OBJECTIVE—Hepatic steatosis is strongly associated with insulin resistance, but a causal role has not been established. In ob/ob mice, sterol regulatory element binding protein 1 (SREBP1) mediates the induction of steatosis by upregulating target genes, including glycerol-3-phosphate acyltransferase-1 (Gpat1), which catalyzes the first and committed step in the pathway of de novo glycerolipid synthesis. We asked whether ob/ob mice lacking Gpat1 would have reduced hepatic steatosis and improved insulin sensitivity.

RESEARCH DESIGN AND METHODS—Hepatic lipids, insulin sensitivity, and hepatic insulin signaling were compared in lean (Lep⁺/⁺), lean-Gpat1⁻/⁻, ob/ob (Lep⁻/obs/obs), and ob/ob-Gpat1⁻/⁻ mice.

RESULTS—Compared with ob/ob mice, the lack of Gpat1 in ob/ob mice reduced hepatic triacylglycerol (TAG) and diacylglycerol (DAG) content 59 and 74%, respectively, but increased acyl-CoA levels. Despite the reduction in hepatic lipids, fasting glucose and insulin concentrations did not improve, and insulin tolerance remained impaired. In both ob/ob and ob/ob-Gpat1⁻/⁻ mice, insulin resistance was accompanied by elevated hepatic protein kinase C-ε activation and blunted insulin-stimulated Akt activation.

CONCLUSIONS—These results suggest that decreasing hepatic steatosis alone does not improve insulin resistance, and that factors other than increased hepatic DAG and TAG contribute to hepatic insulin resistance in this genetically obese model. They also show that the SREBP1-mediated induction of hepatic steatosis in ob/ob mice requires Gpat1.

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Ob/ob mice are leptin deficient and become hyperphagic, obese, and insulin resistant (1). Severe hepatic steatosis in ob/ob mice is secondary to hepatic upregulation of sterol regulatory element binding protein 1 (SREBP1) and its target genes that increase lipogenesis and triacylglycerol (TAG) synthesis (2). Among these target genes is Gpat1, which encodes glycerol-3-phosphate acyltransferase-1, the mitochondrial enzyme that catalyzes the reaction glycerol-3-phosphate + long-chain acyl-CoA → lysophosphatidic acid, the committed step in the pathway of de novo glycerolipid synthesis (3). Four isoforms of glycerol-3-phosphate acyltransferase (GPAT), each encoded by a separate gene, are able to initiate the glycerolipid synthesis (4), but only Gpat1 is regulated by SREBP1 (5). GPAT1 activity and mRNA are highest in tissues with a high capacity for TAG synthesis; in liver, the GPAT1 isoform accounts for 30–50% of total GPAT activity. Hepatic GPAT1 activity and mRNA abundance are increased by insulin via activation of liver X receptor (LXR) and SREBP1c during conditions that promote TAG synthesis (5). Conversely, glucagon, fasting, and streptozotocin-induced diabetes decrease liver GPAT1 mRNA and activity (6).

Elevated hepatic TAG content in humans has been linked to insulin resistance (7–9). In rats increasing hepatic TAG content by adenovirus-mediated overexpression of GPAT1 induced hepatic and peripheral insulin resistance within 5–7 days (10). Conversely, Gpat1⁻/⁻ mice fed a high-fat safflower oil diet for 3 weeks were protected from hepatic steatosis and insulin resistance (11).

Although studies strongly suggest that reducing hepatic steatosis improves hepatic insulin sensitivity (11–14), it is unlikely that the accumulation of hepatic TAG itself, rather than another glycerolipid metabolite such as diacylglycerol (DAG), plays a causal role in impaired hepatic insulin signaling (15). Furthermore, the role of GPAT1 in hepatic steatosis has been questioned because both Gpat1⁻/⁻ mice fed a high-fat/high-sucrose diet for 4 months (16) and an independently derived Gpat1⁻/⁻ mouse strain fed a high-fat diet for 3 months (17) developed hepatic steatosis and became insulin resistant, although both developed in the context of obesity.

Because the insulin-sensitizing effects that occur when deleted Gpat1 reduces hepatic steatosis appear to be highly sensitive to diet composition and duration, we investigated ob/ob mice to determine whether the absence of Gpat1 would improve hepatic steatosis and insulin resistance associated with genetic-induced obesity. Hepatic steatosis in ob/ob mice is associated with a 2.3-fold increase in GPAT1 activity and a 5.5-fold increase in Gpat1 mRNA (18). Because a 5-day 90% adenovirus-mediated knockdown of hepatic Gpat1 in ob/ob mice resulted in a 42% reduction in hepatic TAG and a 30% decrease in plasma glucose (19), we hypothesized that a total lack of Gpat1 in ob/ob mice would prevent both hepatic steatosis and insulin resistance.
RESEARCH DESIGN AND METHODS

