Impaired thermogenesis and sharp increases in plasma triglyceride levels in GPIHBP1-deficient mice during cold exposure

Mikael Larsson,[1,*] Christopher M. Allan,[*] Patrick J. Heizer,[*] Yiping Tu,[*] Norma P. Sandoval,[*] Rachel S. Jung,[*] Rosemary L. Walzem,[†] Anne P. Beigneux,[*] Stephen G. Young,[1,*,§] and Loren G. Fong[1,*]

Departments of Medicine* and Human Genetics,[§] David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA 90095; and Department of Poultry Science and Faculty of Nutrition,[†] Texas A&M University, College Station, TX 77843

Abstract **Glycosylphosphatidylinositol-anchored high density lipoprotein–binding protein 1 (GPIHBP1), an endothelial cell protein, binds LPL in the subendothelial spaces and transports it to the capillary lumen. In *Gpihbp1*$^{-/-}$ mice, LPL remains stranded in the subendothelial spaces, causing hypertriglyceridemia, but how *Gpihbp1*$^{-/-}$ mice respond to metabolic stress (e.g., cold exposure) has never been studied. In wild-type mice, cold exposure increases LPL-mediated processing of triglyceride-rich lipoproteins (TRLs) in brown adipose tissue (BAT), providing fuel for thermogenesis and leading to lower plasma triglyceride levels. We suspected that defective TRL processing in *Gpihbp1*$^{-/-}$ mice might impair thermogenesis and blunt the fall in plasma triglyceride levels. Indeed, *Gpihbp1*$^{-/-}$ mice exhibited cold intolerance, but the effects on plasma triglyceride levels were paradoxical. Rather than falling, the plasma triglyceride levels increased sharply (from ~4,000 to ~15,000 mg/dl), likely because fatty acid release by peripheral tissues drives hepatic production of TRLs that cannot be processed. We predicted that the sharp increase in plasma triglyceride levels would not occur in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice, where LPL activity is higher and baseline plasma triglyceride levels are lower. Indeed, the plasma triglyceride levels in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice fell during cold exposure. Metabolic studies revealed increased levels of TRL processing in the BAT of *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice.**—Larsson, M., C. M. Allan, P. J. Heizer, Y. Tu, N. P. Sandoval, R. S. Jung, R. L. Walzem, A. P. Beigneux, S. G. Young, and L. G. Fong. **Impaired thermogenesis and sharp increases in plasma triglyceride levels in GPIHBP1-deficient mice during cold exposure. *J. Lipid Res.* 2018.** 59: **706–713.**

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Glycosylphosphatidylinositol-anchored high density lipoprotein–binding protein 1 (GPIHBP1), a glycoprotein of capillary endothelial cells, plays three important functions in plasma triglyceride metabolism (1). First, GPIHBP1 captures LPL in the interstitial spaces and transports it to its site of action in the capillary lumen (2, 3). Second, GPIHBP1 expression is crucial for the margination of triglyceride-rich lipoproteins (TRLs) along capillaries (4), allowing LPL-mediated triglyceride hydrolysis to proceed. Finally, in vitro biochemical studies have demonstrated that the binding of LPL to GPIHBP1 stabilizes LPL's hydrolase domain and preserves catalytic activity—even in the presence of angiopoietin-like protein 4 (ANGPTL4), an LPL inhibitor protein (5, 6).

In *Gpihbp1*-deficient mice (*Gpihbp1*$^{-/-}$), LPL is trapped in the interstitial spaces around parenchymal cells, causing impaired lipolytic processing of TRLs and severe hypertriglyceridemia ("chylomicronemia") (2, 3). Human patients with *GPIHBP1* loss-of-function mutations manifest chylomicronemia, with plasma triglyceride levels greater than 1,500 mg/dl (7–9). In *Gpihbp1*$^{-/-}$ mice, the plasma triglyceride levels on a chow diet are ~4,000 mg/dl (2, 3, 10). But while high plasma triglyceride levels in *Gpihbp1*$^{-/-}$ mice have been well documented (1), it is unclear how these

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Abbreviations: ANGPTL4, angiopoietin-like protein 4; BAT, brown adipose tissue; FABP, fatty acid binding protein; GPIHBP1, glycosylphosphatidylinositol-anchored high density lipoprotein–binding protein 1; TRL, triglyceride-rich lipoprotein.

[1]To whom correspondence should be addressed.
e-mail: larsson.lom@gmail.com (M.L); lfong@mednet.ucla.edu (L.G.F.); sgyoung@mednet.ucla.edu (S.G.Y.)

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mice respond to metabolic stress, such as that associated with exposure to the cold.

