Human hormone-sensitive lipase (HSL): expression in white fat corrects the white adipose phenotype of HSL-deficient mice[S]

Mélanie Fortier,* Krishnakant Soni,* Nancy Laurin,[1,*] Shu Pei Wang,* Pascale Mauriège,[†] Frank R. Jirik,[§] and Grant A. Mitchell[2,*]

Division of Medical Genetics,* Research Centre, Sainte-Justine Hospital, Montréal, Québec, Canada; Division of Kinesiology,[†] Department of Social and Preventive Medicine, Laval University, Ste-Foy, Quebec, Canada; and Department of Biochemistry and Molecular Biology,[§] University of Calgary, Calgary, Alberta, Canada

Abstract **In white adipose tissue (WAT), hormone-sensitive lipase (HSL) can mediate lipolysis, a central pathway in obesity and diabetes. Gene-targeted HSL-deficient ($HSL^{-/-}$) mice with no detectable HSL peptide or activity (measured as cholesteryl esterase) have WAT abnormalities, including low mass, marked heterogeneity of cell diameter, increased diacylglycerol content, and low β-adrenergic stimulation of adipocyte lipolysis. Three transgenic mouse strains preferentially expressing human HSL in WAT were bred to a $HSL^{-/-}$ background. One, $HSL^{-/-}$N, expresses normal human HSL (41.3 ± 9.1% of normal activity); two express a serine-to-alanine mutant (S554A) initially hypothesized to be constitutively active: $HSL^{-/-}$ML, 50.3 ± 12.3% of normal, and $HSL^{-/-}$MH, 69.8 ± 15.8% of normal. In WAT, $HSL^{-/-}$N mice resembled $HSL^{+/+}$ controls in WAT mass, histology, diacylglyceride content, and lipolytic response to β-adrenergic agents. In contrast, $HSL^{-/-}$ ML and $HSL^{-/-}$MH mice resembled nontransgenic $HSL^{-/-}$ mice, except that diacylglycerol content and perirenal and inguinal WAT masses approached normal in $HSL^{-/-}$MH mice. Therefore, *1*) WAT expression of normal human HSL markedly improves $HSL^{-/-}$ WAT biochemically, physiologically, and morphologically; *2*) similar levels of S554A HSL have a low physiological effect despite being active in vitro; and *3*) diacylglycerol accumulation is not essential for the development of the characteristic WAT pathology of $HSL^{-/-}$ mice.**—Fortier, M., K. Soni, N. Laurin, S. P. Wang, P. Mauriège, F. R. Jirik, and G. A. Mitchell. **Human hormone-sensitive lipase (HSL): expression in white fat corrects the white adipose phenotype of HSL-deficient mice.** ***J. Lipid Res.*** **2005.** 46: **1860–1867.**

Supplementary key words hormone-sensitive lipase • adipocyte • mouse • mutation • fat metabolism

Hormone-sensitive lipase (HSL; EC 3.1.1.3; gene designation *Lipe*) is principally known for its role in white adipose tissue (WAT), in which HSL can release fatty acids from triglycerides (TGs) and diglycerides (DGs) (1). Gene-targeted HSL-deficient ($HSL^{-/-}$) mice expressing no identifiable HSL demonstrated that HSL is essential for normal WAT morphology and function and also that an HSL-independent lipolytic pathway exists (2–5). $HSL^{-/-}$ mice also revealed the importance of HSL in other organs. Although some intriguing phenotypic differences exist between different independently derived $HSL^{-/-}$ lines, these mice have been reported to have abnormal glucose-stimulated insulin secretion (5–7), cholesteryl ester accumulation in adrenal cortex (8), and male infertility (3, 9).

HSL is regulated by phosphorylation on several serine residues. β-Adrenergic agonists activate HSL by protein kinase A-mediated serine phosphorylation at positions 552, 649, and 650 in human HSL (10, 11). Other kinases can also phosphorylate serine 554 (S554) in vitro, including glycogen synthase kinase-4 and protein kinase II Ca^{2+}/calmodulin-dependent and AMP-activated protein kinase (12–14). Phosphorylation at S600 can be mediated by extracellular signal-regulated kinase (15). In addition to phosphorylation, WAT HSL activity is also enhanced by translocation of HSL from the cytoplasm to the lipid droplet surface (16), by dimerization (17), and by interaction with adipocyte fatty acid binding protein (aP2) (18). HSL also reportedly docks with lipotransin (19).

Several groups have studied S554 and orthologous residues in mammalian HSLs (11, 14, 20, 21). In rat HSL, phosphorylation of S565, which corresponds to S554 in human HSL, was associated with lipolytic inactivity and was reportedly incompatible with phosphorylation at S552 (14).

Manuscript received 28 February 2005 and in revised form 23 May 2005.
Published, JLR Papers in Press, June 16, 2005.
DOI 10.1194/jlr.M500081-JLR200

Abbreviations: aP2, adipocyte fatty acid binding protein; DG, diglyceride; HSL, hormone-sensitive lipase; $HSL^{-/-}$, hormone-sensitive lipase-deficient; TG, triglyceride; WAT, white adipose tissue.

[1] Present address of N. Laurin: Cell and Molecular Biology Division, Toronto Western Research Institute, University Health Network, Toronto, Ontario, Canada M5G 2C4.
[2] To whom correspondence should be addressed.
e-mail: grant.mitchell@recherche-ste-justine.qc.ca

[S] The online version of this article (available at http://www.jlr.org) contains an additional table.

Phosphorylation of S563, orthologous to human S552, correlated with active HSL (14). We hypothesized that replacement of S554 with an alanine in human HSL (S554A) might produce a stable peptide, favor S552 phosphorylation, and constitutively activate HSL in vivo. Two in vitro studies of the corresponding rat HSL mutant, S565A, showed it to possess catalytic activity, although one reported low DG hydrolase activity in CHO cells (65% of normal) (20) and the other reported increased activity in COS cells (135%) (11).

To directly study the physiological effect of normal and mutant S554A human HSL, we created transgenic mice that express HSL primarily in WAT from the aP2 promoter (22) and studied their WAT phenotypes on a HSL$^{-/-}$ background.

RESEARCH DESIGN AND METHODS

Chemicals

Raffinose, DTT, Triton X-100, BSA, and enzymes for glycerol determination were from Roche Diagnostics (Laval, Quebec, Canada). The selective β-3 adrenergic agonist CL316,243 was donated by Wyeth-Ayerst Research Laboratories (Princeton, NJ). Other chemicals were from Sigma-Aldrich (Oakville, Ontario, Canada).

