Am J Physiol Endocrinol Metab 285: E1182–E1195, 2003.
First published September 3, 2003; 10.1152/ajpendo.00259.2003.

Resistance to high-fat diet-induced obesity and altered expression of adipose-specific genes in HSL-deficient mice

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Submitted 11 June 2003; accepted in final form 21 August 2003

Harada, Kenji, Wen-Jun Shen, Shailja Patel, Vanita Natu, Jining Wang, Jun-ichi Osuga, Shun Ishibashi, and Fredric B. Kraemer. Resistance to high-fat diet-induced obesity and altered expression of adipose-specific genes in HSL-deficient mice. *Am J Physiol Endocrinol Metab* 285: E1182–E1195, 2003. First published September 3, 2003; 10.1152/ajpendo.00259.2003.—To elucidate the role of hormone-sensitive lipase (HSL) in diet-induced obesity, HSL-deficient ($HSL^{-/-}$) and wild-type mice were fed normal chow or high-fat diets. $HSL^{-/-}$ mice were resistant to diet-induced obesity showing higher core body temperatures. Weight and triacylglycerol contents were decreased in white adipose tissue (WAT) but increased in both brown adipose tissue (BAT) and liver of $HSL^{-/-}$ mice. Serum insulin levels in the fed state and tumor necrosis factor-α mRNA levels in adipose tissues were higher, whereas serum levels of adipocyte complement-related protein of 30 kDa (ACRP30)/adiponectin and leptin, as well as mRNA levels of ACRP30/adiponectin, leptin, resistin, and adipsin in WAT, were lower in $HSL^{-/-}$ mice than in controls. Expression of transcription factors associated with adipogenesis (peroxisome proliferator-activated receptor-γ, CAAT/enhancer-binding protein-α) and lipogenesis (carbohydrate response element-binding protein, adipocyte determination- and differentiation-dependent factor-1/sterol regulatory element-binding protein-1c), as well as of adipose differentiation markers (adipocyte lipid-binding protein, perilipin, lipoprotein lipase), lipogenic enzymes (glycerol-3-phosphate acyltransferase, acyl-CoA:diacylglycerol acyltransferase-1 and -2, fatty acid synthase, ATP citrate lyase) and insulin signaling proteins (insulin receptor, insulin receptor substrate-1, GLUT4), was suppressed in WAT but not in BAT of $HSL^{-/-}$ mice. In contrast, expression of genes associated with cholesterol metabolism (sterol-regulatory element-binding protein-2, 3-hydroxy-3-methylglutaryl-CoA reductase, acyl-CoA:cholesterol acyltransferase-1) and thermogenesis (uncoupling protein-2) was upregulated in both WAT and BAT of $HSL^{-/-}$ mice. Our results suggest that impaired lipolysis in HSL deficiency affects lipid metabolism through alterations of adipose differentiation and adipose-derived hormone levels.

adipocyte; differentiation; insulin; leptin; fatty liver

HORMONE-SENSITIVE LIPASE (HSL) mediates the cytosolic hydrolysis of triacylglycerols (lipolysis) and cholesteryl esters (12). HSL is expressed in various tissues, including white (WAT) and brown adipose tissues (BAT), cardiomyocytes, adrenocortical cells, and gonads (11). Because HSL is responsible for the release of free fatty acids (FFA) from stored triacylglycerols in adipose tissues, the enzyme has been proposed to play an essential role in the regulation of body weight and fat mass. We previously reported, however, that the body weight of HSL-deficient ($HSL^{-/-}$) mice generated by homologous recombination fed a normal chow diet did not differ from that of wild-type ($HSL^{+/+}$) mice despite the presence of a markedly suppressed hydrolysis of triacylglycerols and cholesteryl esters in adipocytes. Moreover, the weights of white adipose tissues showed a tendency to be lower in $HSL^{-/-}$ mice than in $HSL^{+/+}$ mice (27).

A combination of genetic and environmental factors causes obesity, which is associated with disorders such as glucose intolerance, diabetes, hyperlipidemia, hypertension, and atherosclerosis. Among the environmental factors, intake of high-fat food is well known to contribute to the development of obesity.

Adipose tissues are not static sites for lipid storage but play active roles in the regulation of lipid and carbohydrate metabolism. Adipocytes are the source of several hormonal factors (18, 20, 21, 38, 39). Adipose differentiation (adipogenesis) is regulated by a series of interactions between specific transcription factors, which result in the expression of adipose-specific proteins (adipose markers) and enzymes for triglyceride synthesis (lipogenesis) as well as insulin-signaling pathways (22, 30, 36). Furthermore, BAT of rodents constitutes the major organ of thermogenesis, which is responsible for energy expenditure (14).

In the present study, we have sought to elucidate the role of HSL in diet-induced obesity by comparing the responses of $HSL^{-/-}$ and $HSL^{+/+}$ mice to normal chow and high-fat diets, focusing mainly on the function of adipose tissues.

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Table 1. *Primers used for real-time RT-PCR*

	Forward	Reverse
ACRP30	5′-CGGCAGCACTGGCAAGTT-3′	5′-CCGTGATGTGGTAAGAGAAGTAGTAGA-3′
Leptin	5′-AACCCTCATCAAGACCATTGTCA-3′	5′-CCTCTGCTTGGCGGATACC-3′
Resistin	5′-GCTGCTGCCAAGGCTGAT-3′	5′-TCTCCTTCCACCATGTAGTTT CC-3′
Adipsin	5′-GCCTGATGTCCTGCATCAACT-3′	5′-GCGCAGATTGCAGGTTGTC-3′
TNFα	5′-CACAAGATGCTGGGACAGTGA-3′	5′-TCCTTGATGGTGGTGCATGA-3′
PPARγ	5′-GCCCACCAACTTCGGAATC-3′	5′-TGCGAGTGGTCTTCCATCAC-3′
PGC-1	5′-GGACAGTCTCCCCGTGGAT-3′	5′-TCCATCTGTCAGTGCATCAAATG-3′
C/EBPα	5′-CGCAAGAGCCGAGATAAAGC-3′	5′-CGGTCATTGTCACTGGTCAACT-3′
ChREBP	5′-GGACAAGATCCGGCTGAACA-3′	5′-CGTCCGTTGCACATATTGAATG-3′
ADD1/SREBP-1c	5′-GGAGCCATGGATTGCACATT-3′	5′-GGAAGTCACTGTCTTGGTTGTTGA-3′
SREBP-1a	5′-ACAGCGGTTTTGAACGACATC-3′	5′-GGAAGTCACTGTCTTGGTTGTTGA-3′
SREBP-2	5′-GCGCCAGGAGAACATGGT-3′	5′-CGATGCCCTTCAGGAGCTT-3′
ALBP	5′-GCGTGGAATTCGATGAAATCA-3′	5′-CCCGCCATCTAGGGTTATGA-3′
Perilipin	5′-CATCTCTACCCGCCTTCGAA-3′	5′-TGCTTGCAATGGGCACACT-3′
LPL	5′-AGTGGCCGAGAGCGAGAAC-3′	5′-CCACCTCCGTGTAAATCAAGAAG-3′
GPAT	5′-CAACACCATCCCCGACATC-3′	5′-GTGACCTTCGATTATGCGATCA-3′
DGAT1	5′-CATGCGTGATTATTGCATCCA-3′	5′-GCCAGGCGCTTCTCAATCT-3′
DGAT2	5′-CCGCAAAGGCTTTGTGAAG-3′	5′-AGGAATAAGTGGGAACCAGATCAG-3′
FAS	5′-TCCTGGAACGAGAACACGATCT-3′	5′-GAGACGTGTCACTCCTGGACTTG-3′
ACLY	5′-TGGAGGCAGCATTGCAAAC-3′	5′-TCTCACAATGCCCTTGAAGGT-3′
INSR	5′-CGCTGTGTGAACTTCAGCTTCT-3′	5′-CAGCCAGGCTTCCGAGAGT-3′
IRS-1	5′-CCTCAGTCCCAACCATAACCA-3′	5′-CCGGCACCCTTGAGTGTCT-3′
GLUT4	5′-CATGGCTGTCGCTGGTTTC-3′	5′-AAACCCATGCCGACAATGA-3′
HMGCS1	5′-GACAAGAAGCCTGCTGCCATA-3′	5′-CGGCTTCACAAACCACAGTCT-3′
HMGCR	5′-TGCACGGATCGTGAAGACA-3′	5′-GTCTCTCCATCAGTTTCTGAACCA-3′
LDLR	5′-GCTCCATAGGCTATCTGCTCTTCA-3′	5′-GCGGTCCAGGGTCATCTTC-3′
ACAT1	5′-TGACTTTGTGACCAACCTCATTG-3′	5′-GAGTGCACACCCACCATTGTC-3′
UCP1	5′-CGATGTCCATGTACACCAAGGA-3′	5′-TCGCAGAAAAGAAGCCACAA-3′
UCP2	5′-GGCCTCTGGAAAGGGACTTC-3′	5′-ACCAGCTCAGCACAGTTGACA-3′
28S rRNA	5′-GAATCCGCTAAGGAGTGTGTAACA-3′	5′-CTCCAGCGCCATCCATTT-3′

ACRP30, adipocyte complement-related protein of 30 kDa; TNF-α, tumor necrosis factor-α; PPARγ, peroxisome proliferator-activated receptor-γ; PGC-1, PPARγ coactivator 1; C/EBPα, CAAT/enhancer-binding protein-α; ChREBP, carbohydrate response element-binding protein; ADD1, adipocyte determination- and differentiation-dependent factor 1; SREBP, sterol-regulatory element-binding protein; ALBP, adipocyte lipid-binding protein; LPL, lipoprotein lipase; GPAT, glycerol-3-phosphate acyltransferase; DGAT, acyl-CoA:diacylglycerol acyltransferase; FAS, fatty acid synthase; ACLY, ATP citrate lyase; INSR, insulin receptor; IRS, insulin receptor substrate; GLUT, glucose transporter; HMGCS, 3-hydroxy-3-methylglutaryl-CoA synthase; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; LDLR, low-density lipoprotein receptor; ACAT1, acyl-CoA:cholesterol acyltransferase 1; UCP, uncoupling protein.

