Targeted deletion of thioesterase superfamily member 1 promotes energy expenditure and protects against obesity and insulin resistance

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Mammalian acyl-CoA thioesterases (Acots) catalyze the hydrolysis of fatty acyl-CoAs to form free fatty acids plus CoA, but their metabolic functions remain undefined. Thioesterase superfamily member 1 (Them1; synonyms Acot11, StarD14, and brown fat inducible thioesterase) is a long-chain fatty acyl-CoA thioesterase that is highly expressed in brown adipose tissue and is regulated by both ambient temperature and food consumption. Here we show that *Them1*$^{-/-}$ mice were resistant to diet-induced obesity despite greater food consumption. *Them1*$^{-/-}$ mice exhibited increased O_2 consumption and heat production, which were accompanied by increased rates of fatty acid oxidation in brown adipose tissue and up-regulation of genes that promote energy expenditure. *Them1*$^{-/-}$ mice were also protected against diet-induced inflammation in white adipose tissue, as well as hepatic steatosis, and demonstrated improved glucose homeostasis. The absence of Them1 expression in vivo and in cell culture led to markedly attenuated diet- or chemically induced endoplasmic reticulum stress responses, providing a mechanism by which Them1 deficiency protects against insulin resistance and lipid deposition. Taken together, these data suggest that Them1 functions to decrease energy consumption and conserve calories. In the setting of nutritional excess, the overproduction of free fatty acids by Them1 provokes insulin resistance that is associated with inflammation and endoplasmic reticulum stress.

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Following uptake into cells, free fatty acids (FFAs) are activated by esterification to CoA (CoASH) molecules by acyl-CoA synthetases (1). Fatty acyl-CoAs may then be oxidized or incorporated into complex lipids; they may also participate in intracellular signaling or as transcription factor ligands. Mammalian cells also express acyl-CoA thioesterases (Acots), which hydrolyze fatty acyl-CoAs and release FFAs plus CoASH. Although postulated to regulate intracellular concentrations of fatty acyl-CoAs, FFAs, and CoASH (2, 3), the biological functions of Acots remain unclear.

Acots are classified into two types. Type 1 enzymes (Acots 1–6) contain N-terminal β-sandwich and C-terminal α/β-hydrolase domains, whereas type 2 (Acots 7–13) contain hot dog-fold thioesterase domains (2). In addition to two hot dog-fold thioesterase domains, Acots 11 and 12 each contain a steroidogenic acute regulatory protein-related lipid transfer (START) domain at the C terminus and are named StarD14 and 15, respectively, within the START domain superfamily (4). START domains bind hydrophobic ligands and participate in lipid sensing (4). Synonyms for Acot11/StarD14 are thioesterase superfamily member 1 (Them1) and the brown fat inducible thioesterase (BFIT).

Them1/StarD14/BFIT/Acot11 (hereafter Them1) was identified as a gene that is regulated by ambient temperature in mouse brown adipose tissue (BAT) (5): Them1 mRNA levels were up-regulated in BAT by cold exposure and down-regulated by warm temperatures. These levels were also higher in BAT of mouse strains that resist diet-induced obesity. These observations led to speculation that Them1 promotes energy expenditure.

To explore its role in energy homeostasis and to gain insights into the metabolic function of an Acot, we created mice lacking Them1. In contrast to expectations, *Them1*$^{-/-}$ mice exhibited marked increases in energy expenditure. When challenged with a high-fat diet, these mice exhibited improved glucose and lipid homeostasis and were protected against diet-induced obesity, inflammation, and hepatic steatosis.

Results

Them1-Deficient Mice Are Resistant to Diet-Induced Obesity. Consistent with prior observations for steady-state mRNA levels (5), Them1 protein was highly expressed in BAT (Fig. S1*A*), but was also expressed at lower levels in other tissues, including liver and white adipose tissue (WAT). Subcellular fractionation of BAT demonstrated that Them1 was localized mainly to cytosol, mitochondria, and the endoplasmic reticulum (ER) (Fig. S1*B*). In contrast, in liver Them1 was enriched in cytosol. As was previously described (5), Them1 mRNA levels were strongly induced in BAT by reductions in ambient temperature, with lowest expression at thermoneutrality (30 °C) and a 90-fold increase at 4 °C (Fig. S1*C*). These changes were reflected by Them1 protein levels (Fig. S1*D*). In keeping with regulation of Them1 by food intake (5), Them1 mRNA and protein expression in BAT was down-regulated by high-fat feeding (Fig. S1 *E* and *F*).

To explore the biological function of Them1, we developed *Them1*$^{-/-}$ mice using a gene-targeting strategy that disrupted both thioesterase domains, as well as the C-terminal START domain (Fig. S2*A*). Mice lacking Them1 mRNA and protein (Fig. S2 *B*–*F*) were viable and exhibited normal development. There were no gross anatomic or histologic abnormalities observed in a comprehensive tissue survey. Body weights did not differ between genotypes at weaning, and growth curves were similar for chow-fed *Them1*$^{-/-}$ and *Them1*$^{+/+}$ mice (Fig. 1*A*). When *Them1*$^{+/+}$ mice were fed the high-fat diet, they gained excess weight over time. In contrast, *Them1*$^{-/-}$ mice were resistant to diet-induced obesity, as evidenced by growth that was more similar to chow-fed animals (Fig. 1*A*): At 21 wk of age, high-fat-fed *Them1*$^{-/-}$ mice weighed 22% less than *Them1*$^{+/+}$ controls. Notably, when fed either diet, *Them1*$^{-/-}$ mice consumed nearly twice as much food as *Them1*$^{+/+}$ mice (Fig. 1*B*). Body compositions of chow-fed *Them1*$^{-/-}$ mice were similar to *Them1*$^{+/+}$ mice, with the exception that *Them1*$^{-/-}$ mice harbored high proportions of BAT (Fig. 1*C*). The high-fat diet increased the percentages of WAT and BAT in

