Reciprocal Metabolic Perturbations in the Adipose Tissue and Liver of GPIHBP1-Deficient Mice

Michael M. Weinstein, Christopher Goulbourne, Brandon S.J. Davies, Yiping Tu, Richard H. Barnes II, Steven M. Watkins, Ryan Davis, Karen Reue, Peter Tontonoz, Anne P. Beigneux, Loren G. Fong, Stephen G. Young

Objective—Gpihbp1-deficient (Gpihbp1−/−) mice lack the ability to transport lipoprotein lipase to the capillary lumen, resulting in mislocalization of lipoprotein lipase within tissues, defective lipolysis of triglyceride-rich lipoproteins, and chylomicronemia. We asked whether GPIHBP1 deficiency and mislocalization of catalytically active lipoprotein lipase would alter the composition of triglycerides in adipose tissue or perturb the expression of lipid biosynthetic genes. We also asked whether perturbations in adipose tissue composition and gene expression, if they occur, would be accompanied by reciprocal metabolic changes in the liver.

Methods and Results—The chylomicronemia in Gpihbp1−/− mice was associated with reduced levels of essential fatty acids in adipose tissue triglycerides and increased expression of lipid biosynthetic genes. The liver exhibited the opposite changes: increased levels of essential fatty acids in triglycerides and reduced expression of lipid biosynthetic genes.

Conclusion—Defective lipolysis in Gpihbp1−/− mice causes reciprocal metabolic perturbations in adipose tissue and liver. In adipose tissue, the essential fatty acid content of triglycerides is reduced and lipid biosynthetic gene expression is increased, whereas the opposite changes occur in the liver. (Arterioscler Thromb Vasc Biol. 2012;32:00-00.)

Key Words: genetically altered mice | lipoproteins | metabolism | GPIHBP1

GPIHBP1, a glycosylphosphatidylinositol-anchored glycoprotein of capillary endothelial cells, shuttles lipoprotein lipase (LPL) from the interstitial spaces to the capillary lumen.1 When GPIHBP1 is absent, the stores of catalytically active LPL within tissues are normal,2 but the LPL is mislocalized to the interstitial spaces and is absent from the capillary lumen.3 The mislocalization of LPL interferes with lipoprotein lipolysis and causes chylomicronemia,2,3 but whether GPIHBP1 deficiency and LPL mislocalization lead to significant perturbations in tissue lipid metabolism has never been examined.

Defects in LPL also cause chylomicronemia,4 and the impact of LPL deficiency on lipid metabolism in adipose tissue has been examined by several groups.5–7 When LPL is absent, the levels of essential fatty acids (α-linolenic acid and linoleic acid) in triglycerides are low, whereas levels of palmitoleic acid (16:1) are high. These compositional abnormalities have been interpreted as indicating increased de novo synthesis of triglycerides.5–7 Mice that produce LPL exclusively in skeletal muscle do not have elevated plasma triglyceride levels,8 but they nevertheless exhibit low levels of essential fatty acids in adipose tissue triglycerides9 and increased de novo lipogenesis within adipose tissue.9

In the current study, we sought to investigate 2 mysteries surrounding lipid metabolism in Gpihbp1−/− mice. The first was whether GPIHBP1 deficiency, like LPL deficiency, would perturb adipose tissue lipid composition and metabolism. One could argue that the very existence of chylomicronemia in Gpihbp1−/− mice suggests that tissue lipid metabolism must be perturbed. But on the other hand, one could argue that the large stores of catalytically active LPL in the extravascular spaces of Gpihbp1−/− mice might mitigate, or entirely prevent, significant perturbations in tissue lipid metabolism. The notion that extravascular LPL might influence tissue lipid metabolism was lent credibility by a transgenic mouse study showing that extravascular LPL can lead to increased lipid accumulation in parenchymal cells.10 In that study, the expression of a glycosylphosphatidylinositol-anchored LPL on the surface of cardiomyocytes actually caused increased lipid accumulation in cells, presumably by promoting uptake of lipids from lipoproteins within the interstitial fluid.

