An upstream enhancer regulates Gpihbp1 expression in a tissue-specific manner.

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Abstract Glycosylphosphatidylinositol-anchored high density lipoprotein–binding protein 1 (GPIHBP1), the protein that shuttles LPL to the capillary lumen, is essential for plasma triglyceride metabolism. When GPIHBP1 is absent, LPL remains stranded within the interstitial spaces and plasma triglyceride hydrolysis is impaired, resulting in severe hypertriglyceridemia. While the functions of GPIHBP1 in intravascular lipolysis are reasonably well understood, no one has yet identified DNA sequences regulating Gpihbp1 expression. In the current studies, we identified an enhancer element located ~3.6 kb upstream from exon 1 of mouse Gpihbp1. To examine the importance of the enhancer, we used CRISPR/Cas9 genome editing to create mice lacking the enhancer (Gpihbp1Enh/−). Removing the enhancer reduced Gpihbp1 expression by >90% in the liver and by ~50% in heart and brown adipose tissue. The reduced expression of GPIHBP1 was insufficient to prevent LPL from reaching the capillary lumen, and it did not lead to hypertriglyceridemia—even when mice were fed a high-fat diet. Compound heterozygotes (Gpihbp1Enh/+ mice) displayed further reductions in Gpihbp1 expression and exhibited partial mislocalization of LPL (increased amounts of LPL within the interstitial spaces of the heart), but the plasma triglyceride levels were not perturbed. The enhancer element that we identified represents the first insight into DNA sequences controlling Gpihbp1 expression.—Allan, C. M., P. J. Heizer, Y. Tu, N. P. Sandoval, R. S. Jung, J. E. Morales, E. Sajti, T. D. Troutman, T. L. Saunders, D. A. Cusanovich, A. P. Beigneux, C. E. Romanoski, L. G. Fong, and S. G. Young.

The intravascular processing of triglyceride-rich lipoproteins (TRLs) by LPL is the central event in plasma lipid metabolism, providing nutrients for vital tissues and generating the lipoprotein remnants that play a causal role in atherosogenesis (1, 2). LPL is produced by parenchymal cells (e.g., adipocytes, myocytes) and secreted into the interstitial spaces. The interstitial LPL is almost certainly bound by heparan sulfate proteoglycans near the surface of parenchymal cells, but those interactions are weak and transient, making it possible for LPL to move to glycosylphosphatidylinositol-anchored high density lipoprotein–binding protein 1 (GPIHBP1) on the basolateral surface of capillary endothelial cells (3, 4). After being captured by GPIHBP1, the LPL is shuttled across endothelial cells to its site of action in the capillary lumen (5). In the absence of GPIHBP1, LPL remains stranded within the interstitial spaces,

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affixed to heparan sulfate proteoglycans (3, 4). Aside from transporting LPL to the capillary lumen, GPIHBP1 has two other important functions in intravascular lipolysis. First, GPIHBP1 is required for the margination of TRLs within capillaries, allowing the lipolytic processing of TRLs to proceed (6). In the absence of GPIHBP1-bound LPL, TRLs do not stop along capillaries and simply “flow on by” in the bloodstream (6). Second, the GPIHBP1–LPL interaction prevents unfolding of LPL’s N-terminal catalytic domain and thereby preserves catalytic activity (7). Homozygous deficiency of GPIHBP1, either in mice or in humans, impairs TRL processing, resulting in severe hypertriglyceridemia (cholesterinemia) (2, 8). Heterozygosity for Gpihbp1 deficiency lowers tissue levels of GPIHBP1 by 50% (9–11), but half-normal amounts of GPIHBP1 appear to be quite sufficient for normal triglyceride metabolism.