Nomenclature of HSL serine residues

Human, rat, and mouse HSLs differ by in-frame deletions/insertions (23). We designate residues by their positions in human HSL unless stated otherwise.

Transgenic mice

HSL vectors (**Fig. 1**) were constructed. hHSL(1.3)/blue (a generous gift of C. Holm), containing a 1.3 kb human HSL cDNA 3′ fragment, was subcloned into pBluescript (SK). Two synonymous mutations were introduced by PCR mutagenesis, A1250C and C1253A, creating a *Nde*I site at nucleotide 1,250. Then, a fragment spanning nucleotides −18 to 1,260 of human HSL and flanked by the introduced *Nde*I site and a 5′ cloning *Sal*I site was amplified by RT-PCR from human adipocyte RNA and inserted in the *Nde*I and *Sal*I sites of hHSL(1.3)/pBluescript (SK), giving a full-length human HSL cDNA. One vector contained the normal HSL sequence. In the other, a T→G transversion at nucleotide 1,662 was introduced by PCR mutagenesis, producing the S554A mutation. The SV40 polyadenylation sequence was cloned after amplification from the SVCMVexPA vector (a generous gift from E. Cohen) (24), and cloned into the *Eco*RI site, permitting cloning in the *Eco*RI site immediately 3′ to the end of the HSL coding sequence. The introduced sequences were verified by sequencing of both strands. The normal and mutant HSL cDNAs were cloned downstream of the 5.4 kb aP2 promoter in pBluescript (SK) vector (22). For microinjection, the *Hind*III-*Sac*II fragment was purified (Qiaquick gel extraction kit; Qiagen, Mississauga, Ontario, Canada) and introduced to the pronuclei of fertilized (B6×CBA) F2 oocytes, which were implanted in pseudopregnant females.

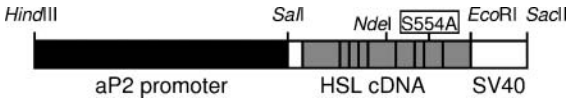

Fig. 1. The human hormone-sensitive lipase (HSL) transgenes contain, from left to right, a 5.4 kb adipocyte fatty acid binding protein (aP2) promoter fragment (black); an 18 bp 5′ untranslated region (white); the complete coding sequence of human HSL (gray), in which the positions of the nine HSL exons are shown; and a 3′ untranslated region (white), including the SV40 polyadenylation sequence. The site of the serine-to-alanine mutation (S554A) is shown within exon 8.

Three lines were obtained, one expressing the normal human transgene (N) and two expressing either low or high levels of the mutant S554A cDNA (ML and MH, respectively). Transgene copy number was evaluated semiquantitatively by genomic Southern blots as one copy for transgenic N, seven for transgenic MH, and three for transgenic ML (data not shown). Initial evaluations of the transgenes in HSL$^{+/+}$ mice were performed on a mixed B6×CBA background. All studies presented here of transgenic mice on a HSL$^{-/-}$ background used mice bred for at least six generations to a C57BL/6 background. All procedures were approved by the Institutional Committee for Animal Protection at the Research Centre of Ste-Justine Hospital.

Genotyping

HSL genotyping was as described (2). The presence of the human transgene was assessed by genomic Southern blotting with a 1.3 kb *Eco*RI-*Sst*I fragment of phHSL(1.3) spanning nucleotides 1,263 to 260 bases 3′ to the stop codon. On genomic *Eco*RI digests, the transgene generates a 5 kb fragment.

Organ weights of transgenic mice

Mice were maintained on Teklad Mouse Breeder Diet 8626 (Harlan, Madison, WI) and a 12 h light/dark cycle. At age 6 months, mice were killed after overnight fasting using pentobarbital sodium anesthesia, 6.5 μg/10 g body weight intraperitoneally (Somnotol; MTC Pharmaceuticals, Hamilton, Ontario, Canada). After cardiac puncture, organs were rapidly removed, weighed, and then frozen or used for cell isolation.

Leptin concentration

Plasma leptin levels was measured by ELISA (Mouse Leptin Quantikine ELISA Kit; R&D Systems, Minneapolis, MN) according to the manufacturer's recommendations.

Histology

Formol-fixed perigonadal fat fragments were embedded in paraffin. Tissue sections (5 μm) were stained with hematoxylin-phloxine-safran. Histological images were stored using the SPOT program (Diagnostic Instruments, Inc., Sterling Heights, MI) and analyzed with Image Pro software (Media Cybernetics, Carlsbad, CA). To determine cell diameter, points were randomly chosen in regions of good histological quality. Starting from these points, the maximal diameters was measured consecutively for 150–200 cells in a centrifugal spiral excluding blood vessel and connective tissue cells. For each genotype, six or more nonoverlapping patches were studied (800–1,000 adipocytes).

RNA quantification

Total RNA was extracted from perigonadal fat (Trizol; Gibco-Life Technologies, Burlington, Ontario, Canada). To increase yield, homogenates were incubated at 37°C for 10 min, then vortexed before continuing.

Total RNA served as the template for first-strand synthesis using poly(dT) primers and Superscript II reverse transcriptase (Invitrogen, Burlington, Ontario, Canada). For quantitative real-time PCR, we used the QuantiTect SYBR green PCR kit (Qiagen). Primers were as follows: GAGTTAAGTGGGCGCAAGTC and AAGTCCCTCAGGGTCAGGTT (exons 7 and 8, respectively) for human HSL mRNA; TGAGATGGTAACTGTGAGCC and ACTGAGATTGAGGTGCTGTC (exons 2 and 3) for mouse HSL mRNA; and ACGTTGACATCCGTAAAGACCT and GCAGTAATCTCCTTCTGCATCC for β-actin mRNA, an internal control for RNA quantity. Each reaction yields 100 bp amplicons. PCR conditions were 15 s at

94°C, 20 s at 60°C, and 20 s at 72°C for 45 cycles. After amplification, a melting curve (0.01°C/s) was used to assess product purity.

Enzyme assays

HSL activity was assayed in vitro as neutral cholesteryl esterase using cholesteryl [1-^{14}C]oleate and as TG hydrolase using glycerol tri[9,10(n)-^{3}H]oleate (Amersham Biosciences, Baie d'Urfé, Quebec, Canada) as described (25) using fat-free infranatants of perirenal WAT from 2 month old mice. Protein concentration was estimated using the DC Protein Assay (Bio-Rad, Mississauga, Ontario, Canada).