Mice. Animal protocols were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee. Mice were housed in a pathogen-free barrier facility on a 12-h light/dark cycle with free access to water and food (Prolab 5P76 Isopro 3000; 5.5% fat by weight). To generate Gpat1−/− mice on an obese background, Gpat1+/− mice (20), which have been backcrossed to C57BL/6J mice seven times, were crossed with Lep−/− mice (B6.V-Lepob/J; The Jackson Laboratory, Bar Harbor, ME). After crossing the double heterozygotes, Lep−/−Gpat1−/− or Lep−/−Gpat1+/− mice were then intercrossed to generate Lep−/−Gpat1−/− or Lep−/−Gpat1+/− mice and their respective lean (Lep+/−) littermates. Male mice were used in all experiments. At 16 weeks, total lean and fat mass was measured using an EchoMRI-100 QMRI system (Echo Medical Systems, LLC, Houston, TX). Fasted (12 h) 16-week-old mice were killed by cervical dislocation, and trunk blood was collected. Collated blood was centrifuged at 1,500g for 20 min and sera were stored at −80°C until analyzed. Tissues were quickly harvested, weighed, snap-frozen with liquid nitrogen, and stored at −80°C until analyzed. Liver sections were fixed with 4% paraformaldehyde, paraffin-embedded, sectioned (0.5 μm), and stained with hematoxylin-eosin.

Serum chemistries and lipids. Serum nonesterified fatty acids and total cholesterol (Wako Diagnostics, Richmond, VA), β-hydroxybutyrate (Staibio, Boeime, TN), and triacylglycerol and cholesterol (free cholesterol and triacylglyceride reagents; Sigma-Aldrich). Data are expressed as equivalent extracts were analyzed for TAG by an enzymatic assay (Triglyceride and Cholesterol Reagents, Wako Diagnostics, Richmond, VA). Serum chemistries and lipids, as the net area contained between individual baselines (set by the glucose value at time 0) and curves using the trapezoidal rule (24).

Western blot analysis. Protein kinase Cε (PKCe) membrane translocation was determined by immunoblotting cytosol and membrane protein extracts (20 μg) with rabbit anti-PKCe (1:100; Santa Cruz Biotechnology, Santa Cruz, CA) (25,26). Translocation for each sample was determined as the ratio of the density of PKCe of the membrane fraction over the density of PKCe of the cytosolic fraction.

To evaluate insulin signaling, after an overnight (12 h) fast, insulin (2 units/kg body wt) or PBS was administered intraperitoneally to 6- or 16-week-old mice. After 7.5 min, mice were killed by cervical dislocation, and livers were excised and snap-frozen in liquid nitrogen. Tissues were then homogenized in lysis buffer (20 mmol/l Tris, 150 mmol/l NaCl, 1 mmol/l EDTA, 1 mmol/l dithiorthreitol) with 10 up-and-down strokes with a Teflon-glass motor-driven homogenizer. Homogenates were centrifuged at 100,000g for 1 h to obtain total membrane fractions. Membrane pellets were rehomogenized in Medium I. Gpat specific activity was assayed at room temperature in a 200 μl reaction mixture containing 75 mmol/l Tris-HCl, pH 7.5, 1 μmol/l ATP, 20 μmol/l MgCl2, 1 μmol/l BSA (essentially fatty acid-free), 1 mmol/l dithiorthreitol, 8 mmol/l NaF, 800 μmol/l [3H]glycerol 3-phosphate, and 80 μmol/l palmitoyl-CoA (20). The reaction was initiated by adding 10–30 μg of membrane protein after incubating the membrane protein on ice for 15 min in the presence or absence of 2 mmol/l N-ethylmaleimide (NEM), which inactivates Gpat isoforms 2, 3, and 4. NEM-resistant activity (Gpat1) was calculated by subtracting NEM-sensitive activity from total activity.

Quantitative real-time RT-PCR. RNA was extracted from liver using Trizol (Invitrogen, Carlsbad, CA) and reverse-transcribed with the High-Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA). cDNA was amplified by real-time PCR in a total reaction volume of 25 μl using Absolute QPCR Sybr Green Fluorescein Mix (Thermoscientific, Waltham, MA). Primer sets are identified in supplementary Table 1, available in an online appendix at http://diabetes.diabetesjournals.org/content/early/2010/03/02/db09-1380/suppl/DC1. Target gene expression was normalized to endogenous β-actin and ex-pressed as a percentage of the Gpat1+/− group (21).

Tissue lipid metabolites. Tissues were homogenized in 10× (wt/vol) cold lysis buffer (20 mmol/l Tris base, 50 mmol/l NaCl, 250 mmol/l sucrose, 50 mmol/l NaF, 5 mmol/l Na2HPO4·10 H2O, 1% Triton X-100, and proteinase inhibitors). Lipids were extracted (22), dried under N2 gas, and solubilized in 3:1 (vol/vol/vol) tert-butanol, methanol, and Triton X-100 (23). Tissue lipid extracts were analyzed for TAG by an enzymatic assay (Trygliceride and Free-Glycerol reagents; Sigma-Aldrich). Data are expressed as equivalent

Insulin tolerance tests. At 15 weeks of age, after an overnight (12 h) fast, mice were injected intraperitoneally with insulin (Humulin; R; Eli Lilly, Indianapolis, IN) at either 0.5 units/kg body wt (lean mice) or 1.5 units/kg body wt (ob/ob mice). Glucose in tail vein blood was measured immediately before injection (time 0) and at 15, 30, 60, 90, and 120 min after injection (One Touch Ultra glucometer; LifeScan, Milpitas, CA). Areas of the curves were calculated as the net area contained between individual baselines (set by the glucose value at time 0) and curves using the trapezoidal rule (24).