GPIHBP1 is expressed by capillary endothelial cells in every peripheral tissue that has been tested, but GPIHBP1 is absent from both capillary endothelial cells of the brain and endothelial cells of large blood vessels (5). GPIHBP1 is expressed at high levels in capillaries of heart and brown adipose tissue (BAT), where TRL processing by LPL is robust, but expression is low in tissues where LPL-mediated TRL processing is less active (e.g., liver, kidney, spleen) (8). GPIHBP1 expression is absent in the brain, a tissue that relies primarily on glucose for fuel (8). However, even though GPIHBP1 has been studied for more than a decade, we have minimal insights into the regulation of GPIHBP1 expression. No one understands why GPIHBP1 expression is higher in some tissues than in others—or why GPIHBP1 expression is absent in endothelial cells of the brain and large blood vessels. Also, the DNA sequences that regulate GPIHBP1 expression have not been defined. Davies et al. (9) found that Gpihbp1 transcript levels in mice are altered by fasting/refeeding and by PPARY agonists, but the DNA sequences controlling those responses have not been defined (9).

In the current study, we sought to gain insights into the DNA sequences that regulate Gpihbp1 expression, and in particular whether an enhancer element participates in Gpihbp1 regulation.

**MATERIALS AND METHODS**

**Analysis of mouse and human chromatin accessibility profiles**

To explore the possibility of an enhancer element upstream of Gpihbp1, several lines of experimental data were analyzed. We examined single-cell chromatin accessibility data from mouse brain, heart, kidney, liver, and lung with the assay for transposase-accessible chromatin with sequencing (ChIP-seq). We analyzed public chromatin immunoprecipitation sequencing (ChIP-seq) data for the acetylation of histone H3 on lysine 27 (H3K27ac) in adipocytes and nonadipocytes of BAT and hepatocytes and nonhepatocytes of the liver. Finally, we assessed regulatory marks in the orthologous region of the human genome with DNase1 hypersensitivity sequencing and H3K27ac ChIP-seq data (from human muscle, lung, and liver).

Single-cell ATAC-seq analyses. Single-cell ATAC-seq data were used to define chromatin accessibility in mouse endothelial cells (13). Nine types of endothelial cells have been identified (13). Normalized chromatin accessibility data, measured in counts per million (CPM) reads, were downloaded from the Mouse ATAC-seq Atlas (atlas.gs.washington.edu/mouse-atac) and plotted to the region of interest. To facilitate visualization of data, Gpihbp1 enhancer coordinates were converted to mm9 using liftOver (14). Browser tracks were generated with the Gviz package in R (15).

**FACS isolation of mouse liver sinusoidal endothelial cells and lung endothelial cells.** SPRET/EiJ mice (6–10 weeks old) were purchased from Jackson Laboratories, euthanized with CO2, and nonparenchymal cell preparations were isolated (16–18). Livers were retrograde perfused for 5 min through the inferior vena cava with HBSS without Ca2+ or Mg2+ (Gibco) supplemented with 0.5 mM ethyleneglycol tetraacetic acid (EGTA) at a rate of 5 ml/min. Livers were digested for 5 min in a solution containing low-glucose DMEM, 0.05% Pronase E (Roche), 1 μg/ml DNase1 (Roche), and 2% FBS, followed by a 7 min digestion in DMEM containing 0.05% collagenase D (Roche), 1 μg/ml DNase1 (Roche), and 2% FBS. After passing the cell suspension through a 70 μm cell strainer, two low-speed centrifugation steps (50 g for 2 min) were used to pellet hepatocytes for removal. Next, nonparenchymal cells were collected at 700 g for 7 min, and the erythrocytes removed by osmotic lysis. To remove debris, cells were suspended in 47.5% Opti-Prep (Sigma-Aldrich) and placed beneath a 33% Opti-Prep solution, followed by centrifugation for 20 min at 932 g. Nonhepatocyte cells at the interface were collected, washed, and suspended in 33% isotonic Percoll and centrifuged at 700 g for 10 min to remove digestion debris. Finally, preparations were stained with anti-CD16/CD32 (BioLegend) to block Fc receptors and sorted by FACS on a BD FACS Aria II. The staining cocktail consisted of Zombie Aqua to label dead cells, anti-CD31-PE, anti-CD45-Alexa 488, and anti-CD146-PE/Cy7; gated by LiveSingleCD45+CD146+CD31+. All antibodies were purchased from BioLegend.