Antibody production and Western blotting

For HSL, a N-terminal human HSL cDNA fragment encoding amino acids 1–323 was cloned in pEt-30a(+) (Novagen, Madison, WI). Overexpression was stimulated by isopropyl β-D-1-thiogalactopyranoside as described by the supplier. The expressed HSL fragment was isolated from the inclusion body of cultures incubated to an optical density of 0.6 using an affinity column recognizing the N-terminal histidine tag, as recommended by the company. The histidine tag was removed by enterokinase digestion. The HSL peptide was solubilized in 0.1 M Tris-Cl, pH 8.0, containing 0.1% L-sarcosine, 20% glycerol, and 10 mM 1,4-dithioerythritol. Polyclonal rabbit anti-HSL antibodies were produced (Clontech, Palo Alto, CA).

For Western blotting, fat-free infranatant proteins were used for SDS-PAGE. Western blotting was performed using a 1:8,000 dilution of anti-recombinant HSL serum. Bound antibody was detected by chemiluminescence (POD detection system; Roche) as recommended by the manufacturer. Signal intensity was estimated with ImageJ version 1.31 software (National Institutes of Health, Bethesda, MD).

Lipolysis in isolated adipocytes

Adipocytes were isolated between 9 and 10 AM from male mice with free access to food until the experiment, using collagenase digestion of 250 mg of perigonadal fat (26). Lipolysis was assayed as described (27), both in the nonstimulated (basal) state and in the presence of 10 μM CL316,243. Triplicate measurements were performed for each mouse. At least three mice of each genotype were tested. Lipolysis results were expressed as micromoles of glycerol per 10^6 cells per 2 h.

DG and TG measurements

Thirty milligrams of fat was homogenized in 2 ml of 0.9% NaCl and then extracted for 1 h with shaking in 20 ml of a mixture of chloroform-methanol (2:1) plus 4 ml of 0.9% NaCl. After centrifugation for 10 min at 2,000 *g*, the chloroform phase was removed and then evaporated under nitrogen. Lipids were resuspended in chloroform-methanol (2:1), and thin-layer chromatography was performed (Partisil K5; Whatman International Ltd., Maidstone, England) using hexane-ether-acetic acid (80:20:3) as the mobile phase. Scrapings of the TG and DG spots were extracted with chloroform-methanol (2:1) and quantified (TG GPO-PAP kit; Roche).

Data analysis

Comparisons were performed using the unpaired two-tailed Student's *t*-test. Distributions of adipocyte diameters were analyzed using GraphPad InStat software (GraphPad, San Diego, CA).

RESULTS

Effects of HSL transgenes on WAT mass and circulating metabolites

On a normal (HSL$^{+/+}$) background, fat masses, organ weights, and WAT histology did not differ significantly between the transgenic mice (N, ML, and MH) and normal HSL$^{+/+}$ controls (data not shown). Litter size and survival to adulthood were not affected by transgene expression (data not shown). The transgenes showed Mendelian segregation independent of the endogenous mouse HSL gene.

As reported previously in this strain of HSL$^{-/-}$ mice (2), body mass in 6 month old males was significantly less in HSL$^{-/-}$ (32.6 ± 0.4 g) than in HSL$^{+/+}$ (39.9 ± 0.8 g) mice ($P < 0.001$) (**Fig. 2**). In HSL$^{-/-}$N mice, body, mes-

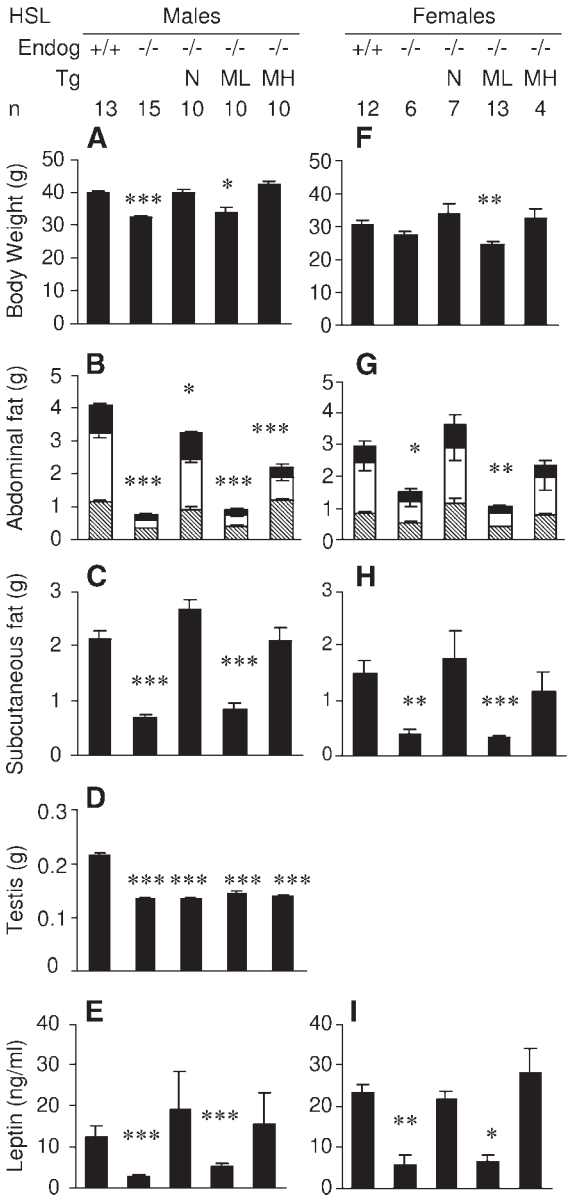

Fig. 2. Effect of HSL genotype on whole body, adipose, and testicular masses and plasma leptin concentrations of 6 month old mice. Endogenous (Endog) or transgenic (Tg) HSL is specified for males and females. For abdominal fat masses (B, G), individual abdominal fat depot masses are indicated as follows: mesenteric (black, error bars directed up); perigonadal (white, error bars down), and perirenal (hatched, error bars up); in these panels, the statistical comparisons refer to total abdominal fat masses. In all panels, statistical differences from wild-type HSL$^{+/+}$ mice are indicated as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. n, number of mice.

enteric, and subcutaneous fat masses were not statistically different from normal, but perirenal and perigonadal fat masses were lower ($P < 0.01$) (Fig. 2; see supplementary table). HSL$^{-/-}$ML mice resemble HSL$^{-/-}$ mice in body mass (31.1 ± 1.1 g; $P < 0.01$) (Fig. 2A) and in intra-abdominal masses (perigonadal, perirenal, mesenteric; Fig. 2B) and subcutaneous inguinal WAT depot masses (Fig. 2C). In contrast, HSL$^{-/-}$MH mice had a mixed profile, with perirenal and subcutaneous fat depot masses similar to those of HSL$^{+/+}$ controls but lower perigonadal and mesenteric fat masses ($P < 0.001$). In females, body weights and fat masses followed similar patterns in relation to HSL genotype (Fig. 2F–H).

