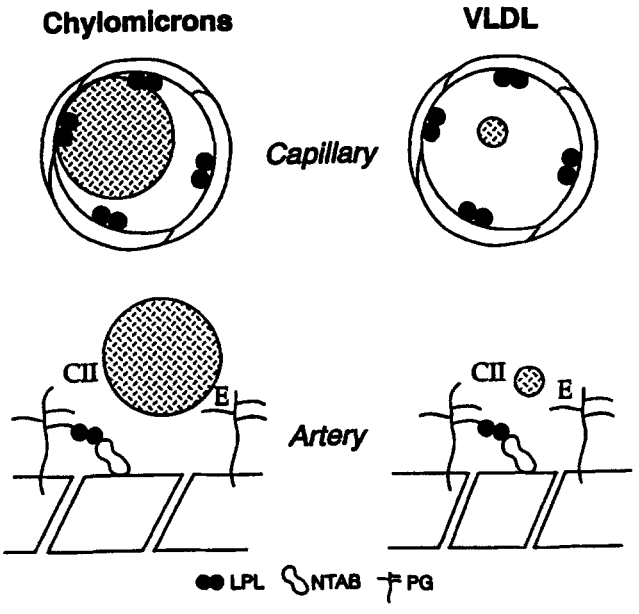

Fig. 1. Lipoprotein lipase regulations of lipolysis. Lipolysis of large TG-rich lipoproteins like chylomicrons and smaller VLDL have different kinetics. Some of these differences may be attributable to differences in lipoprotein composition. Others, as illustrated by comparing the two top panels, are more likely to be due to geometric considerations. As shown by expanding the actual size of the particles, within capillaries the chylomicrons fill a larger cross-sectional area of the luminal. This makes it more likely that they will interact with endothelial cell-associated LPL and more likely that they will encounter more than one LPL molecule during a single capillary passage. A single VLDL with 1/5 the diameter has 1/25 of the chance of interacting with luminal wall LPL. A different situation exists on the wall of the artery, shown in the bottom panels. Both lipoproteins are less likely to be hydrolyzed here unless other factors are involved. One of these may be apoE which has a higher affinity interaction than does apoB for HSPG on the endothelial surface. In the case of both lipoproteins, partial hydrolysis increases the exposure of apoE and makes it more amenable to interaction with HSPG. Also shown is a postulated mechanism for LPL attachment to endothelial cells that involves binding to both HSPG and the second, non-proteoglycan binding protein, NTAB. This second protein is homologous with the NH_2 terminal region of apoB.

chylomicron might be 10 min, that of the smaller VLDL would be 250 min, over 4 h.

A second ramification of this analysis relates to the role of apoE in anchoring lipoproteins to the endothelial surface. Such an action would be less important for chylomicrons within the capillary wall than for lipoproteins contacting the luminal surface of larger arteries (as shown in the lower panels). Moreover, the apoE-requiring process may most important for smaller particles. Thus, the in vitro phenomena, noted above, in which apoE exposure on the lipoproteins is augmented by lipolysis might be a necessary requirement for further, but not initial, lipoprotein lipolysis. Larger TG-rich lipoproteins (e.g., nascent chylomicrons, perhaps the chylomicrons from apoE-knockout mice, and large lipid emulsion particles) would be hydrolyzed during their passage through the capillary without requiring any additional changes in their surface lipid or apolipoproteins. It should be noted that this type of analysis is fraught with assumptions and ignores the actual three-dimensional configuration of the capillary and the lipoprotein, and the changes in LPL actions that occur during lipolysis due to product inhibition and its release from the capillary. Nonetheless, it appears to explain the well-established relationship of size to rate of lipoprotein hydrolysis.

Two additional factors that could affect the lipoproteins interacting with the vessel are the rate of blood flow and the movement of the vessel itself. In the rapid, turbulent flow of large arteries, the insoluble chylomicrons remain in suspension. Within the slower flowing capillaries, however, these particles may begin to separate from the blood. This is analogous to the well-known separation of chylomicrons from plasma when hyperlipidemic blood is allowed to remain undisturbed in the refrigerator. VLDL are in solution and do not separate, hence they are less likely to float to the sides of the capillary. Moreover, the "vibration" and motion of an entire tissue, like exercising muscle, would distort the flow through the vessel and increase the likelihood of an insoluble lipid particle hitting the vessel wall. These theoretical postulates linking the size and density of TG-containing lipoproteins with their lipolytic rate could be tested using model particles perfused through LPL-containing blood vessels.