To obtain lung cells, the five lobes were dissected, minced with a razor blade, and digested in a 5 ml solution of RPMI 1640 medium, collagenase IV (1.6 mg/ml; Worthington), DNase1 (50 U/ml; Roche), and 1 μM flavopiridol (Sigma-Aldrich). The tissue was digested with gentle shaking at 37°C for 15 min, followed by erythrocyte lysis (eBioscience) on ice for 3 min. For FACS sorting, the staining cocktail consisted of Zombie Aqua to label dead cells, anti-CD45-APC-Cy7, anti-CD31-PerCP-Cy5.5, and anti-CD146-FITC; gated by LiveSingleCD45+CD146+CD31-

**ATAC-seq data analysis.** ATAC-seq (12) was performed on 50,000 freshly sorted endothelial cells. After preparing sequencing libraries (19), sequencing was performed on an Illumina HiSeq 4000. Demultiplexed sequence reads were mapped to the Mus musculus genome assembly GRCm38/mm10 with Bowtie2 (20) and default parameters. Sequence alignment map (SAM) files were converted into tag directories with HOMER’s makeTag-Directory function (21). To remove normalization bias associated with different numbers of mitochondrial reads, tags mapping to the mitochondrial genome were removed, and the remaining reads were normalized across samples to 10 million reads. Data were visualized in the University of California, Santa Cruz Genome Browser (14) with the MakeMultiWigHub.pl in HOMER.

**Conservation of DNA sequences.** Conserved sequences from several mammals were retrieved with Mutiliz (22), and sequence motifs were assessed with scanMotifGenomeWide.pl in HOMER (21).

**Public datasets.** Public mouse datasets from the Gene Expression Omnibus (GEO) series GSE92590 (23) were downloaded (www.ncbi.nlm.nih.gov/geo/). Samples of SRR5121144
Gene-expression was calculated with the comparative CT method (7900HT Fast real-time PCR system (Applied Biosystems) (34–36)). (q)RT-PCR measurements were performed in triplicate with a protocol involving TRI reagent (Molecular Research), and quantitative analysis was carried out with software designed for real-time PCR. Brain were flash-frozen in liquid nitrogen. RNA was isolated with TRI reagent (Molecular Research), and quantitative analysis was performed with a comparative CT method.

Quantifying mouse tissue transcripts

Mice were anesthetized with isoflurane and perfused with PBS containing 5 mM EDTA. The heart, liver, and kidney were harvested and homogenized on ice for 12–15 s in homogenization buffer [50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% NP-40, 2.5 mg/ml deoxycholic acid, 0.1% SDS, and Complete EDTA-free protease inhibitor (Roche)]. Samples were centrifuged (15,000 g for 15 min) to remove cellular debris, and the supernatant fluid was collected. ELISA plates were coated overnight with a rat monoclonal antibody against the carboxyl terminus of mouse GPIHBP1 (11A12; 0.5 μg/well). On the next day, the wells were blocked for 4 h with Starting block (Thermo Fisher Scientific). Dilutions of mouse plasma (1:1, 1:2, 1:4) or tissue extracts (100, 50, 25, 12.5 μg of total protein) were added to the wells and incubated overnight at 4°C. A standard curve (from recombinant mouse GPIHBP1) was run in parallel (0–800 pg/well). After washing, the amount of GPIHBP1 captured on the wells was assessed by adding 50 ng of an HRP-labeled GPIHBP1-specific monoclonal antibody (2A8) to each well and incubating for 2 h at 4°C. After washing, wells were incubated on ice for 15–30 min with TIMB substrate (100 μl/well). The reaction was stopped with 2 M sulfuric acid (100 μl/well), and the optical density was read at 450 nm.

Mouse GPIHBP1 was immunoprecipitated from heart (200 μg of protein), BALB/c mouse brain (200 μg of protein), and liver (1 mg of protein) extracts in 500 μl of homogenization buffer by incubating the extracts with 25 μl of agarose beads coated with antibody 11A12 for 90 min at 4°C. Beads were washed with PBS/Ca/Mg containing 0.2% NP-40 and mouse GPIHBP1 was eluted by boiling the beads in 30 μl of SDS sample buffer at 90°C for 10 min. Proteins (25 μl/lane) were size-fractioned by SDS-PAGE, followed by Western blotting with an IRDye680-conjugated monoclonal antibody against mouse GPIHBP1 (11A12, 3 μg/ml) and a goat polyclonal antibody against mouse LPL (10 μg/ml) (37), followed by an IRDye680-conjugated donkey anti–goat IgG (LI-COR). Signals were visualized with an Odyssey infrared scanner (LI-COR).