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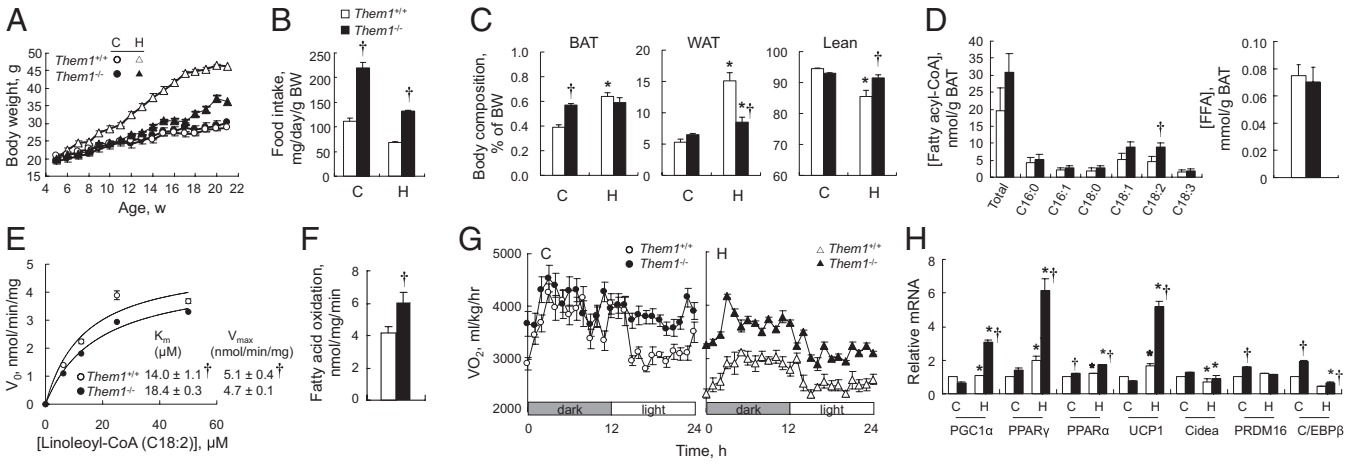

Fig. 1. Resistance to diet-induced obesity and increased energy expenditure in mice lacking Them1. (*A*) Mice were fed chow (C: *Them1*$^{+/+}$, $n = 10$; *Them1*$^{-/-}$, $n = 12$) or the high-fat diet (H: *Them1*$^{+/+}$, $n = 12$; *Them1*$^{-/-}$, $n = 16$) for up to 16 wk. (*B*) Food consumption was measured between 6 and 9 wk for mice in *A*. (*C*) BAT, WAT, and lean body mass (Lean) for 21-wk-old chow-fed (*Them1*$^{+/+}$, $n = 3$; *Them1*$^{-/-}$, $n = 4$) or high-fat-fed mice ($n = 4$ per group) were quantified by computed tomography. (*D*) Tissue concentrations of total and individual long-chain fatty acyl-CoAs, as well as total FFAs were determined for chow-fed mice ($n = 5$ per group). (*E*) Acot activity was determined in homogenates of BAT from chow-fed mice ($n = 3$ per group) using linoleoyl-CoA as the exogenous substrate. (*F*) Fatty acid oxidation rates were measured in BAT of chow-fed mice (*Them1*$^{+/+}$, $n = 6$; *Them1*$^{-/-}$, $n = 5$). (*G*) Values of VO_2 were determined by indirect calorimetry and normalized to lean body mass for mice fed chow ($n = 6$ per group) or the high-fat diet (*Them1*$^{+/+}$, $n = 10$; *Them1*$^{-/-}$, $n = 7$) for 16 wk. (*H*) Relative expression of selected genes was determined in BAT of mice fed high-fat or chow diets for 16 wk ($n = 3$ per group). *$P < 0.05$ high-fat vs. chow diet; †$P < 0.05$ *Them1*$^{-/-}$ vs. *Them1*$^{+/+}$ mice.

Them1$^{+/+}$ mice by 2.7- and 1.7-fold, respectively, with a correspondingly decreased lean mass. In contrast, high-fat-fed *Them1*$^{-/-}$ mice exhibited increases of only 1.3- and 1.1-fold (nonsignificant) in WAT and BAT, respectively, as well as a nonsignificant decrease in percentage of lean body mass. The total lean body mass of high-fat-fed *Them1*$^{-/-}$ mice was modestly lower than *Them1*$^{+/+}$ mice (Fig. S3*A*).

Fatty acyl-CoA Accumulation and Increased Fatty Acid Oxidation in BAT of *Them1*$^{-/-}$ Mice. In BAT of *Them1*$^{-/-}$ mice, there was trend ($P = 0.22$) toward a 59% increase in total concentrations of long-chain fatty acyl-CoAs (Fig. 1*D*). This trend was attributable to a significant increase in the most abundant long-chain fatty acyl-CoA [linoleoyl (C18:2)-CoA] along with nonsignificant increases in each of the other species. Presumably because of an excess of FFAs compared with fatty acyl-CoAs, we were unable to detect a corresponding decrease in steady-state tissue FFA concentrations in BAT (Fig. 1*D*). We did observe decreased sizes of intracellular lipid droplets in BAT of *Them1*$^{-/-}$ mice (Fig. S3*B*).

