GPIHBP1, a GPI-anchored protein required for the lipolytic processing of triglyceride-rich lipoproteins

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Abstract GPIHBP1, a small glycosylphosphatidylinositol-anchored glycoprotein, is required for the lipolytic processing of triglyceride-rich lipoproteins. GPIHBP1 knockout mice exhibit chylomicronemia, even on a low-fat diet, with plasma triglyceride levels of 3,500–5,000 mg/dl. GPIHBP1 is expressed highly in heart, adipose tissue, and skeletal muscle, the same tissues that express high levels of lipoprotein lipase (LPL). In each of these tissues, GPIHBP1 is located in capillary endothelial cells. Chinese hamster ovary (CHO) cells transfected with a GPIHBP1 expression vector bind LPL and chylomicrons avidly. The expression of GPIHBP1 in mice is modulated by fasting and refeeding and is also regulated by peroxisome proliferator-activated receptor (PPAR)γ agonists. Here, we review recent progress in understanding GPIHBP1 and discuss its role in lipolysis.—Beigneux, A. P., B. S. J. Davies, A. Bensadoun, L. G. Fong, and S. G. Young. **GPIHBP1, a GPI-anchored protein required for the lipolytic processing of triglyceride-rich lipoproteins. *J. Lipid Res.* 2009.** 50: **S57–S62.**

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For 50 years, the entire history of the *Journal of Lipid Research*, it has been known that plasma triglycerides can be cleared by a dedicated enzyme, lipoprotein lipase (LPL) (1). The lipolytic processing of triglyceride-rich lipoproteins occurs mainly in heart, skeletal muscle, and adipose tissue (2–4). We now know that LPL is synthesized by myocytes and adipocytes and then finds its way into capillaries, where lipolysis takes place (2, 3). In the absence of LPL (or its cofactor apolipoprotein CII), lipolytic processing of lipoproteins cannot occur, leading to severe hypertriglyceridemia ("chylomicronemia") (2).

For the past few decades, the protagonists in lipolysis (i.e., LPL, apo-CII) have remained the same, and their functions have been studied thoroughly. Along the way, several "supporting actors" (e.g., apo-CIII, apo-AV, ANGPTL3, ANGPTL4) have appeared (5–8). These molecules regulate the efficiency of lipolysis, and deficiencies in these proteins perturb plasma triglyceride levels. Over the past few years, the functions of these molecules have been investigated by multiple laboratories.

Recently, just as we were beginning to think that the broad outlines of lipolysis were understood, a new molecule, GPIHBP1, appeared on the scene (**Fig. 1**) (9–11). GPIHPB1 is required for lipolysis, and a deficiency of this protein causes frank chylomicronemia. Interestingly, GPIHBP1 is synthesized by endothelial cells (9). The discovery of an endothelial cell protein for lipolysis is welcome news, at least in our opinion. Prior to the discovery of GPIHBP1, dogma held that lipolysis, a process occurring within capillaries, actually took place without any truly dedicated endothelial cell protein. That, in our view, seemed odd. With the discovery of GPIHBP1, a void has been filled. No longer can endothelial cells be viewed as playing a passive role in lipolysis, merely "hosting" a lipolytic process controlled by surrounding tissues. With the discovery of GPIHBP1, it is clear that endothelial cells are actively involved in lipolysis. Here, we review progress in understanding GPIHBP1, focusing on its structure, function, and regulation.

The involvement of GPIHBP1 in lipolysis was obvious from the analysis of knockout mice by Beigneux et al. (9). The sole phenotype of *Gpihbp1*$^{-/-}$ mice on a chow diet is chylomicronemia, with milky plasma and triglyceride levels of 3,500–5,000 mg/dl and cholesterol levels of 300–900 mg/dl. Interestingly, the plasma lipid levels

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Abbreviations: CHO, Chinese hamster ovary; GPI, glycosylphosphatidylinositol; LPL, lipoprotein lipase; Ly6, lymphocyte antigen 6; PPAR, peroxisome proliferator-activated receptor; UPAR, urokinase-type plasminogen activator receptor.