LPL interaction with endothelial cells. The amount of LPL on the capillary surface is another potential regulatory event in chylomicron lipolysis. After its synthesis, LPL must be released from its cell of origin, contact the endothelial cells, and then be transported across them. Perhaps during this transit some LPL is inactivated, resulting in the changes in LPL specific activity noted in some conditions (49, 50).

The observations that heparin released LPL into the bloodstream (4) and that LPL binding to endothelial cells was markedly decreased by HSPG-degrading enzymes (51) suggested that LPL is associated with HSPG. HSPG are members of the family of proteoglycans, negatively charged polysaccharides that are components of cell membranes and the extracellular matrix, and are important in cell adhesion and growth. The two major parts of the proteoglycan molecule are the glycosaminoglycans (GAG-carbohydrate chains) and the core proteins. The major classes of sulfated GAG, chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), and keratin sulfate, differ in their component sugars. HS is a polymer composed of repeating disaccharide units of a hexuronic acid (either glucuronic acid or iduronic acid) and glucosamine. The glucosamine residues are either N-acetylated or N-sulfated and both hexuronate and glucosamine residues may be O-sulfated in varying positions (52). This leads to a highly variable structure that depends on tissue of origin, molecular environment, and cell growth state. Heparin differs from HS in extent of N-acetylation, N- and O-sulfation, and content of iduronate.

Vascular endothelial cells synthesize a variety of HSPG whose core proteins are integral membrane proteins or are membrane-associated through a glycosylphosphotidylinositol (GPI) linkage. In other cell types, some proteoglycans associate with cell surfaces by binding to receptors for either the GAG or core protein. Other endothelial cell proteoglycans are released from the cells to incorporate into the basement membrane (53). LPL-binding HSPG from the apical surface of cultured endothelial cells were isolated using LPL-Sepharose affinity chromatography (54). A 220 kDa HSPG containing a core protein of approximately 50 kDa was found. When total cellular HSPG were radiolabeled with inorganic sulfate, a large > 500 kDa protein also was associated with the LPL. This HSPG is named perlecan and is the major HSPG of subendothelial basement membrane (55, 56). LPL binding to endothelial cells is resistant to treatment with phosphotidylinositol specific phospholipase C (PIPLC) which should release proteoglycans bound to cell surfaces via GPI linkages (GPI) (54). In contrast, GPI-linked proteoglycans are present and responsible for some LPL binding to adipocytes (57) and heart mesenchymal cells (58).

Specificity of protein binding to HSPG often depends on the structural features of the GAG chains. Specific oligosaccharides with high affinity for antithrombin and several growth factors have been sequenced. LPL is no exception to this pattern; GAG also determine the affinity of LPL for HSPG. LPL binds more tightly to highly sulfated heparin GAG (59) and treatment of cells with chlorate, which reduces sulfate of GAG, decreases LPL binding to adipocytes (60). The minimal size of the

LPL-binding oligosaccharide was determined by purification of high affinity LPL-binding GAG obtained from partial digestion of endothelial cell proteoglycans. A decasaccharide, 5 repeated disaccharides of iduronic sulfate- (2 O-sulfate) -glucosamine-N-sulfate (6 O-sulfate), has been isolated (61). An LPL-binding oligosaccharide that is the same size and contains the identical sequence has been isolated from commercial heparin (62). This sequence differs from the high affinity antithrombin-binding pentasaccharide, but is similar to that which binds to basic fibroblast growth factor (63), except for the presence of sulfate at the 6th position of the glucosamine.

Most HSPG-binding proteins have a second non-proteoglycan binding protein that is involved with their interaction with cells. This is true for growth factors, apolipoproteins, and some clotting factors; they all bind to HSPG and also have cell surface receptors. These receptors are necessary for internalization or receptor activation. A similar dual binding might be required for LPL. LPL association with endothelial cells must be maintained despite the physical effects of the blood flow and competition for cell binding with a number of other HSPG-binding proteins. A non-proteoglycan LPL-binding protein has been isolated (64). This protein has sequence homology with the amino terminal region of apoB (65). Thus, it was postulated that this region of apoB enhances LPL binding to cells and to apoB-containing lipoproteins.

