Published in final edited form as:

Curr Opin Lipidol. 2009 June ; 20(3): 211–216. doi:10.1097/MOL.0b013e32832ac026.

GPIHBP1 and lipolysis: an update

Anne P. Beigneux^a, Michael M. Weinstein^{a,b}, Brandon S.J. Davies^a, Peter Gin^a, André Bensadoun^c, Loren G. Fong^a, and Stephen G. Young^{a,b}

^a Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, California

^b Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, California

^c Division of Nutritional Science, Cornell University, Ithaca, New York, USA

Abstract

Purpose of review—This review will provide an update on the structure of GPIHBP1, a 28-kDa glycosylphosphatidylinositol-anchored glycoprotein, and its role in the lipolytic processing of triglyceride-rich lipoproteins.

Recent findings—*Gpihbp1* knockout mice on a chow diet have milky plasma and plasma triglyceride levels of more than 3000 mg/dl. GPIHBP1 is located on the luminal surface of endothelial cells in tissues where lipolysis occurs: heart, skeletal muscle, and adipose tissue. The pattern of lipoprotein lipase (LPL) release into the plasma after an intravenous injection of heparin is abnormal in *Gpihbp1*-deficient mice, suggesting that GPIHBP1 plays a direct role in binding LPL within the tissues of mice. Transfection of CHO cells with a GPIHBP1 expression vector confers on cells the ability to bind both LPL and chylomicrons. Two regions of GPIHBP1 are required for the binding of LPL – an amino-terminal acidic domain and the cysteine-rich Ly6 domain. GPIHBP1 expression in mice changes with fasting and refeeding and is regulated in part by peroxisome proliferator-activated receptor- γ .

Summary—GPIHBP1, an endothelial cell-surface glycoprotein, binds LPL and is required for the lipolytic processing of triglyceride-rich lipoproteins.

Keywords

chylomicrons; endothelial; lipoprotein lipase; PPAR γ

Introduction

Lipolysis of triglyceride-rich lipoproteins (chylomicrons, VLDLs) by lipoprotein lipase (LPL) is the central event in plasma lipoprotein metabolism – providing lipid nutrients to vital tissues, giving rise to cholesterol-enriched remnant lipoproteins, and contributing to the formation of HDL. The hydrolysis of lipoprotein triglycerides occurs mainly within heart, skeletal muscle, and adipose tissue [1–3]. LPL is synthesized and secreted by myocytes and adipocytes but then finds its way into the lumen of capillaries, where it hydrolyzes lipoprotein triglycerides, releasing nutrients for use by surrounding tissues.

Correspondence to Dr Anne P. Beigneux, 650 Charles, E. Young Dr South, Los Angeles, CA 90095, USA, Tel: +1 310 825 9422; fax: +1 310 206 0865; abeigneux@mednet.ucla.edu.

There was no conflict of interest.

Recently, Beigneux *et al.* [4] showed that an endothelial cell protein, glycosylphosphatidylinositol (GPI)-anchored high-density lipoprotein-binding protein 1 (GPIHBP1), is required for the lipolytic processing of triglyceride-rich lipoproteins. GPIHBP1 is located within the lumen of capillaries of heart, skeletal muscle, and adipose tissue—precisely where lipolysis is known to occur. With the discovery of GPIHBP1, we now know that capillary endothelial cells are not simply the passive hosts of lipolysis, but rather active participants in the process. Here, we review recent progress in understanding GPIHBP1, focusing on its structure, function, and regulation.

Chylomicronemia in *Gpihbp1* knockout mice

GPIHBP1 was initially identified by expression cloning as a cell-surface protein that binds HDLs [5], but its physiologic importance in mammals was revealed later with the characterization of knockout mice [4]. *Gpihbp1*^{−/−} mice on a chow diet have milky plasma and plasma triglyceride levels of 2500–5000 mg/dl, very similar to those in adult mice that are completely deficient in LPL [6]. The plasma lipid levels in heterozygous knockout mice (*Gpihbp1*^{+/-}) are entirely normal [4].

Beigneux *et al.* [4] found that retinyl palmitate clearance from the plasma was markedly delayed in *Gpihbp1*^{−/−} mice, suggesting defective lipolytic processing of chylomicrons [4]. In keeping with that idea, the diameters of the apoB-containing lipoproteins were much larger in *Gpihbp1*^{−/−} mice, and the plasma levels of apoB-48 were markedly increased [4].