The second issue that we sought to address was whether abnormalities in lipid metabolism in Gpihbp1−/− mice would extend to tissues lacking a significant role in LPL-mediated lipoprotein lipolysis. Specifically, we tested whether perturbations in adipose tissue lipid composition and gene expression in GPIHBP1-deficient mice would be accompanied by reciprocal metabolic changes in the liver, an organ that normally has a negligible role in LPL-mediated processing of triglyceride-rich lipoproteins.
Materials and Methods

Gpihbp1−/− mice1 were housed in a barrier facility and fed a chow diet containing 0.02% cholesterol (LabDiet No. 5001, Purina Mills) or a Western diet containing 21.2% fat (anhydrous milk fat) and low (0.05%) amounts of cholesterol (TD No. 05311, Harlan Teklad, Madison, WI). In some experiments, mice were housed individually in sealed metabolic cages (Oxymax, Columbus Instruments, Columbus, OH). Mice were acclimated to the metabolic cages for 8 hours before data collection (3 cycles of 12 hours light/12 hours dark). Respiratory quotient data were compiled with Oxymax software and analyzed with Microsoft Excel by averaging readings taken every 20 minutes during each light/dark cycle.

Lipids were measured in the plasma and in 3 tissues (gonadal fat pad, liver, lung) of 14-week-old chow-fed Gpihbp1−/− and littermate wild-type mice (n=5/group). All mice were perfused extensively with phosphate-buffered saline before tissues were harvested. We performed identical studies in Gpihbp1−/− and littermate wild-type mice after 5 weeks on a high-fat diet. Lipids were extracted with chloroform and methanol in the presence of internal standards by the method of Folch et al.11 For the separation of neutral lipids, thin-layer chromatography with a solvent system consisting of petroleum ether/diethyl ether/acetonic acid (80/20/1, vol/vol/vol) was used. Individual phospholipids were separated by liquid chromatography (Agilent Technologies 1100 Series). Each lipid class was transesterified in 1% sulfuric acid in methanol in a sealed vial under nitrogen atmosphere at 100°C for 45 minutes. The resulting fatty acid methyl esters were extracted from the mixture with hexane containing 0.05% butyraldehyde and hexane extracts were scaled under nitrogen until analysis by gas chromatography.

To quantify gene expression levels, RNA was prepared from tissues with TRI Reagent (Sigma-Aldrich). After the RNA was treated with DNase I (Ambion), cDNA was prepared with a mixture of random primers, oligo(DT), and Superscript III reverse transcriptase (Invitrogen). Quantitative reverse transcription–polymerase chain reaction studies were performed in triplicate with SYBR Green PCR Master Mix (Quanta) on a 7500 Fast Real-Time PCR System (Applied Biosystems). The primers for 4 lipid biosynthetic genes (acyl-CoA carboxylase [Acc], fatty acid synthase [Fasn], stearoyl-CoA desaturase [Scd], and ATP citrate lyase [Acly]) and endothelial lipase (Lipg) are listed in the Supplemental Table 1, available online at http://atvb.ahajournals.org. We also measured expression of endothelial lipase (Lipg) in adipose tissue. Gene expression levels were calculated with the comparative C<sub>T</sub> method and normalized to levels of β2-microglobulin expression.

Results

The levels of triglycerides and cholesterol in the plasma of Gpihbp1−/− mice were markedly elevated on both chow and high-fat diets, consistent with earlier studies.2,3 The plasma triglyceride and cholesterol levels in Gpihbp1−/− mice on the chow diet were 64.0±19.1 and 12.5±1.6 mmol/L, respectively (versus 1.3±0.4 and 3.2±0.2, respectively, in wild-type control mice [n=6/group]). On a high-fat diet, the triglyceride and cholesterol levels in Gpihbp1−/− mice were 94.8±37.2 and 26.22±3.9 mmol/L, respectively (versus 1.0±0.1 and 5.0±0.3, respectively, in wild-type control mice [n=6/group]). Earlier studies showed that Gpihbp1−/− mice gained less weight after short-term feeding of a high-fat diet.12 Here, we measured body weight and adiposity in littermate male Gpihbp1−/− and wild-type mice that were maintained on chow or high-fat diets until they were 6 months of age. In wild-type mice, the body weights and adiposity were far greater on the high-fat diet, as expected, but with Gpihbp1−/− mice, body weights and adiposity were nearly identical on chow and high-fat diets (Figure 1).