As with Them1 (Fig. S1*C*), mRNA expression of most of the other Acot genes in BAT varied as functions of decreasing ambient temperature (Fig. S4). However, only the transcripts for Acots 9/10, 12, and 13 also increased monotonically, albeit to much lesser extents than for Them1 (Fig. S1*C*). Deletion of Them1 resulted in altered expression of other Acots (Fig. S4), but these displayed similar patterns of response to ambient temperature as were observed in the presence of Them1. In general, these Acots were expressed at relatively higher levels in BAT of *Them1*$^{-/-}$ compared with *Them1*$^{+/+}$ mice, suggesting compensation for the absence of Them1 expression.

Because of the changes in expression of other Acot genes (Fig. S4), we determined the overall influence of Them1 expression on Acot activity in BAT using linoleoyl-CoA as a substrate (Fig. 1*E*). This determination revealed a 31% increase in K_m and an 8% decrease in V_{max} for BAT homogenates prepared from *Them1*$^{-/-}$ mice. To determine whether reduced hydrolysis of fatty acyl-CoA molecules altered their metabolism, we measured rates of fatty acid oxidation. There was a 46% increase in fatty acid oxidation rates of BAT from mice lacking Them1 (Fig. 1*F*). These findings indicate that compensatory changes in gene expression do not restore Acot activity to wild-type levels and that excess fatty acyl-CoAs are targeted for oxidation.

Lack of Them1 Expression Increases Energy Expenditure. In light of the observations that both chow and high-fat-fed mice consumed more food but did not gain excess weight, we used indirect calorimetry to explore evidence for increased energy expenditure in *Them1*$^{-/-}$ mice (Fig. 1*G* and Fig. S3*C*). In chow-fed mice, rates of oxygen consumption (VO_2) values were comparable for the two genotypes during the nocturnal phase, but were elevated during the light phase in *Them1*$^{-/-}$ mice. In high-fat-fed mice, there was a pronounced reduction in VO_2 values for *Them1*$^{+/+}$ mice. This reduction was nearly eliminated, however, in *Them1*$^{-/-}$ mice. Values of heat production (energy expenditure) for *Them1*$^{-/-}$ mice were also higher during the light phase for chow-fed mice and during both dark and light phases for high-fat-fed mice (Fig. S3*C*). For both diets, total and ambulatory activity were similar for *Them1*$^{+/+}$ and *Them1*$^{-/-}$ mice (Fig. S3 *D* and *E*), and there were no differences in plasma concentration of thyroid hormones T3 (chow diet: *Them1*$^{+/+}$ 0.65 ± 0.06 ng/mL, *Them1*$^{-/-}$ 0.43 ± 0.04 ng/mL; high-fat diet: *Them1*$^{+/+}$ 0.82 ± 0.06 ng/mL, *Them1*$^{-/-}$ 0.76 ± 0.14 ng/mL) or T4 (chow diet: *Them1*$^{+/+}$ 1.56 ± 0.32 μg/dL, *Them1*$^{-/-}$ 1.38 ± 0.23 μg/dL; high-fat diet: *Them1*$^{+/+}$ 1.08 ± 0.21 μg/dL, *Them1*$^{-/-}$ 0.61 ± 0.04 μg/dL).

We next examined the possibility that BAT-mediated thermogenesis was increased in *Them1*$^{-/-}$ mice. In BAT, mRNA levels of genes that regulate thermogenesis [peroxisome proliferator-activated receptor-α (PPARα), PPARγ coactivator-1α (PGC-1α), uncoupling protein 1 (UCP1), Cidea] and differentiation [PPARγ, PR domain-containing 16 (PRDM16), and CCAAT/enhancer binding protein β (C/EBPβ)] were similar in chow-fed *Them1*$^{-/-}$ and *Them1*$^{+/+}$ mice. In response to high-fat feeding, there was much more pronounced up-regulation of PGC-1α, PPARγ and UCP1 in *Them1*$^{-/-}$ mice (Fig. 1*H*). Based on the increased PGC-1α observed in high-fat-fed mice, we assayed markers of mitochondrial mass. *Them1*$^{-/-}$ mice fed the high-fat diet exhibited increased mitochondrial contents, as evidenced by higher values of mitochondrial DNA (Fig. S5*A*) and increased activity of citrate synthase (Fig. S5*B*). We further explored the influence of Them1 expression on BAT function and development using cultures of primary brown adipocytes (Fig. S5*C*). As observed in BAT, both thermogenic genes and genes

involved in glucose uptake (GLUT4 and IRS1) were up-regulated in cultured primary brown adipocytes from *Them1*$^{-/-}$ mice. In contrast, there were smaller changes in markers of adipose differentiation (PRDM16 and C/EBPβ) (6). Under these conditions, the UCP-1 inhibitor Cidea (7) was down-regulated.

The increases in fatty acyl-CoA concentrations in BAT and in genes related to glucose uptake in primary brown adipocytes from *Them1*$^{-/-}$ mice prompted us to examine whether Them1 expression might influence energy substrate utilization. Indicative of greater carbohydrate metabolism in the absence of Them1, values of respiratory exchange rate in *Them1*$^{-/-}$ mice were higher during the light phase when fed chow diet, and higher during the dark phase when the fed high-fat diet (Fig. S3*C*). This possibility was supported by an increase in 2-[1,2-^{3}H (N)]-deoxy-D-glucose ([^{3}H]-2-DOG) uptake in primary brown adipocytes that lacked Them1 expression (Fig. S5*D*).