In mice, mutations that impair fatty acid utilization impair thermogenesis during cold exposure, compromising their ability to maintain a normal body temperature. For example, a deficiency in fatty acid binding protein 3 (FABP3) or a combined deficiency of FABP4 and FABP5 impairs fatty acid oxidation in brown adipose tissue (BAT), leading to cold intolerance (11, 12). A deficiency of CD36, a putative fatty acid transporter, also causes cold intolerance (13, 14). However, no one has yet determined whether the processing of TRLs by the GPIHBP1–LPL complex is essential for thermogenesis. Normally, LPL-mediated processing of TRLs by BAT is robust during cold exposure, providing fatty acids for mitochondrial oxidation and leading to lower plasma triglyceride levels (15, 16). The latter observations raise doubts about whether *Gpihbp1*$^{-/-}$ mice, where triglyceride hydrolysis is clearly impaired, would remain euthermic during cold exposure.

In the current study, we had two goals. The first was to test whether intravascular lipolysis by the LPL–GPIHBP1 complex is crucial for thermogenesis during cold exposure. It seemed possible that the impaired intravascular lipolysis in *Gpihbp1*$^{-/-}$ mice would lead to defective thermogenesis and cold sensitivity, but our confidence in making this prediction was limited. Indeed, one could argue that the metabolic flux of triglycerides through the tissues of *Gpihbp1*$^{-/-}$ mice is normal (despite markedly elevated levels of triglycerides in the plasma compartment), and that this flux would provide the fuel required for thermogenesis. One could also argue that the fatty acids released by peripheral tissues during cold exposure (17–19) would be sufficient to fuel thermogenesis. Our second goal was to test whether plasma triglyceride levels in *Gpihbp1*$^{-/-}$ mice would fall during cold exposure—as they do in wild-type mice. Once again, it was difficult to make a firm prediction. On one hand, one could imagine that the defective lipolysis in *Gpihbp1*$^{-/-}$ mice would blunt the fall in plasma triglyceride levels. On the other hand, one could argue that the decline in plasma triglyceride levels during cold exposure might be fairly normal, simply because triglyceride levels fall sharply during cold exposure in *Apoa5*$^{-/-}$ mice (16), where impaired intravascular lipolysis leads to moderate to severe increases in plasma triglyceride levels (20–22). In the current studies, we addressed both experimental goals, assessing cold sensitivity in *Gpihbp1*$^{-/-}$ mice as well as the plasma triglyceride response during cold exposure.

MATERIAL AND METHODS

Genetically modified mice

Gpihbp1$^{-/-}$ mice have been described previously (2). *APOC3* transgenic mice (B6;CBA-Tg(*APOC3*)3707Bres/J) (23) and *Apoc3*$^{-/-}$ mice (B6.129-*Apoc3*^{tm1Unc}/J) (24) were purchased from the Jackson Laboratory. *Apoc3*$^{-/-}$ mice were bred with *Gpihbp1*$^{-/-}$ mice to generate *Gpihbp1*$^{-/-}$*Apoc3*$^{-/-}$ mice. *Angptl4*$^{-/-}$ mice (25) were bred with *Gpihbp1*$^{-/-}$ mice to generate *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice. We also used *Lpl*$^{-/-}$ mice carrying a skeletal muscle–specific human LPL transgene (L0-MCK) (26, 27). Unless otherwise specified, all mice were fed rodent chow and housed in a specific pathogen–free barrier facility with a 12 h light–dark cycle. In some experiments, mice were fed a 62% sucrose/fat-free diet (Envigo, TD.03314). Acute cold exposure studies were performed by subjecting mice to 4 or 6°C for no more than 6 h, or by subjecting mice to 12°C for 2 days and then 6°C for an additional 2 days. Body temperatures were measured twice daily with a rectal thermometer (Physitemp BAT-12). All studies were approved by UCLA's Animal Research Committee.

Triglyceride and apoB measurements

Blood was collected from anesthetized mice by retro-orbital puncture with heparinized capillary tubes (Kimble Chase). Plasma was separated from blood cells by centrifugation (14,000 *g* for 30 s) and stored at –80°C. Plasma triglycerides were quantified with a commercial kit (Sigma, TR0100). To assess relative levels of apoB proteins, plasma samples were delipidated with acetone:ethanol (1:1) and then size-fractionated by SDS-PAGE. apoB was detected by Western blotting with an apoB-specific monoclonal antibody (2G11, 10 μg/ml) (28) and IRDye680- or IRDye800-labeled secondary antibodies (LI-COR; 1:2,000). Antibody binding was detected with an Odyssey infrared scanner (LI-COR).

Distribution of radiolabeled lipids in mouse tissues

Mice were administered, by gastric gavage, [^{3}H]retinol (3.5 μCi) and [^{14}C]oleic acid (5 μCi), or [^{3}H]triolein alone (7.5 μCi) [mixed with 150 μl of 20% Intralipid (Fresenius Kabi)]. Either 3 or 6 h later, the mice were perfused with 15 ml PBS containing 5 mM EDTA, and tissues were collected and weighed. Mouse carcasses, tissues, and perfusates were saponified in 25% KOH (w/v) in 50% ethanol (v/v) at 37°C overnight. Tissue homogenates (400 μl) were added to 4 ml Optiphase HiSafe 3 supplemented with 2.5% (v/v) glacial acetic acid, and radioactivity was quantified by liquid scintillation counting.

