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Absence of Hormone-sensitive Lipase Inhibits Obesity and Adipogenesis in *Lep$^{ob/ob}$* Mice*

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Hormone-sensitive lipase (HSL) plays a crucial role in the hydrolysis of triacylglycerol and cholesteryl ester in various tissues including adipose tissues. To explore the role of HSL in the metabolism of fat and carbohydrate, we have generated mice lacking both leptin and HSL (*Lep$^{ob/ob}$/HSL$^{-/-}$*) by cross-breeding *HSL$^{-/-}$* mice with genetically obese *Lep$^{ob/ob}$* mice. Unexpectedly, *Lep$^{ob/ob}$/HSL$^{-/-}$* mice ate less food, gained less weight, and had lower adiposity than *Lep$^{ob/ob}$/HSL$^{+/+}$* mice. *Lep$^{ob/ob}$/HSL$^{-/-}$* mice had massive accumulation of preadipocytes in white adipose tissues with increased expression of preadipocyte-specific genes (CAAT/enhancer-binding protein β and adipose differentiation-related protein) and decreased expression of genes characteristic of mature adipocytes (CCAAT/enhancer-binding protein α, peroxisome proliferator activator receptor γ, and adipocyte determination and differentiation factor 1/sterol regulatory element-binding protein-1). Consistent with the reduced food intake, hypothalamic expression of neuropeptide Y and agouti-related peptide was decreased. Since HSL is expressed in hypothalamus, we speculate that defective generation of free fatty acids in the hypothalamus due to the absence of HSL mediates the altered expression of these orexigenic neuropeptides. Thus, deficiency of both leptin and HSL has unmasked novel roles of HSL in adipogenesis as well as in feeding behavior.

Excessive cellular accumulation of neutral lipids underlies many diseases such as obesity, type 2 diabetes, and atherosclerosis, all of which are epidemic in industrialized countries. Therefore, elucidating the metabolic pathways that degrade excessive neutral lipids is of extreme importance in the prevention of the diseases caused by lipotoxicity (1).

Hormone-sensitive lipase (HSL)[1] is an intracellular neutral lipase that catalyzes the hydrolysis of cellular triglycerols (TG), diglycerols (DG), monoacylglycerols, and cholesteryl esters as well as other lipids (2, 3). HSL is expressed in a wide variety of organs and cells, including adipose tissues, heart, skeletal muscle, adrenal glands, testes, ovaries, and pancreatic β-cells, and is under neural and hormonal control.

Several laboratories including ours have generated HSL-deficient (*HSL$^{-/-}$*) mice by targeted gene disruption (4–6). Unexpectedly, these mice showed male sterility due to the failure of spermatogenesis (4, 7). Although *HSL$^{-/-}$* mice had a decreased ability to release free fatty acids (FFA) from adipocytes in response to the β-adrenergic stimulation both *in vivo* and *in vitro*, they were neither obese nor cold-sensitive (4, 5). The attenuated development of adipocyte-associated phenotypes may result from the presence of a residual TG lipase activity in adipocytes, which is induced by both β-adrenergic agonist and tumor necrosis factor-α (8). However, it is unknown how HSL deficiency affects adiposity in the setting of obesity, which is commonly associated with type 2 diabetes.

Here we show that mice lacking both HSL and leptin (*Lep$^{ob/ob}$/HSL$^{-/-}$*) show impaired adipogenesis and paradoxical resistance to obesity primarily due to the reduced food intake. These observations in the leptin-deficient state have revealed new functions of HSL in adipogenesis and feeding behavior.

EXPERIMENTAL PROCEDURES

Animals—HSL$^{-/-}$ mice (4), which were backcrossed five times into the C57BL/6J background, were intercrossed with mice heterozygous for leptin deficiency (*Lep$^{+/ob}$*/C57BL/6J; Jackson Laboratories, Bar Harbor, ME) to generate double heterozygotes, which were then interbred to produce *Lep$^{ob/ob}$/HSL$^{-/-}$* mice. Genotyping was performed as described previously (4, 9). Mice were housed in a temperature-controlled environment with a 12-h light/dark cycle and allowed free access to water and a standard chow diet (Oriental MF, Oriental Yeast, Tokyo, Japan). Mice (16 weeks) were sacrificed after a 6-h fast unless otherwise stated. Mice were sacrificed at the end of the light cycle to isolate hypothalamic RNA. All experiments were performed in accord with institutional guidelines.

Biochemical Analyses—Blood was collected from the retro-orbital venous plexus after a 6-h fast. Cholesterol (Determiner TC; Kyowa Medex, Tokyo, Japan), TG and glycerol (TG LH; Wako Chemicals,

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[1] The abbreviations used are: HSL, hormone-sensitive lipase; TG, triglycerol(s); DG, diglycerol(s); FFA, free fatty acid(s); WAT, white adipose tissue; ACTH, adrenocorticotropic hormone.