Immunohistochemistry

Mice were anesthetized with isoflurane and perfused with PBS containing 5 mM EDTA followed by 3% paraformaldehyde. The heart, BAT, liver, and kidney were harvested and embedded in OCT medium on dry ice. In some experiments, mice were anesthetized with ketamine/xylazine and then injected with DyLight488-conjugated tomato lectin (100 μg; Vector Laboratories) via the inferior vena cava. After 20 min, the mice were perfused with PBS containing 5 mM EDTA followed by 3% paraformaldehyde. Tissue sections (7 μm for heart, liver, and kidney; 10 μm for BAT) were fixed with 3% paraformaldehyde at room temperature for 15 min, permeabilized with 0.2% Triton X-100 for 5 min, and blocked at room temperature with 5% donkey serum and 0.2% BSA in PBS/Mg/Ca. Tissues were incubated overnight at 4°C with a goat polyclonal antibody against mouse LPL (12 μg/ml) (37) and a rabbit polyclonal antibody against mouse CD51 (Abcam; 1:50), followed by a 45 min incubation at room temperature with Alexa647-conjugated antibody 11A12 (3 μg/ml), Alexa568-conjugated donkey anti–goat IgG (Thermo Fisher Scientific; 1:200), and Alexa488-conjugated donkey anti–rabbit IgG (Thermo Fisher Scientific; 1:200)
Fig. 1. Epigenetic profiles for a putative Gpihbp1 enhancer. A: Single-cell chromatin accessibility profiles for mouse tissues identified nine types of endothelial cells with distinct accessibility profiles (13). Normalized sequence tag counts are shown at the Gpihbp1 enhancer in counts per million (CPM) reads; 5 CPM is the maximum on the y axis. The dashed box indicates the nucleotides deleted in the Gpihbp1Enh allele. The cell cluster identifier, labeled in the same manner as in the original publication (13), is shown to the right of each
donkey anti–rabbit IgG (Thermo Fisher Scientific; 1:200). For mice injected with tomato lectin, the incubations of sections with the rabbit polyclonal antibody against mouse CD31 and the Alexa488-conjugated donkey anti–rabbit IgG were omitted.

In other experiments, tissue sections were incubated overnight at 4°C with a goat polyclonal antibody against mouse LPL (12 μg/ml) (37), a rabbit polyclonal antibody against mouse β-dystroglycan (Santa Cruz Biotechnology; 1:100), and a rat monoclonal antibody against mouse CD31 (BD Pharmingen; 1:50), followed by a 45 min incubation at room temperature with Alexa488-conjugated donkey anti–rat IgG (Thermo Fisher Scientific; 1:200), Alexa568-conjugated donkey anti–rabbit IgG (Thermo Fisher Scientific; 1:200), and Alexa647-conjugated donkey anti–goat IgG (Thermo Fisher Scientific; 1:200). After washing, the tissues were fixed with 3% paraformaldehyde for 5 min and stained with DAPI to visualize DNA. Images were recorded with an Axiocget 200M microscope and processed with Zen 2010 software (all from Zeiss). The confocal microscope exposure conditions within each experiment were identical.

### Measurements of plasma triglycerides

Blood was collected from 10-week-old mice by retroorbital bleeding. In some experiments, mice were given 100 μl of corn oil by gastric gavage, and blood was collected at baseline and 1, 2, 3, and 4 h post-gavage by retroorbital bleeding. In other experiments, mice were fed a high-fat diet (42% calories from fat; Envigo TD.88137). Blood was collected before initiation of this diet and after 1 month on the diet. Triglycerides were measured in plasma samples with a triglyceride determination kit (Sigma-Aldrich).

