

LIPIDS AND LIPOPROTEINS:

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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*

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Hormone-sensitive lipase (HSL) is believed to play an important role in the mobilization of fatty acids from triglycerides (TG), diglycerides, and cholesteryl esters in various tissues. Because HSL-mediated lipolysis of TG in adipose tissue (AT) directly feeds non-esterified fatty acids (NEFA) into the vascular system, the enzyme is expected to affect many metabolic processes including the metabolism of plasma lipids and lipoproteins. In the present study we examined these metabolic changes in induced mutant mouse lines that lack HSL expression (HSL-ko mice). During fasting, when HSL is normally strongly induced in AT, HSL-ko animals exhibited markedly decreased plasma concentrations of NEFA (–40%) and TG (–63%), whereas total cholesterol and HDL cholesterol levels were increased (+34%). Except for the increased HDL cholesterol concentrations, these differences were not observed in fed animals, in which HSL activity is generally low. Decreased plasma TG levels in fasted HSL-ko mice were mainly caused by decreased hepatic very low density lipoprotein (VLDL) synthesis as a result of decreased NEFA transport from the periphery to the liver. Reduced NEFA transport was also indicated by a depletion of hepatic TG stores (–90%) and strongly decreased ketone body concentrations in plasma (–80%). Decreased plasma NEFA and TG levels in fasted HSL-ko mice were associated with increased fractional catabolic rates of VLDL-TG and an induction of the tissue-specific lipoprotein lipase (LPL) activity in cardiac muscle, skeletal muscle, and white AT. In brown AT, LPL activity was decreased. Both increased VLDL fractional catabolic rates and increased LPL activity in muscle were unable to provide the heart with sufficient NEFA, which led to decreased tissue TG levels in cardiac muscle. Our results demonstrate that HSL deficiency markedly affects the metabolism of TG-rich lipoproteins by the coordinate down-regulation of VLDL synthesis and up-regulation of LPL in muscle and white adipose tissue. These changes result in an “anti-atherogenic” lipoprotein profile.

In mammals, white adipose tissue (WAT)¹ is the most important storage organ of TG. The mobilization of TG during

fasting or periods of increased energy demand, and the release of non-esterified fatty acids (NEFA) is an essential process that supplies non-adipose organs with substrates for energy conversion (1, 2). NEFA absorbed by skeletal and cardiac muscle are predominantly used for oxidation and energy production. In the liver, NEFA are also used for oxidation but, in addition, are utilized for several other metabolic processes. NEFA can be stored as hepatic TG droplets, used for the synthesis of ketone bodies, or incorporated into VLDL (3, 4). Once formed, VLDL particles are secreted from the liver into the vascular system where they are lipolyzed by endothelial cell associated lipoprotein lipase (LPL) (5, 6). This process supplies peripheral tissues such as AT with NEFA, thereby closing an inter-tissue cycle of fatty acid transport.

An important enzyme for the mobilization of TG and NEFA production in AT is hormone-sensitive lipase (HSL). This multifunctional neutral lipase hydrolyzes TG, diglycerides, cholesteryl esters, retinyl esters (7–10), and possibly other, yet unidentified, substrates. Although the highest levels of expression are found in WAT and brown adipose tissue (BAT), the enzyme is also found in many other tissues including muscle (2), macrophages (11), testis (12), and pancreas (13). In the postprandial state, HSL activity accounts for most of the detectable lipolysis in human WAT and thus determines whole-body lipid fuel availability (14, 15). In WAT, the enzyme activity is activated by hormones such as catecholamines. Stimulation of adenylyl cyclase activity (16–20) results in a rise in the intracellular cAMP levels that activate protein kinase A (21). Protein kinase A-mediated phosphorylation of HSL promotes the formation of a stable complex between HSL and lipotransin (22), which translocates the enzyme from the cytosol to the lipid droplet. In response to hormone signals, lipotransin-mediated ATP hydrolysis causes HSL to dissociate and thus gain access to the lipid surface. Insulin, the major antilipolytic hormone, inhibits HSL through phosphodiesterase-3-dependent cAMP degradation and interference with the lipotransin-mediated enzyme translocation.

