Abnormal Patterns of Lipoprotein Lipase Release into the Plasma in GPIHBP1-deficient Mice*

Received for publication, August 6, 2008, and in revised form, September 30, 2008. Published, JBC Papers in Press, October 8, 2008, DOI 10.1074/jbc.M806067200

Michael M. Weinstein‡, Liya Yin‡, Anne P. Beigneux‡, Brandon S. J. Davies‡, Peter Gin‡, Kristine Estrada†, Kristan Melford‡, Joseph R. Bishop∥, Jeffrey D. Esko, Geesje M. Dallinga-Thie‡, Loren G. Fong‡, André Bensadoun‡, and Stephen G. Young‡∥§

From the Departments of ‡Medicine and †Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, California 90095, the ¶Division of Nutritional Science, Cornell University, Ithaca, New York 14853, and the ∥Department of Cellular and Molecular Medicine, University of California, San Diego, California 92093

GPIHBP1-deficient mice (Gpihbp1–/–) exhibit severe chylomicronemia. GPIHBP1 is located within capillaries of muscle and adipose tissue, and expression of GPIHBP1 in Chinese hamster ovary cells confers upon those cells the ability to bind lipoprotein lipase (LPL). However, there has been absolutely no evidence that GPIHBP1 actually interacts with LPL in vivo. Heparin is known to release LPL from its in vivo binding sites, allowing it to enter the plasma. After an injection of heparin, we reasoned that LPL bound to GPIHBP1 in capillaries would be released very quickly, and we hypothesized that the kinetics of LPL entry into the plasma would differ in Gpihbp1–/– mice. Indeed, plasma LPL levels peaked very rapidly (within 1 min) after heparin in control mice. In contrast, plasma LPL levels in Gpihbp1–/– mice were much lower 1 min after heparin and increased slowly over 15 min. In keeping with that result, plasma triglycerides fell sharply within 10 min after heparin in wild-type mice, but were negligibly altered in the first 15 min after heparin in Gpihbp1–/– mice. Also, an injection of Intralipid released LPL into the plasma of wild-type mice but was ineffective in releasing LPL in Gpihbp1–/– mice. The observed differences in LPL release cannot be ascribed to different tissue stores of LPL, as LPL mass levels in tissues were similar in Gpihbp1–/– and control mice. The differences in LPL release after intravenous heparin and Intralipid strongly suggest that GPIHBP1 represents an important binding site for LPL in vivo.

Triglyceride-rich lipoproteins (chylomicrons and very low density lipoproteins) undergo lipolysis along the surface of capillaries, mainly in heart, skeletal muscle, and adipose tissue (1, 2). This lipolytic processing is carried out by lipoprotein lipase (LPL) (2). LPL is synthesized and secreted by parenchymal cells (e.g. myocytes, adipocytes) and then translocated into the lumen of capillaries, where it is widely assumed to bind to cell-surface heparan sulfate proteoglycans (HSPGs) (1–3). The binding of LPL is thought to involve electrostatic interactions between positively charged “heparin-binding domains” in LPL and negatively charged HSPGs. Multiple lines of evidence have supported this concept. Affinity chromatography of endothelial cell extracts on an LPL-Sepharose column resulted in the isolation of a single 220-kDa heparan sulfate proteoglycan (4). The binding of LPL to cultured endothelial cells can be reduced by removing HSPGs from the surface of cells (5). LPL can be released from its in vivo binding sites by heparin (6), and mutation of the principal heparin-binding domain of LPL reduces LPL binding to cells (7).

Recent findings have suggested that the paradigm for lipolysis of lipoproteins by LPL requires updating (8–11). Adult mice lacking glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (Gpihbp1–/–) exhibit chylomicronemia (8), a severe form of hypertriglyceridemia that, in humans, is often caused by a deficiency in LPL (2). GPIHBP1 is expressed on the luminal surface of the endothelial cells in heart, skeletal muscle, and adipose tissue, the same tissues that express LPL (8). The chylomicronemia in Gpihbp1–/– mice and the localization of GPIHBP1 within capillaries suggested that GPIHBP1 might serve a key role in the lipolytic processing of lipoproteins (8, 11).

GPIHBP1 contains a highly negatively charged domain, with 21 of 25 consecutive residues in the human protein being aspartate or glutamate (11). Beigneux et al. (8) suspected that this domain might be capable of binding proteins with positively charged “heparin-binding domains,” such as LPL and/or certain apolipoproteins within chylomicrons (12, 13). Indeed, Chinese hamster ovary cells expressing high levels of GPIHBP1 are capable of binding LPL, and this LPL can be released with heparin (8). GPIHBP1-expressing cells also bind chylomicrons (8).

The ability of GPIHBP1-expressing Chinese hamster ovary cells to bind LPL was intriguing, but its significance in vivo

* This work was supported, in whole or in part, by National Institutes of Health Postdoctoral Fellowship Awards HL66621, HL66600, SP01HL090553 (to P. G.), HL087228 (to S. G. Y.), and GM33063 (to J. D. E.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom correspondence may be addressed. E-mail: lfong@mednet.ucla.edu.