Them1-Deficient Mice Exhibit Reduced Diet-Induced Inflammation in WAT. There were no changes in fatty acyl-CoA concentrations in WAT from chow-fed mice, which expressed Them1 at much lower levels than BAT. However, in WAT, expression of Them1 protein and mRNA was up-regulated by 16 wk of high-fat feeding (Fig. 2 *A* and *B*). Consistent with well-established observations that diet-induced obesity leads to inflammation in WAT (8), we observed the accumulation of macrophages in WAT from *Them1*$^{+/+}$ mice. In contrast, there was no appreciable high-fat diet-induced macrophage infiltration of WAT from *Them1*$^{-/-}$ mice (Fig. 2*C*). In agreement with this finding, the lack of Them1 expression was associated with blunted responses to high-fat feeding of plasma TNF-α and IL-6 concentrations (Fig. 2*D*). Expression levels of mRNAs encoding TNF-α, IL-6, CD11b, and monocyte chemotactic protein-1 (MCP-1) were reduced in WAT of chow and high-fat-fed mice (Fig. 2*E*).

Because Them1 mRNA and protein were both undetectable in bone marrow-derived macrophages from *Them1*$^{+/+}$ mice or LPS-treated macrophage cells, we examined evidence that Them1 expression in WAT could influence macrophage activation in chow-fed mice (i.e., in the absence of secondary metabolic changes that occurred in response to high-fat feeding) (9). Consistent with this possibility, induction of F4/80, IL-6, TNF-α, and MCP-1 gene expression was attenuated when macrophages were cultured in conditioned medium developed from the cultures of primary white adipocytes from chow-fed *Them1*$^{-/-}$ compared with *Them1*$^{+/+}$ mice (Fig. S6*A*). To determine whether the absence of Them1 expression promoted browning of WAT (10), we examined the expression in WAT of genes that regulate differentiation and thermogenesis of BAT. Instead, the absence of Them1 expression tended to decrease expression of several of these genes (Fig. S6*B*). Interestingly, mRNA expression of the adipokine leptin was increased in WAT from chow-fed *Them1*$^{-/-}$ compared with *Them1*$^{+/+}$ mice and tended to be increased in high-fat-fed *Them1*$^{-/-}$ mice (Fig. S6*C*).

***Them1*$^{-/-}$ Mice Are Protected Against Diet-Induced Hepatic Steatosis.** High-fat feeding results in hepatic steatosis (11), and this was the case for *Them1*$^{+/+}$ mice, as evidenced by increased liver-to-body weight ratios (Fig. 3*A*), gross (Fig. S7*A*) and histopathologic (Fig. 3*B*) steatosis, and increased hepatic concentrations of triglycerides and cholesterol (Fig. 3 *C* and *D*). In contrast, mice lacking Them1 were protected against the development of hepatic steatosis when judged by the same parameters. Whereas the high-fat diet did not alter hepatic FFA concentrations in *Them1*$^{+/+}$ mice, these were reduced in livers of *Them1*$^{-/-}$ mice compared with their chow-fed counterparts (Fig. 3*E*). Plasma concentrations of β-hydroxybutyrate, a metric of fatty acid oxidation, tended to increase in both chow and high-fat-fed *Them1*$^{-/-}$ mice, although these changes did not achieve statistical significance (Fig. 3*F*).

Whereas the high-fat diet did not influence plasma triglyceride concentrations in *Them1*$^{+/+}$ mice (Fig. S8*A*), high-fat feeding of *Them1*$^{-/-}$ mice resulted in a threefold increase. The high-fat diet increased plasma cholesterol concentrations in both genotypes, but values remained lower in *Them1*$^{-/-}$ mice (Fig. S8*B*). There were no differences in plasma FFA concentrations in *Them1*$^{+/+}$ and *Them1*$^{-/-}$ mice fed either chow or the high-fat diet (Fig. S8*C*). FPLC elution profiles were similar in chow-fed *Them1*$^{+/+}$ and *Them1*$^{-/-}$ mice, indicating that the reduction in plasma cholesterol was mainly attributable to lower HDL cholesterol concentrations. As previously described (12), the high-fat diet increased HDL concentrations and promoted formation of HDL1 particles in the plasma as observed for *Them1*$^{+/+}$ mice, but this was observed to a lesser extent in *Them1*$^{-/-}$ mice (Fig. S8*D*).

The high concentrations of triglycerides in plasma of high-fat-fed *Them1*$^{-/-}$ mice suggest that protection against hepatic steatosis was caused at least in part by increased hepatic triglyceride secretion. However, we also investigated expression of genes involved in the regulation of hepatic lipogenesis (Fig. 3*G*), fatty acid uptake (Fig. 3*H*), and fatty acid oxidation (Fig. 3*I*). Although similar in chow-fed mice, mRNA levels of sterol regulatory element binding protein 1c (SREBP-1c) were higher in high-fat-fed *Them1*$^{+/+}$ compared with *Them1*$^{-/-}$ mice (Fig. 3*G*). This finding was in keeping with reduced nuclear SREBP-1c protein in livers of high-fat-fed *Them1*$^{-/-}$ compared with *Them1*$^{+/+}$ mice (Fig. S7*B*). The SREBP-1c target genes stearoyl-CoA desaturase-1 and fatty acid synthase were also induced to

