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GPIHBP1 and the intravascular processing of triglyceride-rich lipoproteins

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Abstract

Lipoprotein lipase (LPL) is produced by parenchymal cells, mainly adipocytes and myocytes, but its role in hydrolyzing triglycerides in plasma lipoproteins occurs at the capillary lumen. For decades, the mechanism by which LPL reached its site of action in capillaries was unclear, but this mystery was recently solved. GPIHBP1, a GPI-anchored protein of capillary endothelial cells, picks up LPL from the interstitial spaces and shuttles it across endothelial cells to the capillary lumen. When GPIHBP1 is absent, LPL is mislocalized to the interstitial spaces, leading to severe hypertriglyceridemia. Some cases of hypertriglyceridemia in humans are caused by *GPIHBP1* mutations that interfere with GPIHBP1's ability to bind LPL, and some are caused by *LPL* mutations that impair LPL's ability to bind to GPIHBP1. This review will cover recent progress in understanding GPIHBP1's role in health and disease and will discuss some remaining mysteries surrounding the processing of triglyceride-rich lipoproteins.

Keywords

hypertriglyceridemia; chylomicronemia; GPIHBP1; lipoprotein lipase; endothelial cells; lymphocyte antigen 6 proteins

Introduction

We have known for decades that the lipolytic processing of chylomicrons and very low density lipoproteins (VLDL) by lipoprotein lipase (LPL) occurs at the capillary lumen, mainly in adipose tissue and striated muscle [1-4]. For years, LPL was assumed to be bound to heparan sulfate proteoglycans (HSPGs) on the surface of capillary endothelial cells. This long-held model (Fig. 1A) made sense, given that LPL binds avidly to HSPGs [5-7] and because LPL can be released into the plasma by heparin [8], a highly sulfated proteoglycan. However, some elements of the story were missing from this model. One was why LPL, an enzyme that is secreted by myocytes and adipocytes, would bind preferentially to HSPGs at

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the capillary lumen rather than to HSPGs surrounding myocytes and adipocytes. Also, this model did not explain how LPL was shuttled across endothelial cells to the capillary lumen.

Over the past few years, the model for plasma triglyceride metabolism at the capillary lumen has changed significantly (Fig. 1B). We now know that GPIHBP1 (glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1), a protein of capillary endothelial cells, is the principal binding site for LPL on endothelial cells and that it is responsible for transporting LPL to the capillary lumen [9]. Not surprisingly, GPIHBP1 proved to be relevant to human disease. *GPIHBP1* mutations that interfere with LPL binding and transport cause severe hypertriglyceridemia (chylomicronemia) [10-15], while some *LPL* mutations cause hypertriglyceridemia by interfering with LPL's capacity to bind to GPIHBP1 [16]. In this review, we will discuss GPIHBP1's role in lipolysis and its involvement in hypertriglyceridemia in humans. In addition to discussing recent progress, we will highlight lingering mysteries in the lipolysis field and topics for future research.

The discovery of GPIHBP1 and its role in lipolysis

GPIHBP1 was initially identified, by expression cloning, as a molecule that conferred upon CHO cells the ability to bind high-density lipoproteins [17]. The authors of this first report noted that GPIHBP1 has three noteworthy features [17]. First, GPIHBP1 has a striking acidic domain; 17 of 25 consecutive residues at the amino terminus of mouse GPIHBP1 are glutamate or aspartate. Second, GPIHBP1 has a cysteine-rich lymphocyte antigen 6 (Ly6) motif, similar to CD59 (which regulates complement activation) and urokinase-type plasminogen activator receptor (UPAR). Third, mature GPIHBP1 contains a glycosylphosphatidylinositol (GPI) anchor. GPIHBP1 can be released from the plasma membrane by cleaving the GPI anchor with a phosphatidylinositol-specific phospholipase C. In the initial report, the authors suggested that GPIHBP1 played a role in "the initial entry of HDL cholesterol into scavenger cells for further transportation of cholesterol." They added that understanding GPIHBP1 function would "require the development of knockout mice" [17].

The physiologic function of GPIHBP1 came into focus with the characterization of *Gpihbp1* knockout mice (*Gpihbp1*^{-/-}) [18]. When fed a low-fat chow diet, *Gpihbp1*^{-/-} mice have plasma triglyceride levels of ~2000–5000 mg/dl and plasma cholesterol levels of 300–900 mg/dl [18]; nearly all of these lipids are in the VLDL/chylomicron fraction. By electron microscopy, the lipoprotein particles in *Gpihbp1*^{-/-} mouse plasma are far larger than those of wild-type mice [18]. Also, the clearance of retinyl palmitate from *Gpihbp1*^{-/-} mice was markedly delayed [18], and the plasma levels of apo-B48 were elevated. Each of these findings suggested that the lipolytic processing of triglyceride-rich lipoproteins (TRLs) in *Gpihbp1*^{-/-} mice was defective.