MATERIALS AND METHODS

Animals. $HSL^{-/-}$ mice were generated by homologous recombination as previously described (27). For breeding experiments, mice heterozygous for the deleted HSL allele were used to generate homozygous $HSL^{-/-}$ mice and $HSL^{+/+}$ wild-type littermates. Genotyping was performed by a single-step PCR using three primers, as described previously (27). All experiments reported here were performed with 129/Sv-C57BL6 hybrid descendants. High-fat (35.9% wt/wt lard fat) diet and control normal chow (4.8% wt/wt fat) diet were obtained from Research Diets (New Brunswick, NJ; product nos. D12309 and D12310, respectively). Twelve-week-old male and female $HSL^{+/+}$ and $HSL^{-/-}$ littermate mice were randomized to either high-fat or normal chow diets ad libitum for 15 wk. For the comparison of food intake, $HSL^{+/+}$ and $HSL^{-/-}$ mice were housed individually, and food consumption was measured. For assessment of fasting-induced weight loss, 24-wk-old mice were fasted from 5 PM until 9 AM and body weights measured before and after the fast. For all other conditions, mice were housed in groups of one to four animals.

Temperature measurement. Body temperature was measured with a digital thermometer (model 421501; Extech Instruments, Waltham, MA). Core and surface body temperatures were measured by placing the probe for 30 s in the rectum and on the skin of the midabdominal region, respectively.

Fecal lipid analysis. Feces of 39-wk-old female mice that were fed high-fat diets and housed in metabolic cages were collected for 48 h, weighed, and stored at −80°C. Total lipids in the collected feces and in the consumed diet were extracted by the method of Folch et al. (6), and acylglycerol content was determined using an enzymatic assay kit (Sigma, St. Louis, MO).

Tissue lipid analysis. Animals under anesthesia were killed by exsanguination. Tissues were excised, weighed, quickly frozen, and stored at −80°C. Total lipids were extracted (6), and enzymatic assay kits (Sigma) were used for the determination of triacylglycerol and total cholesterol.

Histological analysis. Tissues were fixed with neutral-buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin. Samples were observed under a Leica DM IRB microscope.

Blood chemistries. Blood samples were drawn by retroorbital puncture from animals in the fed or overnight (>16 h)-fasted state, as indicated in the figure legends. Cardiac puncture was performed at the time of death in the fed state. Enzymatic assay kits were used for the determination of serum glucose, triglyceride, total cholesterol (Sigma), and FFA (Wako, Richmond, VA). Serum insulin, adipocyte complement-related protein of 30 kDa (ACRP30)/adiponectin, and leptin were measured using radioimmunoassay kits (Linco Research, St. Charles, MO).

Analysis of mRNA. Tissues were homogenized in TRIzol reagent (Invitrogen, Carlsbad, CA), and total RNA was extracted. RNA samples were further purified using the RNeasy kit (Qiagen, Valencia, CA) with RNase-free DNase I treatment according to the manufacturer's instructions. Total RNA (1 μg) was reverse-transcribed in a 20-μl reaction containing random primers and Superscript II enzyme (Invitrogen). Real-time PCR was performed with an ABI Prism 8500 system using SYBR Green Master Mix reagent (Applied Biosystems, Foster City, CA) and specific primer pairs (Table 1) selected with Primer Express software (Applied Biosystems). The relative mass of specific RNAs was calculated by the comparative cycle of threshold detection method according to the manufacturer's instructions. Equal PCR efficiency was ensured by control amplification using serial dilutions (1:1–1:128) of reverse-transcribed RNA. We confirmed the absence of genomic DNA amplification or primer dimer formation by control amplifications using non-reverse-transcribed RNA or no addition of DNA as templates. Agarose gel electrophoresis or dissociation curve analysis of RT-PCR products was performed to ensure that a single amplicon was obtained.

Statistical analysis. Results are given as means ± SE, and statistical significance was tested by unpaired two-tailed Student's *t*-test, except where otherwise stated, using StatView (version 4.5; Abacus Concepts, Berkeley, CA) and InStat (version 2.03; GraphPad Software, San Diego, CA) software for Macintosh. Data were analyzed separately for male and female mice and the results combined unless sex differences were observed.

RESULTS

Resistance to high-fat diet-induced obesity in $HSL^{-/-}$ mice. Changes in body weights during 15 wk of conditioned feeding are shown in Fig. 1. Feeding of a normal chow diet resulted in similar small increments in body weight in both male and female mice, without any differences between $HSL^{-/-}$ and $HSL^{+/+}$ mice. In contrast, a high-fat diet was associated with a substantial weight gain in normal mice; however, the absence of HSL resulted in a 20% lower ($P < 0.01$) body weight in male $HSL^{-/-}$ mice than controls (Fig. 1*A*). The body

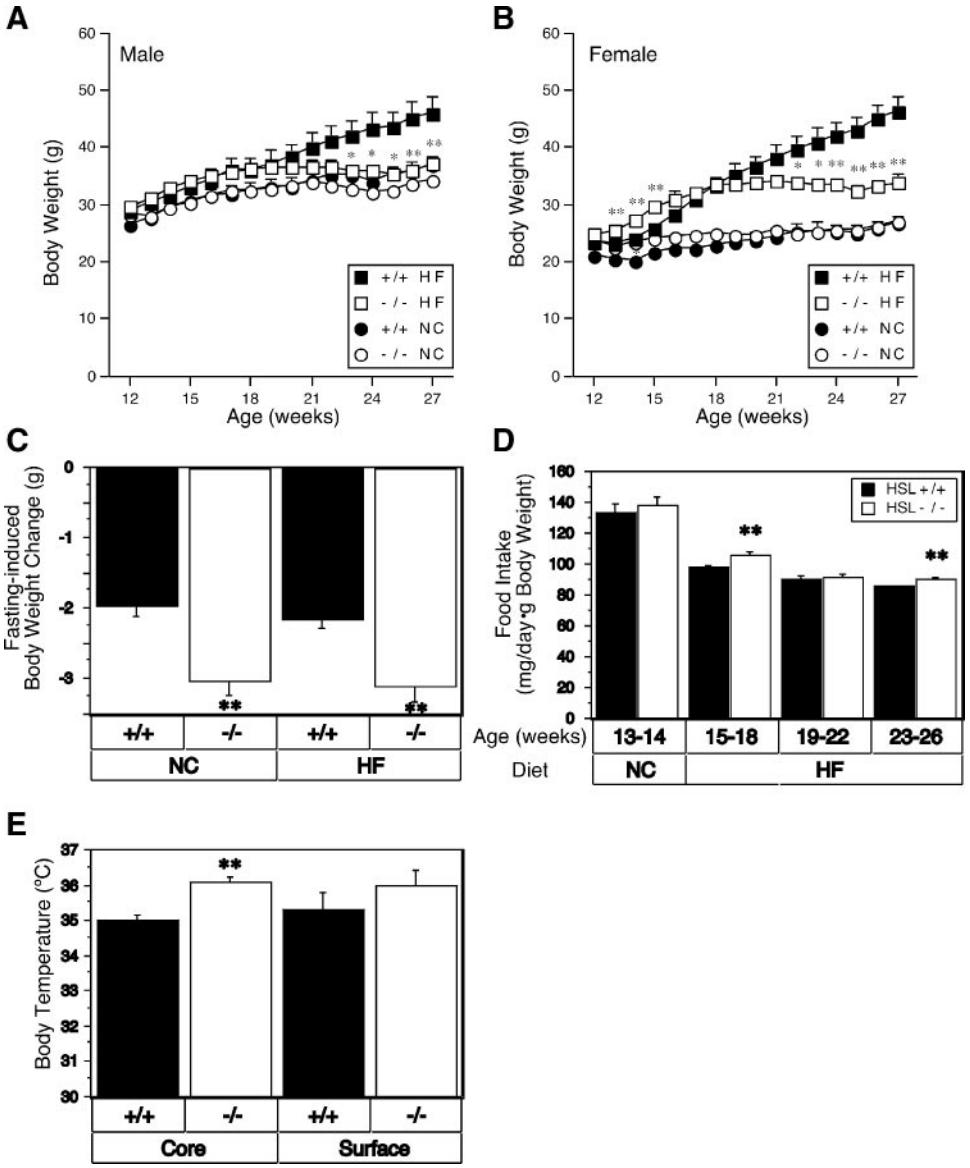

Fig. 1. Body weight, food intake and body temperature of wild-type ($HSL^{+/+}$, closed symbols) and hormone-sensitive lipase (HSL)-deficient ($HSL^{-/-}$, open symbols) mice. Twelve-week-old mice were fed either normal chow (NC, 4.8% wt/wt fat, circles) or high-fat (HF, 35.9% wt/wt fat, squares) diet for 15 wk. Body weight values of male (*A*) and female (*B*) mice. *C*: loss of body weight after overnight fasting. Twenty-four-week-old mice were fasted overnight, and differences in body weights after the fast are shown; $n = 15$–18. *D*: food intake of $HSL^{+/+}$ and $HSL^{-/-}$ female mice fed NC or HF diets ($n = 8$–30). *E*: core and surface body temperature of 44-wk-old $HSL^{+/+}$ and $HSL^{-/-}$ female mice fed HF diets ($n = 6$). Results are means ± SE. $^*P < 0.05$, $^{**}P < 0.01$ compared with $HSL^{+/+}$ mice under corresponding condition.

weight of female $HSL^{-/-}$ mice was slightly higher than that of $HSL^{+/+}$ mice during the first 3 wk of a high-fat diet (Fig. 1*B*); however, after 10 wk of high-fat feeding, body weight gain was markedly reduced in female $HSL^{-/-}$ mice compared with $HSL^{+/+}$ mice. Final body weight of high-fat-fed female $HSL^{-/-}$ mice was 26% lower ($P < 0.001$) than that of $HSL^{+/+}$ mice.