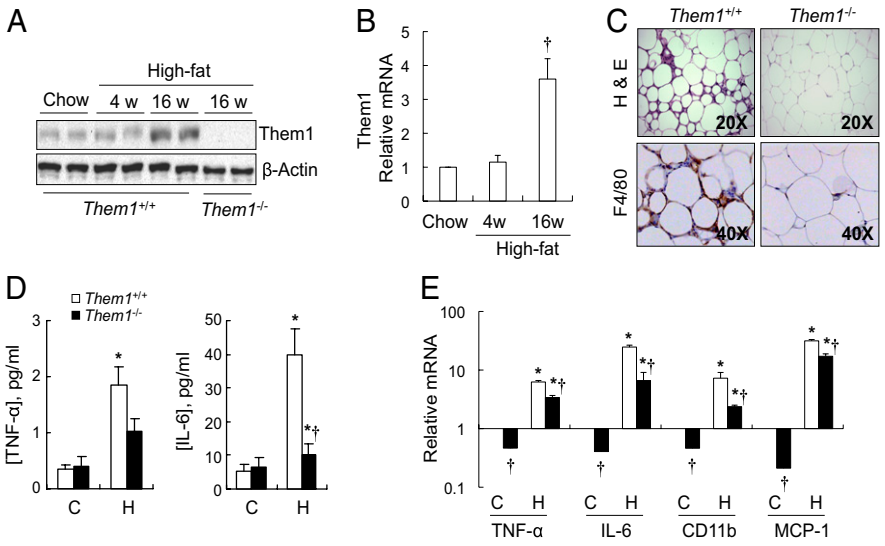

Fig. 2. High-fat diet-induced inflammation in WAT is attenuated by loss of Them1 expression. Influence of the high-fat feeding on (*A*) Them1 protein and (*B*) mRNA expression ($n = 3$ per group) in WAT. (*C*) Representative light microscopic images of epididymal fat pad sections from mice after 16 wk of high-fat feeding and stained with H&E or immunostained for F4/80. (*D*) Plasma concentrations of inflammatory cytokines in mice ($n = 5$ per group) fed chow or the high-fat diet for 16 wk. (*E*) Relative expression of selected inflammatory genes in epididymal WAT ($n = 3$ per group). *$P < 0.05$ high-fat vs. chow; †$P < 0.05$ *Them1*$^{-/-}$ vs. *Them1*$^{+/+}$.

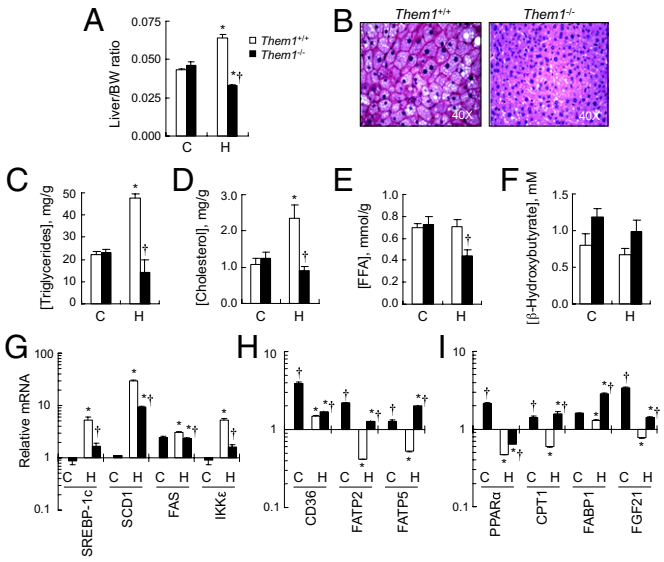

Fig. 3. Resistance to diet-induced hepatic steatosis in mice lacking Them1. (*A*) Liver-to-body weight ratios were determined for mice fed chow or 16 wk of the high-fat diet (*Them1*$^{+/+}$, $n = 4$ per group; *Them1*$^{-/-}$, $n = 3$ per group). (*B*) Representative light microscopic images of H&E-stained sections of livers following 16 wk of high-fat feeding. Hepatic concentrations (n = 3–5 per group) of (*C*) triglycerides, (*D*) cholesterol, and (*E*) FFA, as well as (*F*) plasma concentrations of β-hydroxybutryate. Relative expression of hepatic genes (n = 3 per group) that regulate (*G*) lipogenesis, as well as fatty acid (*H*) uptake and (*I*) oxidation in mice fed with chow or 16 wk of the high-fat diet. $^{*}P < 0.05$ high-fat vs. chow; $^{\dagger}P < 0.05$ *Them1*$^{-/-}$ vs. *Them1*$^{+/+}$.

a lesser extent by high-fat feeding in *Them1*$^{-/-}$ compared with *Them1*$^{+/+}$ mice. The same finding was true for IKKε, which has been identified as an important regulator of lipid biosynthesis in liver and WAT (11). Genes that encode the fatty acid transporters CD36, FATP2, and FATP5 were expressed to greater extents in livers of both chow- and high-fat-fed *Them1*$^{-/-}$ mice (Fig. 3*H*). Although PPARα and its target genes CPT1, FABP1, and FGF21 (Fig. 3*I*) were more highly expressed in livers of *Them1*$^{-/-}$ mice, we were unable to detect an associated increase in rates of hepatic fatty acid oxidation (*Them1*$^{+/+}$ 2.45 ± 0.34 nmol/mg/min; *Them1*$^{-/-}$ 1.96 ± 0.40 nmol/mg/min).

Hepatic lipid accumulation may initiate inflammation (13), and this can be observed in high-fat-fed mice (11). Whereas F4/80-positive Kupffer cells were readily detected in livers of *Them1*$^{+/+}$ mice, these were rarely observed in livers of *Them1*$^{-/-}$ mice (Fig. S7*C*). In keeping with reduced hepatic inflammation, steady-state mRNA levels of TNF-α, IL-6, and MCP-1 were also lower in livers of high-fat-fed *Them1*$^{-/-}$ mice (Fig. S7*D*).