The finding of chylomicronemia in *Gpihbp1*^{−/−} mice suggested that GPIHBP1 must play a crucial role in LPL-mediated processing of triglyceride-rich lipoproteins. Consistent with that idea, the tissue pattern of GPIHBP1 expression is almost identical to that of LPL, with very high levels of expression in adipose tissue and heart. In addition, GPIHBP1 is found on the luminal surface of capillary endothelial cells, where the hydrolysis of triglycerides occurs [4]. It was found that LPL binds avidly ($K_d = 3.6 \times 10^{-8}$ mol/l) to GPIHBP1-expressing pgsA-745 CHO cells [4,7]. Chylomicrons ($d < 1.006$ g/ml lipoproteins from *Gpihbp1*^{−/−} mice) also bind avidly to GPIHBP1. Apolipoprotein AV-containing liposomes bind strongly to GPIHBP1, but it is quite possible that other apolipoproteins within chylomicrons are equally or more important in mediating the binding of chylomicrons to GPIHBP1. The fact that GPIHBP1-expressing CHO cells bind both LPL and chylomicrons prompted us to speculate that GPIHBP1 might serve as a ‘platform’ for lipolysis on the surface of endothelial cells.

GPIHBP1 domains required for lipoprotein lipase binding

GPIHBP1 is a short protein, only 184 amino acids in length (228 amino acids in the mouse), and has four principal domains: a signal peptide, an amino-terminal acidic domain, a cysteine-rich Ly6 (lymphocyte antigen 6) domain, and a hydrophobic carboxyl-terminal motif that is replaced within the endoplasmic reticulum by a GPI anchor (Fig. 1) [4,5,7]. GPIHBP1 is a member of a large group of mammalian GPI-anchored Ly6 proteins, the best known of which are urokinase-type plasminogen activator receptor (UPAR) and CD59 (an inhibitor of the complement cascade). All family members contain a cysteine-rich Ly6 domain (in which eight to 10 cysteines have a characteristic spacing pattern); however, GPIHBP1 is unique in having the strongly acidic amino-terminal domain. Recent evidence suggests that both the acidic domain and the Ly6 domain are important for the binding of LPL and chylomicrons [8••].

GPIHBP1’s acidic domain contains a striking stretch of negatively charged amino acids (Fig. 1). In human GPIHBP1, 21 of 26 consecutive residues are aspartate or glutamate, and in mouse GPIHBP1, 17 of 25 residues are aspartate or glutamate. This region also contains a tyrosine that is predicted to be sulfated, a modification that would further add to the negative charge of this domain. The acidic domain is essential for the binding of LPL and chylomicrons [8••]. A

mutant GPIHBP1 lacking the acidic domain cannot bind LPL or chylomicrons, nor can a GPIHBP1 mutant in which approximately one-half of the aspartates and glutamates within the acidic domain were changed to alanine. An antiserum against GPIHBP1's acidic domain prevented the binding of both LPL and chylomicrons to GPIHBP1-expressing CHO cells. In addition, the binding of LPL to GPIHBP1 could be blocked by heparin, polyaspartate, or polyglutamate, suggesting that electrostatic interactions play an important role in ligand binding [8•]. In addition to being important for the binding of ligands, the acidic domain may be required for the trafficking of GPIHBP1 to the cell surface. A GPIHBP1 mutant lacking the acidic domain is expressed at lower levels on the surface of cells [8•].

Lipoprotein lipase contains a positively charged 'heparin-binding domain' near its carboxyl terminus that has been shown to be important for the binding of LPL to heparan sulfate proteoglycans (HSPGs) [9]. This same domain is important for LPL's ability to bind GPIHBP1 [8•]. When three positively charged amino acids within LPL's principal heparin-binding domain (amino acids 430–441) were changed to alanine, LPL was unable to bind to GPIHBP1 [8•]. Of course, it remains possible that other regions of LPL – in addition to amino acids 430–441 – are also important for the binding of LPL to GPIHBP1.