LPL binding to endothelial cells is not a static situation: some LPL dissociates from the cells and some LPL is internalized and recycled to the cell surface (66). Lipolysis causes LPL to dissociate from the cell surface (19) and probably for this reason plasma LPL is correlated with plasma free fatty acid levels (67).

Role of LPL as a receptor ligand. Aside from its enzymatic actions, LPL has been postulated to be a receptor ligand for lipoprotein removal. Felts, Itakura, and Crane (68) first postulated that LPL was a signal for hepatic removal of chylomicron remnants. Beisiegel, Weber, and Bengtsson-Olivecrona (69) renewed interest in this concept by showing that LPL is a ligand for the LDL receptor-related protein (LRP). They also demonstrated that this process was markedly reduced when HSPG-deficient cells were studied. Several laboratories have confirmed this and have shown that the carboxyl terminal region of LPL is required for LPL to bind to LRP (70–72). More recently, it was reported that LPL is also a ligand for the VLDL receptor (73). To prove that LPL-mediated lipoprotein uptake is physiologically relevant for chylomicron removal, several issues remain to be resolved. These include proving that a sufficient amount of plasma LPL exists and that it effectively competes for LRP binding with the multiplicity of other LRP ligands. An additional putative chylomicron receptor termed the lipolysis-stimulated receptor or LSR, has also been described (74) and LPL actions that generate free fatty acids could be a mechanism of its activation.

LPL converts VLDL to IDL

VLDL metabolism parallels that of chylomicrons in many aspects. Like all lipoproteins, VLDL are a collection of particles that differ in size, and apolipoprotein and lipid composition. Their metabolism has been studied by subdividing them into classes that can be separated by ultracentrifugation. Although other methods of VLDL subclass analysis might be preferable, the exchange of apolipoproteins (E and Cs) in vivo prevents easy analysis of such experimental data. Catabolism of larger VLDL begins with an LPL-mediated step and more of these large particles are removed from the bloodstream before their conversion to LDL (75). More small VLDL are converted to LDL. There may be one of three reasons for this. *1)* The VLDL to LDL conversion requires multiple lipolytic steps. After each round of lipolysis the enzyme must then interact with a lipoprotein that has escaped liver clearance; more interactions lead to a lower percentage of VLDL that survive in the bloodstream. *2)* Only some subclasses of VLDL may be destined to be transformed into LDL (76); this theory has been termed metabolic channeling. *3)* Smaller, but not larger, VLDL are also substrates for hepatic triglyceride lipase (HTGL) (77). Therefore, the intravascular metabolic cascade may be favored, over receptor removal, by the additional hydrolysis performed by this second enzyme.

The LPL-initiated conversion of VLDL to LDL could lead to a more or less atherogenic lipoprotein profile. The relationship of hypertriglyceridemia to coronary artery disease (CAD) risk is still nebulous and probably depends on the population studied and the prevalence of other atherogenic factors (78). The relationship between LDL and atherogenesis is well established. There are several clinical situations in which LPL actions are increased, TG levels are reduced, but LDL increases. Examples of this include weight loss of obese subjects (79), treatment of diabetes mellitus (80), and fibric acid therapy (81). Whether the conversion from a hypertriglyceridemic to a more LDL-rich lipoprotein profile is atherogenic is unclear.

It has been postulated that LPL can regulate the production of apoB-containing lipoproteins by non-enzymatic mechanisms. The original hypothesis stemmed from the observation that some families that were obligate carriers of LPL genetic defects had an increased incidence of familial combined hyperlipoproteinemia (82). Williams et al. (83) showed that addition of LPL to hepatocytes would increase the uptake of apoB-contain-

ing lipoprotein by these cells and postulated that a defect in this pathway could lead to the overproduction of lipoproteins found in familial combined hyperlipoproteinemia. In contrast, Huff et al. (84) using lower amounts of LPL found that hepatocyte uptake of TG, but not cholesterol, increased and this required the enzymatic actions of LPL. LPL is not made in the adult liver (85), and LPL arriving in the liver from the circulation may be rapidly degraded (86). In addition, hepatic LPL that is located on endothelial cells (87), rather than hepatocytes, might not be able to interact with nascent lipoproteins. Experiments in a physiologic model are needed to test whether hepatic LPL regulates LDL or apoB synthesis exclusive of its actions as a lipolytic enzyme.