Three additional observations lent support to the idea that GPIHBP1 is important for lipolysis. The first was that GPIHBP1 is expressed highly in heart and adipose tissue, the same tissues that express high levels of LPL. The second was that GPIHBP1, when expressed in cultured cells, binds LPL avidly. The third was that GPIHBP1 is found exclusively in capillary endothelial cells, where lipolysis occurs. These findings led Beigneux *et al.* [18] to speculate that GPIHBP1 was a binding site for LPL within capillaries.

In their initial report, Beigneux *et al.* [18] observed that GPIHBP1 binds apo-AV and TRL particles. Later, Gin *et al.* [19] investigated these findings in more detail. GPIHBP1's interaction with apo-AV depends on apo-AV's heparin-binding domain and GPIHBP1's

acidic domain [20] but not on GPIHBP1's Ly6 domain [19]. Gin *et al.* [19] also showed that TRL binding to GPIHBP1-expressing CHO cells is mediated by the hamster LPL secreted by CHO cells [19]. The hamster LPL binds to GPIHBP1, and the LPL promotes binding of TRLs.

Because the plasma of *Gpihbp1*^{-/-} mice contains elevated levels of apo-B48, Beigneux *et al.* [18] raised the possibility that GPIHBP1 might play a special role in processing apo-B48-containing lipoproteins. However, subsequent studies showed that this is not the case; the plasma triglyceride levels in apo-B48-only *Gpihbp1*^{-/-} mice (*Gpihbp1*^{-/-}*Apob*^{48/48}) and apo-B100-only *Gpihbp1*^{-/-} mice (*Gpihbp1*^{-/-}*Apob*^{100/100}) are elevated to the same degree [21].

Metabolic consequences of impaired lipolysis in *Gpihbp1*^{-/-} mice

In a recent study, Weinstein *et al.* [22] found that the ratio of 18:2 and 18:3 fatty acids to 16:1 fatty acids in adipose tissue was lower in *Gpihbp1*^{-/-} mice than in wild-type mice, consistent with increased *de novo* lipogenesis. The triglycerides in the liver exhibited reciprocal changes: the 18:2,18:3/16:1 fatty acid ratio of liver triglycerides was higher in *Gpihbp1*^{-/-} mice than in wild-type mice [22]. The expression of lipid biosynthetic genes was significantly higher in adipose tissue of *Gpihbp1*^{-/-} mice than in wild-type mice, both on chow and high-fat diets [22]. In the liver, the expression of lipid biosynthetic genes was lower in *Gpihbp1*^{-/-} mice than in wild-type mice. These studies suggested that impaired delivery of lipids to peripheral tissues of *Gpihbp1*^{-/-} mice is associated with increased uptake of lipids by the liver [22].

GPIHBP1 transports LPL to the capillary lumen

Two experimental findings prompted us to consider the possibility that GPIHBP1 is involved in LPL transport. The first was that tissue stores of LPL are essentially identical in wild-type and *Gpihbp1*^{-/-} mice [18, 23]; the second was that *Gpihbp1*^{-/-} mice have very respectable levels of catalytically active LPL in the plasma after an intraperitoneal injection of heparin [18]. These observations raised an obvious question: Why do *Gpihbp1*^{-/-} mice have chylomicronemia, given that they have large amounts of catalytically active LPL? Weinstein *et al.* [23] proposed that the LPL in *Gpihbp1*^{-/-} mice was likely mislocalized away from the intravascular compartment. In support of this idea, the entry of LPL into the plasma compartment after an injection of heparin was delayed in *Gpihbp1*^{-/-} mice [23]. Also, an injection of triglyceride emulsion particles failed to release LPL into the plasma of *Gpihbp1*^{-/-} mice.

Davies *et al.* [9] used microscopy to test the notion that LPL was mislocalized in *Gpihbp1*^{-/-} mice. They found that the LPL in wild-type mice is largely associated with capillaries, while the LPL in *Gpihbp1*^{-/-} mice was mislocalized to the interstitial spaces surrounding parenchymal cells (Fig. 2) [9]. This was the case in multiple tissues—heart, skeletal muscle, mammary gland, and brown adipose tissue. Imaging cross-sections of capillaries containing endothelial cell nuclei was also informative. In wild-type mice, LPL was present, along with GPIHBP1, in the capillary lumen; in *Gpihbp1*^{-/-} mice, LPL was absent from the capillary lumen (Fig. 3) [9]. These studies supported the idea that GPIHBP1 functions as an LPL transporter.

Direct support for GPIHBP1's role as a transporter was obtained with transport assays across confluent monolayers of endothelial cells. GPIHBP1-expressing endothelial cells transported a GPIHBP1-specific monoclonal antibody from the basolateral to the apical face of cultured endothelial cells [9]. The antibody transport could be eliminated when GPIHBP1 at the basolateral face of cells was clipped off with a phosphatidylinositol-specific

phospholipase C. GPIHBP1-expressing endothelial cells also transported LPL from the basolateral to the apical face of cells [9].