Because HSL has been reported to be expressed in the small intestine and it has been suggested that HSL might affect the absorption of lipids, especially of cholesteryl esters (7), fecal lipid content was measured to assess the potential malabsorption of dietary acylglycerols. No differences were found between $HSL^{-/-}$ and control mice in fecal appearance or weight (48 ± 3 vs. 60 ± 7 mg/g food intake; $n = 4$, $P > 0.05$). Furthermore, fecal acylglycerol content was not different between $HSL^{-/-}$ and control mice (0.1 ± 0.0 vs. 0.3 ± 0.1% of dietary acylglycerols; $n = 4$, $P > 0.05$), confirming that both $HSL^{-/-}$ and control mice absorbed >99.5% of dietary acylglycerols. Thus malabsorption of acylglycerols is not responsible for the resistance to high-fat diet-induced obesity in $HSL^{-/-}$ mice. The amount of fasting-induced weight loss, which provides a simple approximation of energy expenditure because of the elimination of energy intake, is shown in Fig. 1*C*. $HSL^{-/-}$ mice lost ~1.5-fold more weight compared with controls. Consistent with the higher energy expenditure in $HSL^{-/-}$ mice, food intake per body weight was higher in $HSL^{-/-}$ mice than in controls (Fig. 1*D*). Core body temperatures were also higher in $HSL^{-/-}$ mice (Fig. 1*E*). We (27) previously reported no differences in oxygen consumption, respiratory quotient, or core body temperature between $HSL^{-/-}$ and wild-type mice on a normal chow diet, which is consistent with similar body weights of $HSL^{-/-}$ and wild-type mice on a normal chow diet (Fig. 1, *A* and *B*). The present results, however, suggest that, on a high-fat diet, $HSL^{-/-}$ mice display increased energy expenditure through elevated thermogenesis.

We next examined the weights of various internal organs, including several different adipose tissue depots (Table 2). Even though total body weights were similar on a normal chow diet, total WAT mass, reflective of each of the fat depots (inguinal, femoral, scapular, perigonadal, and retroperitoneal), was statistically reduced ($P < 0.05$) in $HSL^{-/-}$ mice compared with controls. A high-fat diet resulted in large increases in WAT mass in all fat depots in normal mice; however, there were few or no changes in WAT mass in $HSL^{-/-}$ mice. Thus, with a high-fat diet, all measured WAT depots were significantly smaller in $HSL^{-/-}$ mice than in $HSL^{+/+}$ mice, with total WAT weight >70% lower in $HSL^{-/-}$ mice than in $HSL^{+/+}$ mice ($P < 0.0001$). The differences in total WAT weights were 5.12 g in male and 8.24 g in female mice, which contribute 56 and 68% of the lower body weights, respectively. Differences in the percent weight of WAT vs. body weight were still significant between $HSL^{-/-}$ and $HSL^{+/+}$ mice although total body weights were lower in $HSL^{-/-}$ mice on a high-fat diet (Fig. 2*A*). In contrast to WAT, interscapular BAT was larger in $HSL^{-/-}$ mice than in controls (Table 2). This resulted in BAT representing a greater percentage of total body weight in male and female $HSL^{-/-}$ mice than in controls on either a normal chow or high-fat diet (Fig. 2*B*).

In view of the reduction in WAT weight in the presence of similar body weights on a normal chow diet, most of the internal organs, such as liver, spleen, pancreas, heart, and ovary (Table 2), had a tendency to be larger in $HSL^{-/-}$ mice than in $HSL^{+/+}$ mice. This was also true on a high-fat diet. For example, livers of high-fat-fed $HSL^{-/-}$ male mice were ~1.5-fold larger than those in the corresponding controls ($P < 0.05$), whereas spleens were 1.5- to 3.2-fold larger ($P < 0.05$ to $P < 0.01$) in high-fat-fed $HSL^{-/-}$ mice than in controls. One exception was the testis, which was ~30% smaller in $HSL^{-/-}$ mice than in $HSL^{+/+}$ mice under both normal chow and high-fat conditions, consistent with previous reports of severe oligo/azospermia in $HSL^{-/-}$ mice (3, 27).

Lipid composition and histology of tissues. Although WAT weight was lower in $HSL^{-/-}$ mice and these differences from controls were accentuated by high-fat feeding, triacylglycerol content per gram of WAT tended to be similar in female $HSL^{-/-}$ and control mice (Fig. 2*C*); however, triacylglycerol content of WAT from high-fat-fed male $HSL^{-/-}$ mice was 42% lower than that of $HSL^{+/+}$ mice ($P < 0.01$). Likewise, BAT,

Table 2. *Weights of organs*

	Normal Chow			High Fat		
	$HSL^{+/+}$	$HSL^{-/-}$	Fold increase	$HSL^{+/+}$	$HSL^{-/-}$	Fold increase
No. of mice and sex	8M, 8F	8M, 7F		7M, 8F	9M, 9F	
Total WAT	3,131 ± 389	1,980 ± 170*	0.6	9,287 ± 919	2,478 ± 213†	0.3
Interscapular BAT	404 ± 53	673 ± 66†	1.7	837 ± 98	1,012 ± 77	1.2
Liver	1,417 ± 101	1,785 ± 192	1.3	1,900 ± 158	2,589 ± 218*	1.4
Spleen	99 ± 6	173 ± 17†	1.7	112 ± 7	274 ± 42†	2.4
Pancreas	188 ± 11	227 ± 24	1.2	195 ± 18	237 ± 14	1.2
Heart	147 ± 6	170 ± 11	1.2	173 ± 8	196 ± 6*	1.1
Testis (male)	256 ± 18	154 ± 7*	0.6	249 ± 12	166 ± 7*	0.7
Ovary (female)	8 ± 1	20 ± 5*	2.4	17 ± 5	32 ± 3*	1.9

Results are means ± SE (in mg) of indicated no. of mice aged 27 wk in the fed state. WAT and BAT, white and brown adipose tissue, respectively. Fold increase compares hormone-sensitive lipase (HSL)-deficient ($HSL^{-/-}$) with wild-type ($HSL^{+/+}$) mice. Total WAT mass is the sum of inguinal, femoral, scapular, perigonadal, and retroperitoneal WAT depots. *$P < 0.05$, †$P < 0.01$ compared with $HSL^{+/+}$ mice.

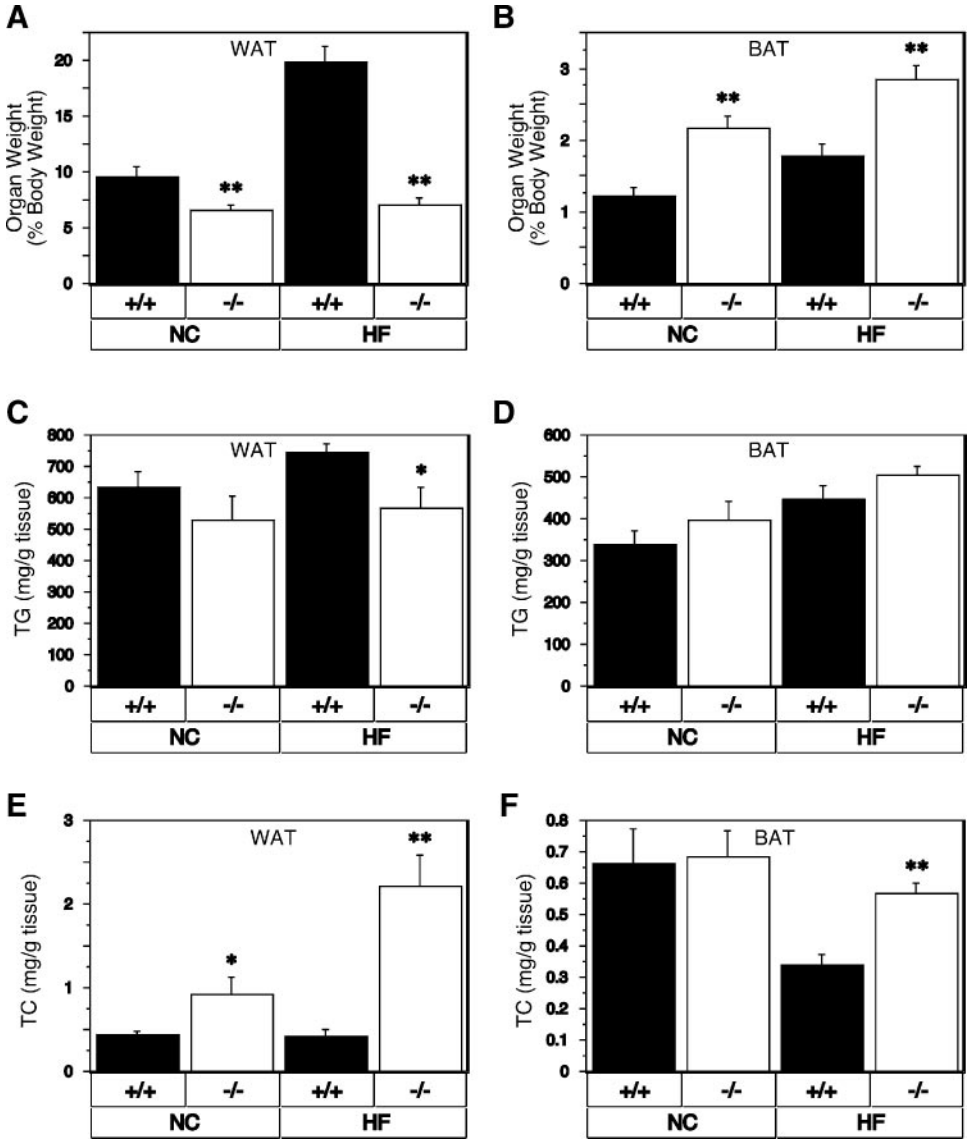

Fig. 2. Percent weight of fat depots and tissue lipid content. Percent organ weights of total white (WAT; *A*) and interscapular brown adipose tissue (BAT; *B*); $n = 15–18$ mice. Triacylglycerol (TG; *C* and *D*) and total cholesterol (TC; *E* and *F*) content in WAT (*C* and *E*) and BAT (*D* and *F*) of 27-wk-old fed mice. Results are means ± SE; $n = 8$ mice. *$P < 0.05$, **$P < 0.01$ compared with $HSL^{+/+}$ mice under corresponding condition.

although tending to have greater weight in $HSL^{-/-}$ mice, displayed similar triacylglycerol content per gram of tissue in $HSL^{-/-}$ and control mice (Fig. 2*D*). In contrast to triacylglycerol content, cholesterol content in adipose tissues (WAT and BAT) tended to be higher in $HSL^{-/-}$ mice on a normal chow diet (Fig. 2, *E* and *F*). High-fat feeding accentuated these differences, particularly in WAT, where cholesterol content was about fivefold higher in male and female $HSL^{-/-}$ mice ($P < 0.01$). These elevations in cholesterol content are compatible with the absence of neutral cholesteryl ester hydrolase (CEH) activity in adipose tissue (WAT and BAT) of $HSL^{-/-}$ mice (27).