LPL actions in the periphery, rather than in the liver, are more likely to be correlated with hepatic lipoprotein production. Defects in the amount of LPL activity in muscle and adipose tissue reduce chylomicron TG hydrolysis in the periphery and should lead to more postprandial TG returning to the liver. This, in turn, should stimulate the production of hepatic TG-rich lipoproteins. This scenario for the role of peripheral LPL in hepatic production of lipoproteins is also relevant to lipoprotein production in the postprandial state. Postprandially, VLDL synthesis rates are increased (88, 89). LPL deficiency may increase the concentrations of both chylomicrons and apoB-100 particles in the postprandial period.

LPL regulation of HDL

LPL activity regulates HDL cholesterol levels in at least three ways. *1)* During LPL-mediated hydrolysis of TG-rich lipoproteins, surface lipids and apolipoproteins are transferred to HDL (90). *2)* The amount of HDL cholesterol that is exchanged for TG is modulated by the amount of VLDL in the bloodstream. Therefore, by decreasing plasma TG, LPL limits cholesteryl ester transfer protein (CETP)-mediated HDL cholesterol reduction. *3)* By altering the core lipid composition of HDL, the catabolic rate of HDL apoA-I is altered. If HDL become TG-enriched, the TG can be more rapidly removed resulting in production of smaller lipid-poor apoA-I (91, 92). TG-containing HDL are better substrates for HTGL; lipid-poor apoA-I are more rapidly cleared from the circulation. Such a pathway would lead to the increased fractional catabolic rates and low HDL levels reported in hypertriglyceridemic humans (93, 94) and the increased apoA-I catabolic rate found during LPL inhibition (95).

In some, but not all humans, LPL activity is positively correlated with HDL levels (96, 97). Similarly, HDL is often (98, 99), but not always (100, 101), inversely correlated with postprandial lipemia, an in vivo measurement of LPL actions. Via this indirect analysis, HDL levels are sometimes viewed as an index of the activity of LPL in vivo.

POTENTIAL ATHEROGENIC EFFECTS OF LPL

LPL via its actions on circulating fasting and postprandial lipoproteins is generally viewed as an antiatherogenic enzyme. This is illustrated by the protective actions of a compound that increased LPL in an atherosclerosis-susceptible strain of rats (102). However, several lines of experimental data also suggest that LPL is involved in the atherogenic process. Like many other factors implicated in atherogenesis, LPL may be pathogenic only in certain settings. Several postulated atherogenic actions of LPL are extrapolations from in vitro experimental data reviewed below.

LPL on the endothelial surface

The human and animal data that support the hypothesis that chylomicron remnants are atherogenic has recently been reviewed elsewhere (101). The present review will concentrate on how these remnants may be atherogenic, and how arterial LPL contributes to this process. First, the relationship of postprandial lipemia to arterial wall lipolysis will be considered and then the data supporting the atherogenicity of this process will be reviewed.

The amount of postprandial lipemia is regulated by a number of factors. Chief among them is the amount of peripheral lipolysis of the chylomicron, a necessary step preceding uptake of the particle by the liver. This lipolysis is performed by LPL associated with capillaries within muscle, adipose tissue, and lung. Decreased peripheral (muscle and adipose) LPL leads to postprandial hyperlipoproteinemia.

How then does the amount of chylomicrons, presumably non-atherogenic particles, affect the number of atherogenic remnants interacting with the artery wall? Decreased peripheral lipolysis and postprandial lipemia should increase the actions of arterial wall, as opposed to capillary, LPL. The amount of lipolysis that occurs at any individual area is a function of the amount of LPL, the association between LPL and the circulating TG-containing particles, and the amount of LPL substrate. In vitro assays of LPL are performed by adding LPL to a TG-containing emulsion. In this situation, a concentration of 1.5–5 mM of TG, approximately 132–440 mg/dl, is required for maximal free fatty acid generation (103, 104). The higher of these concentrations is similar to that shown by Brunzell et al. (105) to lead to competition between chylomicrons and VLDL for cell surface LPL. Therefore, under normal physiologic conditions the

production of lipolysis products along the vessel wall is largely determined by substrate availability. A major determinant of the amount of lipolysis and remnant production that occurs along the vessel wall must be the rate of clearance of postprandial lipoproteins, which in turn is determined by LPL in the muscle and adipose depots and the composition of the circulating lipoproteins.

Pathologic effects of lipolysis along the artery wall

How could postprandial lipoproteins cause atherosclerosis? Either the lipoproteins themselves become a constituent of the lesion, or metabolic processes involved in their formation may be pathogenic. There are at least three processes that could account for this.