The low levels of adiposity in Gpihbp1−/− on the high-fat diet heightened our suspicion that we might find perturbed lipid metabolism in adipose tissue. To address this issue, we examined the composition of triglycerides in the adipose tissue in Gpihbp1−/− and wild-type mice on a chow diet containing 1.2% palmitoleic acid (16:1), 44.3% linoleic acid (18:2), and 5.0% α-linolenic acid (18:3). In adipose tissue, the ratio of 18:2 and 18:3 fatty acids to 16:1 fatty acids was lower in Gpihbp1−/− mice than in wild-type mice (Figure 2A). Also, the absolute levels of 18:2 and 18:3 fatty acids in adipose tissue triglycerides were lower in Gpihbp1−/− mice than in wild-type mice, whereas levels of 16:1 fatty acids were higher (Figure 2B). Interestingly, the composition of triglycerides in the liver exhibited reciprocal changes: the 18:2/18:3/16:1 fatty acid ratio in the liver triglycerides was actually higher in Gpihbp1−/− mice than in wild-type mice (Figure 2C). Also, the amounts of 18:2 fatty acids in liver triglycerides tended to be higher, whereas 16:1 fatty acids tended to be lower (Figure 2D). The amounts of triglycerides in adipose tissue and liver, per gram of tissue, were similar in Gpihbp1−/− and wild-type mice (Supplemental Figure I).

We also observed reciprocal differences in the composition of free fatty acids in adipose tissue and liver. The ratio of 18:2 and 18:3 free fatty acids to 16:1 free fatty acids in adipose tissue was...
lower in Gpihbp1−/− mice than in wild-type mice (Figure 3A and 3B), whereas in the liver, the ratio was higher in Gpihbp1−/− mice than in wild-type mice (Figure 3C and 3D).

The 18:2,18:3/16:1 fatty acid ratio in the plasma triglycerides was higher in Gpihbp1−/− mice than in wild-type mice (Figure 3E), mirroring the compositional changes in liver triglycerides (Figure 2C). In a recent study, we reported that the lung has very high levels of GPIHBP1 expression, facilitating LPL’s role in lipid metabolism in that tissue.13 Interestingly, the 18:2,18:3/16:1 fatty acid ratio in triglycerides of the lung was lower in Gpihbp1−/− mice than in wild-type mice (Figure 3F), similar to results with adipose tissue triglycerides (Figure 2A).

We also identified reciprocal changes in phospholipid composition in adipose tissue and liver. The phospholipids in the adipose tissue of Gpihbp1−/− mice had a lower-than-wild-type 18:2,18:3/16:1 fatty acid ratio (Figure 3G), whereas the phospholipids in the liver had a higher-than-wild-type 18:2,18:3/16:1 fatty acid ratio (Figure 3H).

The composition of triglycerides in the adipose tissue (Figure 2A) and plasma (Figure 3E) of Gpihbp1−/− mice was very different, whereas the composition of triglycerides in the liver (Figure 2C) and plasma (Figure 3E) of Gpihbp1−/− mice was quite similar. These findings led us to predict that the composition of adipose tissue triglycerides in Gpihbp1−/− mice would change minimally, if at all, in mice fed a saturated fat–rich diet, whereas the composition of liver triglycerides would change dramatically. Indeed, when we placed Gpihbp1−/− mice on a high-fat diet (65.3% saturated fat and 4.5% polyunsaturated fat) for 5 weeks, the levels of saturated fatty acids and polyunsaturated fatty acids in the adipose tissue triglycerides were no different than those in chow-fed mice. In contrast, the composition of the adipose tissue in wild-type mice reflected the new diet, with higher levels of saturated fatty acids and lower levels of polyunsaturated fatty acids (Figure 4A and 4B). Reciprocal changes were observed in the liver: the composition of triglycerides in the liver of Gpihbp1−/− mice changed markedly on the high-fat diet, with increased levels of saturated fat and lower levels of polyunsaturated fat (Figure 4C and 4D). The composition of the triglycerides in the liver of wild-type mice also changed, but the changes were smaller than those in Gpihbp1−/− mice (Figure 4C and 4D). In both wild-type and Gpihbp1−/− mice, the plasma triglycerides contained higher

**Figure 2.** Reciprocal changes in triglyceride composition in the adipose tissue and liver of Gpihbp1−/− and wild-type mice on a chow diet. A, Ratio of 18:2 and 18:3 fatty acids to 16:1 fatty acids in the triglycerides of adipose tissue. B, Levels of 16:1, 18:2, and 18:3 fatty acids in adipose tissue triglycerides. C, Ratio of 18:2 and 18:3 fatty acids to 16:1 fatty acids in liver triglycerides. D, Levels of 16:1, 18:2, and 18:3 fatty acids in liver triglycerides. n=9/group. **P<0.01, ***P<0.001.