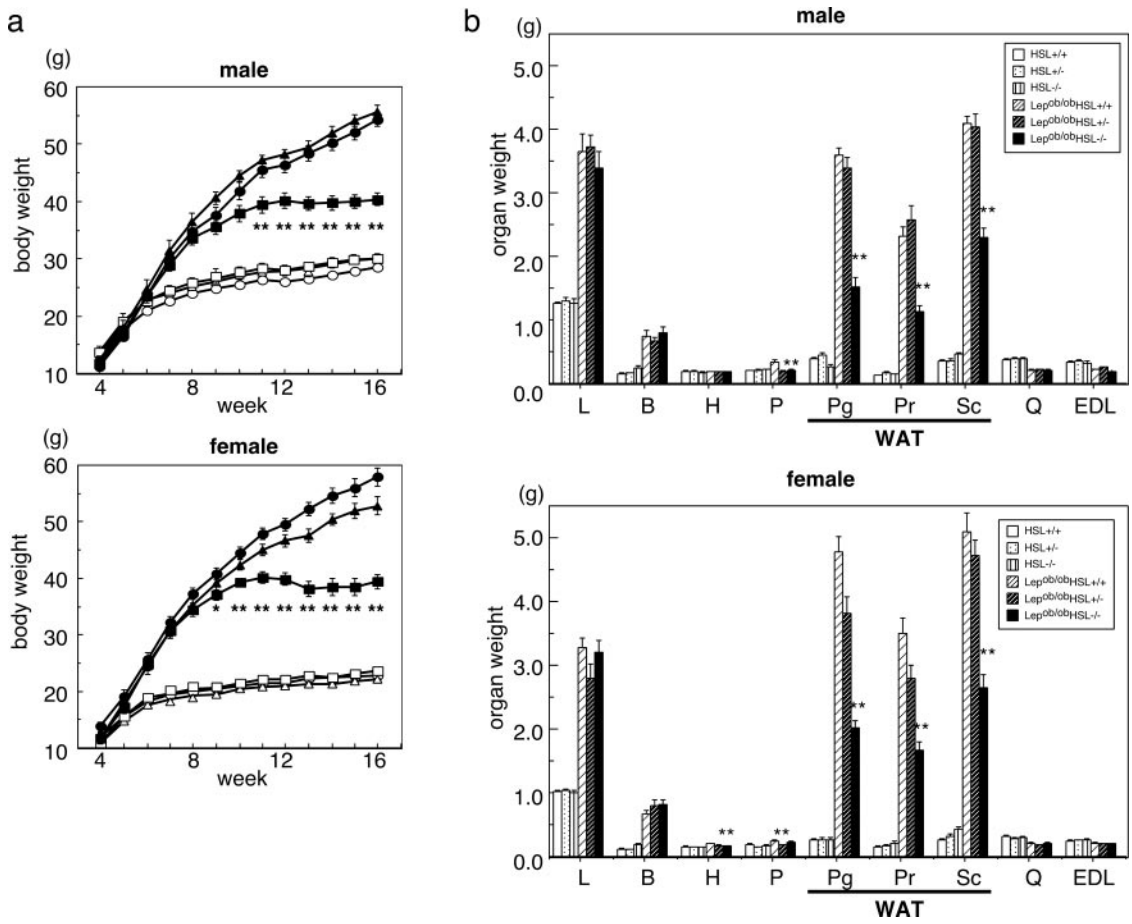

FIG. 1. ***HSL$^{-/-}$* mice are resistant to genetic obesity.** *a*, body weight of each mouse was measured weekly from 4 to 16 weeks ($n = 10$). ○, *Lep*$^{+/+}$/*HSL*$^{+/+}$; □, *Lep*$^{+/+}$/*HSL*$^{+/-}$; □, *Lep*$^{+/+}$/*HSL*$^{-/-}$; ●, *Lep*$^{ob/ob}$/*HSL*$^{+/+}$; ▲, *Lep*$^{ob/ob}$/*HSL*$^{+/-}$; ■, *Lep*$^{ob/ob}$/*HSL*$^{-/-}$. *b*, tissue weights of each mouse were measured at 16 weeks of age after a 6-h fast ($n = 10$). *L*, liver; *B*, brown adipose tissue; *H*, heart; *P*, pancreas; *Pg*, paragonadal WAT; *Pr*, perirenal WAT; *Sc*, subcutaneous WAT; *Q*, quadriceps; *EDL*, extensor digitorum longus. Each value represents the mean ± S.E. * and **, significance at $p < 0.05$ and $p < 0.01$, respectively, *versus HSL*$^{+/+}$ mice in the same *Lep* background.

Tokyo, Japan), and FFA (NEFA C; Wako Chemicals) were measured enzymatically. Plasma glucose was measured by ANTSENSE II (Bayer Medical, Tokyo, Japan); plasma insulin was measured by the mouse insulin ELISA kit (Morinaga, Tokyo, Japan); and plasma corticosterone was measured by the EIA kit (Diagnostic Systems Laboratories, Inc., Webster, TX).

Immunoblot Analysis—Hypothalamic regions were excised from 8-month-old male mice, homogenized in 0.25 M sucrose buffer (20 mM Tris-HCl, pH 7.4, 0.25 M sucrose, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 2.8 μg/ml aprotinin), and centrifuged at 15,000 rpm for 15 min. Fifty μg of the supernatant fraction was subjected to SDS/PAGE analysis. S100 protein (10 μg) from BAT of wild type mouse was used as a positive control. Immunoblot analysis was performed using the ECL kit (Amersham Biosciences) as described previously (4).

Northern Blot Analysis—Total RNA was isolated by Trizol reagent (Invitrogen). Hypothalamic RNA was isolated as described previously (10). Five μg of hypothalamic RNA from each mouse or 7.5–10 μg of white adipose tissue (WAT) RNA equally pooled from 3–5 mice of each genotype were electrophoresed in a 1% agarose gel containing formaldehyde and transferred to a nylon membrane. The membranes were hybridized with probes, which were labeled with [α-^{32}P]dCTP using the Megaprime DNA labeling system kit (Amersham Biosciences) in Rapidhyb Buffer (Amersham Biosciences) or ULTRAhyb hybridization buffer (Ambion, Austin, TX), and analyzed by a BAS2000 bioimaging analyzer (Fuji Photo Film, Tokyo, Japan). Loading was normalized by the expression of 36B4.