2 To whom correspondence may be addressed: Division of Nutritional Sciences, 321 Savage Hall, Cornell University, Ithaca, NY 14853. Tel.: 607-255-1927; Fax: 607-255-2033; E-mail: ab55@cornell.edu.

3 To whom correspondence may be addressed: 675 Charles E. Young Dr. South, Los Angeles, CA 90095. Tel.: 310-825-4934; Fax: 310-206-0865; E-mail: sgyoung@mednet.ucla.edu.

4 The abbreviations used are: LPL, lipoprotein lipase; GPIHBP1, glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1; HSPG, heparan sulfate proteoglycan.
remains unclear. On one hand, the increased LPL binding to GPIHBP1-expressing cells might actually represent a major clue regarding the actual in vivo function of GPIHBP1. On the other hand, a skeptic could argue that the increased LPL binding to cultured cells is simply an in vitro artifact resulting from the overexpression of a highly negatively charged protein on the surface of cells.

Physiologists have long recognized that an intravenous injection of heparin releases LPL from its binding sites, allowing it to enter the plasma and circulate in the bloodstream (6). We reasoned that the intravascular pool of LPL, the pool of LPL bound to the surface of endothelial cells, would likely be released quickly after an injection of heparin. We hypothesized that if GPIHBP1 on endothelial cells truly plays a significant role in binding LPL in vivo, then the kinetics of LPL appearance in the plasma after heparin might differ in Gpihbp1−/− and control mice. Similarly, we hypothesized that the release of LPL into the plasma after an injection of a triglyceride emulsion (i.e., Intralipid) would be abnormal in Gpihbp1−/− mice. In the current study, we tested these hypotheses.

**EXPERIMENTAL PROCEDURES**

*Genetically Modified Mice—Gpihbp1−/− mice (>90% C57BL/6, <10% 129/Sv) have been described previously (8). Endothelial cell-specific Ndst1 knock-out mice (Ndst1fl/fl TekCre+; TekCre−) and littermate Ndst1fl/fl control mice were also described previously (14). All mice were fed rodent chow (LabDiet number 5001) and housed in a specific pathogen-free barrier facility with a 12-h light/dark cycle. Genotyping of Gpihbp1−/− mice was performed as previously described (8). Genotyping of Ndst1fl/fl TekCre+ and Ndst1fl/fl control mice was performed as described (14). All studies were approved by institutional Animal Research Committees.*

*Blood Collection and Processing—Mice were anesthetized with isoflurane, and blood was collected by retroorbital puncture (BD Biosciences). After sedimenting red blood cells by centrifugation (6000 × g for 2 min), plasma was separated and frozen in liquid nitrogen. Plasma samples were stored at −80 °C. Triglyceride and cholesterol levels were measured on plasma samples with the Serum Triglyceride Determination Kit (Sigma) and Cholesterol E kit (Wako).*  

LPL mass in plasma was determined by enzyme-linked immunosorbent assay with immunopurified goat antibodies against mouse LPL (15). A full-length mouse LPL cDNA (16) was subcloned into the pQE32 vector, and a His6-tagged protein was expressed in *Escherichia coli*. Bacterial extracts were applied to a TALON metal affinity resin (Clontech, Mountain View, CA), and bound LPL was eluted with a 60-ml linear gradient of imidazole (0–250 mM). LPL was further purified by preparative gel electrophoresis and electroelution. The purity of the electroeluted LPL was monitored by SDS-PAGE, and fractions containing a single band were pooled and used to immunize a goat. The identity of the electroeluted protein was confirmed by sequencing of tryptic peptides by mass spectrometry. The mouse LPL was also coupled to an Affi-Prep 10 matrix (Bio-Rad) for immunoglobulin purification. To generate immunopurified high affinity goat antibodies, 2.0 ml of goat serum was diluted 3-fold with a high-salt equilibration buffer (0.5 M NaCl, 0.2 M Tris-HCl buffer, pH 8.0) and applied to the mouse LPL affinity column at a rate of 0.2 ml/min. The column was washed with 120 ml of equilibration buffer, and LPL was eluted with a low-pH buffer (0.5 M NaCl, 0.2 M glycine-HCl, pH 2.8). Immunoglobulins were stored at −20 °C in 50% glycerol. The protein concentration of immunoglobulin fractions was measured as described previously (17).