Hepatic triacylglycerol content was higher in male $HSL^{-/-}$ mice than in controls ($P < 0.05$); however, no significant differences were observed in female mice (Fig. 3*A*). High-fat feeding increased triacylglycerol content further in both $HSL^{-/-}$ and control male mice. Hepatic cholesterol content was generally higher in male and female $HSL^{-/-}$ than in control mice whether fed normal chow or high fat (Fig. 3*B*). Consistent with the changes in lipid content, histological examination of the liver revealed lipid accumulation in hepatocytes of male $HSL^{-/-}$ mice (Fig. 3, *C-F*). On a normal chow diet, hepatocyes of male $HSL^{-/-}$ mice were larger and showed lipid vacuolation (Fig. 3*D*), which resembles that of high-fat-fed $HSL^{+/+}$ control male mice (Fig. 3*E*). Lipid accumulation was even more pronounced in high-fat-fed $HSL^{-/-}$ male mice (Fig. 3*F*). There were no substantial morphological changes in female mice (data not shown), compatible with the analysis of the triacylglycerol content.

Serum biochemical measurements. Serum biochemical values associated with lipid and carbohydrate metabolism are shown in Table 3. Fasting blood glucose values tended to be lower in $HSL^{-/-}$ mice, reaching statistical significance, however, only in male mice fed normal chow (40% decrease, $P < 0.05$). Fasting triglyceride and FFA levels were lower in $HSL^{-/-}$ mice fed a normal chow diet, as previously reported (29, 35), and

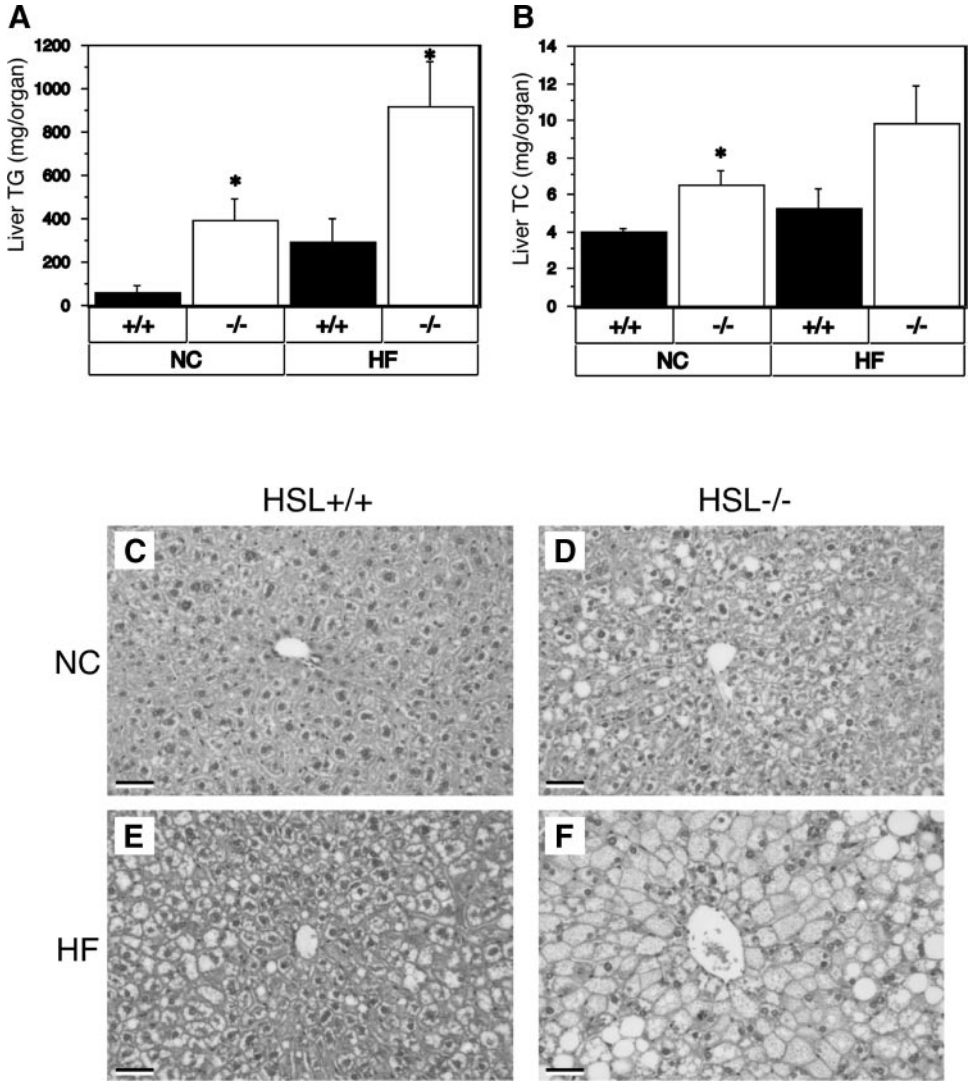

Fig. 3. Biochemical and histological changes in livers. TG (*A*) and TC (*B*) content in livers of $HSL^{-/-}$ and $HSL^{+/+}$ mice. Total lipids were extracted from livers of 27-wk-old fed male mice. Results are means ± SE; $n = 4$ mice. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with $HSL^{+/+}$ mice under corresponding condition. Hematoxylin-eosin stained liver samples from NC-fed $HSL^{+/+}$ (*C*), NC-fed $HSL^{-/-}$ (*D*), HF-fed $HSL^{+/+}$ (*E*), and HF-fed $HSL^{-/-}$ (*F*) mice. Original magnification, ×20. Scale bars, 50 μm.

these differences persisted after high-fat feeding. Total cholesterol levels were not different between $HSL^{-/-}$ and control mice. The high-fat diet caused no significant changes in fasting glucose, triglyceride, FFA, or cholesterol concentrations in normal or $HSL^{-/-}$ mice. Fasting insulin concentrations were not significantly different between control and $HSL^{-/-}$ mice. In contrast, insulin values obtained in the fed state tended to be higher in $HSL^{-/-}$ mice, reaching statistical significance ($P < 0.05$) in normal chow-fed male animals;

Table 3. *Serum biochemical values*

	Normal Chow			High Fat		
	$HSL^{+/+}$	$HSL^{-/-}$	Fold increase	$HSL^{+/+}$	$HSL^{-/-}$	Fold increase
No. of mice and sex	8M, 8F	8M, 7F		7M, 8F	9M, 9F	
Glucose, mg/dl	119 ± 12	88 ± 12	0.7	124 ± 14	92 ± 8*	0.7
Triglyceride, mg/dl	82 ± 6	39 ± 3†	0.5	83 ± 6	50 ± 8†	0.6
Total cholesterol, mg/dl	157 ± 4	157 ± 11	1.0	182 ± 10	183 ± 8	1.0
FFA, mmol/l	1.32 ± 0.10	0.96 ± 0.05†	0.7	1.08 ± 0.06	0.80 ± 0.07†	0.7
No. of mice and sex	8M, 8F	8M, 6F		7M, 8F	8M, 9F	
Insulin, fasted, ng/ml	0.68 ± 0.10	0.54 ± 0.06	0.8	1.24 ± 0.29	1.04 ± 0.18	0.8
No. of mice and sex	4M, 5F	4M, 5F		3M, 4F	3M, 4F	
Insulin, fed, ng/ml	2.06 ± 0.36	9.70 ± 2.50	2.3	7.43 ± 2.28	9.92 ± 2.34	1.3

Results are means ± SE of indicated no. of mice. Fold increase compares $HSL^{-/-}$ with $HSL^{+/+}$ mice. Insulin values in the fed state were obtained from 27-wk-old mice. Other samples were obtained from 24-wk-old mice after an overnight fast. $^{*}P < 0.05$, †$P < 0.01$ compared with $HSL^{+/+}$ mice.

insulin increased similarly in both control and $HSL^{-/-}$ mice with the high-fat diet. There were no significant differences in serum glucose, triglyceride, total cholesterol, or FFA levels between control and $HSL^{-/-}$ mice in the fed state (data not shown). Serum insulin values in the fed state were positively correlated with liver weights (Spearman $r = 0.71$, $P < 0.0001$).

Serum values of ACRP30/adiponectin (10, 15, 31) and leptin (39), two adipocyte-derived hormones, were measured to evaluate adipose tissue function. Serum ACRP30/adiponectin levels were 75–90% lower ($P < 0.01$) in both male and female $HSL^{-/-}$ mice compared with controls fed either normal chow or high-fat diets (Fig. 4*A*). When ACRP30/adiponectin values were plotted against body weights, $HSL^{-/-}$ and control mice distributed as two distinct clusters (Fig. 4*B*), with log-transformed ACRP30 values linearly correlated with body weights in $HSL^{-/-}$ mice (Pearson $r = -0.65$, $P < 0.001$). In contrast, ACRP30/adiponectin values of both $HSL^{-/-}$ and $HSL^{+/+}$ mice were distributed along one regression line when plotted against either liver weights (Fig. 4*C*; Pearson $r = -0.66$, $P < 0.0001$) or hepatic triacylglycerol contents (Pearson $r = -0.61$, $P < 0.001$). Serum ACRP30/adiponectin values were negatively correlated with insulin values in the fed state (Spearman $r = -0.52$, $P < 0.01$).