Transgene expression had no detectable effect on testis weight (Fig. 2D), consistent with the tissue specificity of transgene expression. Also, plasma leptin levels (Fig. 2E, I) were proportional to fat masses. For males, they were similar among HSL$^{-/-}$N mice (21.8 ± 1.8 ng/ml), HSL$^{-/-}$MH mice (28.2 ± 0.8 ng/ml), and HSL$^{+/+}$ controls (23.1 ± 2.3 ng/ml) and lower in HSL$^{-/-}$ males (5.8 ± 2.2 ng/ml; $P < 0.001$) and HSL$^{-/-}$ML males (6.8 ± 1.7 ng/ml; $P < 0.001$) (Fig. 2E). The pattern in females was similar (Fig. 2I).

WAT histology

As previously reported (2), the WAT of HSL$^{-/-}$ mice shows more heterogeneity of cell size and increased interstitial volume than normal WAT (**Fig. 3A**, **B**). In perigonadal WAT, the modal cellular diameter in WAT from HSL$^{-/-}$ mice (55–60 μm; Fig. 3G) is less than that of HSL$^{+/+}$ WAT (105–110 μm; Fig. 3F). In HSL$^{-/-}$N mice, WAT appears morphologically normal and the modal cell diameter is between 105 and 110 μm (Fig. 3C, H), although a small peak is identifiable at ~50 μm as in HSL$^{-/-}$ WAT. In contrast, both mutant transgenic strains resembled HSL$^{-/-}$ WAT morphologically (Fig. 3D, E), with low modal cell diameters (HSL$^{-/-}$ML, 65–70 μm; HSL$^{-/-}$MH, 55–60 μm) (Fig. 3I, J) and a cell diameter profile resembling that of nontransgenic HSL$^{-/-}$ mice.

HSL expression in WAT

To determine HSL mRNA levels in WAT, we used 2 month old mice, which have similar WAT histology in all HSL genotypes (data not shown). The level of mouse HSL mRNA in WAT of HSL$^{-/-}$ mice is <3% of normal, as reported (2). Compared with the intensity of the mouse HSL mRNA expression in HSL$^{+/+}$ adipocytes (**Fig. 4A**), the levels of human transgenic HSL mRNA are 13 ± 2% in HSL$^{-/-}$N, 18 ± 3% in HSL$^{-/-}$ML, and 75 ± 28% in HSL$^{-/-}$MH mice (Fig. 4B).

Western blotting (Fig. 4C, D) shows a similar pattern to RNA expression. Immunoreactive HSL is undetectable in HSL$^{-/-}$ WAT, 15 ± 3% of normal in HSL$^{-/-}$N, 36 ± 19% in HSL$^{-/-}$ML, and 89 ± 23% in HSL$^{-/-}$MH.

TG and cholesteryl ester hydrolase assays

Cholesteryl esterase activity (Fig. 4F) was 1.6 ± 0.4% of normal in HSL$^{-/-}$ WAT, 42 ± 9% in HSL$^{-/-}$N, 50 ± 12% in HSL$^{-/-}$ML, and 70 ± 16% in HSL$^{-/-}$MH. Cholesteryl esterase activity was undetectable in testis in all HSL$^{-/-}$ transgenic animals (data not shown). TG hydrolase activity in WAT (Fig. 4E) was 22 ± 8% of normal in nontransgenic HSL$^{-/-}$ mice, versus 47 ± 15% in HSL$^{-/-}$N mice, 54 ± 15% in HSL$^{-/-}$ML mice, and 67 ± 12% in HSL$^{-/-}$MH mice.

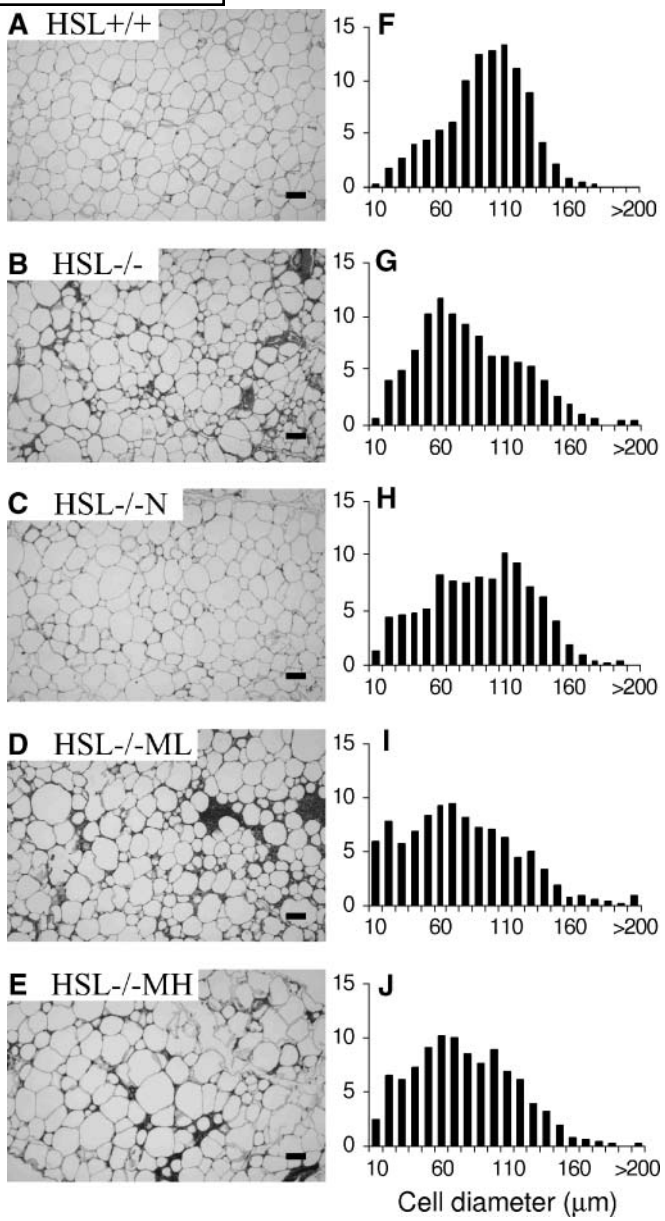

Fig. 3. Effect of HSL genotype on histology (A–E) and distribution of maximal cell diameter (F–J) of perigonadal white adipose tissue (WAT) of 6 month old male mice. Representative sections are shown. The bars in the left panels correspond to 100 μm. In the histograms, 10 μm intervals indicate cell diameters, the upper limits of which are indicated on the *x* axis.