To measure LPL levels, plasma samples were first preincubated in 1.2 M guanidium HCl, 0.8 M NaCl, 1% bovine serum albumin, 0.05% Tween 20, 10 mM sodium phosphate, pH 7.4, for 1 h at 4 °C. The samples were then diluted to 0.24 M guanidium HCl prior to transfer to microtiter wells coated with capture immunoglobulins specific for mouse LPL (1 μg/well). The samples were incubated overnight at 4 °C and the microtiter wells were then washed with an automated plate washer six times with phosphate-buffered saline containing 0.05% Tween 20. This was followed by an incubation with biotinylated goat anti-mouse LPL immunoglobulins overnight at 4 °C. The microtiter wells were then washed nine times and subsequently incubated with streptavidin-coupled horseradish peroxidase for 2 h at 37 °C. The nonspecifically bound streptavidin-horseradish peroxidase was then removed by washing 12 times with phosphate-buffered saline, 0.05% Tween 20 before color development with o-phenylenediamine dihydrochloride and reading at OD490. The multiple washes at every step contributed to a low blank value (OD490 = 0.056 ± 0.005, n = 3) that was subtracted from each reading. A standard curve ranging from 0.02 to 2.0 ng of recombinant mouse LPL was included on each microtiter plate, with the 2 ng of sample giving an OD490 of 0.921 ± 0.060 (n = 3). The standard curve was fitted to a quadratic function (r = 0.998 ± 0.0002, n = 3).

We considered the possibility that the immunoassay might be affected by the high plasma lipoprotein levels in Gpihbp1−/− mice. To explore this possibility, we “spiked” postheparin plasma from wild-type mice with either normal saline or the d < 1.006 g/ml lipoproteins from Gpihbp1−/− mice (so as to bring the plasma triglyceride concentrations to the level observed in Gpihbp1−/− mice). The d < 1.006 g/ml lipoproteins were prepared by ultracentrifugation and did not contain detectable levels of LPL, as judged by immunoblotting. The LPL levels in samples spiked with normal saline and those spiked with d < 1.006 g/ml lipoproteins were similar, providing no evidence that chylomicrons systematically lower plasma LPL mass measurements.

*Release of LPL into the Plasma with Intravenous Injections of Heparin or Intralipid—Age- and sex-matched Gpihbp1−/−, Gpihbp1−/+ mice, and Gpihbp1+/+ mice were anesthetized with isoflurane, and sodium heparin (Baxter) (0.5–50 units in 150 μl of 0.9% sodium chloride) was injected into the caudal vein. In some experiments, mice were injected with 7.5 ml/kg 20% Intralipid (18). Blood samples were collected for LPL mass measurements and LPL specific activity measurements during the next 15 min. Plasma triglyceride levels in response to heparin were assessed in multiple experiments. In one experiment, Gpihbp1−/− mice were given an initial injection of 100 units of
heparin both subcutaneously and intraperitoneally, followed by an additional 100 units of heparin subcutaneously 90 min later. Control mice were given the same volumes of normal saline. Plasma triglyceride levels were measured at multiple time points. In another experiment, a single dose of heparin (50 units) was given intravenously, and plasma triglyceride levels were measured at multiple time points. In a third experiment, subcutaneous heparin (100 units) was given at baseline and 8 and 16 h later, and plasma triglyceride levels were measured at multiple time points.

Assessing LPL Specific Activity—To determine the specific activity of LPL, 200 μl of plasma was collected 1 or 15 min after an injection of heparin (50 units), diluted with an equal volume of buffer A (0.25 M NaCl, 10 mM sodium phosphate, pH 6.5) containing 30% glycerol and 1% bovine serum albumin, and applied to a 1-ml heparin-Sepharose column (GE Healthcare). The column was washed with 15 ml of buffer A. Hepatic lipase and LPL in the plasma were eluted with a 0.25 to 1.5 M NaCl gradient in buffer A (19). Thirty fractions were collected, and lipase activity in each fraction was determined with a [3H]triol-5-enin substrate (500,000 cpm/μl) by the method of Hocquette et al. (20). The LPL activity peak spanned 5 of the 30 fractions. The LPL mass in each of the five fractions was determined by enzyme-linked immunosorbent assay (15). LPL specific activity in each of the 5 fractions was expressed as nanomole of fatty acid released/h/ng of LPL.

Measurement of LPL in Tissues—Mice were fasted overnight. After restoring food for 1 h, the mice were fasted for 4 h before being euthanized for tissue collection (gonadal fat pad, liver, heart, and skeletal muscle). Tissues samples were collected and weighed. A total of 100 mg of tissue was homogenized with a Fisher Scientific PowerGen 125 in 1.0 ml of lysis solution composed of 25 mM ammonium chloride, pH 8.2, 5 mM EDTA, 0.8% Triton X-100, 0.01% SDS, 5 units/ml heparin, 10 mM 6-O-(N-heptylcarbamoyl)-methyl-a-d-glucopyranoside, 1 μg/ml pepstatin, 10 μg/ml leupeptin, and 0.017 trypsin inhibitory units/ml aprotinin. Samples were centrifuged at 20,000 × g for 30 min at 4 °C. The supernatant fractions were collected and stored at −80 °C. Mouse LPL levels in these samples were determined by enzyme-linked immunosorbent assay as described earlier.

To determine the effect of heparin on tissue LPL levels, mice were injected with either 50 units of sodium heparin in 150 μl of 0.9% sodium chloride or the sodium chloride solution alone. The mice were euthanized 2 min later. Before the tissue was collected, the mice were perfused for 4 min with 0.9% sodium chloride. Tissue LPL levels were then measured as described earlier.