Phenotypic differences in GPIHBP1-deficient and LPL-deficient mice

Because LPL cannot reach the capillary lumen in the absence of GPIHBP1, one might have predicted that *Gpihbp1*^{-/-} mice would closely resemble *Lpl* knockout mice (*Lpl*^{-/-}). However, this is not the case. Newborn *Lpl*^{-/-} mice have markedly elevated plasma triglyceride levels (~20,000 mg/dl) and die within 24 h [24]. In contrast, suckling *Gpihbp1*^{-/-} mice are healthy with plasma triglyceride levels of ~120 mg/dl [18].

Transient expression of LPL (from an injection of an LPL adenovirus) rescues LPL-deficient mice from perinatal lethality, allowing them to survive into adulthood [25, 26]. Adult *Lpl*^{-/-} mice have markedly elevated triglyceride levels, very much like adult *Gpihbp1*^{-/-} mice.

Why are the phenotypes of suckling *Lpl*^{-/-} and *Gpihbp1*^{-/-} pups so different, while the phenotypes of adult *Lpl*^{-/-} and *Gpihbp1*^{-/-} mice are so similar? The answer likely relates to the fact that suckling mice, but not adult mice, produce large amounts of LPL in the liver [18]. The fenestrated capillaries of the liver presumably allow unfettered access of LPL to TRLs in the plasma—even when GPIHBP1 is absent.

Atherosclerosis in *Gpihbp1*^{-/-} mice

Chylomicrons are often considered to be nonatherogenic, in part because of earlier observations indicating minimal atherogenicity of very large lipoproteins in rabbits and because of a limited ability of very large lipoproteins to enter the arterial wall [27]. To our surprise, however, chow-fed *Gpihbp1*^{-/-} mice developed lipid- and macrophage-rich atherosclerotic lesions in the aortic root and coronary arteries [21]. The lesions occurred in both male and female mice; they were relatively small at 11–12 months but larger by 16–22 months of age. These lesions were far smaller than those in *ApoE*^{-/-} mice [28–31], but their very existence demonstrated that large TRLs can be atherogenic. These findings were consistent with aortic lesions in *Lpl*-deficient mice [26] and reports of atherosclerotic disease in LPL-deficient humans [32].

Regulation of GPIHBP1 expression

Fasting leads to higher levels of *Lpl* expression in heart, but lower levels of *Lpl* expression in white adipose tissue [33]. This physiologic response makes sense, in that it would serve to promote triglyceride delivery to the heart and away from adipose tissue during fasting [34–36]. In contrast, fasting leads to increased *Gpihbp1* expression in both heart and adipose tissue [33]. Why *Gpihbp1* expression increases in white adipose tissue during fasting is unclear, but one possibility is that increased expression of GPIHBP1 somehow facilitates LPL regulation by ANGPTL4.

PPAR γ agonists increase *Gpihbp1* expression in adipose tissue, heart, and skeletal muscle, whereas PPAR α and PPAR δ agonists have little or no effect. Sequences upstream of exon 1 in *Gpihbp1* contain a PPAR binding site that exhibits activity in a luciferase reporter assay [33]. Also, a knockout of PPAR γ in endothelial cells lowers *Gpihbp1* transcript levels in brown and white adipose tissue, suggesting that PPAR γ regulates *Gpihbp1* expression levels *in vivo* [33]. However, whether GPIHBP1 expression in humans is altered by PPAR γ agonists is not known.

In mice, the expression of LPL in the liver can be induced with dietary cholesterol [37]. Consistent with this observation, the plasma triglyceride levels in *Gpihbp1*^{-/-} mice are lower on a high-cholesterol diet than on a low-cholesterol diet [38]. Conversely, the plasma triglyceride levels in *Gpihbp1*^{-/-} mice are significantly higher when mice are treated with ezetimibe, which lowers cholesterol absorption and leads to lower levels of *Lpl* expression in the liver. Again, the responsiveness of plasma triglyceride levels to changes in LPL expression in the liver is likely explained by fenestrated capillaries in the liver, which would give LPL ready access to TRLs in the bloodstream.

GPIHBP1 expression in different tissues

In general, the *tissue* pattern of GPIHBP1 expression is similar to that of LPL. For example, *Gpihbp1* and *Lpl* transcripts are both found at high levels in heart and brown adipose tissue and at lower levels in skeletal muscle [18]. There are, however, exceptions to this rule. First, GPIHBP1 is absent from the capillaries of the brain, even though certain regions of the brain, for example the hippocampus, express large amounts of LPL [39-43]. The explanation for this discrepancy is not clear, but one possibility is that LPL actually has an extravascular function in the brain.