1) Deposition of surface lipid. Extracellular lipid not associated with LDL apoB, and hence not within LDL, is found in atherosclerotic lesions. This includes myelin-like lipid (106) and lipoproteins that are the size of VLDL and contain TG (107). One possible origin of the extracellular, non-lipoprotein lipids is from the lipolysis products that are shed during LPL-mediated lipolysis of TG-rich lipoproteins. Chung et al. (108) have shown that such material is biochemically similar to that found within the plaque. The products of lipolysis include a number of bioactive compounds including free fatty acids and lysolecithin. Moreover, lipolysis-generated surface lipids may be a substrate for the generation of lysolecithin either via the actions of an extracellular arterial phospholipase, LPL (an A-I phospholipase) or via lysosomal enzymes that are released from apoptotic or necrotic cells found within lesions. Within the reduced albumin environment of the artery wall or within microenvironments, lysolecithin and fatty acids could stimulate macrophage chemotaxis, promote expression of growth factors and adhesion molecules, and cause alterations in vasodilatory NO actions.

2) Local production of smaller remnant lipoproteins. Rather than being rapidly cleared by the liver, remnant lipoproteins produced along the artery surface might directly infiltrate the vessel wall. Remnants produced in capillaries are primarily removed from the bloodstream by the liver or other cells. Only in metabolic disorders of apoE or HTGL do these particles accumulate in the blood stream in large numbers. Thus, the concentration of remnant lipoproteins along the vessel is usually quite low. However, if the remnants are produced at the arterial surface, it would increase the effective concentrations to which the artery is exposed.

3) Lipolysis alteration of endothelial barrier function. Transport of lipoproteins across the endothelium should be affected by the size of the particles, apolipoprotein factors that increase lipoprotein association with the artery, and the permeability of the artery wall. Lipolysis products alter the endothelial barrier in vitro. This process is illustrated in **Fig. 2.** When endothelial cell monolayers were incubated in increased concentrations of free fatty acids, the permeability of the monolayers increased (109). The amount of free fatty acids that are generated and are present along the artery wall is difficult to assess, however, it should be noted that VLDL lipolysis (in the presence of 3% albumin) also altered the endothelial barrier (110). LDL entry into the artery could also be augmented by an increase in arterial permeability.

LPL within the artery wall

Nearly two decades ago, Zilversmit (111) observed that cholesterol-rich, more atherosclerotic areas of rabbit aorta had increased LPL activity. The original hypothesis was that local LPL activity promoted the formation of atherogenic remnant lipoproteins from chylomicrons. Interest in LPL and atherogenesis was reawakened when investigators in both San Diego (112) and Seattle (113) showed that the LPL gene was expressed by macrophages within the vessel wall. This expression appeared to be confined to only some subclasses of macrophages (114). As with many other proteins produced in greater amounts in atherosclerotic lesions, the question remains whether LPL is pro- or anti-atherogenic. The intriguing observation that LPL

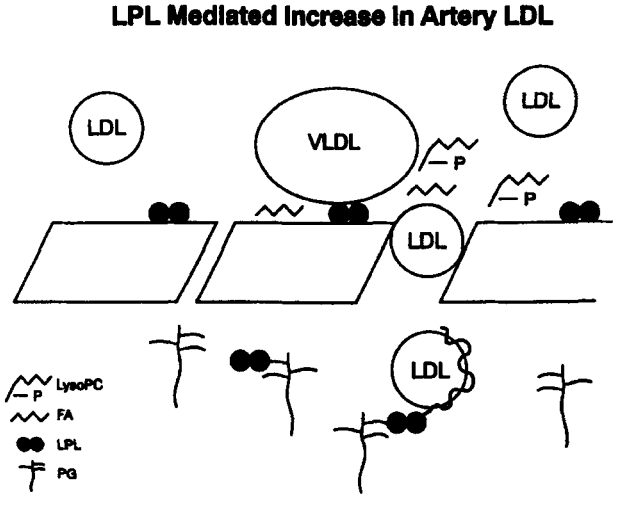

Fig. 2. Lipolysis mediated atherogenesis. LPL attached to endothelial surface proteoglycans (PG) creates lipolytic products such as lysolecithin (denoted Lyso-PC) and fatty acids (denoted FA) as it interacts with circulating triglyceride containing lipoproteins (VLDL and chylomicrons). In addition to decreasing the size of these lipoproteins, hence increasing their ability to cross the endothelial cell barrier, in vitro data suggest that endothelial cell barrier function might be altered during this process. A second LPL-mediated process, retention of lipoproteins in the subendothelial space, occurs via non-enzymatic actions of LPL. LPL, it is hypothesized, can act as a bridge between proteoglycans and lipoproteins.

activity in mouse macrophages correlates with their propensity to develop atherosclerosis (115), however, support the histological association of macrophage LPL and disease promotion. The discussion below will consider only potential pathological effects of LPL.