Statistical Analysis—All results are shown as the mean ± S.E. Differences in plasma LPL levels were analyzed with a Student’s t test (Microsoft Excel) or with a repeated measures analysis of variance test (SAS/STAT software). Comparisons of Gpihbp1+/−, Gpihbp1+/+, and Gpihbp1−/− mice were analyzed with a three-way analysis of variance test. All statistical analyses were reviewed by the Department of Biomathematics at UCLA.

RESULTS

Plasma Lipid Levels in Gpihbp1-deficient Mice—The plasma triglyceride levels in chow-fed Gpihbp1−/− mice (>8 weeks old) were 2872 ± 245 (n = 15), and the plasma was grossly lipemic. The plasma triglyceride levels in Gpihbp1+/− mice (31.8 ± 5.8, n = 17) were far lower and similar to those in Gpihbp1+/+ mice (32.8 ± 8.7, n = 7) (p < 0.00001 compared with Gpihbp1−/− mice). The plasma cholesterol levels in Gpihbp1−/− mice were 480 ± 51.9, far higher than in Gpihbp1+/+ and Gpihbp1+/− mice (79.7 ± 6.1 and 87.2 ± 5.8, respectively) (p < 0.00001 compared with Gpihbp1−/− mice). Body weights of the three groups of mice were not significantly different.

Delayed Appearance of LPL into the Plasma after an Intravenous Injection of Heparin—We hypothesized that the Gpihbp1 in capillaries could play a physiologic role in binding LPL in vivo, and further hypothesized that the kinetics of LPL appearance in the plasma after an injection of heparin might differ in Gpihbp1−/− and control mice. LPL release after intravenous heparin was examined in 10–16-week-old Gpihbp1+/+ (n = 7), Gpihbp1+/− (n = 9), and Gpihbp1−/− (n = 7) mice. The appearance of LPL in the plasma after intravenous heparin was delayed in Gpihbp1−/− mice (Fig. 1A). Plasma LPL levels in Gpihbp1−/− mice were significantly lower than those in Gpihbp1+/+ and Gpihbp1+/− mice at baseline (p = 0.015) and 1 and 3 min after hepargin (p = 0.000001 and 0.00003, respectively) (Fig. 1A), although the plasma LPL levels were similar at the 15-min time point. The change in LPL levels between the earliest (1-min) and latest (15-min) time points was significantly greater in Gpihbp1−/− mice than in Gpihbp1+/+ and Gpihbp1+/− mice (p = 4.26 × 10−5) (Fig. 1B).

These differences in the kinetics of LPL release after heparin in adult mice were highly reproducible. In two additional experiments, LPL levels early after heparin administration were substantially different in Gpihbp1−/− mice and control mice. In both experiments, the differences in plasma LPL levels between the earliest time point and the 15-min time point were very large and achieved high degrees of statistical significance (p = 0.0002 and p < 0.00001 in the two experiments). The lower LPL levels at early time points in Gpihbp1−/− mice were not an immunoenzyme artifact resulting from lipemia, as “spiking” chylomicrons (from Gpihbp1−/− plasma) into wild-type mouse plasma did not lower LPL mass measurements (not shown).

Given the slow release of LPL into the plasma of Gpihbp1−/− mice after heparin (Fig. 1), we predicted that the plasma triglyceride levels in Gpihbp1−/− mice would not fall rapidly in the first 15 min after intravenous heparin. Indeed, plasma triglyceride levels were minimally altered, if at all, in Gpihbp1−/− mice in the first 15 min after heparin (Fig. 2A). We also predicted that we would observe very different results in wild-type mice, where the release of LPL into the plasma after heparin was very prompt. Indeed, when wild-type mice were given Intralipid (so as to acutely increase plasma triglyceride levels) and heparin, the plasma triglyceride levels fell from 2573 ± 646 to 11 ± 3 mg/dl within 10 min (p < 0.00001 between the 1- and 10-min time points) (Fig. 2B). This dramatic drop in triglyceride levels is consistent with the rapid release of LPL in wild-type mice (Fig. 1). Even without heparin, the plasma triglyceride levels in wild-type mice fell dramatically in the first 10 min after an injection of Intralipid (Fig. 2B).
A Role for GPIHBP1 in Lipolysis

We suspected that the differences in the kinetics of LPL entry into plasma might disappear with lower doses of heparin, but this was not the case. When 0.5, 2, or 10 units of heparin were administered to age- and sex-matched Gpihbp1−/− and Gpihbp1+/+ mice, a similar pattern was observed, with delayed appearance of LPL in Gpihbp1−/− mice and a greater increase in plasma LPL levels between the 1- and 15-min time points (Fig. 3).

We considered the possibility that the differences in the kinetics of LPL release were somehow secondary to the lipemia in Gpihbp1−/− mice rather than to the absence of GPIHBP1 per se. To explore this issue, we examined the kinetics of LPL release after injecting 50 units of heparin intravenously into young (<6-week-old) Gpihbp1−/− mice, which have no visible lipemia and have far lower plasma triglyceride levels (409 ± 60 mg/dl, n = 5). Despite lower lipid levels, the delayed appearance of LPL after heparin in Gpihbp1−/− mice was equally apparent (Fig. 4).