Histology—Sixteen-week-old mice were sacrificed by decapitation. Tissues were excised, fixed in 10% neutral buffered formalin, embedded in paraffin, and stained with hematoxylin-eosin. Cell size was estimated by NIH Image. After blocking with 0.5% goat serum, sections of epididymal fat pads were incubated with anti-mouse S-100 antibody (Sigma) overnight and were incubated with an anti-rabbit secondary antibody followed by staining with the avidin-biotin complex-alkaline phosphatase method and counterstaining with Harris's hematoxylin. Apoptotic cells were detected by terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling using an *in situ* apoptosis detection kit (Takara Biomedicals Otsu, Japan) according to the manufacturer's instructions, with counterstaining with methyl green (Wako Chemicals). Proliferating cells were detected by bromodeoxyuridine immunostaining. Two h before sacrifice, bromodeoxyuridine (Sigma) was administered intraperitoneally at 50 mg/kg body weight in phosphate-buffered saline. After fixation with 10% neutral buffered formalin, mouse epididymal fat was embedded in paraffin. Immunostaining was performed by a bromodeoxyuridine *In-Situ* Detection Kit (PharMingen, San Diego, CA) and counterstained with Mayer's hematoxylin (Wako Chemicals).

Detection of DNA Ladder—Adipose tissue DNA was extracted as described previously (4). Five μg of DNA end-labeled with [α-^{32}P]dCTP by Klenow was subjected to electrophoresis in a 1.5% agarose gel and then transferred to nylon membranes, as described previously (11). As a control, mouse thymocyte apoptotic DNA was loaded. In brief, mouse thymus was ground over a mesh, and the isolated thymocytes were induced to undergo apoptosis by culturing in RPMI containing 10% calf serum and 1 μM dexamethasone at 37 °C for 6 h.

Food Intake and Body Temperature—Food intake and body weight were measured for 3 days after the mice were individually housed and adapted for 1 week. Feeding efficiency was defined as an increase in body weight divided by food intake during 3 days ($n = 8$–10). Body temperature was measured by an NK-YSI precision N550 thermometer and its probe (Nikkiso-YSI, Japan). These experiments were performed at the end of light cycle at the age of 14 weeks.

Oxygen Consumption—After a 1-day acclimation period, oxygen and bicarbonate expired by mice (14 weeks old) were measured every 7 min for 24 h by a calorimetric system (Alco System model, Chiba Japan). Oxygen consumption was normalized by body weight raised to the 0.7