### Statistical analyses

All statistical analyses were performed with an unpaired two-tailed Student’s t-test.

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**Fig. 2.** *Gpihbp1* and *Lpl* transcript levels in 10-week-old *Gpihbp1*Enh/Enh and wild-type mice (*Gpihbp1*+/+). *Gpihbp1* (A), *Cd31* (B), *Cd36* (C), and *Lpl* (D) transcript levels were measured by qRT-PCR (n = 10/group). *Gpihbp1* expression was normalized to the expression of cyclophilin A. gWAT, gonadal white adipose tissue. Data show mean ± SD. *P* < 0.01; **P** < 0.001; ***P*** < 0.0001.
RESULTS

Several lines of evidence led to the identification of an ∼325 bp enhancer located ∼3.6 kb upstream of exon 1 of Gpihbp1 (Fig. 1). Single-cell ATAC-seq data on mouse endothelial cells (15) was used to define regions of accessible chromatin. A region of increased chromatin accessibility (a putative Gpihbp1 enhancer) was identified in endothelial cells from heart, liver, and lung (but not brain) (Fig. 1A). These findings were corroborated with bulk ATAC-seq profiles of mouse liver and lung endothelial cells (isolated by FACS) (Fig. 1B). We also examined public ChIP-seq data for H3K27ac (a mark of active enhancers) in adipocytes and nonadipocytes of BAT and hepatocytes and nonhepatocyte cell populations (16) (Fig. 2A). We also examined the binding of an LPL-specific antibody to tissues of Gpihbp1Enh/Enh mice (not shown), as reported earlier for wild-type mice (8).

We used the GPIHBP1 sandwich ELISA to measure plasma GPIHBP1 levels in Gpihbp1Enh/Enh, Gpihbp1Enh/+, and Gpihbp1+/− mice. In earlier studies (42), we found lower plasma GPIHBP1 levels in mutant mice that had reduced amounts of GPIHBP1 on capillary endothelial cells (42). Consistent with those studies and with the finding of reduced amounts of GPIHBP1 in tissues of Gpihbp1Enh/Enh mice (Fig. 3), the plasma GPIHBP1 levels were lower in Gpihbp1Enh/Enh mice than in wild-type mice (supplemental Fig. S2).

To further characterize GPIHBP1 expression in Gpihbp1Enh/Enh mice, immunohistochemistry studies were performed on tissue sections with a fluorescently labeled GPIHBP1-specific monoclonal antibody (11A12). GPIHBP1 was virtually undetectable in kidney and liver capillary endothelial cells of Gpihbp1Enh/Enh mice but was easily detectable in the kidney and liver of wild-type mice (Fig. 4A, B). In BAT and heart, GPIHBP1 staining was less intense in Gpihbp1Enh/Enh mice than in wild-type mice (Fig. 4C, D). In both Gpihbp1Enh/Enh and wild-type mice, GPIHBP1 was present in capillaries but was absent in endothelial cells of larger blood vessels (arterioles, venules) (Fig. 4C, D). GPIHBP1 expression was also absent in brain capillaries of Gpihbp1Enh/Enh mice (not shown), as reported earlier for wild-type mice (8).

We also examined the binding of an LPL-specific antibody to tissues of Gpihbp1Enh/Enh mice (Fig. 4). In earlier studies, we found that nearly all of the LPL in the heart and BAT of wild-type mice is located on capillaries (5, 42), whereas it is mislocalized within the interstitial spaces in Gpihbp1−/− mice. In the current studies, we extended these experiments to include Gpihbp1Enh/Enh mice (Fig. 4). In BAT and heart of Gpihbp1Enh/Enh mice, LPL was located almost exclusively on capillaries (colocalizing with GPIHBP1), and the intensity of antibody staining appeared similar to that in wild-type mice. Also, despite lower levels of GPIHBP1 expression in Gpihbp1Enh/Enh mice, we found no evidence for partial mislocalization of LPL within tissues (i.e., we did not observe increased amounts of LPL within the interstitial spaces). Given that nearly all of the LPL in the BAT of Gpihbp1Enh/Enh mice was associated with capillaries and colocalized with GPIHBP1, we predicted that we would find LPL in the capillary lumen of Gpihbp1Enh/Enh mice. Indeed, this was the case (Fig. 5).