Circulating serum leptin values were not significantly different between control and $HSL^{-/-}$ mice fed normal chow (Fig. 4*D*). Serum leptin values increased with the high-fat diet in control mice; however, no

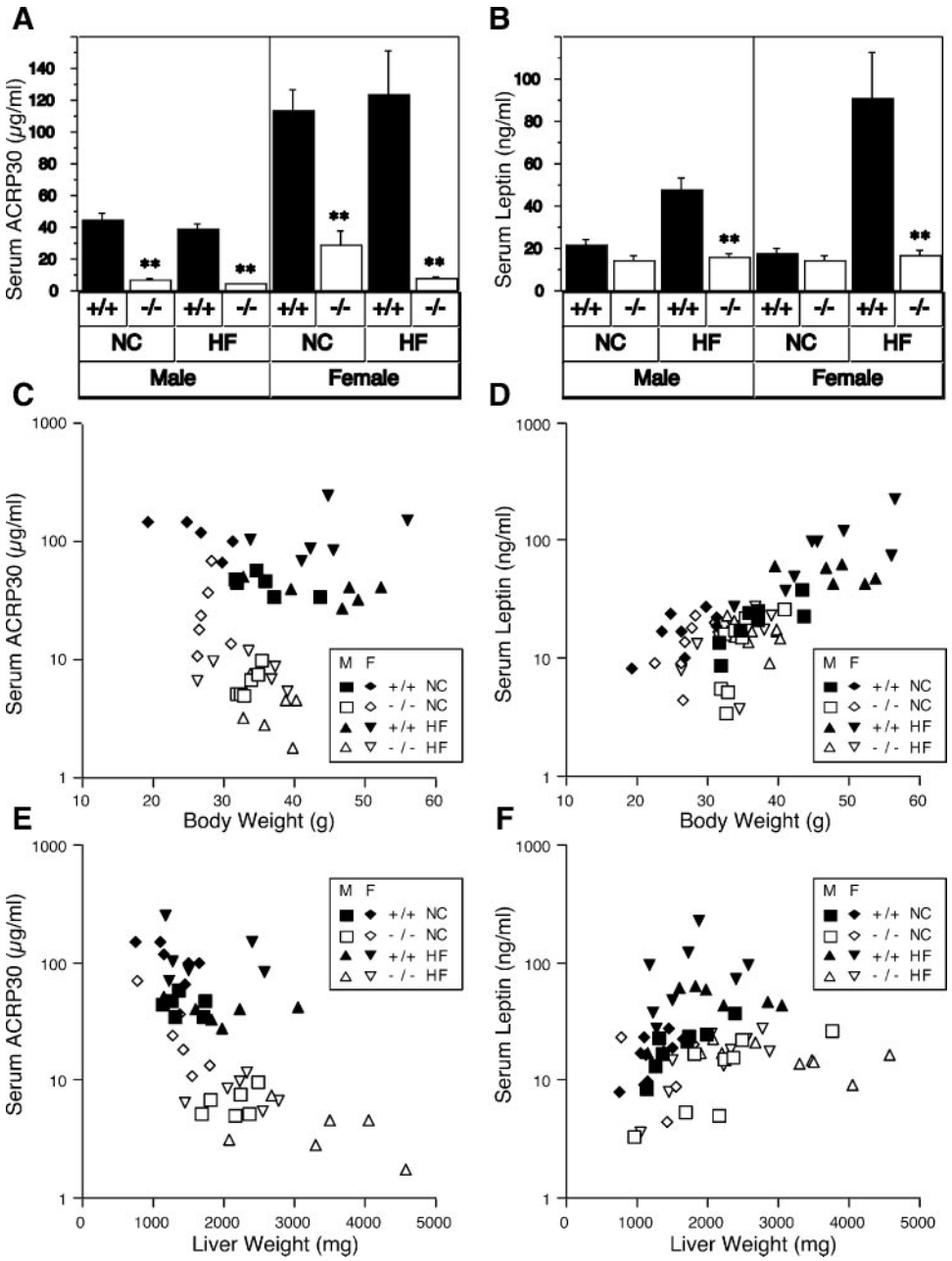

Fig. 4. Serum values of adipocyte complement-related protein of 30 kDa (ACRP30) and leptin. Serum ACRP30/adiponectin (*A*) and leptin (*B*) from 24-wk-old mice ($n = 6$) after overnight fast and from 27-wk-old fed mice ($n = 7$–9), respectively. Results are means ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with $HSL^{+/+}$ mice under corresponding condition. Serum values of ACRP30 (*C* and *E*) and leptin (*D* and *F*) are plotted against body weights (*C* and *D*) and liver weights (*E* and *F*).

changes were observed in $HSL^{-/-}$ mice, resulting in serum leptin concentrations that were 66 and 82% lower in male and female $HSL^{-/-}$ mice on a high-fat diet ($P < 0.01$ and < 0.0001, respectively). Log-transformed leptin values of control mice correlated linearly with body weights (Fig. 4*E*; Pearson $r = 0.83$, $P < 0.0001$). Leptin values observed in $HSL^{-/-}$ mice with either normal chow or high-fat feeding were significantly lower than the values predicted by this correlation (paired *t*-test, $P < 0.01$ and < 0.001, respectively). Therefore, the decrease in leptin values in $HSL^{-/-}$ mice was more than could be explained by the decrease in body weights. In contrast to ACRP30, no significant correlation was observed between serum leptin values and either liver weights (Fig. 4*F*; Pearson $r = 0.14$, $P = 0.27$) or hepatic triacylglycerol contents (Pearson $r = -0.13$, $P = 0.46$). Serum insulin values in the fed state were not correlated with serum leptin values (Spearman $r = 0.20$, $P = 0.12$).

Expression of genes in WAT and BAT of $HSL^{-/-}$ mice. In view of the lower circulating concentrations of ACRP30 and leptin in $HSL^{-/-}$ mice, expression levels of ACRP30/adiponectin (Fig. 5*A*) and leptin (Fig. 5*B*) mRNA in WAT were assessed by real-time RT-PCR. Consistent with the circulating values, both ACRP30/adiponectin and leptin mRNA levels were ~75% lower in $HSL^{-/-}$ mice on normal chow or high-fat diets. Significant correlation was observed between circulating values and mRNA levels for both ACRP30/adiponectin (Spearman $r = 0.85$, $P < 0.0001$) and leptin (Spearman $r = 0.82$, $P < 0.0001$), suggesting that decreased production of these hormones in $HSL^{-/-}$ mice contributed to the lower circulating values. In contrast to WAT, expression of ACRP30/adiponectin in BAT was not different between $HSL^{-/-}$ and control mice (data not shown). Expression of leptin in BAT of $HSL^{-/-}$ mice was lower than in controls only with high-fat feeding (data not shown).

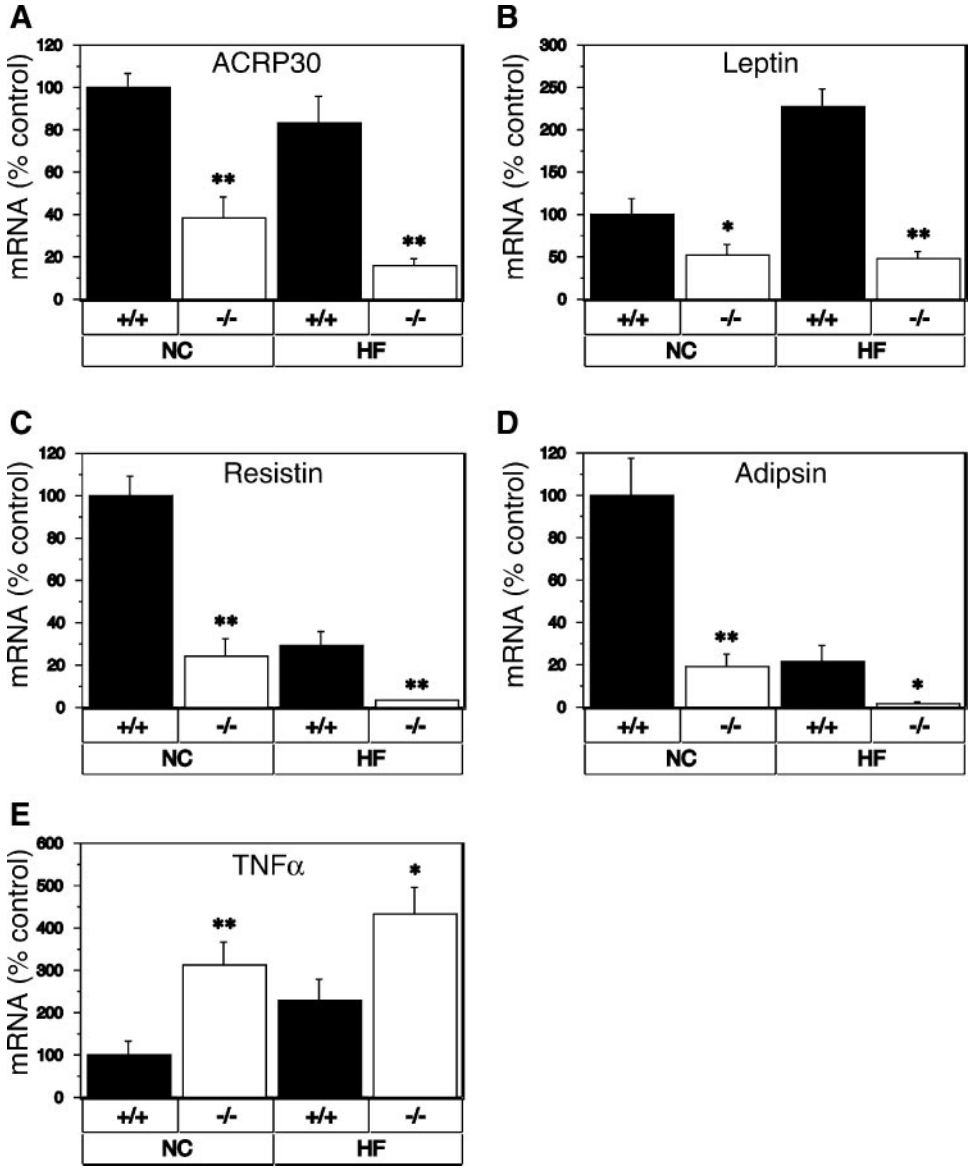

Fig. 5. Expression of adipose-derived humoral factors. Total RNA extracted from perigonadal WAT of 27-wk-old fed mice was used for gene expression analysis of ACRP30 (*A*), leptin (*B*), resistin (*C*), adipsin (*D*), and TNF-α (*E*) by real-time RT-PCR. Levels of 28S rRNA were used for normalization of sample loading. Level of mRNA expression observed in wild-type mice on the NC diet was set as 100% control. Results are means ± SE; $n = 4$ male and 4 female mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with $HSL^{+/+}$ mice under corresponding condition.