Recently, the rate-limiting function of HSL in the catabolism of WAT TG was challenged by studies in HSL knock-out (ko) mice (23). HSL deficiency was compatible with normal body weight and fat mass, suggesting that at least one alternative lipase must exist in WAT to compensate for the hydrolysis of TG in the absence of HSL. Nonetheless, HSL-ko mice exhibited increased mass of BAT and marked changes in the lipid com-

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¹ The abbreviations used are: WAT, white adipose tissue; HSL, hor-

none-sensitive lipase; TG, triglycerides; AT, adipose tissue; BAT, brown adipose tissue; NEFA, non-esterified fatty acids; ko, knock-out; wt, wild type; LPL, lipoprotein lipase; TC, total cholesterol; HDL, high density lipoprotein; VLDL, very low density lipoprotein; FCR, fractional catabolic rate; ACR, absolute catabolic rate; PL, phospholipid; FPLC, fast protein liquid chromatography; wt, wild type.

position of WAT, BAT, and other tissues of the body (23, 24). Additionally, *in vitro* lipolysis studies with isolated WAT revealed decreased NEFA and glycerol release when HSL was absent, thus arguing for a crucial role of HSL in the catabolism of TG and diglycerides and the subsequent release of NEFA (24). As a result, the plasma levels of NEFA are decreased in fasting HSL-deficient mice (23).

Depending on the nutritional condition, plasma NEFA strongly affect lipid synthesis and utilization in hepatic and peripheral tissues (25). Accordingly, decreased NEFA release in HSL-ko mice is expected to result in decreased hepatic VLDL synthesis and other metabolic changes. To test this hypothesis, we studied the role of HSL deficiency on the metabolism of plasma lipids and lipoproteins in HSL-ko mice. We demonstrate that decreased plasma NEFA levels in HSL-deficient mice are associated with decreased hepatic VLDL synthesis, decreased ketogenesis, and the depletion of TG stores in the liver. Additionally, the catabolism of plasma VLDL is increased because of the coordinate up-regulation of the tissue-specific LPL activity in muscle and WAT, which results in drastically reduced plasma TG levels and increased HDL cholesterol concentrations in fed and fasted HSL-ko mice.

EXPERIMENTAL PROCEDURES

Animals—HSL-ko mice were generated by targeted homologous recombination as previously described (24). For breeding experiments mice heterozygous for the deleted HSL allele were used to generate homozygous HSL-ko mice. Mice were maintained on a regular light-dark cycle (14 h light, 10 h dark) and kept on a standard laboratory chow diet (4.5% w/w fat). Genotyping of HSL-ko mice was performed by a single-step PCR using three primers as described previously (24).

Blood Parameters—Samples were drawn by retro-orbital puncture from animals in the fed and fasted state. Blood was obtained from mice in the mornings after they had normal access to food (fed samples) and from mice after they were fasted for a period of 16 h. Total cholesterol (TC), HDL cholesterol, TG, and NEFA were determined using commercial kits (Roche Molecular Biochemicals; Sigma; and Wako Chemicals, Neuss, Germany). Ketone bodies (β -hydroxybutyrate) were determined using a commercial kit from Sigma.

Lipoprotein Analysis—Plasma samples of five fasted HSL-ko and five control mice were pooled. TG, TC, and PL contents were quantitated enzymatically using commercial kits (Sigma; bioMérieux Inc., Durham, NC). Lipoproteins were isolated by fast protein liquid chromatography (FPLC) using a Pharmacia FPLC system and a Superose 6 column (Amersham Biosciences). Plasma samples (250 μ l) were applied to FPLC analysis and eluted with 10 mM Tris-HCl, 1 mM EDTA, 154 mM NaCl, and 0.02% Na₂S₂O₃ (pH 7.4). Fractions of 0.5 ml each were collected and enzymatically assayed for TG, TC, and PL contents.

Cryosections and TG Determination in the Liver and Cardiac Muscle—After perfusion with 0.9% NaCl for removal of blood lipids, organs from fed and fasted mice were instantly frozen in liquid nitrogen. Sections (5 μ m) were prepared at -20°C from tissues embedded in OCT compound (Sakura) and transferred to commercial glass slides. To visualize neutral lipids, the cryosections were stained with Oil Red O (Sigma). Nuclei were stained with hemalaun (Sigma-Aldrich) following a standard protocol (26). The stained sections were analyzed by light microscopy and photographed (3 CCD color video camera, Sony, Power HAD).