**Figure 3.** Reciprocal changes in the composition of lipids in adipose tissue and liver of Gpihbp1−/− and wild-type mice on a chow diet. A, Ratio of 18:2 and 18:3 free fatty acids to 16:1 free fatty acids in adipose tissue. B, Levels of 16:1, 18:2, and 18:3 free fatty acids in adipose tissue. C, Ratio of 18:2 and 18:3 free fatty acids to 16:1 fatty acids in the liver. D, Levels of 16:1, 18:2, and 18:3 fatty acids in the liver. E and F, Ratio of 18:2 and 18:3 fatty acids to 16:1 fatty acids in the plasma (E) and lung (F) triglycerides. G and H, Ratio of 18:2 and 18:3 fatty acids to 16:1 fatty acids in the phospholipids of adipose tissue (G) and liver (H). n=5 mice/group. *P<0.05, **P<0.01, ***P<0.001.
levels of saturated fatty acids and lower levels of unsaturated fatty acids (Figure 4E and 4F), but similar to the findings in the liver, those changes were more exaggerated in Gpihbp1−/− mice.

The lower levels of essential fatty acids in adipose tissue triglycerides in Gpihbp1−/− and wild-type mice fed a chow diet or a high-fat diet for 5 weeks. A to D, Levels of saturated fatty acids (SFA) (A and C) and polyunsaturated fatty acids (PUFA) (B and D) in adipose tissue triglycerides (A and B) and liver triglycerides (C and D) from Gpihbp1+/+ and wild-type mice (n=5/group). E and F, SFA (E) and PUFA (F) in the plasma triglycerides of Gpihbp1+/+ and wild-type mice fed the chow or high-fat diets.

Figure 4. The composition of adipose tissue and liver triglycerides in Gpihbp1−/− and wild-type mice fed a chow diet or a high-fat diet for 5 weeks. A to D, Levels of saturated fatty acids (SFA) (A and C) and polyunsaturated fatty acids (PUFA) (B and D) in adipose tissue triglycerides (A and B) and liver triglycerides (C and D) from Gpihbp1+/+ and wild-type mice (n=5/group). E and F, SFA (E) and PUFA (F) in the plasma triglycerides of Gpihbp1+/+ and wild-type mice fed the chow or high-fat diets.

The reduced adiposity in Gpihbp1−/− mice (Figure 1), the striking differences in lipid composition in the adipose tissue of Gpihbp1−/− mice (Figures 2–4), and the increased expression of lipid biosynthetic genes in the adipose tissue of Gpihbp1−/− mice (Figure 5) suggested that Gpihbp1−/− mice might exhibit significant defects in the utilization of dietary lipids and preferentially use carbohydrates. In line with this prediction, the respiratory quotient in Gpihbp1−/− mice was significantly higher than in wild-type mice during the inactive period (a period of increased lipid utilization). These differences were particularly striking in mice fed the high-fat diet (Figure 6).

Discussion

In the current study, we asked whether lipid composition and gene expression are perturbed in the adipose tissue of Gpihbp1−/− mice—as they are in the setting of LPL deficiency—and whether the large stores of catalytically active LPL in the tissues of Gpihbp1−/− mice might protect them from these changes. Our experiments provided a definitive answer. Like the levels of essential fatty acids in LPL-deficient adipose tissue,5–7 those in the triglycerides of adipose tissue were far lower in Gpihbp1−/− mice than in wild-type mice, and the expression of lipid biosynthetic genes in adipose tissue was higher. No one, as far as we are aware, has assessed the expression of lipid biosynthetic genes in the adipose tissue of LPL-deficient mice with chylomicronemia, but the expression of these genes is clearly increased in the adipose tissue of normolipidemic mice that express LPL exclusively in skeletal muscle.9 Thus, reduced amounts of
catalytically active LPL in capillaries of adipose tissue, whether caused by a deficiency in LPL itself or a deficiency in GPIHBP1, perturbs adipose tissue composition and gene expression. The catalytically active LPL within the interstitium of adipose tissue in Gpihbp1−/− mice2,3 is incapable of preventing the perturbations in adipose tissue lipid composition or gene expression.

The second issue that we addressed is whether compositional and metabolic changes in the adipose tissue of Gpihbp1−/− mice would be accompanied by reciprocal metabolic changes in tissues lacking a prominent role in LPL processing, namely the liver. We reasoned that, at steady state, large amounts of triglycerides must enter the plasma of Gpihbp1−/− mice, exactly as in wild-type mice, and that the “triglyceride entry” must be matched by equal amounts of triglyceride clearance. And we further suspected that evidence of reduced triglyceride uptake in the adipose tissue of Gpihbp1−/− mice (as reflected by lipid compositional abnormalities and increased expression of lipid biosynthetic genes) would be accompanied by reciprocal metabolic perturbations in the liver, a tissue that has a negligible role in LPL-mediated lipoprotein processing but a large role in the uptake of remnant lipoproteins.15 Our studies provided strong support for the “reciprocal metabolic changes” concept. Gpihbp1−/− mice did not have elevated hepatic triglyceride stores, but the levels of essential fatty acids in liver triglycerides were higher in Gpihbp1−/− mice than in wild-type mice (changes opposite to those occurring in adipose tissue).