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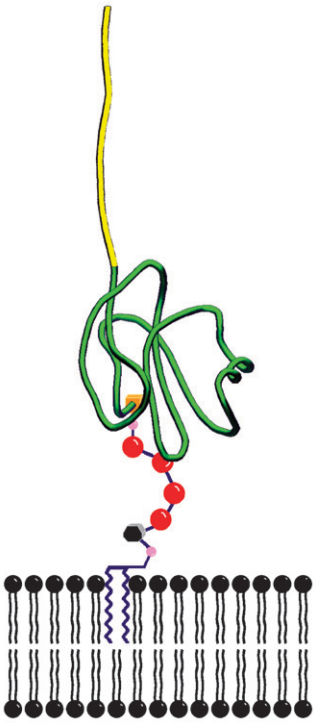

Fig. 1. Model of GPIHBP1 structure depicting a highly acidic N-terminal domain (yellow), a cysteine-rich lymphocyte antigen 6 (Ly6) domain (green), and a glycosylphosphatidylinositol (GPI)-anchor (deep purple) that tethers GPIHBP1 to the plasma membrane.

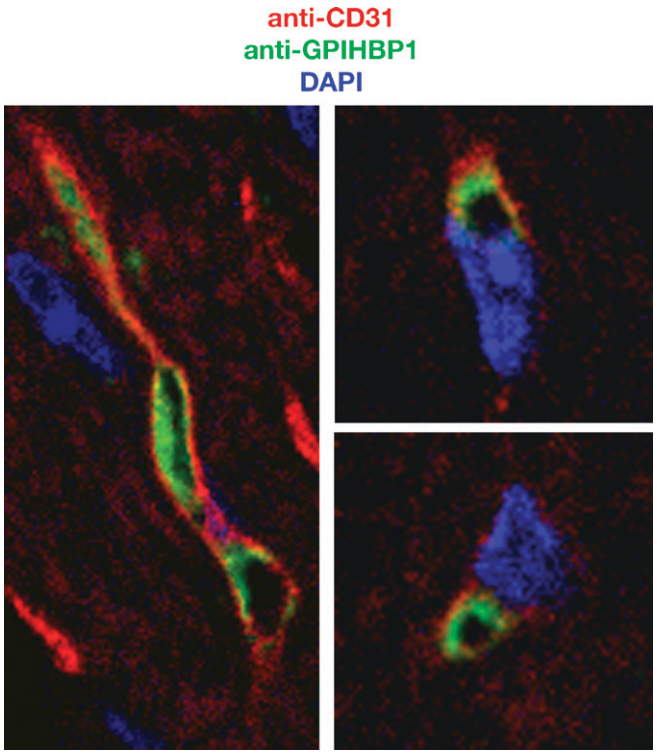

Fig. 2. GPIHBP1 is expressed within the lumen of the capillaries of the mouse heart. Reproduced, with permission, from Beigneux et al. (9).

in *Gpihbp1*$^{+/-}$ and *Gpihbp1*$^{+/+}$ mice were indistinguishable (9).

There had been just one earlier report about GPIHBP1 in the literature, a study by Ioka et al. (12). In that study, GPIHBP1 was identified, by expression cloning, as a molecule that conferred upon Chinese hamster ovary (CHO) cells the ability to bind high-density lipoproteins (HDL), but no functional studies were performed in mouse models.

Beigneux et al. (9) found that nearly all of the lipids in the plasma of *Gpihbp1*$^{-/-}$ mice are contained in large lipoproteins within the $d < 1.006$ g/ml fraction. The plasma also contained increased amounts of apo-B48 (9). The clearance of retinyl palmitate from the plasma was dramatically delayed in *Gpihbp1*$^{-/-}$ mice, strongly suggesting a defect in lipolysis (9). Suckling *Gpihbp1*$^{-/-}$ pups did not die during the perinatal period (9), unlike *Lpl*$^{-/-}$ mice (13). The plasma triglyceride and cholesterol levels in suckling *Gpihbp1*$^{-/-}$ mice were ~120 mg/dl (about 5-fold higher than in wild-type controls).