Immunohistochemistry

BAT was collected from mice that had been perfused with 15 ml of PBS containing 5 mM EDTA, followed by 5 ml of freshly prepared 3% paraformaldehyde in PBS. Interscapular BAT was harvested, embedded in OCT compound, and frozen on dry ice. Tissue sections (10 μm) were fixed in ice-cold methanol, permeabilized with 0.2% Triton X-100, blocked with 5% FBS and 0.2% BSA in PBS, and incubated overnight at 4°C with primary antibodies [a rabbit polyclonal antibody against CD31 (Abcam, ab28364; 1:100); a goat polyclonal antibody against mouse LPL (20 μg/ml) (2); and a rat monoclonal antibody against GPIHBP1 (3 μg/ml) (29)]. Secondary antibodies [Alexa488–donkey anti-rabbit IgG (Thermo Fisher, A21206); Alexa555–donkey anti-goat IgG (Thermo Fisher, A21432); and Alexa647–donkey anti-rat IgG (Jackson ImmunoResearch, 712-605-153)] were used at a 1:200 dilution and incubated with sections at room temperature for 45 min. Nuclei were stained with DAPI (10 μg/ml), and the coverslips were mounted onto slides with ProLong Gold antifade (Thermo Fisher). Images were obtained with an Axiovert 200M microscope (Zeiss) and processed with Zen 2010 software. Within each experiment, exposure conditions were identical.

Statistical analyses

Statistical analyses were performed with GraphPad Prism software. Differences in triglyceride levels before and after cold exposure were analyzed by paired two-tailed *t*-tests. All other statistical calculations were performed by unpaired two-tailed *t*-tests.

RESULTS

To assess cold sensitivity in the setting of GPIHBP1 deficiency, we housed *Gpihbp1*$^{-/-}$ and littermate wild-type mice in the cold (4°C) for 6 h. Three of seven *Gpihbp1*$^{-/-}$ mice, but no wild-type mice, developed hypothermia (**Fig. 1A**, B). We also housed *Gpihbp1*$^{-/-}$ and littermate wild-type mice at 12°C for 2 days and then at 6°C for an additional 2 days. After reducing the temperature to 6°C, the body temperature of *Gpihbp1*$^{-/-}$ mice was significantly lower than in wild-type mice (Fig. 1C).

When wild-type mice were housed at 12°C for 2 days and then 6°C for an additional 2 days, the plasma triglyceride levels fell (Fig. 1D), consistent with earlier studies (16). Plasma triglyceride levels also fell in *Gpihbp1*$^{+/-}$ mice, where the plasma triglyceride levels are normal (2) (supplemental Fig. S1). The plasma triglyceride response in *Gpihbp1*$^{-/-}$ mice during cold exposure was paradoxical. Rather than declining, the triglyceride levels increased sharply (from 3,000–5,000 mg/dl to 13,000−19,000 mg/dl) (Fig. 1D). As expected, the food intake during cold exposure increased in both wild-type and *Gpihbp1*$^{-/-}$