Also, unlike the situation in adipose tissue, the composition of triglycerides in the liver changed dramatically when Gpihbp1−/− mice were fed a high-fat diet, and the magnitude of those changes was greater in Gpihbp1−/− mice than in wild-type mice. Finally, the expression of lipid biosynthetic genes in the liver was lower than normal in chow-fed Gpihbp1−/− mice, whereas the expression levels of these same genes in adipose tissue was higher than normal. As far as we are aware, our studies are the first to propose and document the existence of reciprocal metabolic perturbations in liver and adipose tissue in the setting of defective lipolysis.

Our studies suggest that the liver is capable of removing triglyceride-rich lipoproteins from the plasma under conditions when lipolysis is absent in adipose tissue (eg, GPIHBP1 deficiency), leading to higher-than-normal levels of essential fatty acids in the liver and lower-than-normal expression of lipid biosynthetic genes. The liver’s role in triglyceride metabolism under these circumstances is hardly efficient, given the strikingly elevated plasma triglyceride levels in Gpihbp1−/− mice (and the complete absence of hepatic steatosis in these mice), but increased triglyceride uptake by the liver likely compensates, at least to a degree, for the loss of LPL-mediated triglyceride delivery to peripheral tissues.

The ability to investigate the reciprocal metabolic changes hypothesis was facilitated by the existence of Gpihbp1−/− mice, which are healthy and can be bred in unlimited numbers. Similar studies in LPL-deficient human subjects would be next to impossible, given ethical barriers to obtaining human liver samples for elective scientific experiments. However, we believe that the concept set forth in the current study—reciprocal metabolic changes in adipose tissue and liver in the setting of defective lipolysis—should be of interest to lipidologists. The pathogenesis of many cases of severe hypertriglyceridemia is unclear. Most often, patients with chylomicronemia have no obvious mutations in LPL, GPIHBP1, or APOCII,16 and it is often unclear whether hypertriglyceridemic patients actually have bona fide defects in LPL-mediated processing of lipoproteins in capillaries or whether they actually have lipoprotein overproduction or defects in remnant uptake. In the future, it could be interesting to determine whether reciprocal metabolic changes in adipose tissue and liver, as delineated in this article, represents a “metabolic signature” for severely defective LPL-mediated lipolysis along the capillary wall. Perhaps reciprocal metabolic changes similar to those found in Gpihbp1−/− mice are present only in patients with chylomicronemia due to LPL, APOCII, or GPIHBP1 deficiency (or yet-to-be-defined defects in LPL action) and are largely absent in many garden variety cases of hypertriglyceridemia. In the future, it might be possible to examine this issue in human patients characterizing adipose tissue and liver lipid synthesis profiles with stable isotope studies17,18 rather than with the tissue biopsy experiments used in our mouse studies.

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Disclosures
None.

References
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Supplement Material

“Reciprocal Metabolic Perturbations in the Adipose Tissue and Liver of GPIHBP1-deficient Mice” by Weinstein et al.

Table I

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<th>PCR Primers for Quantitative RT-PCR Studies</th>
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Legends for Supplementary Figures

**Figure I.** Similar levels of triglycerides in the tissues of *Gpihbp1*−/− and wild-type control mice, regardless of diet. (A) Adipose tissue triglyceride levels per gram of tissue. The levels were similar in all groups, although the adipose tissue of wild-type mice on the high-fat diet exhibited a slight but significant decrease in triglyceride content (per gram of tissue). (B) Liver triglyceride levels per gram of tissue. Liver triglyceride content increased in response to the high-fat diet, but there was no significant difference in liver triglycerides between *Gpihbp1*−/− and wild-type mice on either diet. **P < 0.01**

**Figure II.** Increased expression of endothelial lipase, as judged by qRT-PCR, in the adipose tissue of *Gpihbp1*−/− mice. **P < 0.01**
Figure II

Relative Expression of Endothelial Lipase

- Gpihbp1$^{+/+}$
- Gpihbp1$^{-/-}$

Chow

High-fat