The tissue TG content was determined from blood free tissues. After anesthesia, mice were perfused with a 0.9% NaCl solution. Livers and hearts were excised, weighed, and frozen. Total lipids were extracted from organs by the method of Folch (27). Lipid extracts were incubated in a buffer containing 4 units/ml *Candida rugosa* lipase (Sigma), 50 mM Tris-HCl pH 7.4, 5% bovine serum albumin for 3 h at 37°C to achieve complete TG hydrolysis. Subsequently the released glycerol was quantitated with a commercially available TG-kit (GPO-Trinder) obtained from Sigma.

In Vivo Synthesis of VLDL—Hepatic TG synthesis rates were determined after blocking the lipolytic degradation of TG-rich lipoproteins with Triton WR1339 (28) according to a standard protocol (29). Anesthetized fasted mice were injected intravenously with 500 mg of Triton WR1339 (Sigma)/kg of body weight as a 15 g/dl solution in 0.9% NaCl. Blood samples were drawn before injection of Triton WR1339 and 1, 2, 3, and 4 h after injection. TG values were determined from plasma

using an enzymatic kit (GPO-Trinder, Sigma).

In Vivo Clearance of Labeled VLDL—For clearance studies, VLDL was labeled *in vivo* in control mice. Fasted mice were injected intravenously with [1,2-³H]palmitate (200 μ Ci) and bled 35 min after the injection. Plasma samples were ultracentrifuged to obtain the VLDL fraction. TLC on silica gel G plates using a hexane-diethylether-acetic acid solution (70:29:1) as the solvent revealed that 98% of the radioactivity was in the TG fraction. Anesthetized mice were injected intravenously with 400,000 cpm of radiolabeled VLDL, and the disappearance of radioactivity was determined from plasma samples drawn 0.5, 1, 2, 3, 5, 10, 15, and 30 min after the injection. Data were analyzed for individual animals using a two-pool model, and the fractional catabolic rate (FCR) as well as the absolute catabolic rate (ACR) were calculated (30, 31).

Assay of Tissue Lipoprotein Lipase Activity—LPL activity in tissue was determined in 3-month-old female mice. Enzyme activities were determined in both the fed and the fasted state. The tissues were surgically removed from the animals and put into ice-cold tubes containing 1 ml of Dulbecco's modified Eagle's medium with 2% bovine serum albumin and 2 units/ml heparin. After the tissue was minced with scissors, it was incubated in medium for 1 h at 37°C . Enzyme activities of LPL were assayed as described earlier (32).

Analysis of LPL mRNA in WAT, BAT, and Cardiac Muscle—Tissues from fasted mice were surgically removed, weighed, and subsequently frozen in liquid nitrogen. Wet tissues were homogenized in 5 ml TRI reagent (Molecular Research Center, Inc., Karlsruhe, Germany), and total tissue RNA was prepared as described previously (33). For Northern blotting analysis, 10 μ g of total RNA were separated by 1% formaldehyde-agarose gel electrophoresis and blotted overnight onto nylon membranes (Hybond N+, Amersham Biosciences). Subsequently, the RNA was cross-linked to the membrane by ultraviolet irradiation. Blots were prehybridized for 4 h at 65°C in a buffer containing 0.15 M sodium phosphate (pH 7.2), 1 mM EDTA, 7% SDS, and 1% bovine serum albumin. Membranes were hybridized in the same buffer at 65°C overnight with a specific mouse LPL cDNA probe as previously described (34). After hybridization, the blots were washed, and specific hybridization was visualized by a 3-h exposure to a phosphorimaging screen (Apbiotech, Freiburg, Germany). Signal intensities were quantified in relation to the 28 S rRNA corresponding band by ImageQuant 5.1 Software (Apbiotech).

Statistical Analysis—Results are given as the mean \pm S.D. Statistical significance was tested using two-tailed Student's *t* test.