GPIHBP1 and LPL expression levels are also discrepant in the lung [44]. *Lpl* transcript levels are negligibly low in the lung while *Gpihbp1* transcript levels are very high (in the same range as brown adipose tissue and heart). Consistent with high *Gpihbp1* transcript levels, GPIHBP1 protein levels are quite high in lung capillaries. Indeed, when GPIHBP1-specific monoclonal antibodies are injected into mice, antibody binding to lung capillaries is very high, similar to the levels of antibody binding in heart and brown adipose tissue [44]. Interestingly, while *Lpl* transcript levels in the lung are extremely low, one can detect LPL protein in the lung. We suspect that GPIHBP1 in lung capillaries scavenges LPL produced by other tissues. While most of the LPL secreted by myocytes and adipocytes is bound by GPIHBP1 or HSPGs, we suspect that some LPL escapes and finds its way into the lymph and eventually into the venous circulation. That LPL, we believe, is likely to be captured by GPIHBP1 in lung capillaries. Consistent with this idea, we found that the amount of LPL protein in the lung was lower in *Gpihbp1*^{-/-} mice than in wild-type mice [44]. Also, there are relatively high levels of *human* LPL in the lungs of transgenic mice that express human LPL exclusively in skeletal muscle [44].

Delineating LPL–GPIHBP1 interactions

LPL has been long recognized to have positively charged domains that bind to heparin [45-48], and we suspected that those same domains would interact with GPIHBP1's amino-terminal acidic domain. In support of this idea, wild-type GPIHBP1 binds LPL avidly, but an acidic domain GPIHBP1 mutant (in which the aspartates and glutamates in the second half of the acidic domain were changed to alanines) was unable to bind LPL. Also, a rabbit antiserum against the GPIHBP1 acidic domain markedly reduced LPL binding to cells expressing wild-type GPIHBP1 [20]. These findings implied that electrostatic interactions play a role in LPL–GPIHBP1 interactions. Consistent with this idea, the binding of LPL to GPIHBP1 could be blocked by polyaspartate, polyglutamate, and heparin [20]. Also, the binding of LPL to GPIHBP1 was reduced when positively charged residues in LPL's carboxyl-terminal heparin-binding domain (K403, R405, K407, K413, K414) were changed to alanine [20].

In addition to the acidic domain, GPIHBP1's Ly6 domain is crucial for LPL–GPIHBP1 interactions. The Ly6 domain is an ~80-amino acid motif containing 10 cysteines arranged in a characteristic spacing pattern. Each of the 10 cysteines is disulfide-bonded, generating a

three-fingered structural motif [49]. The involvement of GPIHBP1's Ly6 domain in LPL binding is supported by several observations. First, a chimeric protein containing GPIHBP1's acidic domain and the Ly6 domain of another Ly6 family member (CD59) lacks the ability to bind LPL [20]. Second, changing any of the 10 cysteines in GPIHBP1's Ly6 domain to alanine reduces the ability of GPIHBP1 to bind LPL [50]. Thus, intact disulfide bonds and the maintenance of GPIHBP1's three-fingered structure are important for LPL binding. Finally, Beigneux *et al.* [51] mutated each amino acid of the Ly6 domain (Cys-65 to Cys-136) and tested the impact of these mutations on LPL binding. In addition to the conserved cysteines, 12 residues were essential for LPL binding, nine of which were clustered in finger 2 of GPIHBP1's three-fingered Ly6 domain [51]. The mutant GPIHBP1 proteins that could not bind LPL in cell-based binding assays also lacked the ability to transport LPL across cultured endothelial cells [51].

An example of a western blot assay testing the binding of LPL to mutant forms of GPIHBP1 is shown in Fig. 4. In this experiment, CHO cells were transfected with a wild-type or a mutant human GPIHBP1 expression vector; 24 h later, the cells were incubated with LPL for 2 h at 4°C in the presence or absence of heparin. After washing the cells, the amount of LPL bound to cells was assessed by western blotting. In this experiment, wild-type GPIHBP1 bound LPL avidly, as did GPIHBP1-D112A and GPIHBP1-W109S, but virtually no LPL bound to cells expressing GPIHBP1-C110A.

Mouse GPIHBP1 contains a single N-linked glycosylation site, and glycosylation of that site is important for the transport of mature, GPI-anchored GPIHBP1 to the cell surface [52]. However, an absence of N-linked glycosylation has little or no effect on the secretion of a *soluble* version of GPIHBP1 (*i.e.*, a version lacking the GPI anchor) from cells [52]. The absence of the N-linked glycan does not prevent GPIHBP1 from binding LPL [52].

Early studies had suggested that the LPL binding to GPIHBP1 might require intact LPL homodimers [19], but this is not the case [53]. The carboxyl-terminal half of LPL (residues 298–448)—acting as a monomer and free of LPL's amino-terminal catalytic domain—binds avidly to GPIHBP1 [53].