GPIHBP1's Ly6 domain is approximately 80 amino acids in length and contains 10 cysteines (Fig. 1). In the case of other 'Ly6 proteins,' for example, CD59, all of the cysteines are disulfide bonded, so it seems overwhelmingly likely that the same is the case for GPIHBP1. The Ly6 domain of GPIHBP1 contains an N-linked glycosylation site (Asn-76 in mouse GPIHBP1). Beigneux *et al.* [10•] showed that glycosylation of Asn-76 is very important for the trafficking of GPIHBP1 to the cell surface. When that Asn is replaced with an isoleucine, or when N-linked glycosylation is inhibited with tunicamycin, GPIHBP1 accumulates in the endoplasmic reticulum and little reaches the cell surface.

The Ly6 domain of GPIHBP1 also appears to be important for binding LPL. Gin *et al.* [8•] produced a GPIHBP1 mutant containing the Ly6 domain from CD59. The chimeric protein retained GPIHBP1's acidic domain and reached the cell surface normally; however, the mutant protein was unable to bind LPL. The fact that the Ly6 domain of GPIHBP1 participates in ligand binding is not particularly surprising, because the Ly6 domain of other Ly6 family members (e.g., CD59, UPAR) is crucial for the binding of ligands [11,12].

Postheparin lipoprotein lipase levels in the setting of GPIHBP1 deficiency

In the initial description of *Gpihbp1*^{-/-} mice [4], LPL levels were measured in plasma after a single intraperitoneal injection of heparin. The postheparin plasma LPL levels [as judged by an enzyme-linked immunosorbent assay (ELISA)] were approximately half-normal, as were enzymatic activity levels. Finding half-normal levels of LPL in the postheparin plasma of mice with chylomicronemia was puzzling, particularly as half-normal levels of LPL in heterozygous LPL knockout mice do not lead to severe hypertriglyceridemia [13]. The key question is: Why do *Gpihbp1*^{-/-} mice display severe chylomicronemia when they have 'respectable' levels of LPL in the postheparin plasma? Weinstein *et al.* [14•] speculated that there might be at least two pools of heparin-releasable LPL – an intravascular pool bound to GPIHBP1 that is highly relevant to lipolysis and an extravascular pool that is essentially irrelevant to lipolysis. They hypothesized that the intravascular pool was virtually absent in *Gpihbp1*^{-/-} mice and further hypothesized that the LPL that is released into the plasma by heparin in *Gpihbp1*^{-/-} mice might originate from the extravascular pool (the pool that is essentially irrelevant to lipoprotein lipolysis).

Weinstein *et al.* [14•] reasoned that the intravascular, GPIHBP1-bound pool of LPL might be released into the plasma very quickly after an injection of heparin, whereas the LPL released from extravascular pools might enter the plasma more slowly. And they predicted that if

GPIHBP1 were to play a significant role in binding LPL in capillaries, then the appearance of LPL in the plasma after an injection of heparin might be more rapid in wild-type mice than in *Gpihbp1*^{-/-} mice. To explore this issue, they administered an intravenous (i.v.) injection of heparin to *Gpihbp1*^{-/-} and control mice and then monitored the time course of LPL appearance in the plasma. As they predicted, the entry of LPL into the plasma was delayed in *Gpihbp1*^{-/-} mice. In control mice, plasma LPL levels peaked within 1 min after heparin [14•], whereas in *Gpihbp1*^{-/-} mice, the levels of LPL in the plasma were much lower 1 min after heparin, and the levels increased slowly over 15 min (Fig. 2). Consistent with that result, plasma triglyceride levels in control mice fell sharply within 10 min after an injection of heparin, whereas the triglyceride levels in *Gpihbp1*^{-/-} mice changed little within the first 15 min after an injection of heparin [14•]. After the 15-min time point, however, the plasma triglyceride levels in *Gpihbp1*^{-/-} mice fell dramatically. Within 2 h after heparin, plasma triglyceride levels in *Gpihbp1*^{-/-} mice fell by as much as 80%, and repeated doses of heparin resulted in sustained lowering of triglycerides for 24 h [14•].

LPL can also be released into the circulation with an i.v. injection of Intralipid (a triglyceride emulsion, Fresenius Kabi, Bad Homburg, Germany) [15]. Because Intralipid particles are quite large (>200 nm in diameter) [16], they would not be expected to readily leave the intravascular compartment. Thus, Intralipid would not be expected to be able to release LPL from extravascular pools. As expected, plasma LPL levels increased significantly in *Gpihbp1*^{+/+} and *Gpihbp1*^{+/-} mice after an injection of 7.5 ml/kg Intralipid 20%. However, LPL levels did not increase in *Gpihbp1*^{-/-} mice, suggesting that there was little or no LPL within the intravascular compartment (i.e., that all of the LPL was inaccessible to Intralipid).