RESULTS

Plasma Lipid and Lipoprotein Analysis—The consequences of HSL deficiency with regard to plasma lipid levels were investigated in the fed and the fasted states (Table I). In the fed state, plasma TG and NEFA concentrations were not significantly altered in HSL-ko mice compared with controls. In the fasted state, plasma TG and NEFA levels of HSL-ko mice were markedly decreased by 63 and 39%, respectively. Independent of the dietary status, plasma cholesterol levels were increased in HSL-ko mice by 30–40%. This increase was mainly because of increased HDL cholesterol levels. Essentially identical results were obtained from three independent experiments (data not shown).

To investigate the lipid distribution among lipoprotein subclasses, plasma samples of fasted HSL-ko animals and controls were subjected to FPLC analysis (Fig. 1). TG measurements in FPLC subfractions revealed a 41% reduction of the VLDL-TG fraction of HSL-ko mice. In contrast, HDL cholesterol was increased by 48% in HSL-ko animals compared with controls. LDL-cholesterol levels were barely detectable in both HSL-ko and wt mice.

Decreased Hepatic Fatty Acid Utilization for VLDL Synthesis, TG Storage, and Ketogenesis in HSL-ko Mice—To study the underlying cause of the reduced TG levels in HSL-ko mice, we investigated liver TG synthesis in fasted HSL-ko animals and control mice. Hepatic TG synthesis was determined *in vivo* after blocking the lipolytic catabolism of TG-rich lipoproteins with Triton WR1339. The injection of Triton WR1339 resulted in a linear increase in plasma TG concentrations between 1 and

TABLE I
Plasma TG, FFA, total cholesterol, and HDL cholesterol concentrations

Data represent means \pm S.D. Blood was drawn from 14–16-week-old female mice in the fed or fasted state. n_1 = number of animals used for measurements.

Genotype	n_1	TG	FFA	Total cholesterol	HDL cholesterol
mg/dl					
Fed					
HSL-wt	6	149 \pm 52	7 \pm 2	119 \pm 19	93 \pm 18
HSL-ko	6	116 \pm 61	6 \pm 2	145 \pm 38	127 \pm 20 ^a
Fasted					
HSL-wt	6	103 \pm 27	28 \pm 7	70 \pm 8	62 \pm 11
HSL-ko	6	38 \pm 11 ^a	17 \pm 4 ^b	96 \pm 14 ^a	83 \pm 15 ^b

^a $p < 0.01$ compared with HSL-wt.
^b $p < 0.05$ compared with HSL-wt.

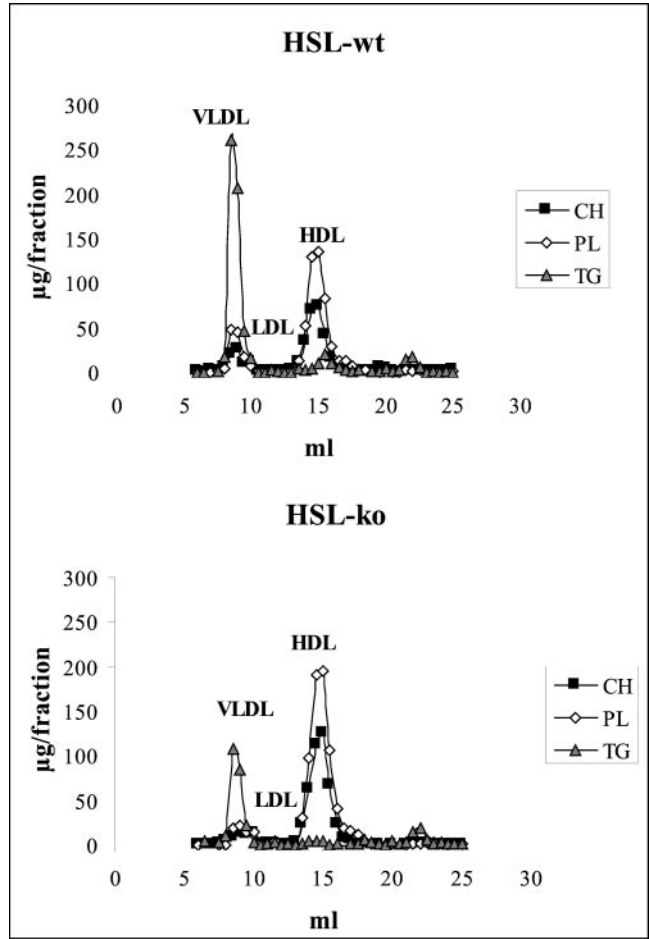


FIG. 1. **Lipoprotein profile.** Lipoprotein profile of plasma pools from five fasted HSL-wt and 5 HSL-ko mice at the age of 14 weeks. Plasma lipoproteins were separated using a Pharmacia FPLC system with a Superose 6 column. FPLC fractions were collected, and TG, TC, and PL concentrations were measured in each fraction enzymatically.