Lipolysis in isolated adipocytes

With β-adrenergic stimulation, lipolysis in HSL$^{-/-}$ adipocytes increased 1.6-fold, versus 18.0-fold in HSL$^{+/+}$ adipocytes. For HSL$^{-/-}$ transgenic adipocytes, mean enhancements were 8.1-fold in HSL$^{-/-}$N, 2.7 in HSL$^{-/-}$ML, and 3.7 in HSL$^{-/-}$MH. Adrenergic-stimulated lipolysis in HSL$^{-/-}$N

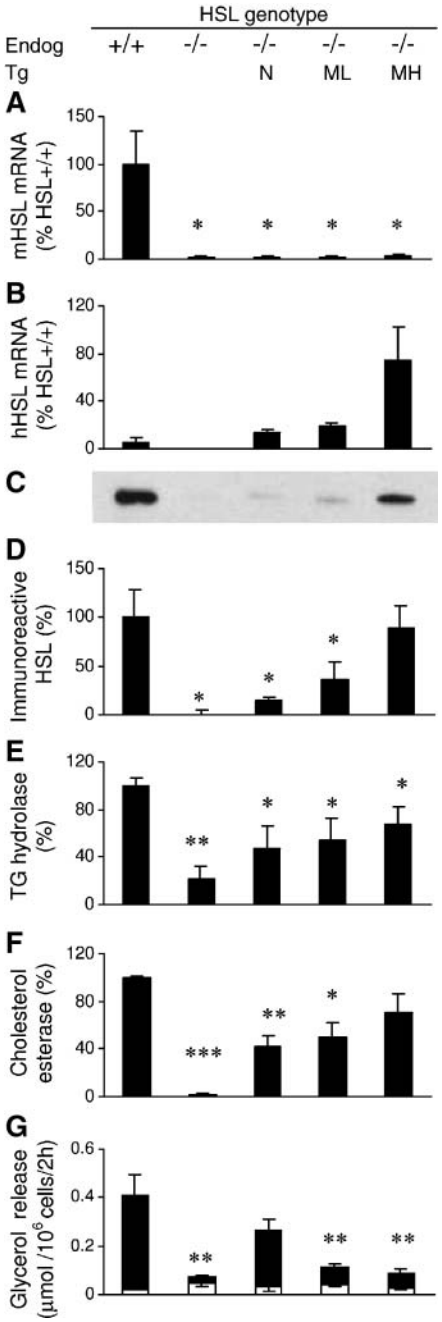

Fig. 4. Effect of HSL genotype on WAT HSL expression, in vitro activity, and lipolysis. Endogenous (Endog) or transgenic (Tg) HSL is specified. A, B: mRNA levels of perirenal fat of 2 month old mice were determined using primers specific for mouse HSL (mHSL) (A) or human HSL (hHSL) (B). Data were normalized to β-actin RNA level and compared with the HSL level of normal $HSL^{+/+}$ WAT (n = 3 mice). C: Representative Western blot analysis of 20 μg of protein from perirenal adipose tissue infranatants. D: Quantification of protein expression using HSL-specific antibody (n = 5 mice). E, F: Activities of triglyceride (TG) hydrolase (E) and cholesteryl esterase (F) (n = 5 mice). G: Glycerol release from isolated adipocytes under basal (open bars) or adrenergic-stimulated conditions (closed bars) (n = 3 mice). Differences from wild-type $HSL^{+/+}$ mice are designated as in Figure 2. In G, statistical comparisons refer to adrenergic-stimulated lipolysis; comparisons between basal lipolysis revealed no statistically significant differences. The absolute values for TG hydrolase and cholesteryl esterase activities in $HSL^{+/+}$ control WAT were 24.4 ± 1.5 and 44.4 ± 14.1 nmol/mg/h, respectively.

cells attained 58% of the maximal rate measured in normal $HSL^{+/+}$ cells.

DG and TG contents of WAT

WAT DG contents (**Fig. 5A**) fell into two groups, low and high. $HSL^{+/+}$, $HSL^{-/-}$N, and $HSL^{-/-}$MH were similar, with 7.9 ± 2.5, 13.5 ± 1.7, and 14.8 ± 3.2 nmol/mg fat tissue, respectively. $HSL^{-/-}$ and $HSL^{-/-}$ML mice had significantly greater DG contents, 52.2 ± 5.1 and 56.2 ± 3.8 nmol/mg fat tissue, respectively. TG levels were not significantly different between strains (Fig. 5B). DG/TG ratios showed a similar pattern to total DG content (Fig. 5C).

DISCUSSION

Adipocyte lipolysis can be determined both by the capacity of adipocyte lipases, of which HSL is a major component, and by the control of lipase access to the lipid droplet. Transgenic $HSL^{-/-}$ mice are a useful and physiologically relevant model for the study of the lipase component of adipocyte lipolysis. In this discussion, we first mention some technical considerations important for the interpretation of the work, then we discuss the results in relationship to the current literature.

Some technical considerations apply to comparisons between $HSL^{-/-}$ mice expressing human HSL trans-

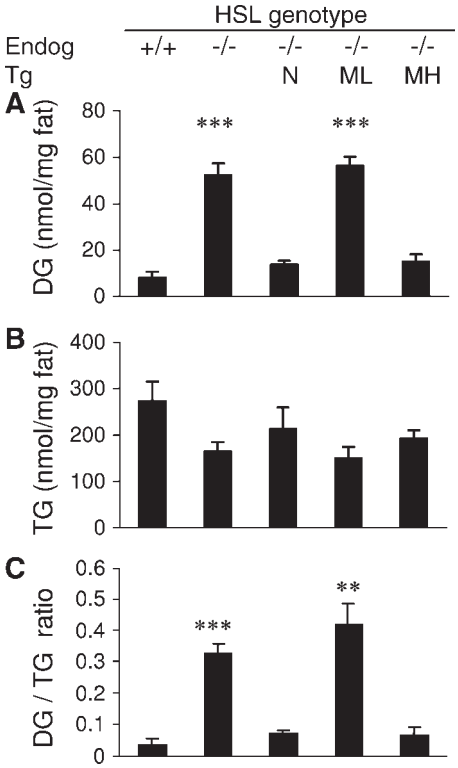

Fig. 5. Effect of HSL genotype on diacylglycerol and triacylglycerol contents of perigonadal fat of 6 month old male mice. Endogenous (Endog) or transgenic (Tg) HSL is specified. Shown are diglyceride (DG) content (A), TG content (B), and DG/TG ratio (C) in perigonadal fat (n = 5 mice). Differences from $HSL^{+/+}$ controls are designated as in Figure 2.