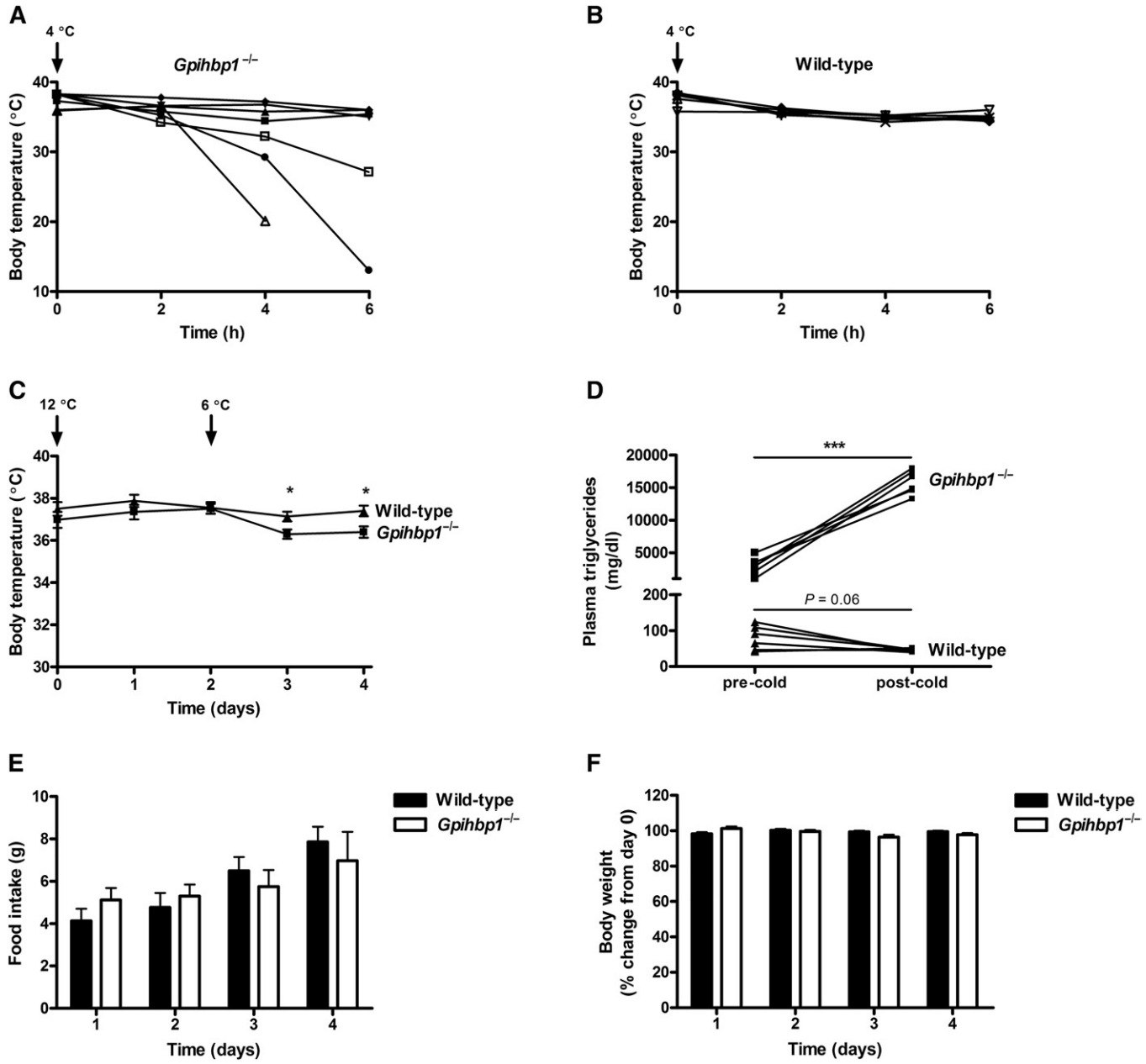

Fig. 1. Lower body temperatures and higher plasma triglyceride levels in *Gpihbp1*$^{-/-}$ mice during cold exposure. Body temperatures of *Gpihbp1*$^{-/-}$ (A) and wild-type (B) mice (n = 6−7/group) when mice were housed in the cold (4°C) for 6 h. C: Body temperatures of *Gpihbp1*$^{-/-}$ and wild-type mice (n = 6/group) when they were housed for 2 days at 12°C followed by 2 days at 6°C. Plasma triglyceride levels (D), food intake (E), and body weights (F) in the same mice used for the studies in panel C. Data in panels C, E, and F show mean ± SEM. *$P < 0.05$; ***$P < 0.001$.

mice—and to a similar degree (Fig. 1E). Body weight during cold exposure was stable in both groups of mice (Fig. 1F).

To be confident that the higher plasma triglyceride levels in *Gpihbp1*$^{-/-}$ mice during cold exposure were not due to increased food consumption, we performed a follow-up study in which food consumption was restricted to 3 g/day (the average amount consumed by *Gpihbp1*$^{-/-}$ mice at room temperature). Despite the restricted intake of food, the plasma triglyceride levels in *Gpihbp1*$^{-/-}$ mice increased sharply during cold exposure (**Fig. 2A**). The plasma triglyceride levels in *Gpihbp1*$^{-/-}$ mice also increased when the mice were fed a fat-free diet (Fig. 2B). We strongly suspect that the higher plasma triglyceride levels in *Gpihbp1*$^{-/-}$ mice during cold exposure were due to increased hepatic production of TRLs—simply because the release of fatty acids from adipose tissue during the cold would be expected to drive hepatic TRL production (30) and because TRL processing in *Gpihbp1*$^{-/-}$ mice is defective (1). Of note, biomedical scientists often infer changes in TRL production when plasma triglyceride levels change after inhibiting LPL activity with Triton WR-1339 (31, 32). LPL-mediated TRL processing is profoundly defective in *Gpihbp1*$^{-/-}$ mice at baseline; hence, studies with Triton WR-1339 would make little sense. We suspected that increased TRL production in cold-exposed *Gpihbp1*$^{-/-}$ mice would lead to higher plasma apoB levels. Indeed, this was the case (Fig. 2C, supplemental Fig. S2).

We also examined the relevance of apoC-III to the plasma triglyceride levels during cold exposure. apoC-III retards LPL-mediated TRL processing, limiting access of LPL to TRL substrates (33). In transgenic mice that overexpress human apoC-III, TRL processing by the LPL–GPIHBP1 complex is impaired (34), causing hypertriglyceridemia. When human apoC-III transgenic mice were exposed to the cold, the plasma triglyceride levels fell sharply (**Fig. 3A**), suggesting that the higher LPL activity in BAT during cold exposure (35, 36) outweighs the ability of apoC-III to limit LPL accessibility to triglyceride substrates (33, 34). We also examined mice deficient in both apoC-III and GPIHBP1 (*Gpihbp1*$^{-/-}$*Apoc3*$^{-/-}$). In those mice, the baseline plasma triglyceride levels on a chow diet (1,248 ± 424 mg/dl) were lower than in *Gpihbp1*$^{-/-}$ mice (3,966 ± 576 mg/dl). Nevertheless, the plasma triglyceride levels in *Gpihbp1*$^{-/-}$*Apoc3*$^{-/-}$ mice increased sharply during cold exposure (Fig. 3B), as in *Gpihbp1*$^{-/-}$ mice (Fig. 3C). Thus, apoC-III deficiency has little effect on the plasma triglyceride response of *Gpihbp1*$^{-/-}$ mice during cold exposure.