RESULTS

The absence of Gpat1 diminished hepatic steatosis in ob/ob mice. Hepatic steatosis in ob/ob mice is mediated by SREBP1c, which increases the expression of lipogenic genes (27), including Gpat1 (5). To determine the contribution of Gpat1 to the development of hepatic steatosis in ob/ob mice, we intercrossed ob/ob and Gpat1−/− mice. Compared with lean littermates, total hepatic Gpat specific activity in ob/ob mice was 33% higher, and both Gpat1 activity (NEM-resistant Gpat1; Fig. 1A) and mRNA expression (Fig. 1A) were reduced to levels observed in lean mice, and Gpat1 activity was diminished. The absence of Gpat1 induced a 28% increase in NEM-sensitive Gpat activity (Gpat2, Gpat3, and Gpat4) in ob/ob mice (Fig. 1A). This increase in NEM-sensitive Gpat activity was likely due to increases in both Gpat3 and -4 expression, although only the increase of Gpat3 mRNA expression in ob/ob mice was significant (supplementary Fig. 1B and C). Collectively, the data suggest that elevated Gpat1 activity is required for the increase in TAG accumulation in ob/ob liver (28). Furthermore, the slight increase in activity and message of Gpat3 and Gpat4 did not compensate for the decrease in total Gpat activity in Gpat1−/− mice.

As previously reported (28), ob/ob mice developed grossly steatotic livers with macrosteatosis and microsteatosis that weighed four times more than livers from lean littermates (Table 1) and accumulated nearly five times as much TAG (Fig. 2A and B). In ob/ob-Gpat1−/− mice, the lack of Gpat1 reduced liver weight by 28% and hepatic TAG content by 59%, evidenced by the absence of large lipid droplets. In ob/ob livers, the lipogenic genes fatty acid synthase (Fasn) and stearyl-CoA desaturase 1 (Scd1) were increased 4- and 6.4-fold, respectively. However, neither Fasn and Scd1 nor transcription factors that regulate lipogenesis, SREBP1 (Srebf1) and ChREBP (Mlxipl), were affected by the lack of Gpat1 (supplement-
FIG. 1. A: Total, NEM-resistant (NEM-R), and NEM-sensitive (NEM-S) Gpat1 specific activities. Liver total particulate fractions (n = 3–4) were assayed for Gpat1 activity in the presence or absence of 2 mmol/L NEM as described in the “Research Design and Methods” section. B: Hepatic gene expression of Gpat1 was determined by quantitative RT-PCR and expressed as 2−ΔΔct relative to the endogenous control 18S rRNA and the lean-Gpat1+/− group (n = 4). Lean (Lep+/+) and ob/ob (Lep−/−) mice. Data are LSM ± SE. Significant differences (P < 0.05) are denoted by different letters. SA, specific activity.

Table 1

| Physiologic parameters | Lean (Lep+/+) | Gpat1+/− | Gpat1−/− | ob/ob (Lep−/−) | Gpat1+/− | Gpat1−/− | Factors (P value) |
|------------------------|--------------|---------|---------|----------------|---------|---------|------------------|
| Final body wt (g)      | 28.8 ± 1.0a  | 28.1 ± 0.9a | 57.2 ± 1.0b | 55.0 ± 0.9b | <0.001 | 0.133 | 0.407 |
| Lean mass (% body wt)  | 86.3 ± 0.9a  | 86.3 ± 1.3a | 44.5 ± 0.9b | 41.9 ± 1.4b | <0.001 | 0.473 | 0.090 |
| Fat mass (% body wt)   | 13.0 ± 0.9a  | 11.8 ± 1.3a | 54.9 ± 0.9b | 57.8 ± 1.4b | <0.001 | 0.274 | 0.257 |
| Liver weight (g)       | 1.04 ± 0.14a | 1.09 ± 0.17a | 3.90 ± 0.14a | 2.67 ± 0.16b | <0.001 | <0.001 | <0.001 |

Serum analytes

| Nonesterified fatty acids (mmol/l) | 1.03 ± 0.09 | 0.97 ± 0.09 | 1.17 ± 0.09 | 1.19 ± 0.1 | 0.056 | 0.826 | 0.672 |
| Triacylglycerol (mmol/l)           | 1.04 ± 0.14b | 0.81 ± 0.16a | 1.42 ± 0.14b | 1.43 ± 0.16b | 0.004 | 0.464 | 0.448 |
| Glycerol (mmol/l)                  | 0.46 ± 0.06b | 0.32 ± 0.07a | 0.56 ± 0.06b | 0.54 ± 0.07b | 0.019 | 0.215 | 0.378 |
| Total cholesterol (mmol/l)         | 3.11 ± 0.39a | 2.96 ± 0.39a | 6.86 ± 0.39a | 5.81 ± 0.39b | <0.001 | 0.129 | 0.254 |
| β-hydroxybutyrate (mmol/l)         | 0.99 ± 0.08c | 0.83 ± 0.07a | 0.44 ± 0.07a | 0.52 ± 0.08b | 0.001 | 0.141 | 0.710 |