Improved Glucose Homeostasis in *Them1*$^{-/-}$ Mice. The altered body composition, increased energy metabolism, and decreased markers of inflammation in *Them1*$^{-/-}$ mice prompted us to examine whether lack of Them1 expression influenced glucose homeostasis (Fig. 4). Glucose tolerance tests carried out after 16 wk of chow or high-fat feeding revealed more rapid glucose clearance in *Them1*$^{-/-}$ mice fed either diet (Fig. 4*A*). Similarly, plasma glucose concentrations declined more rapidly during insulin tolerance tests in chow- and high-fat-fed *Them1*$^{-/-}$ mice (Fig. 4*B*). *Them1*$^{-/-}$ mice exhibited reduced fasting glucose concentrations (Fig. 4*C*) in plasma in both chow and high-fat diets in the absence of differences in plasma insulin concentrations (Fig. 4*D*). Plasma leptin concentrations were not affected by genotype, but were increased by high-fat feeding in both *Them1*$^{-/-}$ and *Them1*$^{+/+}$ mice (Fig. 4*E*). Plasma adiponectin concentrations were influenced by neither diet nor genotype (Fig. 4*F*).

Attenuated ER Stress Response in the Absence of Them1 Expression. As an Acot, Them1 activity could contribute to insulin resistance and fatty liver by generating FFAs, which in turn promote ER stress (14). Suggestive of a role for Them1 in the generation of ER stress, 8 wk of high-fat feeding induced the expression of Grp78 in the livers of *Them1*$^{+/+}$ mice, but not *Them1*$^{-/-}$ mice (Fig. 5*A*). In mouse livers or cultured mouse embryonic fibroblast cells (MEFs), which also express Them1 (Fig. S9*A*), Them1 mRNA was increased by 5.4- and 1.5-fold, respectively, following tunicamycin treatment (Fig. 5*B* and Fig. S9*B*). In response to tunicamycin and thapsigargin, up-regulation of Grp78 was reduced in MEFs lacking Them1 expression (Fig. S9*C*). Moreover, in livers of tunicamycin-treated *Them1*$^{+/+}$ mice, as well as *Them1*$^{+/+}$ MEFs and hepatocytes treated with tunicamycin or thapsigargin, there was induction of genes that mediate the ER stress response: Chop, Grp78, ATF6, and spliced XBP-1 (Fig. 5*C*, and Figs. S9 *D–F* and S10). The induction of these genes was attenuated in the absence of Them1 expression.

Because ER stress induces lipogenesis and lipid droplet formation (15), we explored the role of Them1 in ER stress-mediated lipogenesis in vivo. After 2 d of tunicamycin administration, there was marked induction of lipid droplet formation in livers of *Them1*$^{+/+}$ but not *Them1*$^{-/-}$ mice (Fig. 5*D*). Upon induction of ER stress, hepatic triglyceride concentrations were increased by sixfold in *Them1*$^{+/+}$ mice, but only fourfold in *Them1*$^{-/-}$ mice (Fig. 5*E*). Although concentrations of FFA in liver tended to be lower in *Them1*$^{-/-}$ mice, these changes did not achieve statistical significance (Fig. 5*F*).

Discussion

BAT mediates nonshivering thermogenesis in mice (16) and may regulate energy homeostasis in humans (17). The up-regulation of Them1 in BAT by cold exposure along with the higher expression levels in obesity-resistant strains (5), suggested that *Them1*$^{-/-}$ mice might be obesity-prone. Instead, these mice exhibited increased energy expenditure and resistance to diet-induced obesity. Consistent with a BAT-mediated effect, there was greater up-regulation of thermogenic genes in *Them1*$^{-/-}$ mice, as well as elevated rates of fatty acid oxidation and glucose uptake. Interestingly, Cidea, which limits UCP1 activity (7), was down-regulated. These findings suggest that Them1 regulates whole body O_2 consumption, which is consistent with observations that BAT may consume more than 50% of both caloric intake and O_2 (16). Although it is possible that other oxidative tissues contributed to the changes in VO_2 observed in *Them1*$^{-/-}$ mice, Them1 expression levels in these tissues are quite low. Moreover, there was no associated increase in the physical activity of the mice or increase in thyroid function. Taken together, these data indicate that Them1 limits energy expenditure following the activation of BAT.

In agreement with its putative function as an Acot (5), the absence of Them1 expression in BAT led to increased fatty acyl-CoA concentrations. The substantial changes in gene expression of other Acots suggest that Them1 plays a role in regulating fatty acyl-CoA concentrations, but could reflect transcriptional responses to changes in BAT metabolism. Nevertheless, we could demonstrate a measurable impact of Them1 expression on the overall Acot activity of BAT homogenates, which supports a role for Them1 in the hydrolysis of fatty acyl-CoAs.

The interconversion of FFAs and fatty acyl-CoAs is important for the control of thermogenesis in BAT. Esterification of FFAs to form fatty acyl-CoAs facilitates their mitochondrial oxidation (18). Fatty acyl-CoAs also play a direct role in the biochemical activation of UCP1 (19). In addition to promoting thermogenesis, UCP1 activation stimulates high rates of glucose uptake and oxidation within BAT (20). This finding presumably explains both the increase in glucose uptake in cultured brown adipocytes that lack Them1 and the higher respiratory exchange rate values observed in *Them1*$^{-/-}$ mice. Finally, interconversion of FFAs and fatty acyl-CoA may determine the intracellular concentrations of ligands responsible for the activation of transcription factors