**FIGURE 1.** LPL levels in the plasma of male Gpihbp1−/− (n = 7), Gpihbp1+/+ (n = 9), and Gpihbp1+/+ (n = 7) mice after an intravenous injection of heparin (50 units). A, plasma LPL levels at baseline (before the injection), and 1, 3, 8, and 15 min after the injection. Each line shows the mean LPL levels in Gpihbp1−/− mice after injecting 50 units of heparin intravenously into plasma might disappear with lower doses of heparin, but this was not the case. When 0.5, 2, or 10 units of heparin were administered to age- and sex-matched Gpihbp1−/− and Gpihbp1+/+ mice, a similar pattern was observed, with delayed appearance of LPL in Gpihbp1−/− mice and a greater increase in plasma LPL levels between the 1- and 15-min time points (Fig. 3).

**FIGURE 2.** Effects of intravenous heparin on plasma triglyceride levels in adult Gpihbp1+/+ and Gpihbp1−/− mice. A, Gpihbp1+/+ mice were given 50 units of heparin or an equivalent volume of normal saline (n = 4 mice/group). No differences in plasma triglyceride levels were observed between the 1- and 15-min time points in either group of mice. B, changes in LPL levels in the plasma of Gpihbp1−/− and Gpihbp1+/+ mice after the injection of 150 µl of 20% Intralipid (so as to increase plasma triglyceride levels to >2000 mg/dl) and either 50 units of heparin or an equivalent volume of normal saline. The plasma in both the heparin and saline groups was grossly lipemic 1 min after the injection of Intralipid. Ten minutes after the injection, the plasma triglyceride levels fell dramatically in both the heparin and saline groups of Gpihbp1+/+ mice (p < 0.0001), although the fall in triglyceride levels was more striking in the heparin group.

**FIGURE 3.** Changes in plasma LPL levels between the earliest (1 min) and latest (15 min) time point after an intravenous injection of 10, 2, or 0.5 units of heparin into adult Gpihbp1+/+ and Gpihbp1−/− mice.
LPL Mass and Activity in Gpihbp1−/− and Control Mice—LPL levels in the postheparin plasma of Gpihbp1−/− and control mice were similar at the 15-min time point (Fig. 1), suggesting that Gpihbp1−/− mice do not have lower tissue stores of LPL. Indeed, LPL mass levels in the gonadal fat, liver, heart, and quadriceps muscle of Gpihbp1+/+, Gpihbp1−/−, and Gpihbp1−/− mice were similar (Fig. 5A). LPL mass levels in tissues did not change appreciably after an injection of heparin (Fig. 5B).

We considered the possibility that the LPL released early (1 min) or late (15 min) after an injection of heparin may have been enzymatically inactive, either in Gpihbp1−/− or control mice, but this was not the case. Plasma samples from Gpihbp1−/− and Gpihbp1+/+ mice (n = 2/group) obtained 1 and 15 min after an injection of heparin were fractionated by heparin-Sepharose chromatography, and LPL activity and mass levels were measured in fractions corresponding to the LPL peak. After dividing LPL activity by LPL mass, some variations in LPL specific activity were observed, but no consistent differences were observed (Fig. 6).

LPL Release Kinetics after Heparin in Mice with a Deficiency of Endothelial Cell Heparan Sulfate Proteoglycans—Many studies have suggested that endothelial cell HSPGs could be an important binding site for LPL (3). Thus, we asked whether mice lacking heparan sulfate chains in endothelial cells might also have an abnormal pattern of LPL release after heparin. Wang et al. (14) recently used Cre/loxP recombination techniques to create mice lacking GlcNAc N-deacetylase/N-sulfotransferase 1 (NDST1) in endothelial cells (Ndst1α/αTekCre+ mice). NDST1 is a key enzyme in the sulfation of heparan sulfate proteoglycans. They documented a substantial decrease in the sulfation of HSPGs in the microvascular endothelial cells of these mice and showed that the reduced sulfation of HSPGs led to impaired L-selectin- and chemokine-mediated neutrophil trafficking (14). Plasma triglyceride levels in Ndst1α/αTekCre+ mice were not perturbed. Nevertheless, we tested the possibility that reduced sulfation of HSPGs in endothelial cells might alter the kinetics of LPL release after heparin. We detected no effect of the knock-out on LPL release; the changes in plasma LPL levels between 1 and 15 min after heparin were similar in Ndst1α/αTekCre+ mice and littermate control mice (Ndst1α/α) (Fig. 7).