The fact that the pattern of LPL release after i.v. heparin and Intralipid differed in *Gpihbp1*^{-/-} and control mice provides strong evidence in support of the idea that GPIHBP1 actually plays a role in binding LPL *in vivo*. The distinct patterns of LPL release also favor the idea that there is normally more than one pool of heparin-releasable LPL. The results suggest that there could be one pool of LPL bound to GPIHBP1 on the surface of capillaries; this pool is quickly releasable by heparin and is likely required for lipolysis. The second pool of enzymatically active LPL is probably extravascular, perhaps bound to HSPGs in subendothelial spaces, and therefore irrelevant to the lipolytic processing of large triglyceride-rich lipoproteins. The authors proposed that the extra-vascular pool is normal in *Gpihbp1*^{-/-} mice, but that the intravascular pool is absent – explaining the delayed appearance of LPL after heparin in *Gpihbp1*^{-/-} mice.

The notion that GPIHBP1 plays a direct role in binding LPL along the surface of capillaries needs to be addressed with more direct experimental approaches. For example, it would be desirable to show, either by immunohistochemistry or electron microscopy, that LPL is absent on the surface of capillaries in *Gpihbp1*^{-/-} mice. Equally importantly, it will be essential to learn more about the behavior of GPIHBP1 in endothelial cells. Does GPIHBP1 internalize LPL and chylomicron particles? Does some of the GPIHBP1 in endothelial cells reach the basolateral surface of the cell? Answers to these questions will be forthcoming in the next few years.

The regulation of *Gpihbp1* expression

Beigneux *et al.* [4] showed that *Gpihbp1* is expressed highly in the capillary endothelial cells of 'lipolytic tissues'. Thus far, however, *Gpihbp1* expression has not been observed in *any* endothelial cell line. Davies *et al.* [17•] could not detect *Gpihbp1* expression in human umbilical vein endothelial cells [17•], bovine aortic endothelial cells, or rat heart microvascular endothelial cells. They found that *Gpihbp1* is expressed in *primary* endothelial cells from white adipose tissue at passage 1 but that nearly all of the *Gpihbp1* expression

disappeared after a single passage (while CD31 expression persisted). The mechanism for *Gpihbp1* silencing in primary cultures has not been established, but the authors proposed that *Gpihbp1* expression might depend on stromal interactions or signals from surrounding parenchymal cells (*i.e.*, adipocytes or myocytes).

Gpihbp1 is not expressed in undifferentiated mouse embryonic stem cells; however, *Gpihbp1* expression is turned on when those cells are differentiated into embryoid bodies. In embryoid bodies, GPIHBP1 is expressed exclusively in CD31-positive endothelial cells that surround beating cardiomyocytes [17••].

In mice, *Gpihbp1* transcript levels change with fasting and refeeding. Beigneux *et al.* [4] found that *Gpihbp1* transcripts in skeletal muscle increase with fasting and then fall to 'fed levels' within 6 h of refeeding. More recently, Davies *et al.* [17••] found higher levels of *Gpihbp1* transcripts in the heart, brown adipose tissue, and white adipose tissue of fasted mice. Once again, *Gpihbp1* levels returned to baseline levels within 6 h of refeeding. Fasting tended to lower *Lpl* transcript levels in white adipose tissue but increased *Lpl* expression in heart and brown adipose tissue. Why the expression of *Gpihbp1* increased in white adipose tissue during fasting while *Lpl* expression levels fell is mysterious. One possibility is that the increased *Gpihbp1* expression in white adipose tissue during fasting serves an as-yet-unknown function that is unrelated to the LPL-mediated lipolytic processing of lipoproteins.

The transcription factors that regulate *Gpihbp1* expression in response to fasting and refeeding are unknown. Peroxisome proliferator-activated receptor- α (PPAR α) expression increases with fasting, and PPAR α expression is thought to play a role in energy regulation during fasting [18]. However, studies by Davies *et al.* [17••] tend to point against an important role for PPAR α in regulating *Gpihbp1*; when wild-type mice were treated with a PPAR α agonist, *Gpihbp1* transcript levels did not change [17••].