Final body weights (n = 12–16) and fat and lean masses (n = 6–13) were measured at 16 weeks. Serum analytes (n = 4–9) were measured from sera of mice fasted for 12 h. Values represent LSM ± SE with significant differences (P < 0.05) within rows denoted by different letters. Factors include the main effects of phenotype (Pheno; lean or ob/ob) and Gpat1 and their interaction (Int).
Changes in hepatic lipids due to lack of Gpat1.

Increases in the content of hepatic lipids such as DAG, acyl-CoA, and ceramide have been proposed to mediate hepatic insulin resistance (29,30). Compared with lean mice, total hepatic DAG in \textit{ob/ob} liver was five times higher (Fig. 3A), and when Gpat1 was absent, DAG content decreased to normal levels. The major DAG species reflected this pattern, with the largest differences apparent in C18:1-C18:1 and C18:1-C16:0 (Fig. 3B).

In Gpat1-null mice, liver acyl-CoA content is elevated twofold because acyl-CoAs are substrates for GPAT1 (11). Although phenotype (lean or \textit{ob/ob}) did not have an effect on total hepatic acyl-CoA content, both lean and \textit{ob/ob} livers deficient in Gpat1 contained 3.3- and 5.3-fold more...
total acyl-CoA than their Gpat1+/+ counterparts, respectively (Fig. 3C). All the reported acyl-CoA species were increased, but the major one affected was 18:1-CoA, with 3.8- and 6.0-fold increases in lean- and ob/ob-Gpat1+/+ mice, respectively (Fig. 3D).

In addition to TAG synthesis and β-oxidation, acyl-CoAs may also be directed toward the synthesis of ceramide, another purported modulator of insulin resistance (31). Compared with lean mice, the content of ceramide in livers from ob/ob mice was 25% lower (Fig. 3E), reflected primarily by fewer long-chain ceramides, C22:0 and C24:0 (Fig. 3F), even though C16:0 ceramide was higher. Gpat1 deficiency did not significantly affect total ceramide content, but decreased C22:0 and C24:0 ceramides by ~52% in lean mice. Thus, total ceramide content was not associated with insulin resistance in this model.

Lack of Gpat1 did not restore hepatic insulin signaling in ob/ob mice. Because both DAG and TAG decreased markedly in Gpat1-deficient liver, we examined the effect of Gpat1 deficiency on insulin signaling. Hepatic steatosis...
contributes to insulin resistance via increased DAG activation of PKCε and subsequent impairment of the insulin signaling pathway (11). To determine whether the improved hepatic lipid profile rescued hepatic insulin signaling, we examined activation of PKCε and Akt. In 16-week-old mice, concomitant with impaired insulin sensitivity, compared with lean mice, ob/ob mice had nearly twofold higher hepatic PKCε activation, marked by the increased ratio of membrane to cytosolic PKCε (Fig. 4A). Despite having a content of hepatic DAG similar to that of lean mice, PKCε activation was elevated in ob/ob-Gpat1−/− liver, although to a lesser extent than in ob/ob mice. At 6 weeks, no differences in hepatic PKCε activation were detected among groups (supplementary Fig. 4A, B). Activated PKCε binds and inactivates the insulin receptor kinase, leading to impaired downstream insulin signaling, such as Akt activation (32). When either 16- or 6-week-old lean mice were stimulated with insulin, the amount of phosphorylated Akt in liver dramatically increased compared with basal expression (Fig. 4B and supplementary Fig. 4B). In ob/ob mice, however, the response to insulin was blunted by at least 65%, and this blunted response was not rescued when Gpat1 was deficient. These data indicate that, although hepatic TAG and DAG content was considerably lower in ob/ob-Gpat1−/− mice, hepatic insulin signaling did not improve.

**Lack of Gpat1 did not reduce genetic obesity.** Insulin resistance both in the liver and in peripheral tissues such as muscle and fat can contribute to whole-body insulin resistance. Although Gpat1 constitutes only 10% of total GPAT activity in adipose tissue, adipose GPAT1 specific activity is high (5), and female Gpat1−/− mice have reduced body and gonadal adipose depot weights (20). We determined whether the absence of Gpat1 would reduce obesity in ob/ob mice. At 16 weeks, ob/ob mice weighed twice as much as their lean littermates (Fig. 5A; Table 1). However, the absence of Gpat1 in lean or ob/ob mice did alter body weights over time or final body weights in either male (Fig. 5A; Table 1) or female (data not shown) mice. Compared with lean mice, gonadal, retroperitoneal, and inguinal white adipose and subscapular brown adipose tissue depots weighed dramatically more in the ob/ob mice, and were unaffected by the absence of Gpat1 (Fig. 5B). Quantitative nuclear magnetic resonance analysis confirmed that the Gpat1 deficiency did not have an effect on total lean or fat mass as a percentage of body weight (Table 1).