LPL can increase LDL accumulation in the vessel wall

After a lipoprotein crosses the endothelium it can interact with several different components of the subendothelial cell matrix leading to extracellular lipid accumulation. These matrix components include a variety of proteoglycans and matrix proteins as well as other proteins/enzymes that may be attached to the matrix. Proteoglycans may be the most important molecules for the binding of lipoproteins, and LDL and proteoglycans colocalize within lesions (116, 117). Prolonged incubations of LDL with arterial proteoglycans in the presence of supra-physiological concentrations of calcium can create insoluble lipoprotein–proteoglycan complexes (118). However, under physiological salt and ion concentrations there appears to be very little binding of lipoproteins with matrix from normal arteries (119).

The missing ingredient required for LDL association with PG may include the following possibilities. First, LDL may be modified to a form that increases its affinity for proteoglycans. Second, although LDL binding to proteoglycans is limited, there may be a subset of vessel wall proteoglycans that interact more avidly with LDL. Two studies showed that LDL bound with high affinity to DS/CS proteoglycans produced by proliferating smooth muscle cells (120) or those synthesized by artery wall of injured rabbits (121). This role of DS and CS in trapping of LDL is of great interest as the artery wall content of these proteoglycans increases during atherosclerosis and has been correlated with increased accumulation of aortic cholesterol (122). Third, rather than direct binding between LDL and proteoglycans, there might be additional intermediate molecules. These molecules should possess domains that bind both proteoglycans and lipoproteins. Both LPL and apoE, proteins expressed in atherosclerotic lesions (123), could function in this way.

Much of the LPL in atherosclerotic lesions is within the vessel wall and not on the luminal endothelial surface and, thus, is unlikely to be involved in its classical role of catabolizing circulating TG-rich lipoproteins. Other non-enzymatic or "bridging" actions for LPL might be more important within the vessel. This is postulated to occur because LPL forms macromolecular aggregates by simultaneously binding to both matrix proteoglycans and the lipoproteins. While studying LDL transport across endothelial cell monolayers, Saxena et al. (110) noted that addition of LPL dramatically increased the amount of lipoproteins that associated with endothelial cell-derived matrix. Eisenberg et al. (124) made a similar observation using fibroblasts. The LPL mediated retention of lipoproteins appears to be limited to apoB containing lipoproteins, VLDL and LDL. Although Eisenberg et al. (124) reported a small increase in HDL retention, these studies were performed in lipoprotein-deficient media, conditions that prevented competition with LDL, a higher affinity ligand for LPL. In fact, apoE-rich HDL decrease LPL-mediated binding of LDL to matrix (125). Aggregation of LDL with sphingomyelinase (126) and perhaps other agents will amplify the LPL-mediated retention phenomena. Rutledge and Goldberg (127) showed that LPL increased LDL retention within the wall of frog mesenteric capillaries, confirming that similar phenomena could occur in blood vessels. The hypothesis yet to be tested in vivo is that LPL potentiates atherosclerosis by preventing LDL efflux from arteries.

Biochemical determinants of the PG-LPL-LDL interaction within the artery. HSPG are a minor proteoglycan component of the atherosclerotic lesion, but LPL can also bind to the other more abundant proteoglycans. LPL increased the association of LDL with CSPG and DSPG derived from normal thoracic aortas of cynomolgus monkeys (128). Monocyte-derived macrophages are the source of some of the artery wall proteoglycans. Edwards et al. (129) showed that monocyte conversion into macrophages increased cellular proteoglycan production and led to production of a subspecies of sulfate-enriched CSPG. These proteoglycans bound more avidly to LPL and are likely to be present in higher concentrations within atherosclerotic lesions.