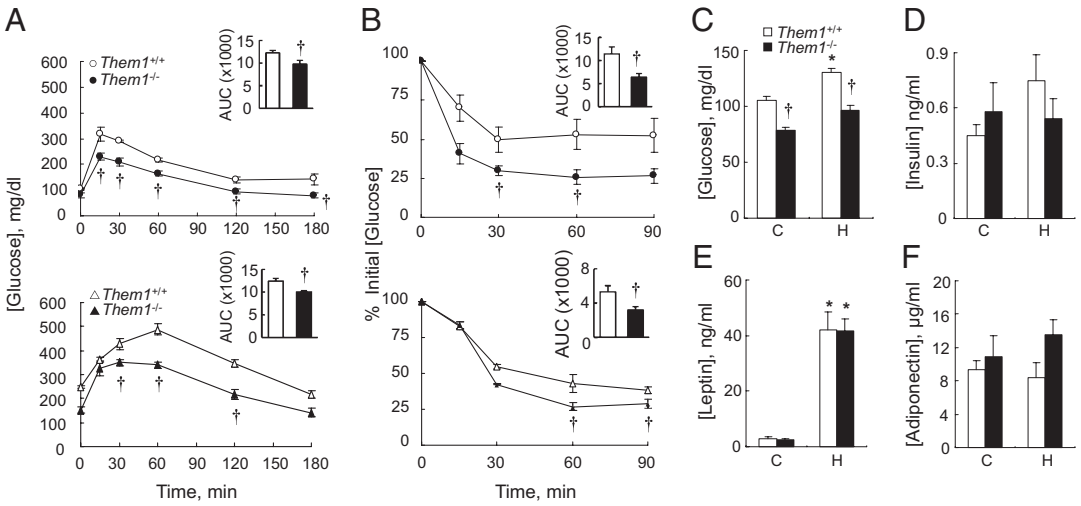

Fig. 4. Improved glucose homeostasis in *Them1*$^{-/-}$ mice. Tolerance tests to (*A*) glucose and (*B*) insulin were performed following a 12-h fast for mice (n = 10 per group) that were maintained on chow (*Upper*) or 16 wk of the high-fat diet (*Lower*). Values of areas under the curve (AUC) are shown as *Inset* barplots. Fasting plasma concentrations (n = 4–10 per group) of (*C*) glucose, (*D*) insulin, (*E*) leptin, and (*F*) adiponectin in mice fed chow or the high-fat diet for 16 wk. *P < 0.05 high-fat vs. chow; $^{\dagger}P$ < 0.05 *Them1*$^{-/-}$ vs. *Them1*$^{+/+}$.

(e.g., PPARγ and PPARα) that control differentiation and thermogenesis in BAT (3, 18). A role for Them1 in transcriptional regulation is supported by the differential regulation of these genes in *Them1*$^{-/-}$ mice.

Obesity is associated with a chronic inflammation in which production of TNF-α and other cytokines leads to infiltration of WAT with macrophages and other immune cells (21). A pathogenic role for Them1 was suggested by the blunted cytokine production and the absence of an inflammatory infiltrate in WAT of high-fat-fed *Them1*$^{-/-}$ mice. Whereas there were no discernable increases in fatty acyl-CoA concentrations in WAT from chow-fed *Them1*$^{-/-}$ mice, the likelihood that Them1 played a pathogenic role in the inflammatory response was supported by its up-regulation in response to high-fat diet feeding. High-fat diet-induced up-regulation of Acots 1, 2, and 7 has been observed in skeletal muscle and heart was interpreted as an adaptive response that protect against fatty acid oversupply (22). It was reasoned that up-regulation of these Acots should protect against lipotoxicity by reducing esterification to form proinflammatory lipid metabolites and by modulating fatty acid oxidation. In contrast, a more mechanistic study revealed a key role for macrophage Acot7 in enabling the inflammatory response, potentially via the generation of proinflammatory lipid mediators (23). Having demonstrated that Them1 was not expressed in macrophages, we provided evidence that its expression in white adipocytes might promote inflammation. The attenuated activation of macrophages following exposure to medium conditioned by white adipocytes from relatively young chow-fed *Them1*$^{-/-}$ mice suggested that differences in WAT inflammation precede the metabolic changes.

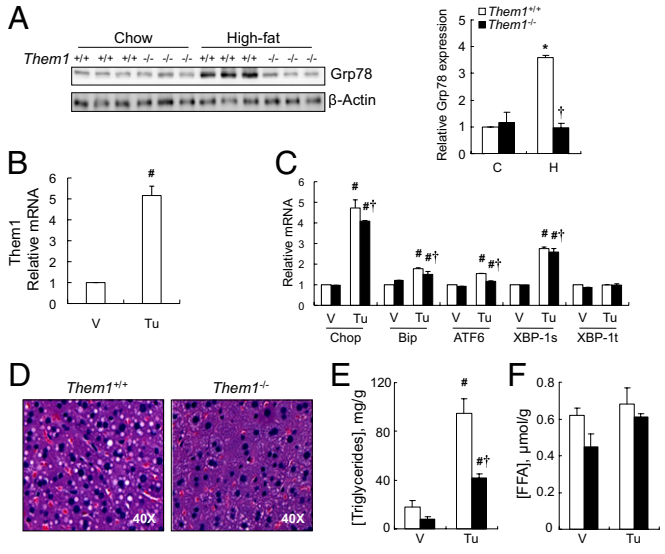

Fig. 5. Attenuated induction of ER stress in livers of *Them1*$^{-/-}$ mice. (*A*) Reduced Grp78 expression in livers of high-fat-fed mice lacking Them1 following 8 wk of chow or high-fat feeding (*Left*, immunoblots; *Right*, densitometric analysis). Mice were treated intraperitoneally with vehicle (V) or tunicamycin (Tu) daily for 2 d and were allowed free access to the chow diet and water before determining hepatic mRNA expression of (*B*) Them1 in *Them1*$^{+/+}$ mice and (*C*) selected genes that mediate ER stress in *Them1*$^{+/+}$ and *Them1*$^{-/-}$ mice. (*D*) Representative light micrographs of H&E-stained liver sections. Hepatic concentrations of (*E*) triglycerides and (*F*) FFA. *P < 0.05 high-fat vs. chow; $^{\dagger}P$ < 0.05 *Them1*$^{-/-}$ vs. *Them1*$^{+/+}$; $^{\#}P$ < 0.05 Tu vs. V.