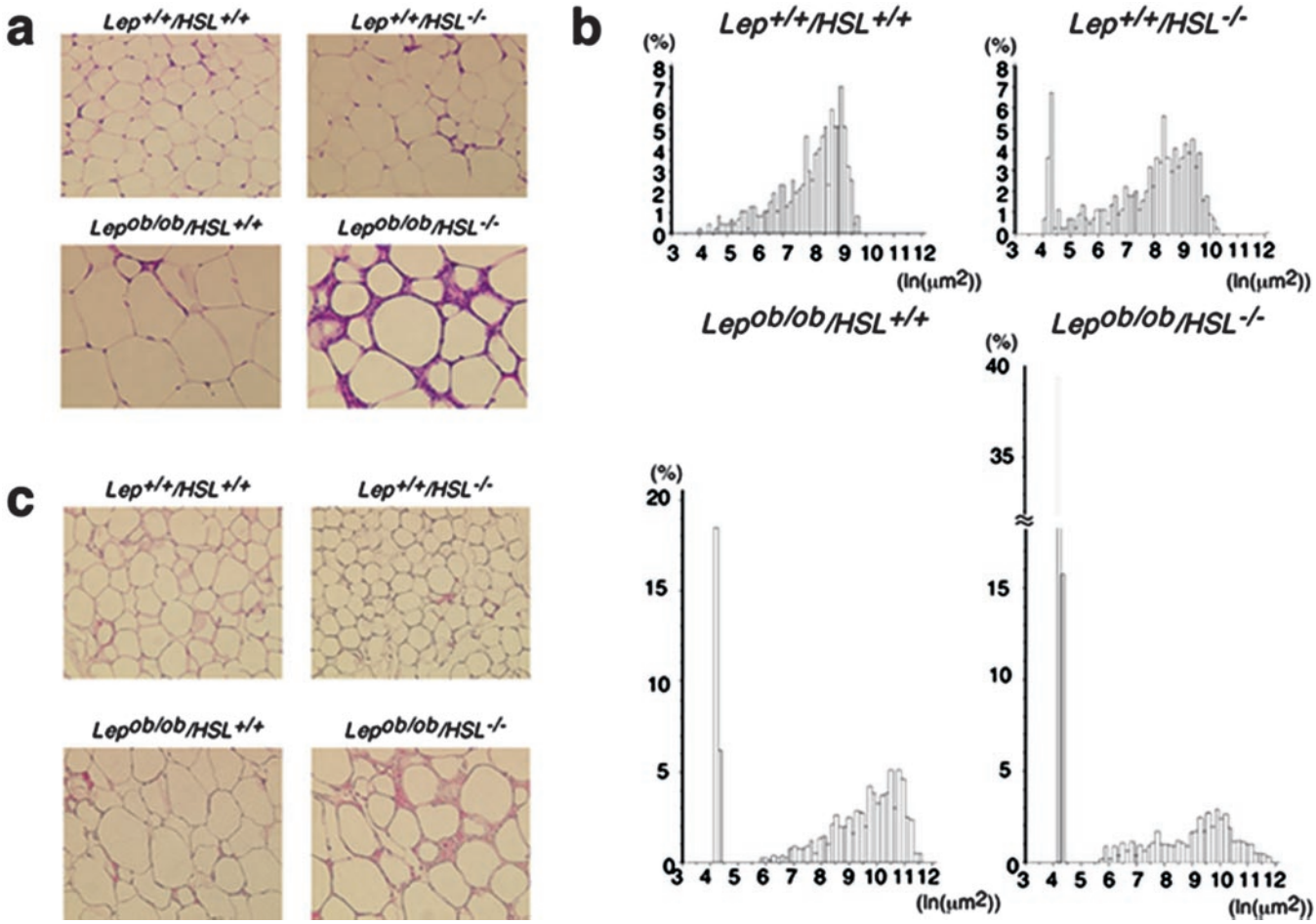

FIG. 2. **Morphological analysis of epididymal WAT.** Mice were sacrificed after a 6-h fast at the age of 16 weeks. *a*, sections of epididymal WAT were stained with hematoxylin-eosin. *b*, cell number is plotted against log of cytoplasmic area. *c*, immunohistochemistry of S100 protein.

power, which is proportional to body surface area.

Statistics—Statistical differences between groups were analyzed by one-way analysis of variance and a *post hoc* Tukey-Kramer test, unless otherwise stated.

RESULTS

HSL Deficiency Ameliorates Obesity in Lep$^{ob/ob}$ Background—In the *Lep*$^{+/+}$ background, there was no difference in body weight among *HSL*$^{+/+}$, *HSL*$^{+/-}$, and *HSL*$^{-/-}$ mice on a normal chow diet (Fig. 1*a*). In the *Lep*$^{ob/ob}$ background, however, body weight of doubly homozygous mice was reduced by 26% compared with the *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ mice at the age of 16 weeks (Fig. 1*a*). The difference was discernible at the age of 11 weeks in males and 9 weeks in females. Furthermore, the age-dependent weight gain was negligible in *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ mice after 10 weeks of age.

At the age of 16 weeks, the weight of WAT in three different regions was selectively reduced in *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ mice compared with *Lep*$^{ob/ob}$/*HSL*$^{+/+}$ mice; paragonadal, perirenal, and subcutaneous WAT were reduced by 58, 51, and 44%, respectively (Fig. 1*b*).

HSL$^{-/-}$ *Mice Have an Increased Number of Preadipocyte-like Cells in WAT*—In the *Lep*$^{+/+}$ background, WAT from *HSL*$^{-/-}$ mice contained clusters of small cells devoid of lipids in addition to lipid-filled mature adipocytes (Fig. 2*a*), as we reported previously (4). In the *Lep*$^{ob/ob}$ background, the small cells, which appeared slightly increased in *Lep*$^{ob/ob}$/*HSL*$^{+/+}$ mice, were robustly increased in *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ mice (Fig. 2, *a* and *b*).

To determine the identity of these small cells, we performed immunohistochemistry for S-100 protein, which is used as a marker of adipocytes (12), particularly of preadipocytes, and neural cells (Fig. 2*c*). The small cells were strongly positive for S-100, indicating that they were preadipocytes. Upon Northern blot analyses of WAT (Fig. 3*a*), the expression of adipose differentiation-related protein (13) and CAAT/enhancer-binding protein β, which is dominant during the early stage of differentiation of adipocytes (14), was increased in *HSL*$^{-/-}$ WAT compared with *HSL*$^{+/+}$ WAT in both *Lep*$^{+/+}$ and *Lep*$^{ob/ob}$ backgrounds. On the other hand, the expression of CAAT/enhancer-binding protein α, which is dominant during the late stage of differentiation (14), adipocyte determination and differentiation factor 1/sterol regulatory element-binding protein-1 (15), fatty acid synthase (16), and peroxisome proliferator activator receptor γ (17) was decreased in *HSL*$^{-/-}$ WAT compared with *HSL*$^{+/+}$ WAT in both *Lep*$^{+/+}$ and *Lep*$^{ob/ob}$ backgrounds. These results strongly support the results of immunohistochemistry and the conclusion that preadipocytes were increased in *HSL*$^{-/-}$ WAT. No significant differences were observed in the expression of lipoprotein lipase and uncoupling protein-2 between *Lep*$^{ob/ob}$/*HSL*$^{+/+}$ and *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ mice.