We next examined the expression of other humoral factors derived from adipose tissues (21). Expression of resistin and adipsin was decreased 60–90% ($P < 0.01$) both in WAT (Fig. 5, *C* and *D*) and BAT (data not shown) of $HSL^{-/-}$ mice. In contrast, expression of TNF-α was upregulated 2- to 3-fold ($P < 0.01$) in WAT (Fig. 5*E*) and 5- to 10-fold ($P < 0.01$) in BAT (data not shown) of $HSL^{-/-}$ mice compared with control.

To further explore the effects of HSL deficiency on adipose metabolism, we examined the expression of transcription factors associated with adipogenesis. Levels of expression of peroxisome proliferator-activated receptor-γ (PPARγ) and CCAAT/enhancer-binding protein-α (C/EBPα), two of the major transcription factors for adipogenesis (30, 36), were suppressed 40–70% ($P < 0.01$) in WAT (Fig. 6, *A* and *B*) of both male and female $HSL^{-/-}$ compared with control mice, although differences were more prominent in male mice (data not shown). Expression levels of lipogenic transcription factors (26, 32, 33) such as carbohydrate response element-binding protein (ChREBP) and adipocyte determination- and differentiation-dependent factor 1/sterol-regulatory element-binding protein-1c (ADD1/SREBP-1c) were also suppressed 50–75% ($P < 0.05$) in WAT of $HSL^{-/-}$ mice (Fig. 6, *C* and *D*). Expression of SREBP-1a, which controls both triglyceride synthesis and cholesterol metabolism, was not affected by HSL deficiency in WAT (data not shown). However, SREBP-2, which controls cholesterol synthesis and uptake, was upregulated twofold ($P < 0.05$) in WAT of $HSL^{-/-}$ mice (Fig. 6*E*). In contrast to WAT, expression levels of PPARγ, C/EBPα, ChREBP, and ADD1/SREBP-1c, as well as expression of PPARγ coactivator-1 (PGC-1), were unaffected by the absence of HSL in BAT (data not shown). However, expression levels of SREBP-1a and SREBP-2 in BAT were increased two- to threefold ($P < 0.01$) in $HSL^{-/-}$ mice (data not shown).

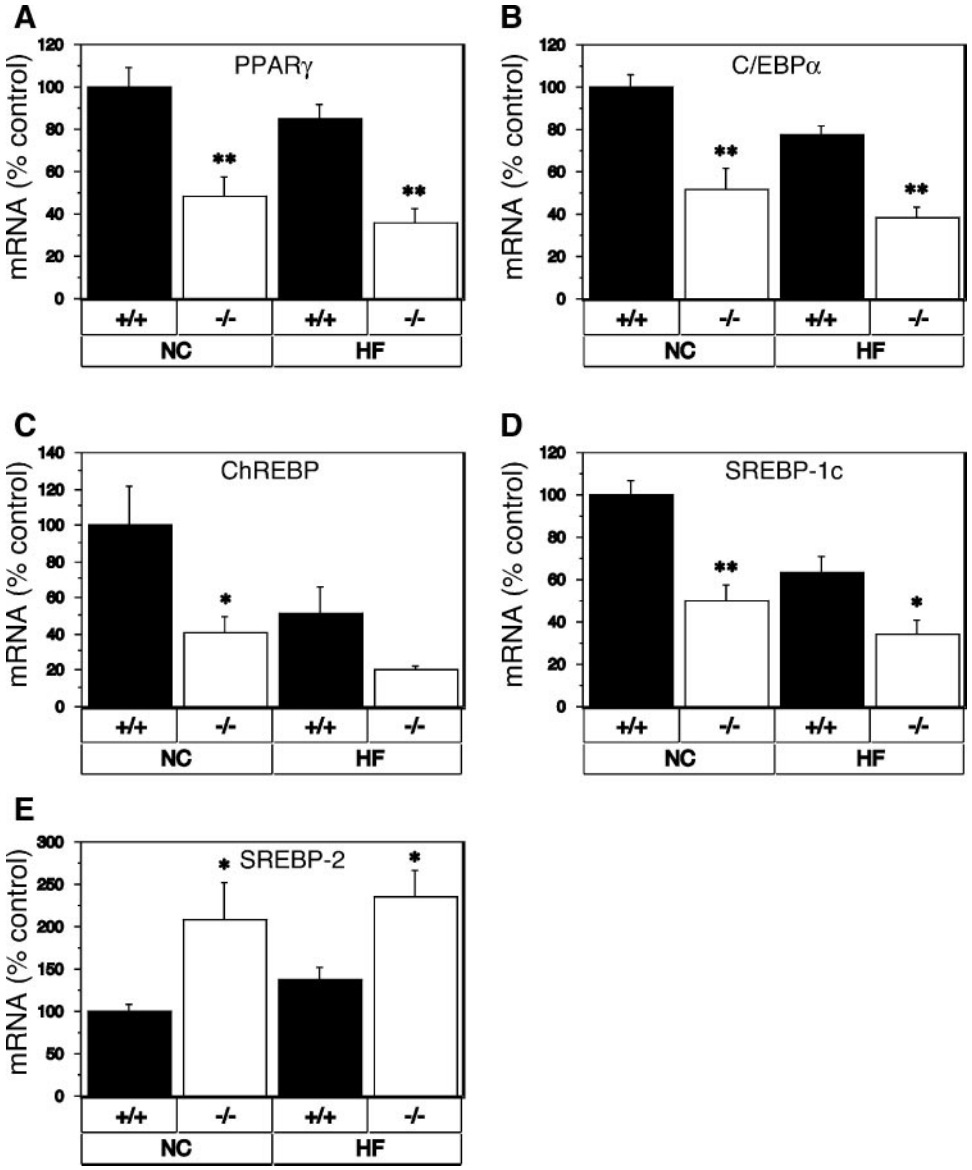

Fig. 6. Expression of transcription factors associated with adipogenesis and lipid metabolism. Total RNA extracted from perigonadal WAT of 27-wk-old fed mice was used for mRNA expression analysis of peroxisome proliferator-activated receptor-γ (PPARγ; *A*), CAAT/enhancer-binding protein-α (C/EBPα; *B*), carbohydrate response element-binding protein (ChREBP; *C*), adipocyte determination- and differentiation-dependent factor 1/sterol-regulatory element-binding protein-1 (ADD1/SREBP-1c; *D*), and SREBP-2 (*E*) by real-time RT-PCR, as described in Fig. 5. Level of mRNA expression observed in wild-type mice on the NC diet was set as 100% control. Results are means ± SE; $n = 4$ male and 4 female mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with $HSL^{+/+}$ mice under corresponding condition.

In parallel with the suppression of adipogenic transcription factors PPARγ and C/EBPα in WAT of $HSL^{-/-}$ mice, expression of adipose differentiation markers, such as ALBP and perilipin, was suppressed 50–80% ($P < 0.01$; Fig. 7, *A* and *B*). Expression of lipoprotein lipase (LPL) was also suppressed 30–40% ($P < 0.05$) in WAT of $HSL^{-/-}$ mice (Fig. 7*C*), with greater reductions observed in male mice. The levels of expression of enzymes for triglyceride synthesis, such as glycerol-3-phosphate acyltransferase (GPAT; Fig. 7*D*) and fatty acid synthase (FAS; Fig. 7*E*), as well as acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1), DGAT2, and ATP citrate lyase (ACLY) (data not shown), were decreased 60–80% ($P < 0.01$) in WAT of $HSL^{-/-}$ mice. Expressions of these adipocyte markers and lipogenic enzymes in BAT were not affected by the absence of HSL (data not shown), in parallel with the levels of PPARγ and C/EBPα mRNA. LPL mRNA levels in BAT were 1.7-fold higher in male $HSL^{-/-}$ mice fed a normal chow diet compared with controls ($P < 0.05$).

Next, we examined the gene expression of some steps in insulin-signaling pathways, since differentiation from preadipocytes to mature adipocytes is known to be associated with an increase in insulin sensitivity (36). In accord with the downregulation of adipogenic transcription factors, expression of insulin receptor (INSR; Fig. 8*A*), insulin receptor substrate-1 (IRS-1; Fig. 8*B*), and glucose transporter 4 (GLUT4) (data not shown) mRNA was decreased 30–80% ($P < 0.01$) in WAT of $HSL^{-/-}$ mice compared with controls. The expression of these genes was not affected in BAT of $HSL^{-/-}$ mice (data not shown).

Because HSL appears to be the only enzyme that has neutral CEH activity in both WAT and BAT (27), the expression of genes involved with cholesterol metabolism was also examined. Expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), the rate-lim-