Reduced Levels of LPL in Gpihbp1−/− Mice after the Injection of a Triglyceride Emulsion (Intralipid)—An intravenous injection of Intralipid releases LPL into the circulation (6). Because Intralipid particles are large (200–1000 nm in diameter) (18), they would not be expected to leave the intravascular compartment rapidly. Gpihbp1+/+, Gpihbp1−/−, and Gpihbp1−/− mice were injected with 7.5 ml/kg 20% Intralipid, a dose that increased plasma triglyceride levels to 7000–17,000 mg/dl. Plasma LPL levels increased significantly in Gpihbp1+/+ and Gpihbp1−/− mice, as expected, but they did not increase in Gpihbp1−/− mice (Fig. 8A). The change in plasma LPL levels between the baseline and 15-min measurements was significantly lower in Gpihbp1−/− mice than in Gpihbp1+/+ and Gpihbp1−/− mice (p < 0.001) (Fig. 8B).
A Role for GPIHBP1 in Lipolysis

A Fall in Plasma Triglyceride Levels in Gpihbp1−/− Mice after an Injection of Heparin—Because Gpihbp1−/− mice have substantial tissue LPL stores (Fig. 5) that can be released into the plasma with heparin (Fig. 1), and because this LPL is enzymatically active (Fig. 6), we predicted that an injection of heparin would reduce plasma triglyceride levels in Gpihbp1−/− mice. This prediction was upheld. We administered 100 units of heparin subcutaneously and intraperitoneally, and then gave an additional 100 units of heparin 90 min later. The plasma triglyceride levels in heparin-treated Gpihbp1−/− mice fell by more than 3500 mg/dl in 135 min (Fig. 9A). We next measured the fall in plasma triglyceride levels in Gpihbp1−/− mice after 50 units of intravenous heparin. Again, plasma triglyceride levels fell dramatically. However, it is noteworthy that the decrease in plasma triglyceride levels at the 20-min time point was small and did not achieve statistical significance (consistent with the findings in Fig. 2A). Finally, we administered 100 units of heparin subcutaneously at baseline and then 8 and 16 h later. Decreased plasma triglyceride levels were sustained over 24 h, compared with mice given the same volume of normal saline (Fig. 9C).

DISCUSSION

We previously showed that Chinese hamster ovary cells expressing high levels of GPIHBP1 bind more LPL than non-transfected cells, and that the GPIHBP1-bound LPL can be released with heparin (8). In the current study, we turned our attention to whether GPIHBP1 might play a role in binding LPL in vivo. To study this issue, we examined the kinetics of LPL appearance in the plasma after intravenous heparin in Gpihbp1−/− mice and littermate control mice. In the control mice, LPL entered the plasma compartment quickly, reaching a peak concentration in 1–3 min. In Gpihbp1−/− mice, the appearance of LPL in the plasma was delayed, and the highest LPL levels were not achieved until 15 min after heparin. In line with these findings, the plasma triglyceride levels changed little in Gpihbp1−/− mice in the 15 min after heparin, whereas the plasma triglyceride levels in control mice fell dramatically within 10 min. The differences in LPL release in Gpihbp1−/− and Gpihbp1+/+ mice could not be ascribed to differences in the tissue stores of LPL, as LPL mass levels in tissues were nearly identical in Gpihbp1−/− and control mice. Nor could the differences be ascribed to markedly elevated lipids in adult mice. However, the findings in Fig. 2A and 2B suggest that GPIHBP1 and LPL are withdrawn from the plasma compartment as LPL is released into the tissue stores. Therefore, we hypothesized that GPIHBP1 might play a role in the binding of LPL to endothelial cells.
A Role for GPIHBP1 in Lipolysis

Gpihbp1−/− mice, as the kinetic differences were equally pronounced in young Gpihbp1−/− mice, where visible lipemia is absent. Interestingly, the kinetics of LPL release also were abnormal after an intravenous injection of Intralipid, a triglyceride emulsion (18). The observed differences in LPL release kinetics after intravenous heparin and Intralipid strongly suggest that GPIHBP1 plays a role in binding LPL in vivo.

Typical plasma triglyceride levels in chow-fed Gpihbp1−/− mice, >2500 mg/dl, are similar to those in adult chow-fed lpl-deficient mice (21). Despite the severe hypertriglyceridemia in Gpihbp1−/− mice, plasma LPL levels in adult Gpihbp1−/− mice 15 min after heparin were similar to those in Gpihbp1−/+ mice. Moreover, the LPL in Gpihbp1−/− mice was enzymatically active. With all of that enzymatically active LPL, why do Gpihbp1−/− mice exhibit chylomicronemia? Our current data, along with earlier work in the field (22–24), suggest a model with more than one pool of heparin-releasable LPL. We suspect that there are two main LPL pools, one relevant to the lipolysis of lipoproteins and one that is largely irrelevant. We suspect that one pool of heparin-releasable LPL is bound to GPIHBP1 on the surface of capillaries in muscle and adipose tissue; this pool is likely important for lipolysis and is probably released quickly after an intravenous injection of heparin. This pool is obviously not present in Gpihbp1−/− mice, explaining the reduced plasma LPL levels 1 min after heparin. We suspect that a second pool of heparin-releasable LPL is extravascular, perhaps bound to HSPGs in subendothelial compartments or on the surface of adipocytes or myocytes, and therefore irrelevant to chylomicron processing. We suspect that the size of the extravascular pool is normal in Gpihbp1−/− mice, given the normal stores of LPL in tissues and the virtually normal plasma LPL levels 15 min after heparin. We further suspect that the delayed appearance of LPL after heparin in Gpihbp1−/− mice is due to the fact that heparin requires more time to reach this pool and to release it into the plasma.