The tissue pattern of GPIHBP1 expression is similar to that of LPL, with high levels in heart and adipose tissue and moderate levels in skeletal muscle. In each of these tissues, GPIHBP1 is expressed in endothelial cells, principally on the luminal face of capillaries (**Fig. 2**) (9).

Beigneux et al. (9) hypothesized that GPIHBP1 might bind LPL or chylomicrons or both. Indeed, GPIHBP1-expressing pgsA-745 CHO cells bound 10–20-fold more LPL than control cells (**Fig. 3**). The affinity of LPL binding to GPIHBP1 was high ($K_d = 3.6 \times 10^{-8}$ M) (9, 11). Also, the $d < 1.006$ g/ml lipoproteins from *Gpihbp1*$^{-/-}$ mice bound avidly to GPIHBP1-expressing cells. Apo-AV clearly is capable of binding to GPIHBP1 (9), but it is possible that other apolipoproteins also mediate binding. Because of GPIHBP1's ability to bind LPL and chylomicrons, Beigneux et al. (9) speculated that GPIHBP1 might serve as an endothelial cell "platform" for lipolysis.

Beigneux et al. (9) found approximately half-normal levels of LPL mass and activity in the plasma of *Gpihbp1*$^{-/-}$ mice 15 min after an intraperitoneal injection of heparin. This heparin-released LPL was enzymatically active. Why would *Gpihbp1*$^{-/-}$ mice have chylomicronemia with these seemingly "respectable" amounts of LPL? The answer is not yet known, but it seems possible that there is more than one pool of heparin-releasable LPL, and that the LPL released by heparin in *Gpihbp1*$^{-/-}$ mice originates from a pool that is irrelevant to lipolysis. This possibility needs investigation.

GPIHBP1 STRUCTURE/FUNCTION

GPIHBP1 contains a signal peptide, an acidic domain, a lymphocyte antigen 6 (Ly6) domain, and a hydrophobic carboxyl-terminal motif that is released and replaced with a glycosylphosphatidylinositol (GPI) anchor in the endoplasmic reticulum (Fig. 1) (9, 11, 12). Like other GPI-anchored proteins, GPIHBP1 can be released from the cell surface by cleaving the GPI anchor with a phosphatidylinositol-specific phospholipase C (9, 11, 12).

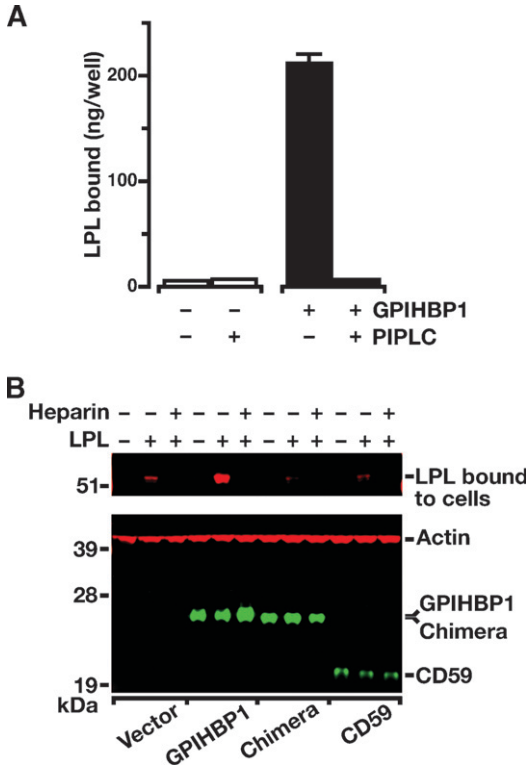

Fig. 3. Lipoprotein lipase (LPL) binds to GPIHBP1 on the surface of transfected pgsA745 Chinese hamster ovary (CHO) cells. A: Binding of LPL (mean ± SD) to mouse GPIHBP1-expressing cells before and after treatment with PIPLC. Reproduced, with permission, from Beigneux et al. (9). B: Binding of human LPL to human GPIHBP1 on pgsA745 CHO cells. Only trace amounts of LPL bound to nontransfected cells. LPL did not bind to GPIHBP1 in which its Ly6 domain was replaced with the Ly6 domain of CD59. Reproduced, with permission, from Gin et al. (23).