GPIHBP1 mutations and chylomicronemia in humans

Finding chylomicronemia in *Gpihbp1*^{−/−} mice [18] prompted interest in determining whether human *GPIHBP1* mutations have a role in the pathogenesis of hypertriglyceridemia. In recent years, a number of clinically significant GPIHBP1 mutations have been uncovered, as discussed below. However, GPIHBP1 mutations are not a common cause of hypertriglyceridemia; even in highly selected “chylomicronemia patients” where mutations in *LPL*, *APOC2*, and *APOA5* have been excluded, GPIHBP1 mutations are uncommon [11, 12, 15, 54].

The first *GPIHBP1* mutation, a Q115P missense mutation, was identified by screening 60 patients with severe hypertriglyceridemia who lacked mutations in *LPL*, *APOC2*, and *APOA5* [12]. The affected patient had lifelong chylomicronemia and was homozygous for a Q115P mutation in *GPIHBP1*. When GPIHBP1-Q115P was expressed in CHO cells, it reached the cell surface normally but it had a markedly reduced capacity to bind LPL [12]. When the same mutation was introduced into mouse GPIHBP1, LPL binding was also markedly reduced [12]. Replacing Q115 in human GPIHBP1 with a lysine (the residue found in canine GPIHBP1) or a glutamate (found in platypus GPIHBP1) did not affect LPL binding [51].

A C65Y mutation in GPIHBP1 was identified in a 3-year-old boy with chylomicronemia [11]. The child had plasma triglyceride levels of >1500 mg/dl and a history of pancreatitis.

When GPIHBP1-C65Y was expressed in cultured cells, it reached the cell surface but could not bind LPL. Later, Coca-Prieto *et al.* [13] analyzed five patients with childhood-onset chylomicronemia and found that one patient had a C68Y mutation in GPIHBP1. Two more GPIHBP1 cysteine mutations were identified in Sweden. Three members of a single family had chylomicronemia, and all three were compound heterozygotes for C65S and C68G alleles [10]. Studies in transfected cells revealed that GPIHBP1-C65S and GPIHBP1-C68G reached the cell surface but were unable to bind LPL. LPL mass and activity levels were normal in the adipose tissue of affected subjects, consistent with the normal stores of LPL in tissues of *Gpihbp1*^{-/-} mice [23]. Interestingly, the breast milk from one of the C65S/C68G compound heterozygotes contained normal amounts of LPL [10].

Drs. Helen Hobbs and Jonathan Cohen identified two family members with chylomicronemia and a homozygous deletion of the entire *GPIHBP1* gene [14]. The proband was an Asian Indian boy who was noted to have severe chylomicronemia at 2 months of age. Array-based copy-number analysis of the genomic DNA uncovered a 17.5-kb deletion that included *GPIHBP1*. A 44-year-old aunt with a history of hypertriglyceridemia and pancreatitis was homozygous for the same deletion [14].

Charrière *et al.* [15] identified two patients with GPIHBP1 mutations among 376 patients with chylomicronemia. A young child with markedly elevated plasma triglyceride levels and a history of pancreatitis, had a C89F mutation in one *GPIHBP1* allele; the other allele had a deletion of the entire *GPIHBP1* gene [15]. The second patient, a 35-year old man with plasma triglycerides >2200 mg/dl, was homozygous for a G175R mutation. The G175R mutation was located in GPIHBP1's carboxyl-terminal domain and likely interfered with the addition of the GPI anchor. In cell transfection experiments, the G175R mutation reduced the amount of GPIHBP1 at the cell surface [15].

Most recently, Surendran *et al.* [54] found a T108R mutation in GPIHBP1 in a 1-year-old baby with hypertriglyceridemia and a history of pancreatitis. Presumably, this mutation reduced LPL binding, but this was not tested. In an earlier study, a T108A mutation had no effect on LPL binding [51].

The levels of LPL in the pre-heparin plasma are extremely low in humans with GPIHBP1 defects [10]. Similarly, plasma LPL levels after a bolus of heparin are quite low. In the Q115P homozygote, postheparin LPL levels were ~10% of those in controls [18]. The postheparin LPL levels in the three C65S/C68G compound heterozygotes were only ~5% of those in control subjects [10]. Postheparin LPL activity levels were also extremely low or undetectable in the G175R homozygote, the patient with the C89F mutation, the C65Y homozygote, and the C68Y homozygote [11, 13, 15]. In all of the kindreds that have been examined so far, GPIHBP1 deficiency is a recessive syndrome; heterozygotes have normal plasma lipid levels [11, 14, 15]. The same is the case in the knockout mice, where one can show that a single GPIHBP1 knockout allele lowers tissue levels of GPIHBP1 expression by 50%. Presumably, half-normal levels of GPIHBP1 are quite sufficient to transport LPL across endothelial cells, both in humans and in mice.