While we did not find partial mislocalization of LPL in the heart of Gpihbp1Enh/Enh mice, we suspected that we might be able to identify partial mislocalization of LPL if the levels of GPIHBP1 expression were reduced below...
those in Gpihbp1\textsuperscript{Enh/Enh} mice. To test this suspicion, we bred Gpihbp1\textsuperscript{Enh/–} mice, where Gpihbp1 transcripts were only one-half as high as in Gpihbp1\textsuperscript{Enh/Enh} mice (supplemental Fig. S3). In the heart of Gpihbp1\textsuperscript{Enh/–} mice, we observed partial mislocalization of LPL (Fig. 6). A substantial fraction of the LPL was located on capillaries, colocalizing with GPIHBP1, but some of the LPL was observed in the interstitial spaces near the surface of parenchymal cells (colocalizing with β-dystroglycan) (Fig. 6). Partial mislocalization of LPL to the interstitial spaces was also observed in the heart of Gpihbp1\textsuperscript{–/–} mice (Fig. 6).

Because the intensity of the LPL staining did not appear to be reduced in the heart and BAT of Gpihbp1\textsuperscript{Enh/Enh} mice (Fig. 4) and because we were able to detect LPL in the
capillary lumen of $Gpihbp1^{Enh/Enh}$ mice (Fig. 5), we were skeptical that we would find elevated plasma triglyceride levels in $Gpihbp1^{Enh/Enh}$ mice. Indeed, the plasma triglyceride levels were similar in $Gpihbp1^{Enh/Enh}$, $Gpihbp1^{Enh/-}$, and wild-type mice (<100 mg/dl) (Fig. 7). Even after administering corn oil to $Gpihbp1^{Enh/-}$ mice by gastric gavage, we did not find elevated plasma triglyceride levels (supplementary Fig. S4A). Also, the plasma triglyceride levels in
DISCUSSION

In the current study, we identified, by ATAC-seq and histone H3K27ac modifications, a Gpihbp1 enhancer in endothelial cells (a ∼325 bp element located ∼3.6 kb upstream of exon 1 of Gpihbp1). The same element was detected, by DNase1-seq and histone H3K27ac modifications, in human tissues. We suspected that the sequences that we identified could be important for regulating levels of Gpihbp1 expression and/or sites of Gpihbp1 expression. To test these possibilities, we created mice lacking the enhancer (Gpihbp1Enh−/−). The enhancer deletion reduced levels of Gpihbp1 transcripts (but not levels of Cd31, Cd36, or Lpl transcripts) in every tissue tested (>90% in the liver, ∼50% in heart and BAT). Reductions in GPIHBP1 protein levels mirrored the decreases in transcript levels. The plasma levels of GPIHBP1 were also lower in Gpihbp1Enh−/− mice. Reduced amounts of GPIHBP1 in Gpihbp1Enh−/− mice were also evident by immunohistochemistry. The fact that the deletion of the enhancer did not eliminate Gpihbp1 expression was not particularly surprising. Gene expression often depends on multiple enhancers (43, 44), and deleting a single enhancer is often insufficient to abolish gene expression (45, 46).

The decrease in Gpihbp1 expression in Gpihbp1Enh−/− mice was quite striking in the liver (where Gpihbp1 expression is normally low) but only moderate in the heart and BAT (where Gpihbp1 expression is high). We don’t understand why the impact of the deletion (as judged by the percentage decrease in Gpihbp1 expression) was greater in the liver, but it is noteworthy that the region of open chromatin (as judged by ATAC-seq) was particularly prominent in liver endothelial cells (Fig. 1).

The expression of Lpl and Gpihbp1 in the liver is normally low, but the expression of Lpl in the liver can be induced substantially by a high-cholesterol diet (47). At this time, the functional importance of GPIHBP1 and LPL in the liver is incompletely understood. Because liver capillaries are fenestrated, GPIHBP1’s role in transporting LPL across endothelial cells is likely superfluous in the liver (8, 47). However, biochemical studies have proven that GPIHBP1 preserves the structural integrity and enzymatic activity of LPL (7, 48), and this function is presumably relevant in all tissues—including the liver.