LPL–lipoprotein association. A second biochemical association that of LPL and LDL is required to retain this lipoprotein within arteries. LPL has a lipid-binding lid structure (3) that allows it to interact with TG-rich particles. Although LPL–TG-rich lipoprotein associations may primarily be between lipid and protein, LPL association with LDL may not. Connally et al. (48) while studying LPL-mediated hydrolysis of VLDL observed that addition of LDL would inhibit the reaction. The LDL acted as a non-competitive inhibitor, suggesting that LPL bound to the LDL. These data were consistent with studies of postheparin plasma that found most LPL associated with LDL size lipoproteins (130, 131). Sivaram et al. (65) noted that LPL associated on ligand blots with a truncated piece of the amino terminal region of apoB found on the surface of cultured endothelial cells. Choi et al. (132) subsequently reported that LPL associates with the amino-terminal region of apoB on LDL. They noted, however, that addition of anti-apoB antibodies did not inhibit LPL-mediated lipolysis of VLDL in a solution assay. Gianturco et al. (133) found

that the interaction between apoB and the VLDL receptor that they were isolating was blocked by addition of LPL. This result presumably was because the LPL associated with the amino-terminal region of apoB on the VLDL and blocked its interaction with the receptor. Together, these data suggest that protein–protein, LPL–apoB interaction allows artery LPL to bind to LDL. In contrast, hydrolysis of TG-rich lipoproteins on the endothelial surface might (as reviewed above) involve other processes.

LPL in macrophages

The function of macrophage LPL is unknown. LPL is produced by mouse peritoneal macrophages (134) and by other macrophages including human monocyte-derived macrophages (135). Macrophage LPL may increase cellular uptake of lipoprotein lipid and fat-soluble vitamins (136, 137), degrade the lipid contained in pathogens and cell debris, or create fatty acids for energy requirements of the cells.

Lipoprotein uptake. A likely role for macrophage LPL is to internalize and degrade lipoproteins. Cholesterol is required for the rapid turnover of the membranes in these metabolically active cells and there are several LPL-mediated pathways that could enhance lipoprotein uptake by cells. Active LPL produced by macrophages enhances chylomicron uptake (138). Thus, the macrophage receptors that interact with apoE-rich particles are better able to internalize the remnant lipoproteins.

LPL will also increase cellular uptake of LDL. Stein et al. (139) first noted that LPL could promote what appeared to be non-LDL receptor-mediated uptake of lipoprotein cholesterol. The original hypothesis was that LPL functioned as a cholesterol transfer protein. Whether LPL can increase lipoprotein uptake via the LDL receptor is unclear. As discussed above, LPL by serving as a ligand for LRP promotes uptake of apoB-containing lipoproteins. Under some conditions, LPL and lipolysis products might lead to aggregation of LDL and increased LDL receptor-mediated uptake of LDL. Aviram, Bierman, and Chait (140) reported that LPL increased LDL receptor-mediated uptake of LDL, perhaps secondary to increasing LDL association with the cell surface (141). In one study, LPL led to less uptake of LDL in LDL receptor-negative cells (141). In another study, however, antibodies against the LDL receptor failed to decrease LPL-mediated lipoprotein uptake (142). Although antibodies against the receptor-binding region of apoB did not decrease LPL-mediated LDL uptake by cells (143), this would not have decreased receptor uptake if, as recently suggested (144), LPL is a ligand for the LDL receptor. Although the relative importance of various receptors and uptake via internalization of cell surface HSPG is unclear, LPL by increasing the proximity of lipoproteins to multiple receptors and cell surface HSPG increases LDL uptake by cultured cells.

Why do remnants increase cholesteryl ester accumulation in macrophages? One hypothesis is that remnant lipoproteins are internalized via a different pathway than LDL and that they affect intracellular cholesterol metabolism differently (145). Because remnant particles contain a significant amount of core TG, one would expect some remnant lipolysis to occur via LPL on the surface of macrophages. Thus, cells incubated with β-VLDL are exposed to both core lipid, taken up with the particles, and dissociated excess surface material containing free cholesterol, phospholipids, and fatty acids. In high concentrations, these lipolysis products are toxic to cells (146). In lower concentrations, fatty acids, as well as cholesterol, stimulate intracellular acyl coenzyme A:cholesterol acyltransferase (147) resulting in increased cholesteryl ester production. Therefore, lipolysis may stimulate foam cell formation.