We suspected that *Angptl4* deficiency, which stabilizes LPL (5, 6) and increases LPL activity in BAT (35, 36), might increase the processing of TRLs in cold-exposed *Gpihbp1*$^{-/-}$ mice and prevent the spike in plasma triglyceride levels. Earlier studies showed that plasma triglyceride levels in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice are only about 10% of those in mice lacking *Gpihbp1* alone (4, 37). We confirmed that finding and went on to show that the plasma triglycerides in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice fall during cold exposure (Fig. 3D). Because LPL activity in BAT increases during cold exposure (36) and because plasma triglyceride levels in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice fall during cold exposure, we suspected that BAT triglyceride hydrolysis would be greater in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice during cold exposure. To test our suspicion, we administered, by gastric gavage, [^{14}C]oleic acid and [^{3}H]retinol to *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice, housed either at room temperature or 6°C. We also tested *Gpihbp1*$^{-/-}$ mice that had been maintained on a fat-free diet for 3 weeks; the baseline plasma triglyceride levels in that group of *Gpihbp1*$^{-/-}$ mice (866 ± 334 mg/dl) were more comparable to those in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice (408 ± 170 mg/dl). We observed more ^{14}C in BAT in the *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice than in *Gpihbp1*$^{-/-}$ mice (**Fig. 4A**). Also, the amount of ^{14}C-labeled fatty acids remaining in the plasma was lower in the *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice than in *Gpihbp1*$^{-/-}$ mice (Fig. 4A). There were no differences in ^{14}C uptake by the liver (Fig. 4A). In our experiments, some of the ^{14}C-labeled fatty acids would have been oxidized and eliminated as carbon dioxide. The recovery of the ^{14}C was lower in cold-exposed *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice than in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice housed at room temperature, consistent with more TRL processing and fatty acid oxidation (supplemental Fig. S3).

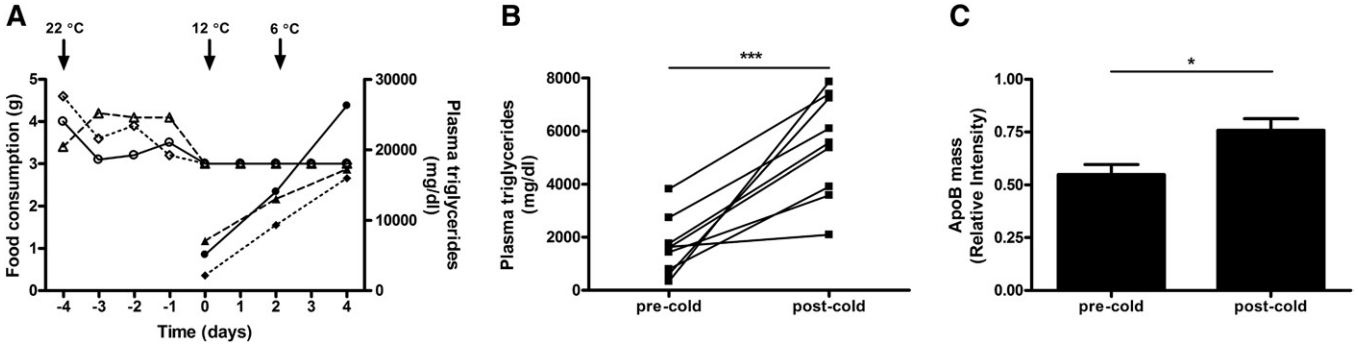

Fig. 2. Sharply increased plasma triglyceride levels in *Gpihbp1*$^{-/-}$ mice during cold exposure, even when food consumption is restricted. A: Food consumption in *Gpihbp1*$^{-/-}$ mice over 4 days (open symbols), after which the mice were exposed to cold and food was restricted to 3 g/day. The plasma triglyceride levels (closed symbols) increased sharply during cold exposure (2 days at 12°C followed by 2 days at 6°C). B: Plasma triglyceride levels in *Gpihbp1*$^{-/-}$ mice (n = 9) maintained on a fat-free diet, before and after cold exposure (2 days at 12°C followed by 2 days at 6°C). C: Bar graph comparing relative amounts of apoB in the plasma before and after cold exposure (using the same mice as in panel B). *$P < 0.05$; ***$P < 0.001$.