In our studies, we found no evidence that the ∼90% decrease in Gpihbp1 expression in the liver and the ∼50% decrease in Gpihbp1 expression in heart and BAT influenced plasma triglyceride levels. The triglyceride levels in Gpihbp1Enh−/− mice were similar to those in wild-type mice. In Gpihbp1Enh−/− mice, where levels of Gpihbp1 expression

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Fig. 6. LPL is partially mislocalized in the heart in Gpihbp1Enh−/− mice, with increased amounts of LPL in the interstitial spaces near the surface of cardiomyocytes. Confocal microscopy studies were performed on sections of heart stained with antibodies for β-dystroglycan (cyan), CD31 (red), and LPL (green). β-Dystroglycan is located along the surface of cardiomyocytes. In comparing confocal images from Gpihbp1+/+ and Gpihbp1Enh−/− mice, we observed more LPL outside of capillaries in Gpihbp1Enh−/− mice (colocalizing with β-dystroglycan) (arrowheads in the CD31/LPL merged image point to several such regions). An even greater amount of interstitial LPL (colocalizing with β-dystroglycan) was observed in sections from Gpihbp1+/− mice (arrowheads). A small amount of LPL was mislocalized to the interstitial spaces in Gpihbp1−/− mice (arrowhead). DNA was stained with DAPI (blue). Scale bar, 10 μm.

Fig. 7. Normal plasma triglycerides in Gpihbp1Enh+/+ and Gpihbp1Enh−/− mice. Plasma triglyceride levels were measured in 10-week-old Gpihbp1+/+ (n = 16), Gpihbp1Enh+ (n = 20), Gpihbp1Enh−/− (n = 17), Gpihbp1−/− (n = 8), and Gpihbp1−/− (n = 6) mice. Data show mean ± SD.
were reduced by ~95% in the liver and ~75% in BAT and heart, LPL was partially mislocalized to the interstitial spaces but the plasma triglyceride levels remained normal—even when the mice were challenged with a corn oil bolus or a high-fat diet. The normal plasma triglyceride levels in Gpihbp1−/− mice stand in contrast to findings with hepatocyte-specific Lpl knockout mice. Eliminating LPL expression in hepatocytes resulted in modestly higher plasma triglyceride levels, both at baseline and after a bolus of corn oil (49).

Filling normal plasma triglyceride levels in Gpihbp1 Enh/Enh mice was not surprising. The plasma triglyceride levels in Gpihbp1−/− mice are entirely normal (8). Also, humans heterozygous for loss-of-function GPIHBP1 mutations have normal plasma triglyceride levels (2). Again, the situation is different with LPL deficiency. Lpl−/− mice have mild–moderate increases in plasma triglyceride levels (50), and humans with one mutant LPL allele have increased plasma triglyceride levels (51, 52) along with an increased risk for coronary heart disease (53). Together, these observations imply that the limiting factor in triglyceride processing is the levels of LPL rather than the levels of GPIHBP1.

Our studies have provided the first insight into the DNA sequences regulating GPIHBP1 expression. We would have to admit, however, that we have only scratched the surface in understanding the regulation of GPIHBP1 expression. We have not yet identified the transcription factors that bind to the upstream Gpihbp1 enhancer, nor do we know whether GPIHBP1 expression is regulated by additional enhancer elements. We also do not understand why Gpihbp1 is expressed at high levels in the heart and BAT but is absent in the brain (5). Remarkably, the promoter for Gpihbp1 has never been defined or studied. Some of these lacunae in our understanding of GPIHBP1 expression will probably disappear with an improved understanding of endothelial cell heterogeneity and the associated heterogeneity in endothelial cell gene expression (54, 55). However, in the end, we suspect that nailing down the DNA sequences controlling GPIHBP1 expression will require additional studies with genetically modified mice, along the lines of the strategy used in the current studies.

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