LPL mutations that interfere with LPL binding to GPIHBP1

The discovery of *GPIHBP1* point mutations that abolish GPIHBP1's ability to bind LPL suggested that there might be "mirror image" *LPL* mutations that abolish LPL's ability to bind GPIHBP1. To investigate this idea, Voss *et al.* [16] took a "second look" at previously described *LPL* mutations associated with chylomicronemia. Two mutations, C418Y and E421K [55, 56], were of particular interest because they were distant from LPL's catalytic domain and had been reported to have little effect on catalytic activity. The C418Y mutation

was discovered in a 30-year-old male with severe hypertriglyceridemia and a history of pancreatitis [56]; the E421K mutation was found in a 24-year-old woman who died of pancreatitis during pregnancy [55].

Voss *et al.* [16] hypothesized that the C418Y and E421K mutations might cause hypertriglyceridemia by interfering with LPL's ability to bind to GPIHBP1. They showed that the enzymatic specific activities of the mutant LPLs were normal and also showed that the mutations did not affect the binding of LPL to heparin. However, both mutations markedly reduced LPL's capacity to bind to wild-type GPIHBP1 [16]. When these mutations were introduced into mouse or chicken LPL, they also reduced binding to GPIHBP1.

The C418Y mutation was intriguing because Cys-418 has been reported to form a disulfide bond with Cys-438 [57]. The inability of LPL-C418Y to bind to GPIHBP1 raised the possibility that the disulfide bond is absolutely essential for GPIHBP1 binding, but this is apparently not the case. LPL-C438A and LPL-C438Y are able to bind to GPIHBP1 (although they appear to bind less avidly than wild-type LPL).

The reduced ability of C418Y-LPL and E421K-LPL to bind to GPIHBP1 suggested that the carboxyl-terminal region of LPL plays an important role in GPIHBP1 binding. In support of that idea, a monoclonal antibody against chicken LPL (cLPL) with an epitope between residues 416 and 435 blocked binding of cLPL to GPIHBP1 while having little effect on cLPL binding to heparin. Mutagenesis studies showed that changing cLPL residues 421–425, 426–430, or 431–435 to alanine reduced cLPL binding to GPIHBP1 and did so without changing cLPL binding to heparin [16].

At this point, it would appear that there are two LPL domains that are important for GPIHBP1 binding. The first is LPL's main heparin-binding domain (K403, R405, K407, K413, and K414). The second is an independent downstream domain encompassing residues ~416–435. It seems likely that LPL's positively charged heparin-binding domain interacts with GPIHBP1's negatively charged acidic domain. The LPL residues that interact with GPIHBP1's Ly6 domain have not been definitively identified, but it is tempting to speculate that this domain interacts with LPL residues ~416–435.

Open issues in the lipolysis field

The identification of GPIHBP1 solved a long-standing mystery in the lipoprotein metabolism field—how LPL reaches the capillary lumen. However, there are many other mysteries in the basic physiology of lipolysis. In the case of GPIHBP1, here are eight topics that we believe need further investigation.

1. Are LPL–GPIHBP1 interactions clinically relevant, aside from the rare mutations that disrupt LPL binding to GPIHBP1?

Most patients with hypertriglyceridemia do not have mutations in GPIHBP1, even when other potential culprit genes have been excluded (*e.g.*, *LPL*, *GPIHBP1*, *APOC2*, *APOA5*) [11, 12, 15, 54, 58]. However, this may not mean that GPIHBP1-mediated LPL transport is irrelevant to the pathogenesis of common forms of hypertriglyceridemia. It is possible, for example, that some cases of hypertriglyceridemia are caused by defects in the cellular machinery for moving GPIHBP1 and LPL across endothelial cells. Also, some cases of hypertriglyceridemia might be caused by inaccessibility of LPL–GPIHBP1 complexes on the surface of endothelial cells. For example, if GPIHBP1–LPL complexes were somehow buried within the glycocalyx along the capillary lumen, the clinical consequences might be quite similar to GPIHBP1 deficiency, where LPL is mislocalized to the subendothelial spaces.

2. Understanding LPL–GPIHBP1 interactions on both sides of endothelial cells

How GPIHBP1 manages to pick up LPL from the subendothelial spaces is still unclear. LPL binds avidly to HSPGs [59, 60]. Even when GPIHBP1 is absent, LPL is tightly bound within the interstitial spaces, presumably attached to HSPGs (the LPL within the subendothelial spaces of *Gpihbp1*^{−/−} mice is readily released by heparin). It will important to understand how GPIHBP1 recruits LPL from HSPG binding sites within the interstitial spaces. It seems possible that GPIHBP1's acidic domain acts as a “lasso,” wresting LPL away from HSPG binding sites (Fig. 5A–B). Once entrained by the acidic domain, it seems possible that interactions with GPIHBP1's Ly6 domain strengthen binding, effectively driving LPL to the surface of endothelial cells (Fig. 5C). Testing this concept will require comparing the affinity of LPL for HSPGs and GPIHBP1.