GPIHBP1 is a member of a family of Ly6 proteins that includes snake venom α-neurotoxins (14), modulators of nicotinic acetylcholine receptors such as SLURP1 and LYNX1, Ly6 alloantigens, the urokinase-type plasminogen activator receptor (UPAR), an inhibitor of the complement cascade (CD59), and a host of novel genes with unknown functions (15). The hallmark of Ly6 proteins is an ~80 amino acid-long Ly6 domain with 8–10 cysteines with characteristic spacing. A crystal structure of CD59 revealed that the cysteines are disulfide bonded (16). Aside from the cysteines, the Ly6 domain is poorly conserved from one family member to another. Most Ly6 proteins contain a single Ly6 motif, but CD177 (17) and RoBo-1 (18) have two, and UPAR has three (19). Several Ly6 proteins, for example snake neurotoxins (14), SLURP1 (20), SLURP2 (14), and the newly identified PATE-like proteins (21), lack a GPI anchor and are secreted.

Similar to many other GPI-anchored Ly6 proteins, mouse GPIHBP1 contains an N-linked glycosylation site within the Ly6 domain (Asn-76). When this site is eliminated, trafficking of the protein to the cell surface is reduced (22).

GPIHBP1 is distinguished from other Ly6 family members by a negatively charged N-terminal domain (17 of 25 consecutive residues in the mouse sequence, and 21 of 26 consecutive residues in the human sequence, are aspartate or glutamate) (9, 12, 23). Several lines of evidence indicate that this acidic domain is important for binding LPL and chylomicrons (23). An antiserum against GPIHBP1's acidic domain interferes with LPL and chylomicron binding (23). A mutant GPIHBP1 lacking the entire acidic domain or only some of the acidic amino acids did not bind LPL or chylomicrons (23). Also, the binding of LPL and chylomicrons to GPIHBP1 can be blocked with polyaspartate or polyglutamate, suggesting that electrostatic interactions are important for ligand binding (23). Consistent with that notion is the observation that LPL binding to GPIHBP1 is abolished when the positively charged residues in LPL's main heparin-binding domain are mutated (23).

GPIHBP1's Ly6 domain also appears to be important for LPL binding (23). Gin et al. (23) generated a chimeric protein in which GPIHBP1's Ly6 domain was replaced with the Ly6 domain of CD59. Even though this protein reached the cell surface and contained the acidic domain, it was incapable of binding LPL (Fig. 3). Thus, LPL binding depends on the acidic domain and the Ly6 domain (23). Interestingly, GPIHBP1's Ly6 motif has been highly conserved in mammalian evolution, more so than the acidic domain. Also, in the case of other GPI-anchored Ly6 proteins (e.g., UPAR and CD59), the Ly6 domain functions as the ligand-binding domain (19, 24).

GPIHBP1 IS A MEMBER OF A FAMILY OF GPI-ANCHORED LY6 PROTEINS

The Ly6 family in humans consists of at least 31 members, concentrated on chromosome 2 (3 members), chromosome 8 (10 members including GPIHBP1 and most LY6 alloantigens), chromosome 11 (6 members including CD59), and chromosome 19 (9 members including UPAR).

Bona fide GPIHBP1's are found in many mammalian species including primates, ruminants, rodents, and the egg-laying platypus (11). Mouse *Gpihbp1* is located on chromosome 15 in a region syntenic to human chromosome 8q24.3 (where human *GPIHBP1* is found). Thus far, no one has identified a fish, amphibian, or avian GPIHBP1. Orthologs for human GPIHBP1 and four neighboring genes (C8orf31, LY6H, GLI4, and ZNF696) are seemingly absent on the syntenic block in chicken. We have also tried, without success, to identify a chicken GPIHBP1 by "blasting" the acidic domain and the Ly6 domain of human GPIHBP1 against the chicken databases. While there is no shortage of GPI-anchored Ly6 domain proteins in the chicken, we have not yet found one that resembles GPIHBP1. The fact that GPIHBP1 is apparently missing in chicken is somewhat surprising because LPL is highly conserved in vertebrates and because chicken LPL binds mammalian GPIHBP1 avidly (9).