Another PPAR, PPAR γ , clearly plays a role in *Gpihbp1* expression. When mice are treated with a PPAR γ agonist, *Gpihbp1* expression levels increase substantially (Fig. 3). In fact, in terms of amplitude of the response to PPAR γ agonists, *Gpihbp1* appeared as responsive as *ap2*, the classic PPAR γ -responsive gene [19]. In addition, baseline *Gpihbp1* expression levels are lower in white adipose tissue, brown adipose tissue, and heart of endothelial cell-specific PPAR γ knockout mice, again suggesting a role for PPAR γ in regulating *Gpihbp1* expression. Several putative PPAR binding sites (PPREs) were identified in the sequences 5' to *Gpihbp1* coding sequences, of which five bound PPAR γ /RXR α heterodimers. In particular, a PPRE located immediately upstream of exon 1 drove the expression of a luciferase reporter construct in a PPAR γ -dependent manner. Although these observations point to a role for PPAR γ in regulating *Gpihbp1*, many more studies are necessary to fully understand PPAR γ -regulation of *Gpihbp1* expression. The luciferase reporter studies of the *Gpihbp1* PPRE involved constructs containing a minimal promoter, and it would clearly be desirable to be able to show that the *Gpihbp1* PPRE actually functions in the context of the endogenous *Gpihbp1* promoter. It will also be important to determine if PPAR γ participates in the regulation of *Gpihbp1* expression in response to fasting and refeeding. Finally, all of the studies performed to date have involved mice treated with a PPAR γ agonist for just a few days. In the future, it would be desirable to determine if increased *Gpihbp1* expression occurs with chronic treatment with a PPAR γ agonist and also determine if the induction of *Gpihbp1* expression occurs in humans treated with PPAR γ agonists (which are commonly used for the treatment of diabetes mellitus).

GPIHBP1 and human hyperlipidemias

Shortly after the discovery of GPIHBP1's role in plasma triglyceride metabolism [4], Wang and Hegele [20] searched for GPIHBP1 mutations in 160 patients with chylomicronemia. They identified two siblings, both with chylomicronemia, who were homozygous for a missense

mutation in GPIHBP1 (p. G56R). This mutation was not found in 600 normolipidemic controls. Glycine-56 is located in a short linker segment between the acidic and Ly6 domains (Fig. 1). Three members of the kindred were heterozygous for the mutation, and all had mild hypertriglyceridemia. Because of an apparent correlation between the presence of the mutation and hyperlipidemia within the kindred, and also because G56R is a nonconservative substitution of a conserved glycine, Wang and Hegele [20] suggested that the mutation might be responsible for the chylomicronemia. However, subsequent studies by Gin *et al.* [21] found no effect of the G56R mutation on chylomicron, apo-AV, or LPL binding to GPIHBP1-expressing CHO cells, raising doubts about whether the G56R mutation affects protein function. However, as Gin *et al.* [21] pointed out, the in-vitro cell culture experiments may have somehow been insensitive to the impact of the G56R mutation. Achieving 100% confidence about the functional relevance of the G56R mutation will probably require the generation of knock-in mice.

The study by Wang and Hegele [20] was important because it showed that GPIHBP1 mutations probably would be an uncommon cause of chylomicronemia in humans. However, additional screening studies would be worthwhile, as it is conceivable that *GPIHBP1* mutations will turn out to be more prevalent in other populations. Even if genetic defects in GPIHBP1 turn out to be extremely rare in humans, it will be important to investigate whether there might be acquired deficiencies in GPIHBP1 in humans. For example, it would be interesting to determine if some spontaneous cases of type V hyperlipoproteinemia in patients with diabetes mellitus are caused by reduced *GPIHBP1* expression in endothelial cells or other endothelial cell abnormalities that interfere with the normal function of GPIHBP1.

Conclusion

GPIHBP1 is a 28-kDa glycoprotein that is required for the lipolysis of triglyceride-rich lipoproteins. Recent studies have shown that mice lacking GPIHBP1 have severe chylomicronemia, even on a low-fat chow diet. In-vitro studies involving transfected CHO cells have revealed that GPIHBP1 binds LPL and chylomicrons avidly. More recent studies have shown that the pattern of LPL release after an injection of heparin is abnormal in *Gpihbp1*^{-/-} mice, strongly suggesting a physiologic role for GPIHBP1 in binding LPL *in vivo*. In mice, GPIHBP1 is regulated, at least in part, by PPAR γ .