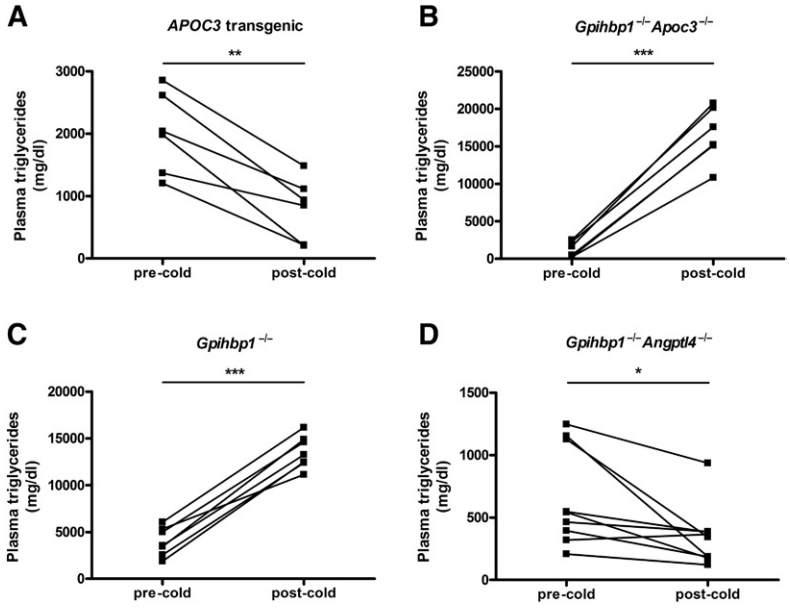

Fig. 3. Plasma triglyceride response during cold exposure (2 days at 12°C followed by 2 days at 6°C). *APOC3* transgenic mice (n = 6) (A); *Gpihbp1*$^{-/-}$*Apoc3*$^{-/-}$ mice (n = 6) (B); *Gpihbp1*$^{-/-}$ mice (n = 7) (C); *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice (n = 9) (D). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Together, our findings support the idea of increased TRL processing in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice, particularly during cold exposure. We found increased [^{3}H]retinol uptake in the BAT of cold-exposed *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice (Fig. 4B), consistent with an earlier study (16) suggesting that TRL particles can be taken up by BAT during cold exposure.

Why is there more TRL processing in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice, given that GPIHBP1 deficiency impairs transport of LPL to the capillary lumen? One possibility is that, despite an absence of GPIHBP1, small amounts of LPL reach the capillary lumen in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice. To test that idea, we performed immunohistochemistry on BAT, focusing on whether small amounts of LPL reach the luminal surface of capillary endothelial cells in the absence of GPIHBP1. The presence (or absence) of LPL in the capillary lumen was judged by imaging capillaries containing endothelial cell nuclei, where the basolateral and luminal surfaces of capillaries are separated and therefore easily visualized by confocal microscopy (3, 29). Nearly all of the LPL in the BAT from wild-type mice was bound to GPIHBP1 on capillary endothelial cells—and was clearly present along the capillary lumen (**Fig. 5A**, B). In *Gpihbp1*$^{-/-}$ mice, the LPL was mislocalized to the interstitial spaces surrounding adipocytes and endothelial cells (Fig. 5A), and no LPL could be detected in the capillary lumen (Fig. 5B). In *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice, the LPL was mislocalized to the interstitial spaces, and as expected the LPL signal was more intense than in *Gpihbp1*$^{-/-}$ mice (Fig. 5A). In contrast to findings in *Gpihbp1*$^{-/-}$ mice, small amounts of LPL were detectable along the capillary lumen in the BAT of *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice (Fig. 5B). In these experiments, we also examined, as an experimental control, the BAT of *Lpl* knockout mice carrying a skeletal muscle–specific human LPL transgene (Fig. 5A, B). In those mice, we could not detect mouse LPL in BAT, either in the interstitial spaces or along the capillary lumen. The absence of mouse LPL in these control studies increased our confidence that the small amounts of LPL along the capillary lumen in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice were not an artifact.

DISCUSSION

We assessed the effects of *Gpihbp1* deficiency on body temperature and plasma triglyceride levels during cold exposure. When *Gpihbp1*$^{-/-}$ and wild-type mice were housed at 4°C, nearly half of the *Gpihbp1*$^{-/-}$ mice, but none of the wild-type mice, developed hypothermia. When the two groups of mice were tested in a milder cold-exposure protocol (2 days at 12°C followed by an additional 2 days at 6°C), the body temperature in the *Gpihbp1*$^{-/-}$ mice was lower than in wild-type mice. These findings show that the defect in intravascular lipolysis associated with *Gpihbp1* deficiency results in cold intolerance. Neither the metabolic flux of triglycerides through tissues of *Gpihbp1*$^{-/-}$ mice nor the utilization of plasma fatty acids or glucose is sufficient for maintaining body temperature. Our findings provide support for the notion that the TRL-mediated delivery of fatty acid fuel to BAT is important for thermogenesis. It is now clear that defective intravascular lipolysis (e.g., GPIHBP1 deficiency), defective entry of fatty acids into mitochondria (e.g., CPT-1 deficiency) (38), defects in intracellular fatty acid trafficking (e.g., FABP3 deficiency) (11), and deficiencies in mitochondrial enzymes that metabolize fatty acids (e.g., LCAD) (39) cause cold intolerance. A deficiency of CD36, a putative fatty acid transporter, also leads to cold intolerance (13, 14). In the current study, we had hoped to determine whether a combined deficiency of GPIHBP1 and CD36 would cause an exaggerated cold intolerance phenotype. Unfortunately, despite exhaustive breeding efforts, only one *Gpihbp1*$^{-/-}$*Cd36*$^{-/-}$ mouse survived to weaning, and that mouse was very small and died before any experiments could be performed.