To rule out the possibility that the small cells are apoptotic due to overaccumulation of intracellular TG, we performed terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling staining and DNA ladder detection. Although *Lep*$^{ob/ob}$ WAT contained an increased number of terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling-positive apoptotic cells and increased DNA ladder formation compared with *Lep*$^{+/+}$ WAT, there was no difference between *HSL*$^{+/+}$ and *HSL*$^{-/-}$ WAT (Fig. 3*b*). Furthermore, we performed *in vivo* bromodeoxyuridine labeling to determine

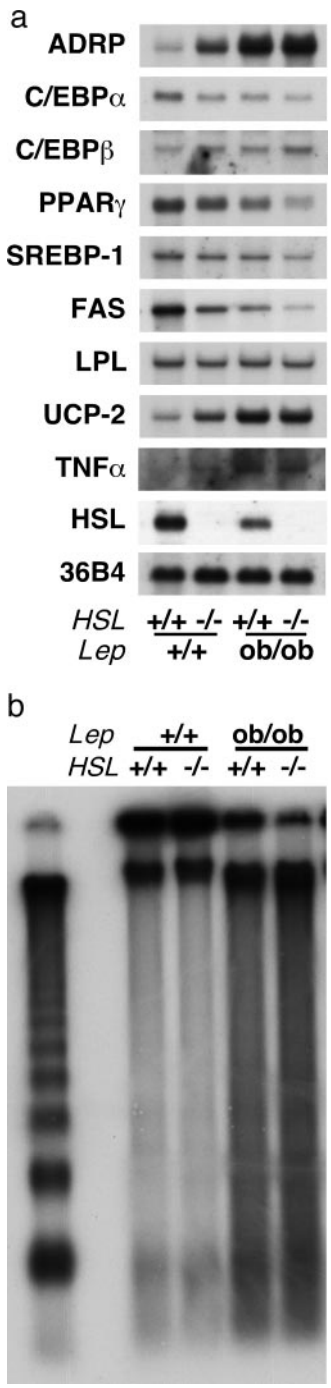

FIG. 3. **Northern blot analysis and DNA ladder of epididymal WAT.** *a*, Northern blot analysis. Total RNA was isolated and pooled equally from three mice. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0 (*ADRP*)) was used as a loading control. The *numbers* in the *figure* represent relative intensities of the bands compared with those of $Lep^{+/+}/HSL^{+/+}$. *b*, DNA ladder. *Left lane*, DNA from mouse thymocyte that had been treated with dexamethasone. Labeled DNA (5 μg) was loaded in each lane. *TNFα*, tumor necrosis factor α. *C/EBP*, CAAT/enhancer-binding protein; *PPAR*, peroxisome proliferator activator receptor.

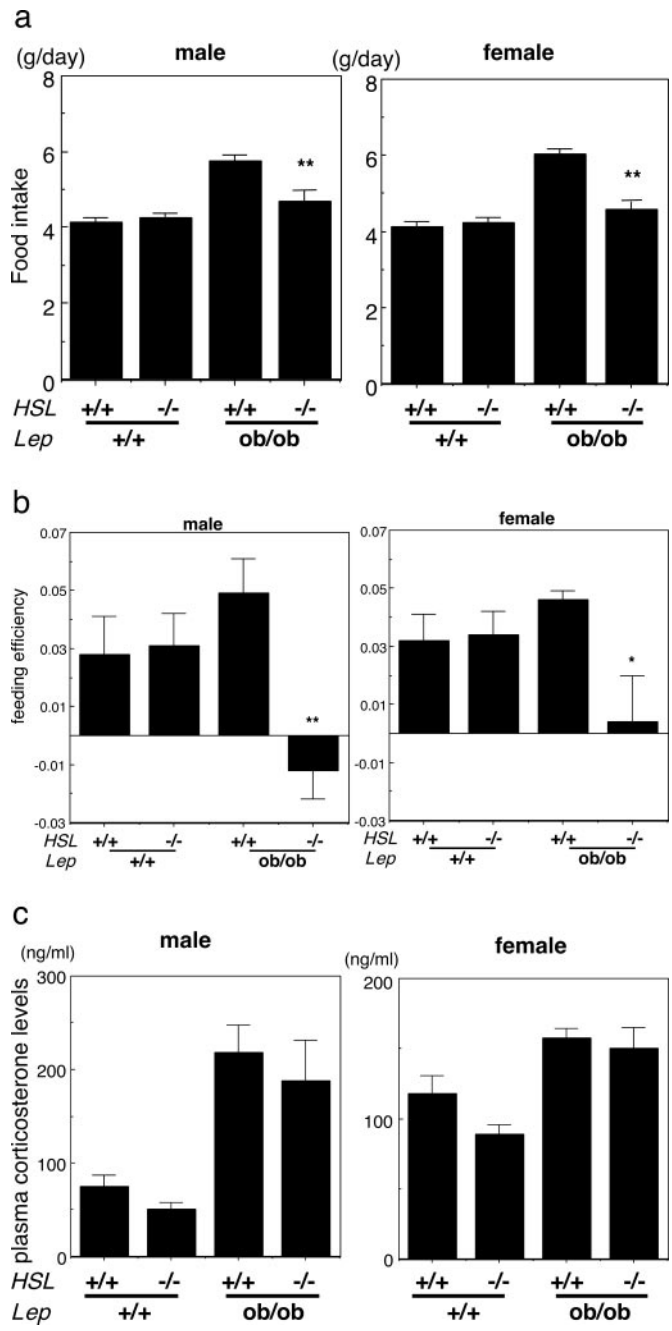

FIG. 4. **Food intake and plasma corticosterone levels.** *a*, food intake was measured at the age of 14 weeks ($n = 10$). *b*, feeding efficiency was determined at the age of 14 weeks ($n = 8$–10). *c*, basal plasma corticosterone levels at the age of 9 weeks ($n = 10$).

whether the small cells have increased proliferative activity. No increase in bromodeoxyuridine-positive cells was observed in $HSL^{-/-}$ WAT (data not shown).