High-fat-fed *Them1*$^{-/-}$ mice also exhibited markedly reduced hepatic steatosis. Whereas genes that regulate fatty acid uptake were up-regulated in the absence of Them1 expression, FFA concentrations in the liver were reduced. Upon esterification to form fatty acyl-CoAs, these molecules were presumably delivered to mitochondria for β-oxidation. In support of this possibility, genes involved in β-oxidation were up-regulated in livers of *Them1*$^{-/-}$ mice, and plasma concentrations of β-hydroxybutyrate tended to be higher. However, we failed to detect increases in β-oxidation rates in livers of chow-fed *Them1*$^{-/-}$ mice. We also observed decreased activation of SREBP-1c in high-fat-fed *Them1*$^{-/-}$ mice. Finally, diet-induced elevations of plasma triglycerides in *Them1*$^{-/-}$ mice suggest that higher export rates of triglycerides from the liver or decreased rates of hepatic uptake may have helped to diminish steatosis.

Improved glucose homeostasis in both chow- and high-fat-fed *Them1*$^{-/-}$ mice may be explained by complementary mechanisms. Glucose consumption was increased in the absence of Them1 expression, presumably in support of greater heat production. Decreased lipid deposition, reduced inflammation, relative increases in plasma leptin concentrations, and diminished ER stress most likely contributed to increased insulin sensitivity. The accumulation of diacylglycerol molecules leads to insulin resistance (24). Although not measured in our study, the decrease in fatty acid synthesis combined with the increase in oxidation that occurred in the absence of Them1 would be expected

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to reduce hepatic diacylglycerol concentrations (25). Inflammation also plays an important role in development of insulin resistance (21, 26), and we observed reduced inflammation in WAT and in livers of high-fat-fed *Them1*$^{-/-}$ mice. Because FFAs are proinflammatory (21), decreased hydrolysis of fatty acyl-CoAs was the most likely protective mechanism. In the setting of reduced WAT in high-fat-fed *Them1*$^{-/-}$ mice, the absence of a proportional decrease in plasma leptin concentrations was apparently because of up-regulation of leptin gene expression. This relative increase in circulating leptin in relation to body mass may have helped to preserve hepatic insulin sensitivity (27). In contrast, concentrations of adiponectin, which also sensitizes the liver to insulin action (28), were not influenced by Them1 expression. For unclear reasons, the high-fat diet failed to suppress plasma adiponectin concentrations in *Them1*$^{+/+}$ mice, as has been reported in wild-type mice (29).

Finally, the ER stress response, which contributes to both hepatic steatosis and insulin resistance (30), can be activated by increased intracellular concentrations of FFAs (14, 31). The absence of Them1 attenuated the responses to both diet- and chemical-induced ER stress. Because *Them1*$^{-/-}$ mice were protected against hepatic steatosis, it was possible that the reductions in Grp78 expression in livers of high-fat-fed *Them1*$^{-/-}$ mice were secondary to reduced tissue lipid concentrations. However, when primary cultured hepatocytes or MEFs lacking Them1 were exposed to either tunicamycin or thapsigargan, activation of ER stress pathways was also reduced. When taken together with the observations that Them1 was up-regulated in both liver and MEFs in response to tunicamycin and that lipid accumulation was attenuated in livers of tunicamycin-treated mice, these findings support a mechanistic contribution of Them1 to ER stress. Recently, it has been shown that membrane phospholipid composition can regulate the ER stress response by modulating the activity of the calcium transporter sarco-(endo)plasmic reticulum calcium ATPase 2b (32). Considering its influence on cellular fatty acid metabolism, Them1 could regulate ER stress by modulating the fatty acyl species of ER membrane phospholipids.

Because of its disproportionately high expression in BAT, we anticipated that a global knockout strategy would primarily reveal functions of Them1 in this tissue. Indeed, a potential explanation of reduced hepatic steatosis and improved glucose metabolism in high-fat-fed *Them1*$^{-/-}$ mice is simply that they did not become obese. However, the transcriptional and metabolic changes that occurred in chow-fed mice and in cultured cells suggest mechanistic roles for Them1, even in tissues in which it is expressed at relatively low levels. This possibility is supported by the observation that Them1 is up-regulated in WAT and liver in response to high-fat feeding and ER stress. A conditional knockout strategy would be expected to help clarify these possibilities.

Overall, our data suggest that Them1 functions to decrease energy consumption in BAT, leading to conservation of calories within the mouse. In an animal with a high basal metabolic rate, a mechanism to actively reduce caloric consumption following resolution of a metabolic stress would presumably favor long-term survival. However, in the setting of nutritional excess, caloric conservation leads to obesity, and the overproduction of FFAs by Them1 provokes insulin resistance, inflammation and ER stress. Considering that the human *THEM1* gene has been linked to adiposity (5), these findings suggest THEM1 as a unique target for the management of the metabolic syndrome.

Materials and Methods

Using bacterial artificial chromosome recombineering techniques (33), *Them1*$^{-/-}$ mice were generated and subjected to diets as detailed in *SI Materials and Methods*. Analytical techniques and metabolic measurements are described in *SI Materials and Methods*, as are experiments using primary cell cultures. Primers for quantitative real-time PCR are listed in Table S1.

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