When we embarked on our studies, we suspected that the plasma triglyceride levels in *Gpihbp1*$^{-/-}$ mice during

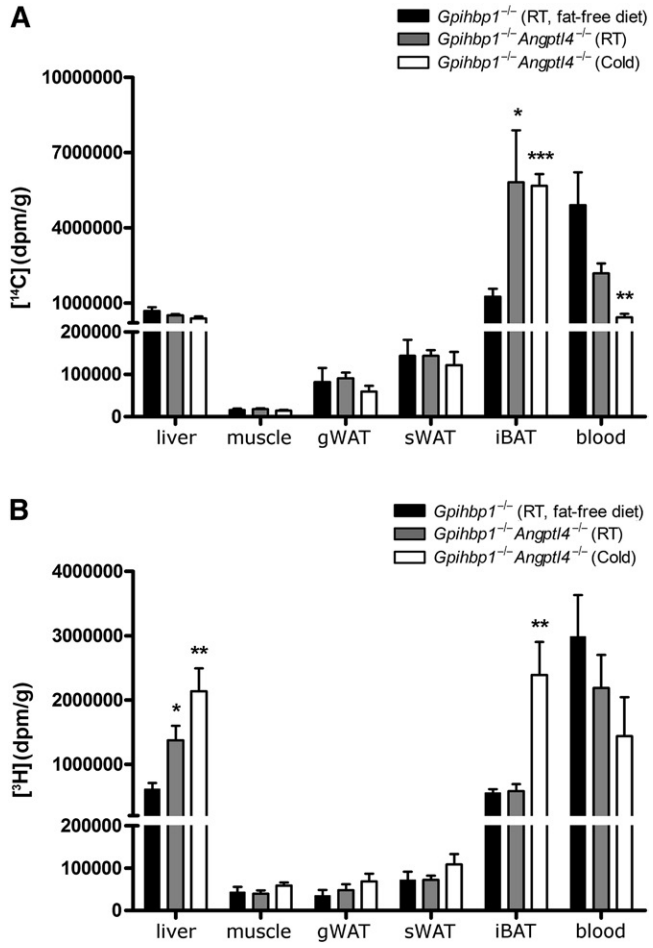

Fig. 4. Increased uptake of TRL-derived fatty acids in the BAT of *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice. Tissue distributions of [^{14}C]oleic acid (A) and [^{3}H]retinol (B) in mice housed at room temperature (RT) or in the cold (2 days at 12°C followed by 2 days at 6°C), 3 h after administering the radioisotopes by gastric gavage. *Gpihbp1*$^{-/-}$ (RT) (black bars, n = 5); *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ (RT, gray bars, n = 4), *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ (cold) (white bars, n = 5). Graphs show mean ± SEM. gWAT, gonadal white adipose tissue; iBAT, interscapular BAT; sWAT, subcutaneous white adipose tissue. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