Food Intake Is Reduced in Obese $HSL^{-/-}$ Mice—To determine the causes of the lower adiposity in $Lep^{ob/ob}/HSL^{-/-}$ mice, we examined food intake. In the $Lep^{+/+}$ background, there was no difference in food intake between $HSL^{+/+}$ and $HSL^{-/-}$ mice. In the $Lep^{ob/ob}$ background, food intake, which was increased compared with the $Lep^{+/+}$ background, was reduced by 19% in $HSL^{-/-}$ mice compared with $HSL^{+/+}$ mice at the age of 14 weeks (Fig. 4*a*). The reduced food intake was apparent at least after 8 weeks of age (data not shown). $Lep^{ob/ob}/HSL^{-/-}$ mice had significantly lower feeding efficiency than $Lep^{ob/ob}/HSL^{+/+}$ mice (Fig. 4*b*), indicating that the reduced food intake was not the only mechanism contributing to the leanness of $Lep^{ob/ob}/HSL^{-/-}$ mice.

Adrenalectomy reduces food intake (18, 19) in $Lep^{ob/ob}$ mice, which are in hyper-ACTH status, and HSL deficiency reduces the corticosterone response to ACTH (20, 21). Therefore, we measured basal plasma corticosterone levels to examine whether adrenal insufficiency underlies the reduced food intake. There was no significant decrease in the plasma corticos-

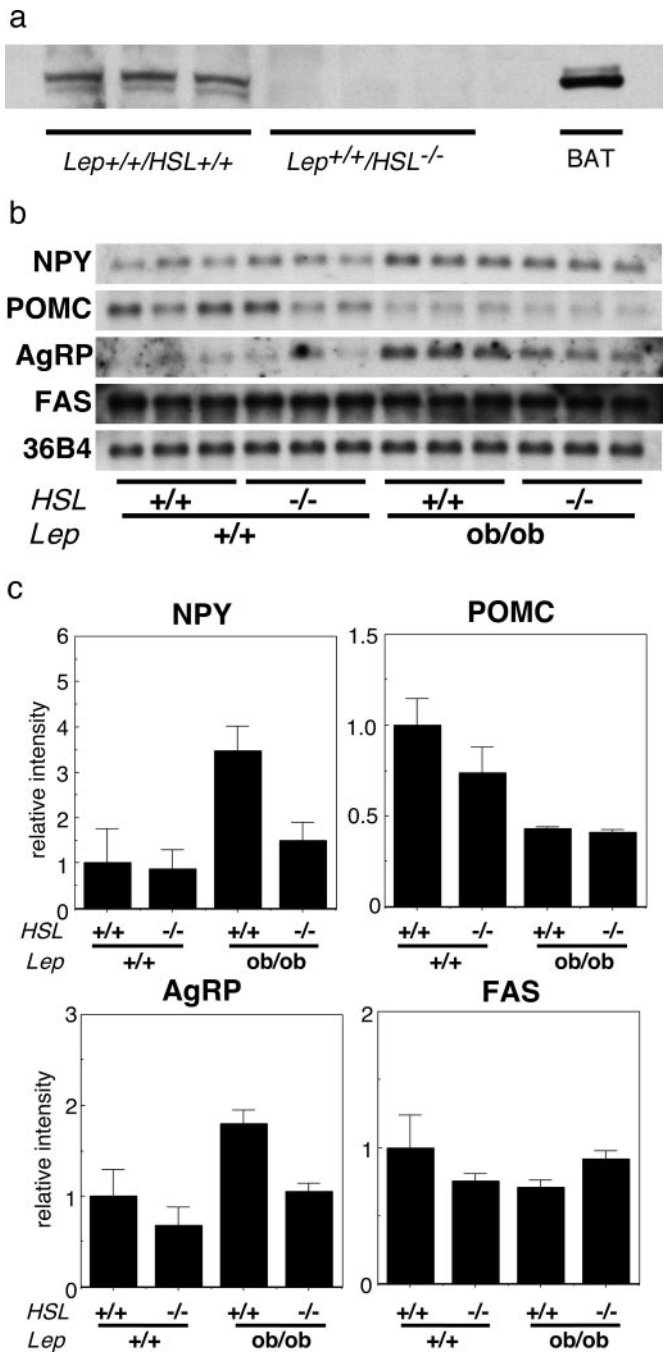

FIG. 5. **Hypothalamic expression of HSL and genes encoding orexigenic and anorexigenic neuropeptides and fatty acid synthase (*FAS*).** *a*, Western blot analysis of HSL protein in the hypothalamic regions of either Lep$^{+/+}$/HSL$^{+/+}$ or Lep$^{+/+}$/HSL$^{-/-}$ mice. Wild-type brown adipose tissue (*BAT*) was used as a positive control. *b*, Northern blot analyses of the hypothalamic genes. Mice aged 16 weeks were sacrificed at the end of light cycle. RNA from three mice was loaded in each lane. 36B4 (acidic ribosomal phosphoprotein P0) was used as a loading control. *c*, the radioactivity was measured by the PhosphorImager and statistically analyzed. Each value represents the mean ± S.E. *POMC*, pro-opiomelanocortin.