It is possible that GPIHBP1 will eventually be uncovered in birds and amphibians as databases are updated. On the other hand, it is possible that GPIHBP1 is a new molecule

that arose in mammals to facilitate the metabolism of triglyceride-rich lipoproteins (9).

GPIHBP1 EXPRESSION IS REGULATED

Beigneux et al. (9) showed that *Gpihbp1* is expressed highly in heart and adipose tissue and showed that GPIHBP1 is found mainly in endothelial cells. We expected to observe *Gpihbp1* expression in endothelial cell lines, but this has not been the case. Davies et al. (25) were unable to detect *Gpihbp1* expression in rat heart microvascular endothelial cells, human umbilical vein endothelial cells (25), or bovine aortic endothelial cells (unpublished observations). Davies et al. (25) also reported that primary endothelial cells from white adipose tissue lost nearly all *Gpihbp1* expression after a single passage. We do not understand the absence of *Gpihbp1* expression in these cell lines, but we suspect that *Gpihbp1* expression may depend on stromal factors and that gene expression is silenced in the absence of surrounding parenchymal cells.

Gpihbp1 expression is absent in undifferentiated mouse embryonic stem cells but can be induced by differentiating these cells into embryoid bodies. In embryoid bodies, GPIHBP1 is found in CD31-positive endothelial cells surrounding beating cardiomyocytes (25).

The expression of *Gpihbp1* is regulated by feeding state. Beigneux et al. (9) reported that *Gpihbp1* expression in skeletal muscle increases with fasting and returns to fed levels by 6 h after refeeding. Recently, Davies et al. (25) further explored the effects of fasting/refeeding on *Gpihbp1* and *Lpl* and found higher levels of *Gpihbp1* expression in the heart, brown adipose tissue, and white adipose tissue after fasting. *Gpihbp1* expression levels returned to baseline 6 h after refeeding. Fasting increased *Lpl* expression in heart and brown adipose tissue, but lowered *Lpl* expression slightly in white adipose tissue. These *Lpl* expression changes make sense, as they would enhance fuel delivery to vital tissues such as the heart during periods of energy deprivation. Why *Gpihbp1* expression increases in adipose tissue during fasting is not clear. It seems that mice may respond to fasting by increasing *Gpihbp1* expression in all tissues and rely on differential expression of *Lpl* to regulate the delivery of lipid nutrients. It is also possible that higher levels of *Gpihbp1* expression in the adipose tissue of fasted mice serves another purpose unrelated to lipolysis.

The molecular mechanisms involved in controlling *Gpihbp1* expression in response to fasting and refeeding are not known. It will be interesting to determine if changes in *Gpihbp1* expression in endothelial cells depends on signals from surrounding parenchymal tissues or from factors in the plasma. The transcription factors involved in the dietary regulation of *Gpihbp1* are not known. Peroxisome proliferator-activated receptor (PPAR)α expression levels increase with fasting and PPARα is known to play a key role in energy management during fasting (26). However, Davies et al. (25) found no change in *Gpihbp1* expression levels when mice were given a PPARα agonist.

GPIHBP1 APPEARS TO BE A TARGET OF PPARγ REGULATION

In the mouse, *Gpihbp1* is a PPARγ target gene. Davies et al. (25) treated mice with PPARα, PPARδ, and PPARγ agonists, and found that *Gpihbp1* expression increased in response to a PPARγ agonist but not PPARα or PPARδ agonists. In adipose tissue, the magnitude of the increase in *Gpihbp1* expression in response to the PPARγ agonist was similar to that observed with the classic PPARγ target *ap2* (although *Gpihbp1* is expressed in endothelial cells, while *ap2* is expressed in adipocytes) (27). A PPARγ agonist also increased the level of *Gpihbp1* expression in embryoid bodies. Davies et al. (25) also found significantly lower levels of *Gpihbp1* expression in white adipose tissue, brown adipose tissue, and heart of endothelial cell-specific PPARγ knockout mice.