**Lack of Gpat1 did not improve muscle lipids.** Obesity-mediated increases in muscle TAG content correlate strongly with whole-body insulin resistance (29). Even though GPAT1 specific activity is very low in muscle (5), we examined lipids in muscle to determine whether changes in muscular lipid could affect whole-body insulin resistance. In gastrocnemius muscle, the content of TAG and DAG in ob/ob mice was 4- and 2.3-fold higher, respectively (supplementary Fig. 5A and B). The absence of Gpat1 in lean or ob/ob mice had no effect on muscle TAG or DAG content or on the DAG species present (supplementary Fig. 5C). Muscle acyl-CoA and ceramide content and molecular species in ob/ob mice were also unaffected by the absence of Gpat1 (supplementary Fig. 5D–G).

**The absence of Gpat1 did not alter serum lipids.** Corresponding with the development of obesity, serum cholesterol and TAG were elevated in ob/ob mice (33), whereas in Gpat1−/− mice, serum cholesterol is normal and serum TAG was reduced (11). The absence of Gpat1,
however, did not diminish serum lipids in ob/ob mice (Table 1). As reported previously in Gpat1<sup>−/−</sup> mice (16), β-hydroxybutyrate was higher in both lean and ob/ob-Gpat1<sup>−/−</sup> mice, although this effect was not statistically significant.

**DISCUSSION**

Several studies have demonstrated that reducing hepatic TAG content decreases hepatic insulin resistance (9,11–14). In the liver, loss- and gain-of-function studies with Gpat1 support a role for hepatic steatosis in the development of insulin resistance (4). Gpat1<sup>−/−</sup> mice are protected from high-fat diet–induced hepatic steatosis and insulin resistance (11), whereas rats that overexpress Gpat1 in liver have increased hepatic TAG accumulation and hepatic insulin resistance (10). Here, we show that the absence of Gpat1 in both 6- and 16-week-old ob/ob mice prevents severe hepatic steatosis but does not protect against obesity-associated insulin resistance and impaired hepatic insulin signaling.

The absence of Gpat1 reduced hepatic TAG content by 59% in ob/ob mice; however, the TAG content was still nearly double that of lean, insulin-sensitive mice. Similarly, in ob/ob-Srebp<sub>1c</sub>−/− mice, the hepatic TAG content is less than half that of ob/ob mice, but still almost twice that of lean mice (2). The reduced hepatic TAG content in ob/ob-Srebp<sub>1c</sub>−/− mice occurs simultaneously with a 70% reduction of mRNA for hepatic Gpat1, a direct target of SREBP1 (5). Like our ob/ob-Gpat1<sup>−/−</sup> mice, despite marked decreases in hepatic steatosis, ob/ob-Srebp1<sup>−/−</sup> mice also remain obese and insulin resistant. In ob/ob mice, the absence of either Srebp1 or Gpat1 results in similar decreases in hepatic TAG. Thus, GPAT1 appears to be responsible for most of SREBP1-regulated hepatic TAG accumulation.

Although the hepatic TAG content of 16-week-old ob/ob-Gpat1<sup>−/−</sup> mice was similar to levels present in mice with diet-induced insulin resistance (16,34,35), even at 6 weeks, ob/ob-Gpat1<sup>−/−</sup> mice were hyperinsulinemic, insulin resistant, and had impaired hepatic insulin signaling despite the fact that their hepatic TAG content was lower than that of their insulin-sensitive lean littermates. These results demonstrate that in ob/ob mice, preventing hepatic steatosis did not protect against insulin resistance, which is consistent with other studies reporting a dissociation between hepatic steatosis and insulin resistance (2,16,36–38).

Because cellular TAG is stored in lipid droplets, and thus segregated from signaling events, it is believed that other, perhaps related, lipid metabolites interfere with insulin signaling pathways (15,39). Although the accumulation of acyl-CoAs has been associated with insulin resistance (40,41), mice that overexpress diacylglycerol acyltransferase-2 in liver and high-fat–fed Gpat1<sup>−/−</sup> mice have increased hepatic acyl-CoA content but do not develop insulin resistance (11,36). Hepatic acyl-CoA content is normal in ob/ob mice and increases when Gpat1 is absent, but the extent of insulin resistance does not change. Thus, in ob/ob liver, increased acyl-CoA content itself does not impair insulin signaling. Similarly, the absence of GPAT1 increases the content of palmitoyl-CoA, the substrate for the first step in ceramide biosynthesis. However, the absence of Gpat1 did not increase hepatic ceramide content; thus, ceramide did not contribute to hepatic insulin resistance in this model.