One prediction of this two-pool model is that there would be minimal LPL release in Gpihbp1−/− mice after an injection of Intralipid (large triglyceride particles that would not be expected to leave the intravascular compartment). Indeed, we observed no release of LPL with Intralipid in Gpihbp1−/− mice.

A second prediction of this model is that the severe hypertriglyceridemia in Gpihbp1−/− mice would disappear when enzymatically active LPL from the extravascular pool was released into the plasma with an injection of heparin. This prediction was also upheld. The plasma triglyceride levels in Gpihbp1−/− mice fell by >3500 mg/dl in the 135 min after subcutaneous and intraperitoneal injections of heparin. After a single intravenous injection of heparin, the plasma triglyceride levels fell by >2600 mg/dl within 60 min. Multiple subcutaneous injections of heparin resulted in a sustained decrease in triglyceride levels over 24 h.

The fact that an injection of heparin did not significantly deplete the tissue stores of LPL was not surprising. Previously, Wu et al. (24) found that an injection of heparin into rats led to only a trivial reduction in adipose tissue LPL activity and mass. They concluded that the size of the extravascular pool of LPL far exceeds the size of the LPL pool on the surface of capillaries. That conclusion is consistent with our studies.
A Role for GPIHBP1 in Lipolysis

For years, the assumption has been that HSPGs lining capillaries play a role in binding LPL (3). It is still possible that some LPL is bound to cell-surface HSPGs in capillaries of Gpihbp1−/− and control mice; however, given the severity of the chylomicronemia in Gpihbp1−/− mice, one would have to argue that this LPL pool is small and/or not particularly active against triglyceride-rich lipoproteins. Also, other observations tend to point against a crucial role for endothelial cell HSPGs in binding LPL. Wang et al. (14) used Cre-loxP recombination techniques to inactivate Ndst1 in endothelial cells (a genetic modification that reduces sulfation of endothelial cell proteoglycans). Subsequent studies found no effect of the endothelial cell Ndst1 knock-out on plasma triglyceride levels (25). In the current study, we confirmed the latter finding and also found no effect of the endothelial cell Ndst1 knock-out on the kinetics of LPL release after an intravenous bolus of heparin.

The delayed LPL release after heparin in Gpihbp1−/− mice is somewhat reminiscent of the pattern of LPL release in a patient with chylomicronemia described by Dr. John Brunzell (26). That patient had markedly elevated plasma triglyceride levels (3410 mg/dl), no plasma LPL activity after a bolus of heparin, but substantial LPL activity in adipose tissue. With a continuous heparin infusion, plasma LPL levels were low during the first 60 min, but increased 5-fold after 5 h. At 5 h, plasma LPL levels were in the range observed in normal control subjects. The authors speculated that the delayed appearance of LPL in the plasma was due to slow release of LPL from adipose tissue (26). Whether their findings were related to a defect in GPIHBP1 is unknown.

Familial chylomicronemia in humans is a recessive syndrome often caused by mutations in LPL or apolipoprotein CII, an activator of LPL. Recently, Peterfy et al. (27) showed that mutations in lipase maturation factor 1 (LMF1) can also cause chylomicronemia. However, in many cases of chylomicronemia, the etiology is unclear (2, 28). We suspect that some cases of chylomicronemia will ultimately be tied to homozygous loss of GPIHBP1, although studies by Wang and Hegele (28) indicate that such cases will be rare. They sequenced the exons of Gpihbp1 in 160 patients with severe hypertriglyceridemia and found only one homozygous Gpihbp1 mutation (a G56R substitution in two siblings). Residue 56 is located in a linker segment between the acidic and Ly-6 domains of GPIHBP1. The functional significance of the G56R mutation is unclear, however, as Gin et al. (29) expressed a GPIHBP1-G56R mutant in Chinese hamster ovary cells and found no abnormality in LPL or chylomicron binding.

In the future, we suspect that patients with chylomicronemia due to Gpihbp1 null mutations will be uncovered. We believe that the current studies will be a useful guide for the characterization of such patients. Our studies suggest that abnormal kinetics of LPL release after heparin and Intralipid are a “signature” of GPIHBP1 deficiency, and we strongly suspect that humans with Gpihbp1 null mutations will exhibit similar phenotypes. Also, based on our current studies, we suspect that an injection of heparin would reduce plasma triglyceride in humans with GPIHBP1 deficiency.