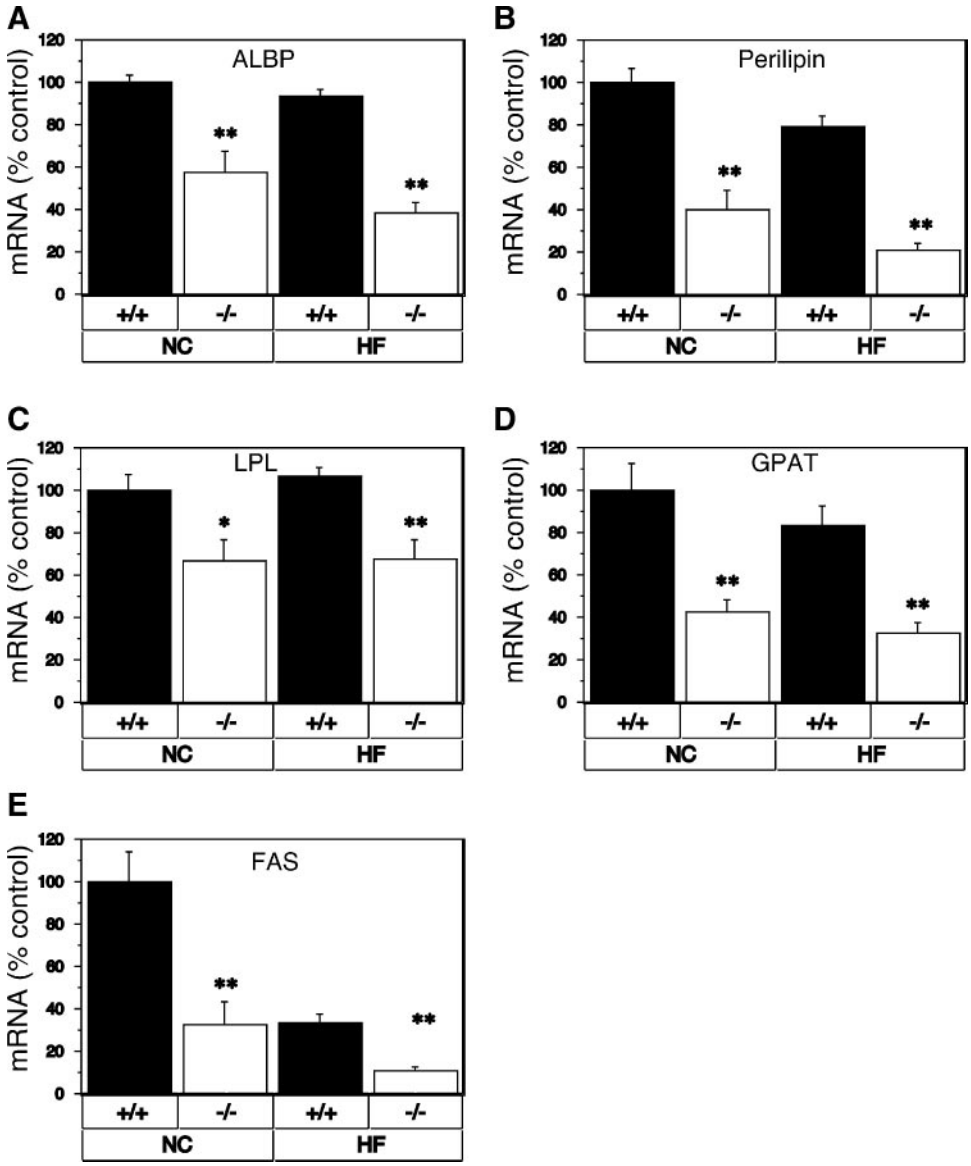

Fig. 7. Expression of genes associated with triglyceride metabolism. Total RNA extracted from perigonadal WAT of 27-wk-old fed mice was used for mRNA expression analysis of adipocyte lipid-binding protein (ALBP; *A*), perilipin (*B*), lipoprotein lipase (LPL; *C*), glycerol-3-phosphate acyltransferase (GPAT; *D*), and fatty acid synthase (FAS; *E*) by real-time RT-PCR, as described in Fig. 5. Level of mRNA expression observed in wild-type mice on the NC diet was set as 100% control. Results are means ± SE; $n = 4$ male and 4 female mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with $HSL^{+/+}$ mice under corresponding condition.

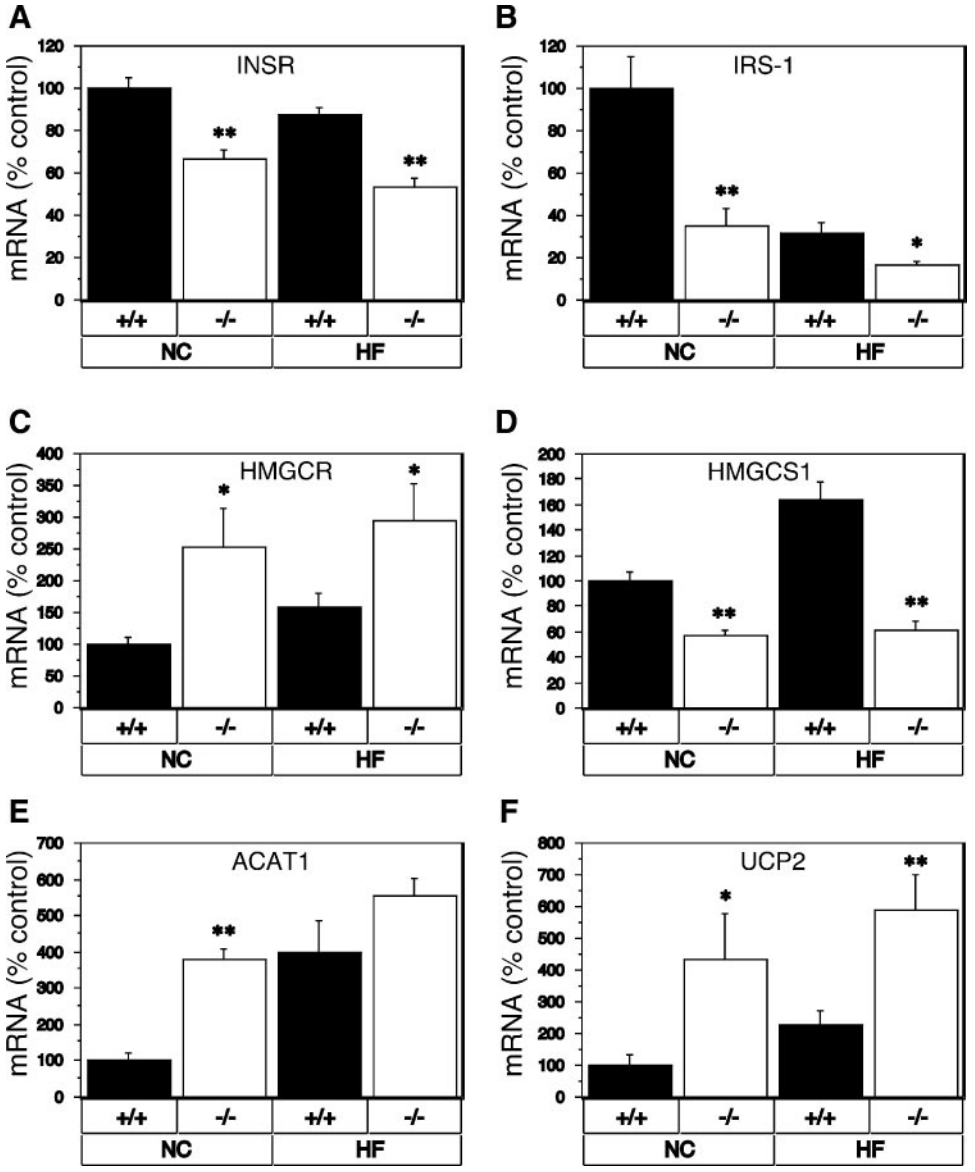

Fig. 8. Expression of genes associated with insulin signaling, cholesterol metabolism, and thermogenesis. Total RNA extracted from perigonadal WAT (*A*-*E*) and interscapular BAT (*F*) of 27-wk-old fed mice was used for mRNA expression analysis of insulin receptor (INSR; *A*), insulin receptor substrate-1 (IRS-1; *B*), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR; *C*), 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1; *D*), acyl-CoA:cholesterol acyltransferase 1 (ACAT1; *E*), and uncoupling protein-2 (UCP2; *F*) by real-time RT-PCR, as described in Fig. 5. Level of mRNA expression observed in wild-type mice on the NC diet was set as 100% control. Results are means ± SE; $n = 4$ male and 4 female mice per group. *$P < 0.05$, **$P < 0.01$ compared with $HSL^{+/+}$ mice under corresponding condition.

iting enzyme for cholesterol synthesis, was upregulated about twofold ($P < 0.05$) in WAT and BAT of $HSL^{-/-}$ mice in parallel with SREBP-2 (Fig. 8*C*). However, 3-hydroxy-3-methylglutaryl-CoA synthase-1 (HMGCS1), an enzyme upstream of HMGCR, was downregulated 50–70% ($P < 0.01$) in WAT, but unchanged in BAT (data not shown), of $HSL^{-/-}$ mice (Fig. 8*D*); expression of low-density lipoprotein receptor (LDLR) mRNA was not affected by HSL deficiency in either WAT or BAT (data not shown). Surprisingly, expression of acyl-CoA:cholesterol acyltransferase 1 (ACAT1), the enzyme that mediates the esterification of cholesterol to cellular cholesteryl esters, was increased two- to fourfold ($P < 0.01$) in WAT and five- to eightfold ($P < 0.01$) in BAT (data not shown) of $HSL^{-/-}$ mice (Fig. 8*E*).

Finally, we examined the expression of uncoupling proteins (UCP), since it appears that $HSL^{-/-}$ mice expend more energy in thermogenesis than control mice. UCP2 is known to be expressed in several organs including WAT and BAT, whereas UCP1 is expressed specifically in BAT. Expression of UCP2 in WAT was not altered by the absence of HSL in male mice but was increased in female $HSL^{-/-}$ mice (data not shown). However, expression of UCP2 in BAT was increased three- to fourfold ($P < 0.01$) in both male and female $HSL^{-/-}$ mice (Fig. 8*F*), whereas expression of UCP1 was not changed (data not shown). The observed amount of fasting-induced loss of body weight correlated with UCP2 expression (Pearson $r = 0.65$, $P < 0.0001$) but not with UCP1 expression (Pearson $r = 0.01$, $P = 0.97$) in BAT.

DISCUSSION

$HSL^{-/-}$ mice have a substantial defect or complete absence of glycerol release from isolated adipose cells when stimulated by catecholamines; although FFA re-

lease occurs, it is significantly attenuated (8, 27, 35). This defective release of glycerol and FFA from adipose cells is accompanied by a marked accumulation of diacylglycerol (8). Thus HSL appears to be the rate-limiting enzyme for diacylglycerol hydrolysis and to be essential for hormone-stimulated lipolysis. Even though lipolysis and the ability to release stored triacylglycerol from adipose tissue are dramatically reduced in $HSL^{-/-}$ mice, these animals are not obese on a normal chow diet, although their adipose cells tend to be hypertrophic and to display size heterogeneity.