Mysteries also exist at the luminal face of endothelial cells. It has been known for years that the carboxyl-terminal domain of LPL is important for its ability to bind to triglyceride emulsion particles [23, 61]. It is also clear that an infusion of triglyceride emulsion particles releases LPL into the plasma. It would be interesting to determine if TRL particles wrest LPL away from GPIHBP1 (Fig. 6A–B), or whether the LPL–GPIHBP1 interaction is simply disrupted by the fatty acid products of triglyceride hydrolysis (Fig. 6C). Also, it will be important to determine if protein regulators of lipolysis influence LPL–GPIHBP1 interactions. ANGPTL4 inactivates LPL by converting LPL homodimers to monomers [36], but no one knows whether ANGPTL4 serves to detach LPL from GPIHBP1.

3. Cellular mechanisms for LPL transport across endothelial cells

GPIHBP1 is essential for shuttling LPL across endothelial cells, but the cellular mechanisms for this transport process are unclear. Do GPIHBP1–LPL complexes cluster in caveolar invaginations and move across cells in vesicles? Is caveolin-1 important for LPL transport? Is the transport process unidirectional (*i.e.*, towards the capillary lumen), or do GPIHBP1 and LPL move across endothelial cells in both directions? If LPL and GPIHBP1 also move towards the basolateral face of cells, do they move in the same vesicles that carry the fatty acid products of lipolysis? Finally, it is unclear whether the transport process is regulated.

4. Mechanisms for the margination of TRLs along capillaries

The field needs a better understanding of why TRLs stop along the luminal face of capillaries (so that lipolysis can occur) rather than simply “flowing by” in the bloodstream. There are hundreds of studies of leukocyte margination in blood vessels, but few studies on the margination of TRL particles. One possibility—proposed in many reviews [62, 63]—is that interactions between apolipoproteins and endothelial cell HSPGs are crucial for the margination of TRLs. However, it is also possible that the LPL–GPIHBP1 complex is an important factor in TRL margination in capillaries, particularly since LPL binds avidly to TRLs. Over the next few years, it will be important to define which molecular interactions cause TRLs to stop along the capillary wall—so that lipolysis can proceed.

5. Interactions between GPIHBP1 and LPL and apo-AV

LPL binds to GPIHBP1 with great specificity, and some of the amino acids required for this interaction have been uncovered, both for LPL and GPIHBP1 [16, 50, 51]. However, precisely how the two molecules interact is unclear. Does GPIHBP1's acidic domain interact solely with LPL's carboxyl-terminal heparin-binding domain? Does GPIHBP1's Ly6 domain interact directly with the carboxyl-terminal ~30 amino acids of LPL? Finding definitive answers to these questions will require determining the structures of GPIHBP1, the carboxyl terminal region of LPL, and ultimately the LPL–GPIHBP1 complex.

The stoichiometry of LPL–GPIHBP1 interactions also needs investigation. LPL is secreted as a homodimer, and presumably contains two binding sites for GPIHBP1. However, it is unclear whether LPL is bound and escorted across endothelial cells by two GPIHBP1 molecules, or only one. The binding of LPL by two GPIHBP1 molecules would presumably contribute to high-affinity interactions, but whether this occurs is unknown. It is conceivable that the binding of a single GPIHBP1 to one LPL monomer would change the conformation of the partner LPL monomer in such a way that it would lose its ability to bind a second GPIHBP1 molecule.

6. Does GPIHBP1 affect the efficiency of triglyceride hydrolysis?

GPIHBP1 serves as a binding site for LPL at the capillary lumen, but is it more than that? Is GPIHBP1-bound LPL more efficient than unbound LPL in hydrolyzing triglycerides? Does GPIHBP1 participate in the regulation of LPL activity? GPIHBP1 has been reported to protect LPL from inactivation by ANGPTL4 [64], but another group found that ANGPTL3 inactivates LPL in the presence of soluble GPIHBP1 [65]. These issues need to be explored in greater detail.

7. What factors control GPIHBP1 expression in capillary endothelial cells?

GPIHBP1 is located exclusively in capillary endothelial cells and is not found in larger blood vessels. Indeed, when examining GPIHBP1 expression in heart or brown adipose tissue by immunofluorescence microscopy, one quickly observes that GPIHBP1 expression is silenced as soon as the capillary expands into the very smallest venule. Is the restricted expression of GPIHBP1 expression in capillaries governed by *cis*-acting regulatory elements within the GPIHBP1 gene? If so, those elements need to be defined. Also, it is unclear if GPIHBP1 expression is regulated by metabolic cues from surrounding tissues (*i.e.*, paracrine factors secreted by parenchymal cells).