genes. First, measurements of endogenous mouse and transgenic human HSL mRNA and protein levels are not strictly equivalent because of potential differences in cDNA synthesis, PCR efficiency, and immunodetection with the anti-human HSL antibody. Comparison of enzymatic activities avoids these problems, but $HSL^{-/-}$ mice have substantial background TG hydrolase activity (2, 3, 28) as a result of non-HSL lipase(s) in WAT (25). Because HSL provides the vast majority of adipocyte cholesteryl esterase activity (2), cholesteryl esterase assay provides a convenient measure of total HSL activity. These considerations apply to comparisons of wild-type controls and $HSL^{-/-}$ human HSL transgenic animals. However, the effects of different human HSL transgenes on a $HSL^{-/-}$ background can be compared directly (Fig. 4). Practical difficulties in obtaining large cohorts of $HSL^{-/-}$ transgenic mice include the sterility of $HSL^{-/-}$ males and the small fraction of same-sex $HSL^{-/-}$ transgenic animals and nontransgenic $HSL^{-/-}$ controls in litters.

On a $HSL^{+/+}$ background, overexpression of normal or S554A HSL transgenes had no detectable effect on WAT mass or histology. For the normal transgene, this result is compatible with transfection studies of HSL in cultured cells, in which little change in TG content was observed except after massive overexpression of HSL (29). Also, Lucas et al. (30) observed in an independently derived transgenic HSL mouse line that expression of normal human HSL to a 3-fold higher level of HSL activity than in wild-type controls had little detectable impact on adipocyte physiology. In S554A $HSL^{+/+}$ mice, the lack of a detectable WAT phenotype argued against a physiologically important activation of HSL by this mutation. However, to study their effects in the absence of endogenous HSL, we bred each of the three transgenes to a $HSL^{-/-}$ background.

The data in transgenic $HSL^{-/-}$ mice are relevant to at least six aspects of adipocyte physiology. First, the results prove that normal human HSL is physiologically active in mouse adipocytes. In $HSL^{-/-}$ mice, the expression of human HSL dramatically improves WAT mass, cell size distribution, DG content, and lipolytic response to adrenergic stimulation. In $HSL^{-/-}$N mice, this occurred despites subnormal levels of HSL mRNA, protein, and activity, ranging from ~13% to 40% of normal endogenous HSL levels. Of note, heterozygous $HSL^{+/-}$ mice, which have half-normal levels of HSL protein, mRNA, and activity, have normal WAT mass and histology and approximately half the β-adrenergic stimulated increase of lipolysis observed in $HSL^{+/+}$ adipocytes (2). The slightly increased prevalence of small cells in $HSL^{-/-}$N WAT (Fig. 3) suggests that HSL levels in these mice may approach the threshold below which the typical pathology of $HSL^{-/-}$ WAT develops. These observations imply that despite the multiple deletions/insertions by which human and mouse HSL differ (23), human HSL is functional in mouse adipocytes. By extension, the in vivo interaction of human HSL with the mouse orthologs of its protein partners (18, 19) and translocation to the lipid droplet surface can occur.

Second, contrary to initial predictions, the expression of S544A HSL on the $HSL^{-/-}$ background had little effect on WAT histology or lipolysis in intact adipocytes, despite the presence of HSL immunoreactive material and in vitro catalytic activity (Fig. 4) at least as great as those of the wild-type HSL transgene that markedly improved WAT function and anatomy. Interestingly, there were differences between the two mutant transgenic lines. MH mice, with a higher transgene copy number, HSL mRNA, and protein levels, had nearly normal DG content and WAT masses in perirenal and subcutaneous WAT. However, the masses of other fat depots, WAT histology in all depots, and adipocyte lipolysis resemble those of $HSL^{-/-}$ and $HSL^{-/-}$ML WAT. Biological differences among WAT adipose depots are increasingly documented (31–33), but the mechanism in the case of $HSL^{-/-}$MH mice is not apparent. Perhaps the expression of S554A HSL at higher levels than those of $HSL^{-/-}$MH mice might correct the WAT phenotype of $HSL^{-/-}$ mice.

The low physiological activity of S554A HSL could potentially arise from differences in HSL phosphorylation at activating serine residues and/or from disturbed interactions of HSL with other molecules of the lipid droplet surface (34), including perilipin, which is thought to determine access to droplet TGs (35–38). Of note, during the preparation of this article, Su et al. (21) provided a potential explanation for this finding. They studied different HSL mutations using FLAG-tagged rat HSL in 3T3-L1 cells. In that study, S565A HSL, orthologous to S554A in human HSL, did not translocate to the lipid droplet. Together, these observations are consistent with the notion that S554A HSL and its rat ortholog may directly affect the translocation of HSL to the lipid droplet surface.

Third, the WAT pathology of $HSL^{-/-}$ mice appears to be mainly cell autonomous, because it is selectively corrected by HSL expression in adipocytes. This was not previously clear: HSL deficiency is a multisystemic endocrinopathy in which observed abnormalities in WAT could plausibly result either directly from HSL deficiency in WAT or indirectly from abnormalities of other HSL-deficient organs. Of note, the transgenic aP2 promoter also mediates low-level expression in macrophages (39, 40). Surprisingly, in transgenic mice, HSL overexpression in macrophages is associated with increased atherosclerosis (41). The effect, if any, of macrophage HSL expression on the WAT phenotype of transgenic $HSL^{-/-}$ mice cannot be deduced at present. Of note, however, transgenic HSL expression in the mice described in this study had little apparent effect on HSL deficiency in other organs (Fig. 2D). It will now be possible to specifically explore the function of nonadipose HSL-deficient tissues, such as pancreatic β cells, using $HSL^{-/-}$ mice with normal or nearly normal HSL function in WAT.

Fourth, leptin levels were roughly proportional to fat masses (Fig. 2E), with no obvious relationship to HSL genotype or to the presence of typical WAT pathology. This suggests that HSL deficiency does not directly affect leptin production except by influencing total WAT mass.

Fifth, a WAT HSL isoform identical to the major isoform except for an additional 43 residue N-terminal ex-

tension accounts for ~15% of HSL transcripts in mouse WAT (42) and apparently predominates in pancreatic β cells (5). Its properties have not been studied exhaustively. However, it is apparently not essential for WAT function, because expression of the major HSL isoform alone can normalize the properties of $HSL^{-/-}$ WAT.

Finally, HSL has greater specific activity toward DGs than TGs (43), and $HSL^{-/-}$ WAT has a marked selective increase of DG content (28). $HSL^{-/-}$MH mice have normal DG content in WAT but abnormal adrenergic-stimulated lipolysis and WAT histology similar to nontransgenic $HSL^{-/-}$ mice. Therefore, the increased DG content that accompanies total HSL deficiency is not essential for the development of the WAT pathology of $HSL^{-/-}$ mice.