The discovery of GPIHBP1 opens doors to fertile avenues of research. One is to investigate the precise role of GPIHBP1 in lipolysis. The capability of GPIHBP1 to confer upon CHO cells the ability to bind LPL and chylomicrons has led to the speculation that GPIHBP1 could serve as a 'platform' for lipolysis in endothelial cells [4,7]. However, our understanding of GPIHBP1 function in endothelial cells is meager, and many more studies are required to define the cell biology of GPIHBP1 and its precise role in lipolysis. Another major goal should be to determine if genetic or acquired deficiencies in GPIHBP1 underlie some cases of hypertriglyceridemia in humans.

Acknowledgments

This study was supported by grants from the National Heart, Lung, and Blood Institute (5P01HL090553-02 and 5R01HL087228-02; both to S.G.Y.), an American Heart Association Scientist Development Award (to A.P.B.), and a Ruth L. Kirschstein NRSA T32HL69766 award (to M.M.W.).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 247).

1. Brunzell, JD.; Deeb, SS. Familial lipoprotein lipase deficiency, apo C-II deficiency, and hepatic lipase deficiency. In: Scriver, CR.; Beaudet, AL.; Sly, WS., et al., editors. The metabolic and molecular bases of inherited disease. Vol. 8. New York: McGraw-Hill; 2001. p. 2789-2816.
2. Havel, RJ.; Kane, JP. Introduction: structure and metabolism of plasma lipoproteins. In: Scriver, CR.; Beaudet, AL.; Sly, WS., et al., editors. The metabolic and molecular bases of inherited disease. Vol. 8. New York: McGraw-Hill; 2001. p. 2705-2716.
3. Tan MH, Sata T, Havel RJ. The significance of lipoprotein lipase in rat skeletal muscles. *J Lipid Res* 1977;18:363–370. [PubMed: 16978]
4. Beigneux AP, Davies B, Gin P, et al. Glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 plays a critical role in the lipolytic processing of chylomicrons. *Cell Metab* 2007;5:279–291. [PubMed: 17403372]
5. Ioka RX, Kang M-J, Kamiyama S, et al. Expression cloning and characterization of a novel glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein, GPIHBP1. *J Biol Chem* 2003;278:7344–7349. [PubMed: 12496272]
6. Strauss JG, Frank S, Kratky D, et al. Adenovirus-mediated rescue of lipoprotein lipase-deficient mice. Lipolysis of triglyceride-rich lipoproteins is essential for high density lipoprotein maturation in mice. *J Biol Chem* 2001;276:36083–36090. [PubMed: 11432868]
7. Young S, Davies BSJ, Fong LG, et al. GPIHBP1: an endothelial cell molecule required for the lipolytic processing of chylomicrons. *Curr Opin Lipidol* 2007;18:389–396. [PubMed: 17620854]
- 8••. Gin P, Yin L, Davies BSJ, et al. The acidic domain of GPIHBP1 is important for the binding of lipoprotein lipase and chylomicrons. *J Biol Chem* 2008;283:29554–29562. This study describes the complexity of the interaction of GPIHBP1 with its ligands. It shows that GPIHBP1's acidic domain is crucial for binding LPL and chylomicrons. It also suggests that GPIHBP1's Ly6 domain is important for ligand binding. [PubMed: 18713736]
9. Sendak RA, Bensadoun A. Identification of a heparin-binding domain in the distal carboxyl-terminal region of lipoprotein lipase by site-directed mutagenesis. *J Lipid Res* 1998;39:1310–1315. [PubMed: 9643364]
- 10•. Beigneux AP, Gin P, Davies BS, et al. Glycosylation of Asn-76 in mouse 3 GPIHBP1 is critical for its appearance on the cell surface and the binding of chylomicrons and lipoprotein lipase. *J Lipid Res* 2008;49:1312–1321. This study shows that the predicted N-linked glycosylation site in the Ly6 domain of GPIHBP1 is utilized and that glycosylation is necessary for proper trafficking of GPIHBP1 to the cell surface. [PubMed: 18340083]
11. Llinas P, Le Du MH, Gardsvoll H, et al. Crystal structure of the human urokinase plasminogen activator receptor bound to an antagonist peptide. *EMBO J* 2005;24:1655–1663. [PubMed: 15861141]
12. Bodian DL, Davis SJ, Morgan BP, Rushmere NK. Mutational analysis of the active site and antibody epitopes of the complement-inhibitory glycoprotein, CD59. *J Exp Med* 1997;185:507–516. [PubMed: 9053451]
13. Weinstock PH, Bisgaier CL, Aalto-Setälä K, et al. Severe hypertriglyceridemia, reduced high density lipoprotein, and neonatal death in lipoprotein lipase knockout mice. Mild hypertriglyceridemia with impaired low density lipoprotein clearance in heterozygotes. *J Clin Invest* 1995;96:2555–2568. [PubMed: 8675619]
- 14••. Weinstein MM, Yin L, Beigneux AP, et al. Abnormal patterns of lipoprotein 33 lipase release into the plasma in GPIHBP1-deficient mice. *J Biol Chem* 2008;283:34511–34518. This study shows that LPL entry into the plasma after an i.v. injection of heparin is delayed in *Gpihbp1*-deficient mice, suggesting that GPIHBP1 plays a role in binding LPL in mouse tissues. [PubMed: 18845532]
15. Korn ED. Clearing factor, a heparin-activated lipoprotein lipase. II: Substrate specificity and activation of coconut oil. *J Biol Chem* 1955;215:15–26. [PubMed: 14392138]