8. Is LPL transported to the capillary lumen in all vertebrates?

LPL is present in all vertebrate species, including fish, amphibians, birds, and mammals. Thus far, however, GPIHBP1 has only been identified in mammals. Why is GPIHBP1 uniquely important for mammals? Might this relate to the fact that these species nurse their young? Perhaps GPIHBP1-mediated transport of LPL to the capillary lumen is essential for the production of lipid-rich maternal milk by the mammary gland. Equally intriguing is the nature of lipolysis in fish, amphibians, and birds—where GPIHBP1 is absent. Do the endothelial cells of those species have a different LPL transporter? Or is it possible that the LPL in other vertebrates never leaves the interstitial spaces surrounding parenchymal cells? Rather than shuttling LPL to capillary lumen, perhaps other vertebrates shuttle lipoproteins across capillaries to the interstitial spaces where LPL resides. These possibilities need investigation.

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Fig. 1.

Two models for the metabolism of triglyceride-rich lipoproteins (TRLs) by lipoprotein lipase (LPL). (A) The model for lipolysis before the discovery of GPIHBP1. LPL was known to be synthesized and secreted by parenchymal cells (myocytes or adipocytes). LPL was known to be active inside capillaries, but how LPL reached the capillary lumen was unknown. Chylomicron particles and LPL were both assumed to bind to heparan-sulfate proteoglycans (HSPGs) inside capillaries. (B) An updated model of lipolysis. GPIHBP1, a cell-surface protein of capillary endothelial cells "picks up" LPL from the interstitial spaces, transports it across endothelial cells to the capillary lumen, and serves to tether LPL at the surface of endothelial cells. Modified, with permission, from Young et al. [66].

Fig. 2.

Confocal immunofluorescence microscopy showing that LPL is tightly bound within the interstitial spaces in skeletal muscle of *Gpihbp1*^{-/-} mice. Images show β -dystroglycan (a marker of skeletal myocytes) (green), CD31 (a marker of endothelial cells) (purple), and LPL (red) in muscle from a wild-type (*Gpihbp1*^{+/+}) mouse and a *Gpihbp1*^{-/-} mouse. The LPL is largely bound to capillaries in the *Gpihbp1*^{+/+} mouse but is mislocalized to the interstitial spaces surrounding myocytes in the *Gpihbp1*^{-/-} mouse. Reproduced, with permission, from Davies et al. [9].

Fig. 3.

Confocal micrographs demonstrating that LPL is absent from the capillary lumen in *Gpihbp1*^{-/-} mice. Micrographs show GPIHBP1 (purple), LPL (red), and CD31 (green) in brown adipose tissue. Nuclei were stained with DAPI (blue). The luminal (arrowhead) and basolateral (arrow) surfaces of capillaries could be distinguished in cross-sections of capillaries containing an endothelial cell nucleus. GPIHBP1, LPL, and CD31 are found at both the luminal and basolateral surfaces of wild-type (*Gpihbp1*^{+/+}) capillaries; however, LPL could not be found along the luminal surface of *Gpihbp1*^{-/-} capillaries. Note the absence of LPL—but not CD31—in the lumen of the “*Gpihbp1*^{-/-} capillary.” Reproduced, with permission, from Davies et al. [9].

Fig. 4.

A western blot assay of LPL binding to wild-type GPIHBP1 and mutant GPIHBP1 proteins harboring specific missense mutations (D112A, W109S, C110A). CHO-K1 cells were electroporated with expression vectors encoding S-protein-tagged versions of wild-type GPIHBP1 or the GPIHBP1 mutants. After 24 h later, the cells were incubated for 2 h with V5-tagged human LPL $\pm$ heparin (500 U/ml). After washing the cells, the levels of GPIHBP1 expression in cells and the amount of LPL bound to cells were assessed by western blotting. Actin was used as a loading control. LPL bound avidly to cells expressing wild-type GPIHBP1, GPIHBP1-D112A, and GPIHBP1-W109S; LPL did not bind to cells expressing GPIHBP1-C110A.

Fig. 5.

LPL–GPIHBP1 interactions at the basolateral face of endothelial cells. Even in the absence of GPIHBP1, LPL is immobilized in the subendothelial spaces, likely bound to HSPGs (Panel A). We hypothesize that GPIHBP1's acidic domain (the orange-colored domain, Panel B) is responsible for competing with HSPGs and wresting LPL away from HSPGs. Once the LPL is detached from HSPGs, we suspect that LPL interacts fully with both of GPIHBP1's binding domains (the acidic and the Ly6 domains), resulting in high-affinity binding (Panel C).

Fig. 6. LPL–GPIHBP1 interactions at the capillary lumen. (A) LPL is normally bound to GPIHBP1 on the cell surface, but LPL is released into the circulation following an injection of a triglyceride emulsion (Intralipid) [23, 61]. (B) Because LPL binds to triglyceride-rich particles [5], it seems possible that triglyceride-rich lipoproteins may cause some LPL to become detached from GPIHBP1. (C) It is also possible that the fatty acid products of lipolysis weaken LPL–GPIHBP1 interactions and cause LPL to become detached from GPIHBP1.