The authors thank J. Penny for performing the pronuclear microinjections. This research began as a Canadian Genetic Diseases Network collaboration (G.A.M., F.R.J.) and was funded by Canadian Institutes of Health Research operating grant MOP 12625 to G.A.M. F.R.J. holds Alberta Heritage Foundation for Medical Research and Canada Research Chair Awards.

REFERENCES

1. Fredrikson, G., P. Stralfors, N. O. Nilsson, and P. Belfrage. 1981. Hormone-sensitive lipase of rat adipose tissue. Purification and some properties. *J. Biol. Chem.* **256:** 6311–6320.
2. Wang, S., N. Laurin, J. Himms-Hagen, M. A. Rudnicki, E. Levy, M. F. Robert, L. Pan, L. Oligny, and G. A. Mitchell. 2001. The adipose tissue phenotype of hormone-sensitive lipase deficiency in mice. *Obes. Res.* **9:** 119–128.
3. Osuga, J. I., S. Ishibashi, T. Oka, H. Yagyu, R. Tozawa, A. Fujimoto, F. Shionoiri, N. Yahagi, F. B. Kraemer, O. Tsutsumi, et al. 2000. Targeted disruption of hormone-sensitive lipase results in male sterility and adipocyte hypertrophy, but not obesity. *Proc. Natl. Acad. Sci. USA.* **97:** 787–792.
4. Haemmerle, G., R. Zimmermann, J. G. Strauss, D. Kralky, M. Riederer, G. Knipping, and R. Zechner. 2002. Hormone-sensitive lipase deficiency in mice changes the plasma lipid profile by affecting the tissue-specific expression pattern of lipoprotein lipase in adipose tissue and muscle. *J. Biol. Chem.* **277:** 12946–12952.
5. Fex, M., C. F. Olofsson, U. Fransson, K. Bacos, H. Lindvall, M. Sorhede-Winzell, P. Rorsman, C. Holm, and H. Mulder. 2004. Hormone-sensitive lipase deficiency in mouse islets abolishes neutral cholesterol ester hydrolase activity but leaves lipolysis, acylglycerides, fat oxidation, and insulin secretion intact. *Endocrinology.* **145:** 3746–3753.
6. Roduit, R., P. Masiello, S. Wang, H. Li, G. A. Mitchell, and M. Prentki. 2001. A role for hormone-sensitive lipase in glucose-stimulated insulin secretion: a study in hormone-sensitive lipase-deficient mice. *Diabetes.* **50:** 1970–1975.
7. Peyot, M., C. J. Nolan, K. Soni, E. Joly, R. Lussier, B. E. Corkey, S. P. Wang, G. A. Mitchell, and M. Prentki. 2003. Hormone-sensitive lipase has a role in lipid signaling for insulin secretion but is nonessential for the incretin action of glucagon-like peptide 1. *Diabetes.* **53:** 1733–1742.
8. Li, H., M. Brochu, S. P. Wang, L. Rochdi, M. Cote, G. A. Mitchell, and N. Gallo-Payet. 2002. Hormone-sensitive lipase deficiency in mice causes lipid storage in the adrenal cortex and impaired corticosterone response to corticotropin stimulation. *Endocrinology.* **143:** 3333–3340.
9. Wang, S. P., S. Chung, K. Soni, H. Bourdages, L. Hermo, J. Trasler, and G. A. Mitchell. 2004. Expression of human hormone sensitive lipase (HSL) in post-meiotic germ cells confers normal fertility to HSL-deficient mice. *Endocrinology.* **145:** 5688–5693.
10. Holm, C., T. G. Kirchgessner, K. L. Svenson, G. Fredrikson, S. Nilsson, C. G. Miller, J. E. Shively, C. Heinzmann, R. S. Sparkes, T. Mohandas, et al. 1988. Hormone-sensitive lipase: sequence, expression, and chromosomal localization to 19 cent-q13.3. *Science.* **241:** 1503–1506.
11. Anthonsen, M. W., L. Ronnstrand, C. Wernsted, E. Degerman, and C. Holm. 1998. Identification of novel phosphorylation sites in hormone-sensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties in vitro. *J. Biol. Chem.* **273:** 215–221.
12. Stralfors, P., and P. Belfrage. 1983. Phosphorylation of hormone-sensitive lipase by cyclic AMP-dependent protein kinase. *J. Biol. Chem.* **258:** 15146–15152.
13. Olsson, H., P. Stralfors, and P. Belfrage. 1986. Phosphorylation of the basal site of hormone-sensitive lipase by glycogen synthase kinase-4. *FEBS Lett.* **209:** 175–180.
14. Garton, A. J., D. G. Campbell, D. Carling, D. G. Hardie, R. J. Colbran, and S. J. Yeaman. 1989. Phosphorylation of bovine hormone-sensitive lipase by the AMP-activated protein kinase. A possible antilipolytic mechanism. *Eur. J. Biochem.* **179:** 249–254.
15. Greenberg, A. S., W. J. Shen, K. Muliro, S. Patel, S. C. Souza, R. A. Roth, and F. B. Kraemer. 2001. Stimulation of lipolysis and hormone-sensitive lipase via the extracellular signal-regulated kinase pathway. *J. Biol. Chem.* **276:** 45456–45461.
16. Egan, J. J., A. S. Greenberg, M. K. Chang, S. A. Wek, M. C. Moos, and C. Londos. 1992. Mechanism of hormone-stimulated lipolysis in adipocytes: translocation of hormone-sensitive lipase to the lipid storage droplet. *Proc. Natl. Acad. Sci. USA.* **89:** 8537–8541.
17. Shen, W. J., S. Patel, R. Hong, and F. B. Kraemer. 2000. Hormone-sensitive lipase functions as an oligomer. *Biochemistry.* **39:** 2392–2398.
18. Shen, W. J., K. Sridhar, D. A. Bernlohr, and F. B. Kraemer. 1999. Interaction of rat hormone-sensitive lipase with adipocyte lipid-binding protein. *Proc. Natl. Acad. Sci. USA.* **96:** 5528–5532.
19. Syu, L. J., and A. R. Saltiel. 1999. Lipotransin: a novel docking protein for hormone-sensitive lipase. *Mol. Cell.* **4:** 109–115.
20. Shen, W. J., S. Patel, V. Natu, and F. B. Kraemer. 1998. Mutational analysis of structural features of rat hormone-sensitive lipase. *Biochemistry.* **37:** 8973–8979.
21. Su, C. L., C. Sztalryd, J. A. Contreras, C. Holm, A. R. Kimmel, and C. Londos. 2003. Mutational analysis of the hormone-sensitive lipase translocation reaction in adipocytes. *J. Biol. Chem.* **278:** 43615–43619.
22. Ross, S. R., R. A. Graves, A. Greenstein, K. A. Platt, H. Shyu, B. Mellovitz, and B. M. Spiegelman. 1990. A fat-specific enhancer is the primary determinant of gene expression for adipocyte P2 in vivo. *Proc. Natl. Acad. Sci. USA.* **87:** 9590–9594.
23. Sztrolovics, R., S. Wang, P. Lapierre, H. S. Chen, M. F. Robert, and G. A. Mitchell. 1997. Hormone-sensitive lipase (*Lipe*): sequence analysis of the 129v mouse *Lipe* gene. *Mamm. Genome.* **8:** 86–89.
24. Levesque, K., Y. S. Zhao, and E. A. Cohen. 2003. Vpu exerts a positive effect on HIV-1 infectivity by down-modulating CD4 receptor molecules at the surface of HIV-1-producing cells. *J. Biol. Chem.* **30:** 28346–28353.
25. Soni, K., R. Lehner, P. Metalnikov, P. O'Donnell, M. Semache, W. Gao, K. Ashman, A. Pshezhetsky, and G. A. Mitchell. 2004. Carboxylesterase 3 (EC 3.1.1.1) is a major adipocyte lipase. *J. Biol. Chem.* **279:** 40683–40689.
26. Mauriège, P., J. P. Després, D. Prud'homme, M. C. Pouliot, M. Marcotte, A. Tremblay, and C. Bouchard. 1991. Regional variation in adipose tissue lipolysis in lean and obese men. *J. Lipid Res.* **32:** 1625–1633.
27. Fortier, M., S. P. Wang, P. Mauriège, M. Semache, L. Mfuma, H. Li, E. Levy, D. Richard, and G. A. Mitchell. 2004. Hormone-sensitive lipase-independent adipocyte lipolysis during beta-adrenergic stimulation, fasting, and dietary fat loading. *Am. J. Physiol. Endocrinol. Metab.* **287:** E282–E288.
28. Haemmerle, G., R. Zimmermann, M. Hayn, C. Theuss, G. Waeg, E. M. Wagner, W. Sattler, T. M. Magin, E. F. Wagner, and R. Zechner. 2003. Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, testis. *J. Biol. Chem.* **277:** 4806–4815.
29. Sztalryd, C., M. C. Komaromy, and F. B. Kraemer. 1995. Overexpression of hormone-sensitive lipase prevents triglyceride accumulation in adipocytes. *J. Clin. Invest.* **95:** 2652–2661.
30. Lucas, S., G. Tavernier, C. Tiraby, A. Mairal, and D. Langin. 2003. Expression of human hormone-sensitive lipase in white adipose tissue of transgenic mice increases lipase activity but does not enhance in vitro lipolysis. *J. Lipid Res.* **44:** 154–163.
31. Vidal, H. 2001. Gene expression in visceral and subcutaneous adipose tissues. *Ann. Med.* **33:** 547–555.