Other potential roles of macrophage LPL. There are several other LPL-mediated processes that could potentiate atherogenic actions of macrophages. Macrophages differ from granulocytes in several ways. They are more long-lived residents of the inflammatory response. This allows them to produce granulomas at sites of inflammation and may be a reason why they reside at sites of atherosclerosis within the vessel wall. Amongst leukocytes, only macrophages produce LPL. LPL-mediated pathways might increase the catabolism of pathogens and cellular debris in much the same manner as macrophage LPL promotes the uptake of apoB-containing lipoproteins, by forming a molecular bridge to the cell surface proteoglycans. Another possible role of macrophage LPL is to produce free fatty acids for energy. Phagocytosis, migration, proliferation, and secretion are all energy-requiring events. Although macrophages readily use glucose as a source of energy, it is conceivable that in situations in which glucose is in short supply, e.g., within an infected abscess, fatty acids derived from lipoprotein TG might supply the energy needs of the cells. Parallel metabolic conditions might be present within the atherosclerotic plaque, especially in areas of the lesions that are more distant from a source of blood-derived nutrients.

Genetics relationship of LPL and CAD in humans

Although animal models are invaluable for defining molecular mechanisms, the relationships of LPL to atherosclerosis must, ultimately, be shown in humans. Both LPL activity and LPL genotype have been studied in human populations and related to lipoproteins and atherogenesis. One would expect that any genotypes associated with alteration in lipoprotein profiles and

CAD risk would also show changes in LPL expression as assessed by activity or proteins.

A large number of mutations and polymorphisms of LPL have been described in recent years and are summarized in other recent reviews (148). Those mutations that are associated with type 1 hyperlipoproteinemia are clearly of functional significance. Obligate heterozygotes for LPL deficiency have abnormalities in postprandial lipemia (149) despite relatively normal fasting TG. Fasting hypertriglyceridemia in heterozygotes was found only in individuals greater than 40 years of age (150), suggesting that secondary metabolic changes that occur with aging are required to lead to a phenotype. Similarly, the metabolic stress of diabetes or pregnancy (151, 152) will cause more hypertriglyceridemia in heterozygotes with LPL deficiency than in normals. There are several polymorphisms of LPL that have been associated with hypertriglyceridemia and low HDL (153). These include a variation in asp9-asn (154) and an asp291-ser (155). These variants do not produce inactive LPL protein; however, they appear to lead to less severe alterations in LPL. Both variants have been found in subjects with CAD or hyperlipidemia and in normals. Most importantly, data are not yet available relating heterozygous LPL deficiency and CAD risk. Such data will provide a more clear-cut illustration of whether LPL enzymatic actions are, at least in the peripheral tissues, related to CAD.

Although low HDL and elevated TG are generally associated with CAD, there are some situations in which defects in VLDL to LDL conversion might be antiatherogenic. For example, a decrease in LDL production in familial hypercholesterolemia may be beneficial. Recently, a study of individuals with homozygous familial hypercholesterolemia showed that LPL mass was positively correlated with atherosclerosis (156). Similarly, LPL deficiency prevented the LDL elevation in a patient with familial hypercholesterolemia (157). These data underscore the complexity of the relationship between LPL, lipoprotein profiles, and CAD risk.

Final comments

This review was written from the perspective of an LPL biologist and has avoided alternative non-LPL mechanisms for some of the metabolic pathways or atherogenic mechanisms. Although studied for several decades, mechanisms of regulation of lipolysis by endothelial cells and apolipoproteins are still incompletely understood. There are relationships between macrophage LPL, postprandial lipemia and atherogenesis, and a number of hypotheses driven by tissue culture and biochemical studies have been developed to explain these relationships. Recently several laboratories have created genetically altered mice that overexpress (158-161) and do not express LPL (162, 163). The ability to test some of the in vitro generated hypotheses has therefore begun and will, no doubt, lead to additional questions requiring all levels of experimentation.

I wish to acknowledge the experimental and intellectual input of several investigators who have worked in my laboratory, especially Drs. U. Saxena and P. Sivaram. I would also like to thank Dr. W. Virgil Brown who provided me with the enthusiasm to begin my work on lipases and who critiqued this manuscript prior to its submission, and Dr. Iris Edwards who carefully critiqued and edited portions of the manuscript, especially the sections on proteoglycans. Support for the author's salary and for experiments performed in the author's laboratory was from grants HL 21006 SCOR and RO1-45095 from the National Heart, Lung, and Blood Institute.

Manuscript received 5 October 1995 and in revised form 28 December 1995.

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