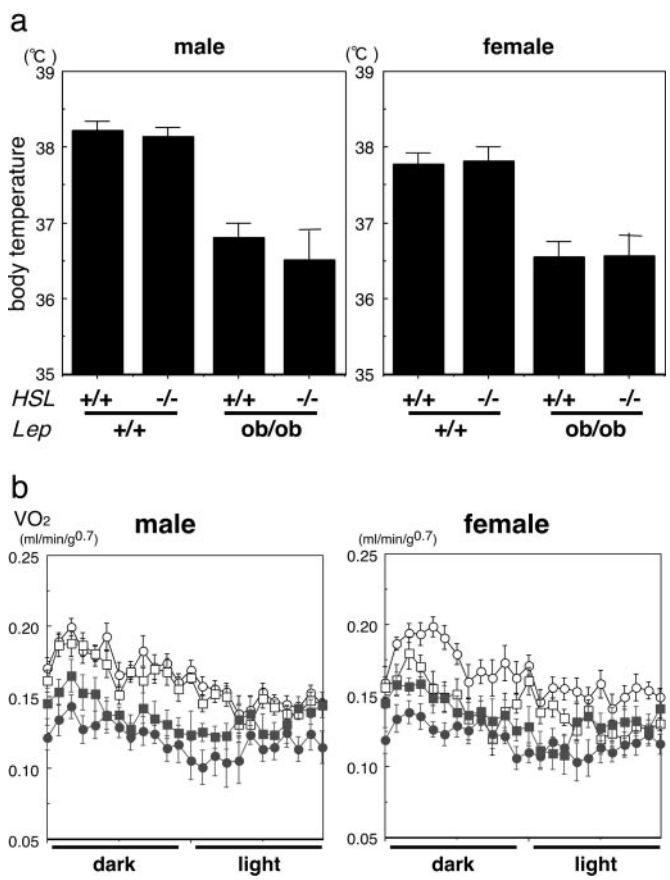

FIG. 6. **Body temperature and oxygen consumption.** *a*, core body temperature was measured at the age of 14 weeks (n = 10). *b*, oxygen consumption was measured under a 12-h light/dark cycle and free access to food and water at the age of 12 weeks (n = 8–9). Oxygen consumption was normalized by body surface area. ○, Lep$^{+/+}$/HSL$^{+/+}$; □, Lep$^{+/+}$/HSL$^{-/-}$; ●, Lep$^{ob/ob}$/HSL$^{+/+}$; ■, Lep$^{ob/ob}$/HSL$^{-/-}$. Each value represents the mean ± S.E.

terone levels in Lep$^{ob/ob}$/HSL$^{-/-}$ mice compared with Lep$^{ob/ob}$/HSL$^{+/+}$ mice (Fig. 4*c*).

Hypothalamus expressed HSL protein in wild-type mice but not in HSL$^{-/-}$ mice (Fig. 5*a*). The suppressed food intake in Lep$^{ob/ob}$ mice led us to examine the expression of various neuropeptides that govern appetite and satiety in the hypothalamus (Fig. 5, *b* and *c*). The expression levels of NPY and AgRP, both of which were induced in Lep$^{ob/ob}$ background, were decreased nearly to the level of lean Lep$^{+/+}$ mice (Fig. 5, *b* and *c*). The expression levels of pro-opiomelanocortin, which were suppressed in the Lep$^{ob/ob}$ background, were not different between HSL$^{+/+}$ and HSL$^{-/-}$ mice (Fig. 5, *b* and *c*).

In addition to food intake, energy expenditure determines adiposity. Body temperature, which was reduced in the Lep$^{ob/ob}$ background, was not significantly different between HSL$^{+/+}$ and HSL$^{-/-}$ mice (Fig. 6*a*). Consistently, the mean oxygen consumption during 24 h was not significantly different between HSL$^{+/+}$ and HSL$^{-/-}$ mice in the Lep$^{ob/ob}$ background when normalized by body surface area (males, 0.120 ± 0.024 *versus* 0.136 ± 0.019 ml/min/g$^{0.7}$, p = 0.18; females, 0.119 ± 0.021 *versus* 0.135 ± 0.025 ml/min/g$^{0.7}$, p = 0.18), although Lep$^{ob/ob}$/HSL$^{-/-}$ mice appeared to have higher oxygen consumption than Lep$^{ob/ob}$/HSL$^{+/+}$ mice (Fig. 6*b*).

*HSL Deficiency Decreases Plasma Levels of FFA and Glycerol—*Lep$^{ob/ob}$/HSL$^{-/-}$ mice showed significant reduction in plasma levels of FFA and glycerol compared with Lep$^{ob/ob}$/HSL$^{+/+}$ mice (FFA, 663 ± 67 *versus* 1,174 ± 17 μM, $p < 0.01$; glycerol, 10.0 ± 2.8 *versus* 43.4 ± 6.0 mg/dl, $p < 0.01$). In the lean Lep$^{+/+}$/HSL$^{-/-}$ mice, plasma cholesterol levels were elevated, whereas plasma TG levels were decreased compared with those in Lep$^{+/+}$/HSL$^{+/+}$ mice, which was due to an increase in high density lipoproteins and a decrease in very low density lipoproteins, respectively (data not shown), as reported previously (22). In the Lep$^{ob/ob}$ background, no significant difference was observed between HSL$^{+/+}$ and HSL$^{-/-}$ mice.

Leanness and low plasma FFA levels are commonly associ-

ated with increased insulin sensitivity, whereas impaired adipogenesis is associated with insulin resistance (23). To estimate insulin sensitivity, we measured blood levels of glucose and insulin. There were no differences in the blood glucose and plasma insulin levels between *Lep*$^{ob/ob}$/*HSL*$^{+/+}$ and *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ mice when fasted (data not shown), indicating that insulin sensitivity was not significantly different between them.