DAG has been most strongly associated with insulin resistance because it activates PKC-θ in the muscle (39,42) and PKCε in the liver (32,43), leading to reductions in downstream insulin signaling. Gpat1<sup>−/−</sup> mice that are protected from safflower oil–induced hepatic insulin resistance have reduced hepatic DAG content and PKCε activation, concomitant with improved insulin signaling (11); conversely, rats that overexpress Gpat1 in the liver showed elevated DAG and PKCε activation coupled with hepatic insulin resistance (10). In the current study, however, despite normalized hepatic DAG content in ob/ob-Gpat1<sup>−/−</sup> mice, hepatic insulin signaling remained impaired. In this study, the decrease in DAG probably reflects a reduction in lipid droplet DAG content rather than membrane DAG (44), because activated PKCε was unaffected. Alternatively, a signal other than DAG that is generated during metabolic dysfunction may be responsible for PKC activation.

Studies linking reduced hepatic steatosis to improved insulin sensitivity also report concomitant decreases in adiposity (14,45). Because adipose mass was not diminished in the ob/ob-Gpat1<sup>−/−</sup> mice, any improvements in either hepatic or whole-body insulin resistance due to lower hepatic lipids could well be overshadowed by other...
factors that interfere with hepatic insulin signaling (46). Rodents deficient in leptin or the leptin receptor secrete excess corticosterone (47,48). Administering a liver-selective glucocorticoid receptor antagonist to leptin receptor-deficient rats increases glucose disposal and decreases hepatic glucose production and fasting glucose (49), demonstrating that increased glucocorticoid signaling impairs hepatic and peripheral insulin sensitivity. Thus, it is likely that elevated plasma glucocorticoid levels or other obesity-related factors may have prevailed over any improvements in insulin sensitivity arising from an improved hepatic lipid profile in this model.

Fasting serum glucose and insulin concentrations were 21 and 48% higher, respectively, in ob/ob-Gpat1+/− mice than in ob/ob mice despite similar insulin sensitivity. Similarly, Gpat1−/− mice fed a high-fat/high-sucrose diet are less glucose tolerant and have 11% higher fasting glucose and 64% higher insulin levels than wild-type mice (16), and ob/ob mice with a liver-specific Gpat1 knockdown have a twofold increase in plasma insulin (19). Hyperinsulinemia can be caused by β-cell hypertrophy and hyperplasia (50), but we did not observe differences in islet size or number (data not shown). Even at 6 weeks, lean and ob/ob-Gpat1+/− mice had 56 and 27% higher fasting insulin levels, respectively, than their Gpat1+/+ counterparts (supplementary Fig. 3C), suggesting that Gpat1−/− mice are hyperinsulinemic, unrelated to the presence of insulin resistance.

In summary, we have demonstrated that Gpat1 is critical for the accumulation of TAG and DAG during the development of hepatic steatosis and is a major enzyme responsible for the lipogenic effect of SREBP1 in ob/ob mice (2). These results suggest that decreasing hepatic steatosis alone does not improve insulin resistance in this genetically obese model, and that factors other than increased hepatic DAG and TAG contribute to hepatic insulin resistance in ob/ob mice.