16. Hansen LM, Hardie BS, Hidalgo J. Fat emulsion for intravenous administration: clinical experience with intralipid 10%. *Ann Surg* 1976;184:80–88. [PubMed: 820291]
- 17••. Davies BSJ, Waki H, Beigneux AP, et al. The expression of GPIHBP1, an 33 endothelial cell binding site for lipoprotein lipase and chylomicrons, is induced by peroxisome proliferator-activated receptor- γ . *Mol Endocrinol* 2008;22:2496–2504. This study provides evidence that, in the mouse, *Gpihbp1* is a PPAR γ target gene. [PubMed: 18787041]
18. Kersten S, Seydoux J, Peters JM, et al. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. *J Clin Invest* 1999;103:1489–1498. [PubMed: 10359558]
19. Tontonoz P, Hu E, Graves RA, et al. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev* 1994;8:1224–1234. [PubMed: 7926726]
20. Wang J, Hegele RA. Homozygous missense mutation (G56R) in glycosyl-phosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPI-HBP1) in two siblings with fasting chylomicronemia (MIM 144650). *Lipids Health Dis* 2007;6:23. [PubMed: 17883852]
21. Gin P, Beigneux AP, Davies B, et al. Normal binding of lipoprotein lipase, chylomicrons, and apo-AV to GPIHBP1 containing a G56R amino acid substitution. *Biochim Biophys Acta* 2007;1771:1464–1468. [PubMed: 17997385]

Figure 1. A schematic illustration of GPIHBP1, showing the amino-terminal acidic domain rich in aspartates and glutamates (dark gray), an Ly6 domain (black) containing 10 cysteines (each of these 10 cysteines is involved in a disulfide bond), and two linker segments (light gray). A carboxyl-terminal hydrophobic domain is clipped off in the endoplasmic reticulum and replaced with a GPI anchor, which tethers the protein to the cell surface.

Figure 2. An abnormal pattern of lipoprotein lipase release into the plasma of *Gpihbp1*^{-/-} mice after an injection of heparin

In control mice, heparin enters the plasma very rapidly, and LPL levels (mean $\pm$ SD) peak within 1 min after the injection. In *Gpihbp1*^{-/-} mice, heparin levels were low at 1 min after the injection and increased slowly over 15 min. *Gpihbp1*^{-/-}, $n = 7$; *Gpihbp1*^{+/-}, $n = 9$; *Gpihbp1*^{+/+}, $n = 7$. LPL, lipoprotein lipase. Reproduced with permission [14••].

Figure 3. Increased expression of *Gpihbp1* ($n = 10$ per group) in the tissues of mice after short-term treatment with a PPAR γ agonist

Bars show the amount of *Gpihbp1*, *Ucp3*, and *ap2* mRNA on a PPAR γ agonist (mean $\pm$ SE), relative to those in vehicle-treated mice. *Gpihbp1* expression in the PPAR γ agonist-treated mice was increased in white adipose tissue (a) as well as brown adipose tissue (b), and heart (c) but not in liver (e). The increased *Gpihbp1* expression in muscle (d) after treatment with a PPAR γ agonist did not reach statistical significance. Reproduced with permission [17••].