32. Jensen, M. D. 1997. Lipolysis: contribution from regional fat. *Annu. Rev. Nutr.* **17:** 127–139.
33. Hellerstein, M. K. 2003. In vivo measurement of fluxes through metabolic pathways: the missing link in functional genomics and pharmaceutical research. *Annu. Rev. Nutr.* **23:** 379–402.
34. Brasaemle, D. L., G. Dolios, L. Shapiro, and R. Wang. 2004. Proteomic analysis of proteins associated with lipid droplets of basal and lipolytically stimulated 3T3-L1 adipocytes. *J. Biol. Chem.* **279:** 46835–46842.
35. Brasaemle, D. L., B. Rubin, I. A. Harten, J. Gruia-Gray, A. R. Kimmel, and C. Londos. 2000. Perilipin A increases triacylglycerol storage by decreasing the rate of triacylglycerol hydrolysis. *J. Biol. Chem.* **275:** 38486–38493.
36. Blanchette-Mackie, E. J., N. K. Dwyer, T. Barber, R. A. Coxey, T. Takeda, C. M. Rondinone, J. L. Theodorakis, A. S. Greenberg, and C. Londos. 1995. Perilipin is located on the surface layer of intracellular lipid droplets in adipocytes. *J. Lipid Res.* **36:** 1211–1226.
37. Greenberg, A. S., J. J. Egan, S. A. Wek, N. B. Garty, E. J. Blanchette-Mackie, and C. Londos. 1991. Perilipin, a major hormonally regulated adipocyte-specific phosphoprotein associated with the periphery of lipid storage droplets. *J. Biol. Chem.* **266:** 11341–11346.
38. Sztalryd, C., G. Xu, H. Dorward, J. T. Tansey, J. A. Contreras, A. R. Kimmel, and C. Londos. 2003. Perilipin A is essential for the translocation of hormone-sensitive lipase during lipolytic activation. *J. Biol. Chem.* **161:** 1093–1103.
39. Makowski, L., J. B. Boord, K. Maeda, V. R. Babaev, K. T. Uysal, M. A. Morgan, R. A. Parker, J. Suttles, S. Fazio, G. S. Hotamisligil, et al. 2001. Lack of macrophage fatty-acid binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis. *Nat. Med.* **7:** 699–705.
40. Layne, M. D., A. Patel, Y. H. Chen, V. I. Rebel, I. M. Carjaval, A. Pellacani, B. Ith, D. Zhao, B. M. Schreiber, S. F. Yet, et al. 2001. Role of macrophage-expressed adipocyte fatty acid binding protein in the development of accelerated atherosclerosis in hypercholesterolemic mice. *FASEB J.* **15:** 2733–2735.
41. Escary, J. L., H. A. Choy, K. Reue, X. P. Wang, L. W. Castellani, C. K. Glass, A. J. Lusis, and M. C. Schotz. 1999. Paradoxical effect on atherosclerosis of hormone-sensitive lipase overexpression in macrophages. *J. Lipid Res.* **40:** 397–404.
42. Laurin, N. N., S. Wang, and G. A. Mitchell. 2000. The hormone-sensitive lipase gene is transcribed from at least five alternative first exons in mouse adipose tissue. *Mamm. Genome.* **11:** 1–7.
43. Fredrikson, G., and P. Belfrage. 1983. Positional specificity of hormone-sensitive lipase from rat adipose tissue. *J. Biol. Chem.* **258:** 14253–14256.