DISCUSSION

In the present studies, we use *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ mice to show that the absence of HSL inhibits adipogenesis and feeding behavior, thereby decreasing adiposity in leptin-deficient obesity. Resistance to obesity was also observed in a high fat diet-induced obesity model (24).

Adipocytes in WAT of *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ mice displayed exaggerated size heterogeneity (Fig. 2, *a* and *b*). In particular, small cells were remarkably increased. Although adipocytes from *Lep*$^{ob/ob}$ WAT are prone to apoptosis upon insulin depletion (25) and indeed *Lep*$^{ob/ob}$ WAT contained a substantial number of apoptotic cells, HSL deficiency did not further increase the number of apoptotic cells in either the *Lep*$^{+/+}$ or *Lep*$^{ob/ob}$ settings (Fig. 3*c*). Since the majority of the small cells were positive for S-100 protein (Fig. 2*c*), we consider the small cells to be preadipocytes. This view is consistent with the gene expression profiles of WAT; adipose differentiation-related protein and CAAT/enhancer-binding protein β, which characterize the early phase of adipocyte differentiation, were up-regulated, and CAAT/enhancer-binding protein α, peroxisome proliferator activator receptor γ, adipocyte determination and differentiation factor 1/sterol regulatory element-binding protein-1, and fatty acid synthase, which characterize the mature stage of adipocyte differentiation, were down-regulated (Fig. 3*a*). Based on these results, we speculate that HSL deficiency inhibits adipogenesis in a certain population of preadipocytes, thereby stimulating their accumulation. Substances released from adipocytes of *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ mice may mediate the inhibition of adipocyte differentiation. In this context, it is of note that FFA, whose production was reduced in *HSL*$^{-/-}$ mice, induces differentiation of preadipocytes (26–29). Moreover, Janke *et al.* (30) have recently reported that angiotensin II secreted by mature adipocytes inhibits differentiation of preadipocytes in culture.

It is also possible that intracellular lipids such as DG, which are accumulated in the absence of HSL (6), directly inhibit adipogenesis. It is tempting to speculate that protein kinase C activation by DG inhibits glycogen synthase kinase-3 (31), thereby enhancing signaling of Wnt, a negative regulator of adipogenesis (32). The impaired adipogenesis observed in *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ mice appears to be inconsistent with our previous findings that *HSL*$^{-/-}$ embryonic fibroblasts are indistinguishable from wild-type embryonic fibroblasts in adipogenic ability (8). In the experiments *in vitro*, a pharmacological dose of insulin, methyl-isobutylxanthine, or dexamethasone in the differentiation induction medium may have overcome the reduced adipogenic potential of *HSL*$^{-/-}$ embryonic fibroblasts. Indeed, it was reported that exposure to methyl-isobutylxanthine or cAMP is sufficient to suppress expression of Wnt10b in 3T3-L1 cells (33).

Hyperphagia of leptin-deficient *Lep*$^{ob/ob}$ is mediated by the up-regulation of the orexigenic neuropeptides, NPY (34) and AgRP (35), with concomitant suppression of the pro-opiomelanocortin gene, which encodes the anorexigenic α-melanocyte-stimulating hormone (36). Since disruption of NPY (37) or its cognate receptor (Y2 receptor) (38) attenuates the obesity of *Lep*$^{ob/ob}$ mice, the reduced expression of NPY and possibly AgRP may account for the decreased food intake in *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ mice (Fig. 5, *b* and *c*). Together with the hypothalamic expression of HSL (Fig. 5*a*), these results strongly indicate the crucial role of hypothalamic metabolism of fatty acids in the control of feeding behavior. In this context, it is noteworthy that inhibition of FFA synthesis by fatty acid synthase inhibitors shows similar suppressing effects on food intake (39) as well as on fasting-induced increase in the hypothalamic expression of NPY and AgRP (40). Likewise, inhibition of hypothalamic carnitine palmitoyltransferase-1 decreases food intake (41). Neither body temperature nor oxygen consumption was different between *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ and *Lep*$^{ob/ob}$/*HSL*$^{+/+}$ mice (Fig. 6). Thus, the reduced food intake as well as the defective differentiation of preadipocytes may account for the lower adipocity in *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ mice at least in mice aged older than 8 weeks (Fig. 1). It is interesting to note that the lower adiposity was not observed in mice younger than 8 weeks. We speculate that non-HSL TG lipase compensates for the deficiency of HSL until about 8 weeks of age in terms of growth and adiposity.

Leanness or resistance to obesity has been reported in other murine models with mutated genes that are instrumental in fatty acid metabolism. For example, mice lacking the RIIβ subunit of protein kinase A (42) or perilipin (43) have increased lipolysis; mice lacking acyl-CoA:diacylglycerol transferase (44) or mitochondrial glycerol-3-phosphate acyltransferase (45) have reduced synthesis of TG. In particular, mice with a mutation in the peroxisome proliferator activator receptor γ-binding site in the gene for the cytosolic form of phosphoenolpyruvate carboxykinase, which lack expression of phosphoenolpyruvate carboxykinase activity in WAT, are similar to *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ mice in that their WAT also has accumulation of lipodystrophy-like small cells dispersed among normal large fat cells (46). The significance of this similarity is currently unknown.

In conclusion, *Lep*$^{ob/ob}$/*HSL*$^{-/-}$ mice have impaired adipogenesis and resistance to obesity at least partially due to the reduced food intake. These findings should provide the basis for understanding the pathophysiology of obesity and can be exploited to develop novel therapy for the endemic disease.

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