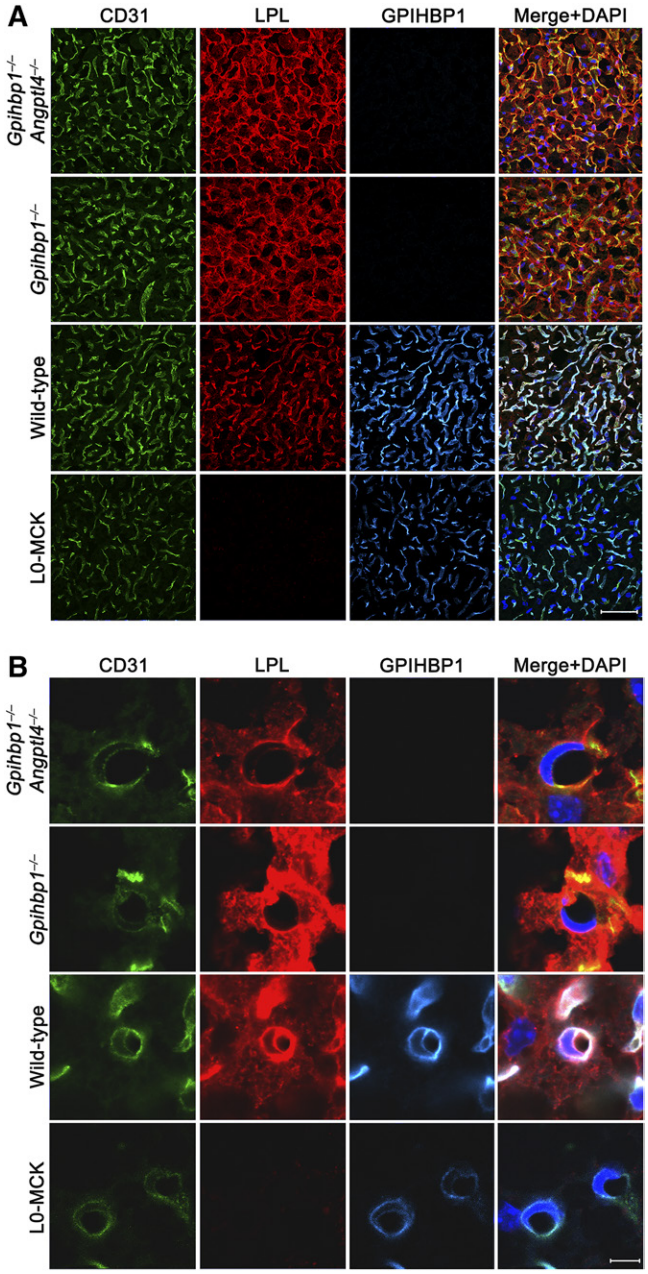

Fig. 5. Small amounts of LPL reach the capillary lumen in BAT from cold-exposed *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice. Immunofluorescence microscopy on sections of BAT from *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$, *Gpihbp1*$^{-/-}$, wild-type, and *Lpl*$^{-/-}$ mice carrying a skeletal muscle–specific human LPL transgene (L0-MCK) after subjecting the mice to the cold (2 days at 12°C followed by 2 days at 6°C). Shown are representative images (two mice per group). Sections were stained with antibodies against CD31 (green), LPL (red), and GPIHBP1 (cyan). DNA was stained with DAPI (blue). A: Images recorded with a 40× objective; scale bar, 50 μm. B: Images recorded with a 63× objective and a 4× digital zoom; scale bar, 5 μm.

cold exposure might fall but that the extent of the fall would be blunted. To our surprise, we observed a sharp increase in plasma triglyceride levels in *Gpihbp1*$^{-/-}$ mice during cold exposure. In hindsight, this finding made perfect sense. Exposure to the cold stimulates lipolysis in adipose tissue (17–19), releasing fatty acids for the production of hepatic TRLs (which in the setting of GPIHBP1 deficiency cannot undergo normal processing). As noted earlier, biomedical scientists who study lipid metabolism in mice often infer changes in hepatic TRL production rates based on changes in plasma triglyceride levels when LPL activity is blocked (31, 32). In *Gpihbp1*$^{-/-}$ mice, we suspect that increased hepatic TRL production, combined with the block in TRL processing (2), explains the sharp increase in plasma triglyceride levels during cold exposure.

The baseline plasma triglyceride levels in *Gpihbp1*$^{-/-}$*Apoc3*$^{-/-}$ mice were lower than in *Gpihbp1*$^{-/-}$ mice, but the absence of apoC-III did not protect *Gpihbp1*$^{-/-}$*Apoc3*$^{-/-}$ mice from the sharp increases in plasma triglycerides during cold exposure. Indeed, the sharp increases in plasma triglyceride levels in cold-exposed *Gpihbp1*$^{-/-}$ and *Gpihbp1*$^{-/-}$*Apoc3*$^{-/-}$ mice were similar, suggesting that apoC-III deficiency has little ability to increase TRL processing in the setting of *Gpihbp1* deficiency.

In contrast to the findings in *Gpihbp1*$^{-/-}$*Apoc3*$^{-/-}$ mice, the plasma triglyceride levels fell in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice during cold exposure. In vitro biochemical studies have shown that ANGPTL4 promotes the unfolding of LPL's hydrolase domain, leading to LPL inactivation (5, 6), and recent studies showed that ANGPTL4 reduces LPL secretion from adipocytes (35, 36). Also, more LPL is released into the plasma of *Angptl4*$^{-/-}$ mice after an injection of heparin (35). In light of these observations, and because baseline plasma triglyceride levels in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice are much lower than in *Gpihbp1*$^{-/-}$ mice (4, 37), it is clear that LPL-mediated TRL processing is greater in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice than in *Gpihbp1*$^{-/-}$ mice. Indeed, we found more oleate uptake in the BAT of *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice. Also, immunohistochemistry studies revealed large amounts of LPL in the interstitial spaces of BAT in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice, along with very small amounts of LPL in the capillary lumen. Our findings suggest that small amounts of LPL are present in the capillary lumen in the absence of GPIHBP1, conceivably by binding to heparan sulfate proteoglycans on the basolateral surface of endothelial cells and then crossing those cells by caveolar transport. The fact that plasma triglyceride levels in adult chow-fed *Lpl*$^{-/-}$ mice (40, 41) are higher than in adult *Gpihbp1*$^{-/-}$ mice (2, 3, 10) is consistent with the idea that small amounts of LPL-mediated TRL processing occur in *Gpihbp1*$^{-/-}$ mice.

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