REFERENCES

1. Havel, R. J., and Kane, J. P. (2001) in The Metabolic and Molecular Bases of Inherited Disease (Scriver, C. R., Beaudet, A. L., Sly, W. S., Valle, D., Childs, B., Kinzler, K. W., and Vogelstein, B., eds) pp. 2705–2716, 8th Ed., McGraw-Hill, New York
2. Brunzell, J. D., and Deeb, S. S. (2001) in The Metabolic and Molecular Bases of Inherited Disease (Scriver, C. R., Beaudet, A. L., Sly, W. S., Valle, D., Childs, B., Kinzler, K. W., and Vogelstein, B., eds) pp. 2789–2816, 8th Ed., McGraw-Hill, New York
3. Goldberg, I. J. (1996) J. Lipid Res. 37, 693–707
4. Saxena, U., Klein, M. G., and Goldberg, I. J. (1991) J. Biol. Chem. 266, 17516–17521
5. Cheng, C. F., Oosta, G. M., Besandaun, A., and Rosenberg, R. D. (1981) J. Biol. Chem. 256, 12893–12898
6. Korn, E. D. (1955) J. Biol. Chem. 215, 15–26
7. Sendak, R. A., and Besandaun, A. (1998) J. Lipid Res. 39, 1310–1315
8. Beigneux, A., Davies, B., Gin, P., Weinstein, M., Farber, E., Qiao, X., Peale, F., Bunting, S., Walzem, R., Wong, J., Blaner, W., Ding, Z., Melford, K., Wong-siriroj, N., Sauvage, F. D., Fong, L., Besandaun, A., and Young, S. (2007) Cell Metab. 5, 279–291
9. Véniant, M. M., Beigneux, A. P., Besandaun, A., Fong, L. G., and Young, S. G. (2007) in Marine Atherosclerosis (Getz, G., ed) Bentham Science Publishers, Oak Park, IL
10. Besandaun, A., Beigneux, A. P., Fong, L. G., and Young, S. G. (2007) in Adipose Tissue in Health and Disease (Leff, T., ed) Wiley, Malden, MA
11. Young, S., Davies, B. S., Fong, L. G., Gin, P., Weinstein, M. M., Besandaun, A., and Beigneux, A. P. (2007) Curr. Opin. Lipidol. 18, 389–396
12. Cardin, A. D., Barnhart, R. L., Witt, K. R., and Jackson, R. L. (1984) Thromb. Res. 34, 541–550
13. Cardin, A. D., Randall, C. J., Hirose, N., and Jackson, R. L. (1987) Biochemistry 26, 5513–5518
14. Wang, L., Fuster, M., Sriramarao, P., and Esko, J. D. (2005) Nat. Immunol. 6, 902–910
15. Page, S., Hudson, A., Melford, K., and Besandaun, A. (2006) J. Biol. Chem. 281, 13901–13908
16. Kirchgesner, T. G., Svenson, K. L., Lusis, A. J., and Schotz, M. C. (1987) J. Biol. Chem. 262, 8463–8466
17. Besandaun, A., and Weinstein, D. (1976) J. Biol. Chem. 251, 11925–11930
18. Hansen, L. M., Hardie, B. S., and Hidalgo, J. (1976) Ann. Surg. 184, 80–88
19. Besandaun, A., Ehnholm, C., Steinberg, D., and Brown, W. V. (1974) J. Biol. Chem. 249, 2220–2227
20. Hocquette, J. F., Graulet, B., and Olivecrona, T. (1998) Comp. Biochem. Physiol. B Biochem. Mol. Biol. 121, 201–212
21. Strauss, J. G., Frank, S., Kratky, D., Hammerle, G., Hrzenjak, A., Kensing, G., von Eckardstein, A., Kostner, G. M., and Zechner, R. (2001) J. Biol. Chem. 276, 36083–36090
22. Obunike, J. C., Lutz, E. P., Li, Z., Paka, L., Katopodis, T., Strickland, D. K., Kozarsky, K. F., Pillarisetti, S., and Goldberg, I. J. (2001) J. Biol. Chem. 276, 8934–8941
23. Saxena, U., Klein, M. G., and Goldberg, I. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2254–2258
24. Wu, G., Olivecrona, G., and Olivecrona, T. (2003) J. Biol. Chem. 278, 11925–11930
25. MacArthur, J. M., Bishop, J. R., Stanford, K. L., Wang, L., Besandaun, A., Witztum, J. L., and Esko, J. D. (2007) J. Clin. Invest. 117, 153–164
26. Brunzell, J. D., Chait, A., Nikkila, E. A., Ehnholm, C., Huttunen, J. K., and Steiner, G. (1980) Metabolism 29, 624–629
27. Peterfy, M., Ben-Zeev, O., Mao, H. Z., Weissglas-Volkov, D., Aouizerat, B. E., Pullinger, C. R., Frost, P. H., Kane, J. P., Malloy, M. J., Reue, K., Pajukanta, P., and DooLittle, M. H. (2007) Nat. Genet. 39, 1483–1487
28. Wang, J., and Hegele, R. A. (2007) Lipids Health Dis. 6, 23
29. Gin, P., Beigneux, A. P., Davies, B., Young, M. F., Ryan, R. O., Besandaun, A., Fong, L. G., and Young, S. G. (2007) Biochim. Biophys. Acta 1771, 1464–1468