Davies et al. (25) identified several potential PPAR binding sites (PPREs) upstream from *Gpihbp1* coding sequences. Several of these putative PPREs bound PPARγ/RXRα heterodimers in electrophoretic mobility shift assays (both direct-binding and competition assays). One of these PPREs, located immediately upstream from exon 1, drove the expression of a luciferase reporter in a PPARγ-dependent manner.

While these data suggest that PPARγ activates *Gpihbp1* expression, additional studies need to be performed. All of the luciferase reporter assays involved reporter constructs with a minimal promoter, rather than constructs containing the endogenous *Gpihbp1* promoter. Davies et al. (25) were unable to identify any promoter activity within the 5 kb of sequences upstream from exon 1 of *Gpihbp1*, at least in HEK293T cells or in bovine aortic endothelial cells (unpublished observations). It is unclear whether those sequences lack crucial regulatory elements, or whether the *Gpihbp1* promoter is simply inactive in these cells. In any case, it would be interesting to show, in the future, whether *Gpihbp1* PPREs are active in the context of the *Gpihbp1* promoter. Also, it would be interesting to determine if PPARγ is crucial for regulating *Gpihbp1* in vivo in response to different metabolic conditions. For example, does the increase in expression observed in fasting mice require PPARγ? Finally, the PPARγ agonist studies of Davies et al. (25) were all short term, and they were obviously performed in mice. It will be important to determine if similar effects are observed with chronic treatment and if these effects are also observed in humans. Thus far, there is no evidence that *GPIHBP1* in humans is regulated by PPARγ.

GPIHBP1 AND HUMAN HYPERLIPIDEMIAS

Wang and Hegele (28) screened 160 human subjects with chylomicronemia for *GPIHBP1* mutations. They identified two siblings with chylomicronemia who were homozygous for a G56R substitution in GPIHBP1; three heterozygotes in the family had mild hypertriglyceridemia. Residue 56 is located in a short linker domain between the

N-terminal acidic domain and the Ly6 domain. Because G56R is a nonconservative substitution and because residue 56 is conserved in the majority of mammalian species, Wang and Hegele (28) suggested that the mutation might be responsible for the chylomicronemia. However, Gin et al. (29) introduced the G56R mutation into a human *GPIHBP1* expression vector, transfected CHO cells, and examined the properties of the mutant protein. The mutant protein reached the cell surface normally and bound LPL, chylomicrons, and apo-AV normally. These studies cast doubt on the proposition that the G56R mutation is a cause of chylomicronemia, although one could argue that additional families (or perhaps a knock-in mouse) would be required to address this issue definitively.

The study by Wang and Hegele (28) suggested that *GPIHBP1* mutations are likely to be uncommon in humans with unexplained hypertriglyceridemia. Nevertheless, we suspect that continued screening of patients with chylomicronemia will yield clinically significant *GPIHBP1* mutations. Also, we would not be surprised if some acquired cases of chylomicronemia were shown to be due to an acquired deficiency of GPIHBP1.

CONCLUDING REMARKS

GPIHBP1 is a new cell-surface glycoprotein that is required for the lipolytic processing of triglyceride-rich lipoproteins. Mice lacking this protein have markedly elevated levels of triglycerides in their plasma, even on a low-fat chow diet. The expression of GPIHBP1 mirrors the expression of LPL, and is highest in "lipolytic tissues." By confocal microscopy, most GPIHBP1 is located on the luminal face of capillaries. Expression of GPIHBP1 in cultured cells confers upon those cells the ability to bind LPL and chylomicrons. Because of these findings, it is tempting to speculate that GPIHBP1 could serve as an endothelial cell "platform" for lipolysis (9, 11). That speculation is attractive; however, our understanding of the cell biology of GPIHBP1 is incomplete, and the precise role of GPIHBP1 in lipolysis needs much more study.

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