In the present work, we have examined whether feeding a high-fat diet would bring out a more apparent phenotype in $HSL^{-/-}$ mice and have explored specific changes in gene expression. Even though $HSL^{-/-}$ mice have body weights similar to controls on a normal chow diet, we found the total amount of WAT to be reduced. Moreover, the amount of body weight gain produced during high-fat feeding, i.e., dietary fat-induced obesity, was markedly reduced in both male and female $HSL^{-/-}$ mice compared with controls. Thus, although the amount of WAT mass increased substantially in controls fed a high-fat diet, there were minimal changes in $HSL^{-/-}$ mice, resulting in markedly lower amounts of WAT in high-fat-fed $HSL^{-/-}$ mice compared with controls. This resistance to weight gain and adiposity was not due to differences in food intake, since $HSL^{-/-}$ mice had higher food consumption than controls when it was expressed as a function of body weight. In contrast to the smaller WAT depots in $HSL^{-/-}$ mice, BAT depots were increased in $HSL^{-/-}$ mice. The increase in BAT in $HSL^{-/-}$ mice was accompanied by higher core body temperatures and an exaggerated weight loss induced by fasting. Therefore, resistance to high-fat diet-induced obesity in $HSL^{-/-}$ mice appears to be due to increased energy expenditure and thermogenesis in BAT. Consistent with this conclusion, the expression of UCP2 mRNA was increased in BAT of $HSL^{-/-}$ mice. Indeed, the observed amount of fasting-induced body weight loss in mice correlated with UCP2, but not with UCP1, expression in BAT. These observations are in contrast to the effects of HSL deficiency in the background of leptin deficiency (Sekiya M, Osuga J, and Ishihashi S, unpublished observations). Whereas the introduction of HSL deficiency into a genetic background of leptin deficiency also results in lower body weights and adiposity, a lower food intake without any effects on energy expenditure appears to be the mechanism responsible in this setting. Thus leptin appears to modulate the mechanistic energy response (consumption vs. utilization) to HSL deficiency.

Although WAT was reduced in $HSL^{-/-}$ mice, WAT was present in all normal depots, and there was no obvious evidence of lipodystrophy. Nonetheless, the levels of expression of transcription factors required for adipocyte differentiation (PPARγ, C/EBPα), markers of adipocyte differentiation (ACRP30/adiponectin, leptin, resistin, adipsin, ALBP, perilipin, and LPL), and enzymes involved in triglyceride synthesis (GPAT, DGAT1, DGAT2, FAS, and ACLY) were reduced in WAT of $HSL^{-/-}$ mice. HSL is presumably not required for adipose differentiation, as embryonic fibroblasts from $HSL^{-/-}$ mice are able to be differentiated into mature adipose cells in vitro (25). However, it is possible that HSL, although not required for initiation or early stages of adipocyte differentiation, is needed under in vivo conditions for terminal differentiation. This suggestion is further supported by the finding of increased numbers of preadipocytes in the WAT depots of $Lep^{ob/ob}HSL^{-/-}$ mice (Sekiya M, Osuga J, and Ishihashi S, unpublished observations). Adipocyte differentiation is a complex process involving a cascade of many transcription factors, such as PPARγ and C/EBPα, and the expression of many adipocyte-specific genes (4, 17). PPARγ, a member of the nuclear hormone receptor family, is known to bind a variety of fatty acids and fatty acid metabolites (1, 24). Because HSL mediates the mobilization of fatty acids by hydrolysis of tri-, di-, and monoacylglycerols, as well as cholesteryl esters, it might play an important role in supplying intrinsic ligands for PPARγ. A relative lack of PPARγ ligands due to the absence of HSL might suppress the mutual activation of PPARγ and C/EBPα, thus affecting adipocyte differentiation in vivo. In this circumstance, the normal differentiation observed of embryonic fibroblasts from $HSL^{-/-}$ mice might be due to the addition of exogenous PPARγ ligands to the cell culture, thereby bypassing the requirement for endogenous PPARγ ligands to be produced by HSL-mediated hydrolysis. Conversely, it is possible that the accumulation of diacylglycerol in WAT of $HSL^{-/-}$ mice interferes with any number of orchestrated events involved in normal differentiation rather than the deficiency of an intrinsic ligand. It is important to note that the expression of not all examined genes was reduced in WAT from $HSL^{-/-}$ mice. For instance, TNF-α, ACAT, HMGCR, and SREBP-2 mRNA levels were all increased in WAT from $HSL^{-/-}$ mice. The elevations in ACAT, HMGCR, and SREBP-2 mRNA levels are interesting in light of the marked increase in cholesterol content of WAT observed in $HSL^{-/-}$ mice that was accentuated by high-fat feeding. It is likely that the absence of neutral CEH activity in WAT of $HSL^{-/-}$ mice causes a reduction in regulatory pools of cellular unesterified cholesterol, leading to upregulation of SREBP-2 and, subsequently, other sterol-regulated genes (2). However, this cannot be the complete explanation, since LDLR mRNA levels are unchanged and HMGCS mRNA levels are actually decreased.

In contrast to WAT, gene expression in BAT was generally unaltered by the absence of HSL, even though BAT depots were increased. Nonetheless, similar to WAT, the ACAT, HMGCR, and SREBP-2 mRNA levels were all elevated in BAT from $HSL^{-/-}$ mice, which occurred concurrently with an increase in cholesterol content, particularly with high-fat feeding. Levels of UCP2 mRNA were elevated in BAT of both male and female $HSL^{-/-}$ mice. The upregulation of UCP2 paralleled the increase in SREBP-2 expression in BAT, which is consistent with the activation of the UCP2 promoter by SREBPs (19). Interestingly, UCP1

mRNA levels were unaltered, as were levels of PGC-1, which has been linked to mitochondrial biogenesis and thermogenesis (28). Thus it is possible that the increased energy expenditure observed in $HSL^{-/-}$ mice is due to the absence of neutral CEH activity in BAT causing a reduction in regulatory pools of cellular unesterified cholesterol, leading to upregulation of SREBP-2 and subsequent upregulation of UCP2 in BAT, resulting in an increase in thermogenesis.

Liver weight was increased in $HSL^{-/-}$ mice. Hepatic content of both triacylglycerol and cholesterol of $HSL^{-/-}$ mice was about twice that of controls. Histological features of the liver clearly supported the accumulation of lipid in hepatocytes of $HSL^{-/-}$ male mice. Because the liver is not an organ where HSL is normally expressed, our findings suggest that HSL deficiency affects hepatic lipid metabolism indirectly. On the basis of the correlation of liver weight with both serum ACRP30/adiponectin and insulin levels, but not with leptin, we speculate that decreased ACRP30/adiponectin levels in $HSL^{-/-}$ mice play a causative role in hepatic fat accumulation, possibly through inactivation of fatty acid oxidation (37), and induction of fatty acid synthesis by hyperinsulinemia in the fed state; the expression of lipogenic enzymes such as FAS, GPAT, and ACLY are increased in the liver of $HSL^{-/-}$ mice (Harada K, Shen W-J, and Kraemer FB, unpublished observations). In contrast to these observations, other investigators (8, 9, 35) have reported that hepatic triacylglycerol content of $HSL^{-/-}$ mice was lower than in controls. The basis for the difference is that these other investigators showed that fasting induced an increase of hepatic triacylglycerol in control mice, but not in $HSL^{-/-}$ mice, where circulating FFA levels failed to rise to the levels seen in controls.

Fasting blood glucose, triglyceride, and FFA levels were lower in $HSL^{-/-}$ mice than in controls on either a normal chow or high-fat diet. There was no difference in fasting insulin level between $HSL^{-/-}$ and control mice, consistent with a previous report (29), and fasting insulin values rose on a high-fat diet similarly in control and $HSL^{-/-}$ mice. However, insulin levels in the fed state were higher in $HSL^{-/-}$ male mice than in controls. This might indicate the existence of insulin resistance in $HSL^{-/-}$ mice, even though they have less adiposity. This would be compatible with the decreased expression of INSR, IRS-1, and GLUT4, accompanied by the decrease of C/EBPα (36), observed in WAT of $HSL^{-/-}$ mice. Lower circulating ACRP30/adiponectin levels in $HSL^{-/-}$ mice might contribute to this, since deficiency of ACRP30/adiponectin has been reported to cause insulin resistance and hyperinsulinemia (13, 16). Another possible mechanism contributing to the potential insulin resistance in $HSL^{-/-}$ mice is the apparent upregulation of adipose tissue TNF-α, a cytokine associated with inflammation and known to cause insulin resistance (34). It is possible that the reduced expression of PPARγ, which has been shown to have an anti-inflammatory effect (5), in WAT of $HSL^{-/-}$ mice might allow the upregulation of TNF-α. Interestingly, we found that the size of pancreatic islets of $HSL^{-/-}$ mice was larger than that of controls (Shen W-J and Kraemer FB, unpublished observations). This enlargement of islets could be a compensatory response to peripheral insulin resistance or could result from abnormalities in insulin secretion due to the absence of β-cell HSL (29). Consistent with our findings, a recent study reported that a different $HSL^{-/-}$ mouse with a hybrid background displayed impaired insulin sensitivity in vivo associated with compensatory hypertrophy of pancreatic islets (23). It is noteworthy that glucose-stimulated insulin secretion has been reported to be intact in one strain of $HSL^{-/-}$ mice (23) but impaired in another $HSL^{-/-}$ mouse with a diabetes-prone C57BL/6 background (29).

In conclusion, $HSL^{-/-}$ mice have reduced quantities of WAT with increased amounts of BAT and are resistant to high-fat diet-induced obesity secondary to an apparent increase in thermogenesis and energy expenditure. Although the accumulation of diacylglycerol in tissues of HSL-deficient mice could be responsible for these effects, the changes in adipose tissue gene expression in $HSL^{-/-}$ mice suggest that abnormalities in the generation of fatty acids or fatty acid metabolites might prevent complete differentiation of WAT, resulting in alterations in adipocyte-derived hormones and cytokines with systemic manifestations. In addition, it is suggested that dysregulation of intracellular cholesterol metabolism due to the inability to hydrolyze cholesteryl esters in adipose tissue of HSL-deficient mice might be the primary abnormality leading to increased thermogenesis. Thus the present experiments establish a potential link between adipose tissue sterol metabolism and energy expenditure. Additional experiments will be needed to prove this.

We thank Shahrad Taheri for suggestions and technical support, Ann Nomoto for microscopic technique, and Eve Reaven, Salman Azhar, Jingwen Liu, Penelope Collins, Yu Liang, and Jenny Wang for helpful discussions.

DISCLOSURES

This work was supported, in part, by research grants from the Research Service of the Department of Veterans Affairs, by Grant DK-46942 from the National Institute of Diabetes and Digestive and Kidney Diseases (to F. B. Kraemer), and by a Research Award from the American Diabetes Association (to W.-J. Shen).

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