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REFERENCES

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372:425–432
2. Yabagi N, Shimano H, Hasty AH, Matsuzuka T, Ide T, Yoshikawa T, Amemiya-Kudo M, Tomita S, Okazaki H, Tamura Y, Izuoka Y, Ohashi K, Osuga J, Harada K, Gotoda T, Nakanishi A, Aihara K, Katsumata H, Itoh K, Ikeda S, Endo H, Itoh N, Ishioka M, Inagaki H, Saito S, Kurihara Y, Kadowaki T, Ouchi N, Yamashita T, Takahashi N, Shinoda Y, Miyazaki H, Ohno T, Takahashi K, Sugita M, Kimura Y, Takahashi T, Hotta K, Kadowaki T, Minokoshi Y, Nakao K, Kihara S, Kadowaki T, Kihara Y, Tanaka K. Downregulation of SREBP-1c in muscle is associated with decreased body weight and fat mass. J Clin Invest 2005;115:1396–1405
3. Gonzalez-Barroso MR, Lewin TM, Coleman RA. Regulation of triglyceride metabolism: II, function of mitochondrial Gpat1 in the regulation of triacylglycerol biosynthesis and insulin action. Am J Physiol Gastrointest Liver Physiol 2007;292:G1105–G1109
4. Wendel AA, Lewin TM, Coleman RA. Glycerol-3-phosphate acyltransferases: rate limiting enzymes of triacylglycerol biosynthesis. Biochim Biophys Acta 2009;179:501–506
5. Coleman RA, Lee DP. Enzymes of triacylglycerol synthesis and their regulation. Prog Lipid Res 2004;43:134–176
6. Shin DH, Paulauskas JD, Moustaid M, Sun HS. Transcriptional regulation of p66Shc with sequence homology to Escherichia coli glycerol-3-phosphate acyltransferase. J Biol Chem 1991;266:23834–23839
7. Kim HH, Kim HJ, Lee KE, Kim DJ, Kim SK, Ahn CW, Lim SK, Kim KR, Lee HC, Huh KB, Cha BS. Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. Arch Intern Med 2004;164:2169–2175
8. Soppla-Lindoors A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Siirtola J, Halavaara J, Yki-Jarvinen H. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J Clin Endocrinol Metab 2002;87:3023–3028
9. Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. Diabetes 2005;54:603–608
10. Nagle CA, An J, Shiota M, Torres TP, Cline GW, Liu ZX, Wang S, Catlin BL, Shulman GI, Newgard CB, Coleman RA. Hepatic overexpression of glycerol-sn-3-phosphate acyltransferase 1 in rats causes insulin resistance. J Biol Chem 2007;282:14807–14815
11. Neschen S, Morino K, Hammond LE, Zhang D, Liu ZX, Romanelli AJ, Cline GW, Pongratz RL, Zhang XM, Choi CS, Coleman RA, Shulman GI. Prevention of diet-induced hepatic steatosis and hepatic insulin resistance in mitochondrial acyl-CoA:glycerol-sn-3-phosphate acyltransferase 1 knockout mice. Cell Metab 2005;2:55–65
12. Savage DB, Choi CS, Samuel VT, Liu ZX, Zhang D, Wang A, Zhang XM, Cline GW, Yu X, Geisler JG, Bhanot S, Monia BP, Shulman GI. Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and 2. J Clin Invest 2006;116:817–824
13. Dentin R, Bhenamed F, Hainault I, Fauveille V, Foufelle F, Dyck JR, Girard J, Pistic C. Liver-specific inhibition of SREBP1 improves hepatic steatosis and insulin resistance in ob/ob mice. Diabetes 2006;55:2191–2197
14. Choi CS, Savage DB, Kulkarni A, Yu X, Liu ZX, Morino K, Kim S, Di Stefano A, Samuel VT, Neschen S, Zhang D, Wang A, Zhang XM, Kahl M, Cline GW, Pantely SK, Geisler JG, Bhanot S, Monia BP, Shulman GI. Suppression of diacylglycerol acyltransferase-2 (DGAT2), but not DGAT1, with antisense oligonucleotides reverses diet-induced hepatic steatosis and insulin resistance. J Biol Chem 2007;282:23267–23288
15. Savage DB, Petersen KF, Shulman GI. Disordered lipid metabolism and the pathogenesis of insulin resistance. Physiol Rev 2007;87:507–529
16. Hammond LE, Neschen S, Romanelli AJ, Cline GW, Ikayeva OR, Shulman GI, Muino DM, Coleman RA. Mitochondrial glycerol-3-phosphate acyltransferase-1 is essential in liver for the metabolism of excess acyl-CoAs. J Biol Chem 2005;280:25629–25636
17. Yazdi M, Ahumark A, William-Olsson L, Suaith M, Turner N, Osla F, Wedin M, Astedt AK, Elmgren A, Bohlooly M, Schreyer S, Linde´n D. The role of mitochondrial glycerol-3-phosphate acyltransferase-1 in regulating lipid and glucose homeostasis in high-fat diet fed mice. Biochem Biophys Res Commun 2008;369:1055–1070
18. Linde´n D, William-olsson L, Rheidn M, Astedt AK, Clapham JC, Schreyer S. Overexpression of mitochondrial Gpat in rat hepatocytes leads to decreased fatty acid oxidation and increased glycerolipid biosynthesis. J Lipid Res 2004;45:1279–1288
19. Xu H, Wilcox D, Nguyen P, Voorbach M, Suhar T, Morgan SJ, An WF, Ge L, Green J, Wu Z, Gimeno RE, Reilly R, Jacobson PB, Collins CA, Landschulz K, Surowy T. Hepatic knockdown of mitochondrial Gpat1 in ob/ob mice improves metabolic profile. Biochem Biophys Res Commun 2006;349:439–448
20. Hammond LE, Gallagher PA, Wang S, Hiller S, Kluckman KD, Posey-Marcos EL, Maeda N, Coleman RA. Mitochondrial glycerol-3-phosphate acyltransferase-deficient mice have reduced weight and liver triacylglycerol content and altered glycerolipid fatty acid composition. Mol Cell Biol 2002;22:8204–8214
21. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001;25:402–408
22. Folch J, Lees M, Sloan-Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226:497–500
23. Dannho Jinchyo B, Budiyanto S, Furukawa Y, Kimura S. A simple enzymatic quantitative analysis of triglycerides in tissues. J Nutr Sci Vitaminol (Tokyo) 1992;38:517–521
24. Wolever TM, Jenkins DJ, Jenkins AL, Josse RG: The glycemic index: methodology and clinical implications. Am J Clin Nutr 1991;54:846–854
25. Qu X, Seale JP, Donnelly R. Tissue and isoform-selective activation of protein kinase C in insulin-resistant obese Zucker rats: effects of feeding. J Endocrinol 1999;162:207–214
26. Donnelly R, Reed MJ, Azhar S, Reaven GM. Expression of the major isoenzyme of protein kinase C in skeletal muscle, nPKC theta, varies with muscle type and in response to fructose-induced insulin resistance. Endocrinology 1994;135:2369–2374
27. Shimomura I, Bashmakov Y, Horton JD. Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes meliitus. J Biol Chem 1999;274:30289–30302
28. Mayer J, Bates MW, Dickie MM. Hereditary diabetes in genetically obese mice. Science 1953;115:746–747
29. Shulman GI. Cellular mechanisms of insulin resistance. J Clin Invest 2000;106:171–176
30. Nagle CA, Klett EL, Coleman RA. Hepatic triacylglycerol accumulation and insulin resistance. J Lipid Res 2009;50(Suppl.):S74–S79
31. Holland WL, Brozinick JT, Wang LP, Hawkins ED, Sargent KM, Liu Y, Narra K, Hoehn KL, Knotts TA, Siesky A, Nelson DH, Karathanasakis SK, Fontenot GK, Birnbaum MJ, Summers SA. Inhibition of ceramide synthesis ameliorates glaucorticoid-, saturated-fat-, and obesity-induced insulin resistance. Cell Metab 2007;5:167–179
32. Samuel VT, Liu ZX, Wang A, Beddow SA, Geisler JG, Kahn M, Zhang XM, Monia BP, Bhanot S, Shulman GI. Inhibition of protein kinase Cepsilon prevents hepatic insulin resistance in nonalcoholic fatty liver disease. J Clin Invest 2007;117:739–745
33. Lindstrom P. The physiology of obese-hyperglycemic mice [ob/ob mice]. ScientificWorldJournal 2007;7:366–385
34. Yu XX, Murray SF, Pandey SK, Booten SL, Bao D, Song XZ, Kelly S, Chen M, Kress RE, Trowell RC, Bain JR, Newgard CB, Farese RV Sr, Farese RV Jr. Overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc Natl Acad Sci USA 2001;98:7523–7527
35. Kim JK, Gimeno RE, Higashimori T, Kim HH, Choi H, Punreddy S, Mozell RL, Song Y, Stricker-Krongrad A, Hirsch DJ, Fillmore JJ, Liu ZX, Dong J, Cline G, Stahl A, Lodish HF, Shulman GI. Inactivation of fatty acid transport protein 1 prevents fat-induced insulin resistance in skeletal muscle. J Clin Invest 2004;113:756–763
36. Griffin ME, Marucci MJ, Cline GW, Bell K, Barucci N, Lee D, Goodyear LJ, Kraegen EW, White MF, Shulman GI. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. Diabetes 1999;48:1270–1274
37. Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, Romanelli AJ, Shulman GI. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. J Biol Chem 2004;279:32345–32353
38. Shin OH, da Costa KA, Mar MH, Zeisel SH. Hepatic protein kinase C is not activated despite high intracellular 1,2-sn-diacylglycerol in obese Zucker rats. Biochim Biophys Acta 1997;1358:72–78
39. Nambu JM, Miyazaki M, Stoehr JP, Liu H, Kendzorski CM, Yandell BS, Song Y, Cohen P, Friedman JM, Attie AD. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. Proc Natl Acad Sci USA 2002;99:11482–11486
40. Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest 2000;106:473–481
41. Livingstone DE, Jones GC, Smith K, Jamieson PM, Andrew R, Kenyon CJ, Walker BR. Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. Endocrinology 2000;141:158–176
42. Matuszak A, Shimano H, Yahagi N, Kato T, Atsumi A, Yamamoto T, Inoue N, Ishikawa M, Okada S, Ishigaki N, Iwasaki H, Iwasaki Y, Karasawa T, Kumadaki S, Matsui T, Sekiya M, Ohashi K, Hasty AH, Nakagawa Y, Takahashi A, Suzuki H, Sone H, Toyoshima H, Osuga J, Yamada K, Nakanishi K. Crucial role of a long-chain fatty acid elongase, Elovl6, in obesity-induced insulin resistance. Nat Med 2007;13:1193–1202
43. Greathurst A, Hoekstra J, Derks TG, Ouwens DM, Bailer JF, Havinga R, Havekes LM, Romijn JA, Kuipers F. Acute hepatic steatosis in mice by blocking beta-oxidation does not reduce insulin sensitivity of very-low-density lipoprotein production. Am J Physiol Gastrointest Liver Physiol 2005;289:G592–G598
44. Yu C, Chen Y, Cline GW, Zhang D, Zong H, Wang Y, Bergeron R, Kim JK, Cushman SW, Cooney GJ, Archeson B, White MF, Kraegen EW, Shulman GI. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. J Biol Chem 2002;277:50230–50236
45. Kim JK, Fillmore JJ, Chen Y, Yu C, Moore IK, Pypaert M, Lutz EP, Kako Y, Velez-Carrasco W, Goldberg LJ, Breslow JL, Shulman GI. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc Natl Acad Sci USA 2001;98:7523–7527
46. Shulman GI. Mechanism by which fatty acids inhibit insulin activation. Diabetes 2005;54:591–602