4 h after injection (Fig. 2A). Linear regression analysis of the increase in plasma TG concentrations between 1 and 4 h after injection (Fig. 2B) revealed that HSL-ko mice produced only 41% of the TG mass found in control mice, suggesting that the impaired NEFA transport to the liver during fasting markedly reduces hepatic VLDL synthesis.

To analyze whether decreased plasma NEFA concentrations also affected the hepatic TG content, cryosections of liver tissues were performed and stained for neutral lipids with Oil Red. As shown in Fig. 3A neutral lipids were barely detectable in the liver of fasted HSL-ko mice, whereas control animals exhibited clearly visible stained lipid droplets. Biochemical determination of intracellular TG concentrations

revealed a drastic 92% reduction of the liver TG content in HSL-ko mice compared with control mice (Fig. 3B). In the fed state, liver TG contents were not altered in HSL-ko animals compared with controls.

HSL deficiency also resulted in a 69 and 78% decrease in plasma ketone body (β -hydroxybutyrate) concentrations (Fig. 4) in the fed and fasted state, respectively, again arguing for reduced utilization of NEFA for hepatic ketogenesis.

Increased VLDL Clearance in HSL-ko Mice—To study the removal of VLDL particles from the circulation, fasted HSL-ko mice and controls were injected intravenously with 400,000 cpm of *in vivo* radiolabeled VLDL TG. As shown in Fig. 5, the VLDL half-time in the circulation was decreased \sim 2-fold in HSL-ko mice compared with controls (7.8 ± 2.0 versus 4.0 ± 1.1 min in controls and HSL-ko mice, respectively). The fractional catabolic rates for VLDL-TG were calculated from the plasma decay curves, assuming a two-pool model, by the method of Mathews (30, 31). The mean FCR were 7.2 ± 1.5 and 12.7 ± 2.3 pools/h (Fig. 5) in HSL-wt and HSL-ko mice, respectively. Despite the 76% increase of the FCR in HSL-ko mice, the absolute catabolic rate as a product of the FCR \times pool size was decreased by 31% because of the drastically decreased plasma TG levels in fasted HSL-ko mice. Considering that under steady state conditions the ACR reflects the production rate (30, 31), these data support the finding of decreased VLDL synthesis in HSL-deficient mice.

Tissue LPL Activities and LPL mRNA Levels—Because the decreased availability of plasma NEFA and VLDL-TG might cause a shortage of NEFA supply in cardiac and skeletal muscle, we considered whether LPL might be induced in these tissues to provide them with additional energy substrate. LPL activities were measured in muscle and AT. As shown in Fig. 6, HSL deficiency led to a highly significant 80, 76, and 48% increase of the LPL activity in cardiac muscle, skeletal muscle, and WAT, respectively, when a group of eight fasted animals was analyzed (Fig. 6A). In BAT, LPL activity was decreased by 73%. In the fed state (Fig. 6B), the increase of LPL activities in cardiac muscle (32%) and skeletal muscle (22%) was less pronounced than in fasted animals. In the WAT and BAT of fed HSL-ko mice, LPL activities were reduced by 56 and 71%, respectively. Northern blot experiments were performed to analyze whether the changes in LPL activities are caused by alterations in LPL mRNA levels. In WAT, BAT, and cardiac muscle of fasted HSL-ko animals and controls, LPL mRNA levels were essentially identical in all tissues despite the marked variation in LPL activities observed in HSL-ko mice compared with controls (Fig. 7). These results, which were confirmed in multiple analyses, suggested that the compensatory up-regulation of LPL activity in cardiac and skeletal muscle might contribute to the low plasma TG levels observed in HSL-ko mice.

Decreased Levels of Neutral Lipids in Cardiac Muscle of HSL-ko Mice—The decreased ACR and the low substrate

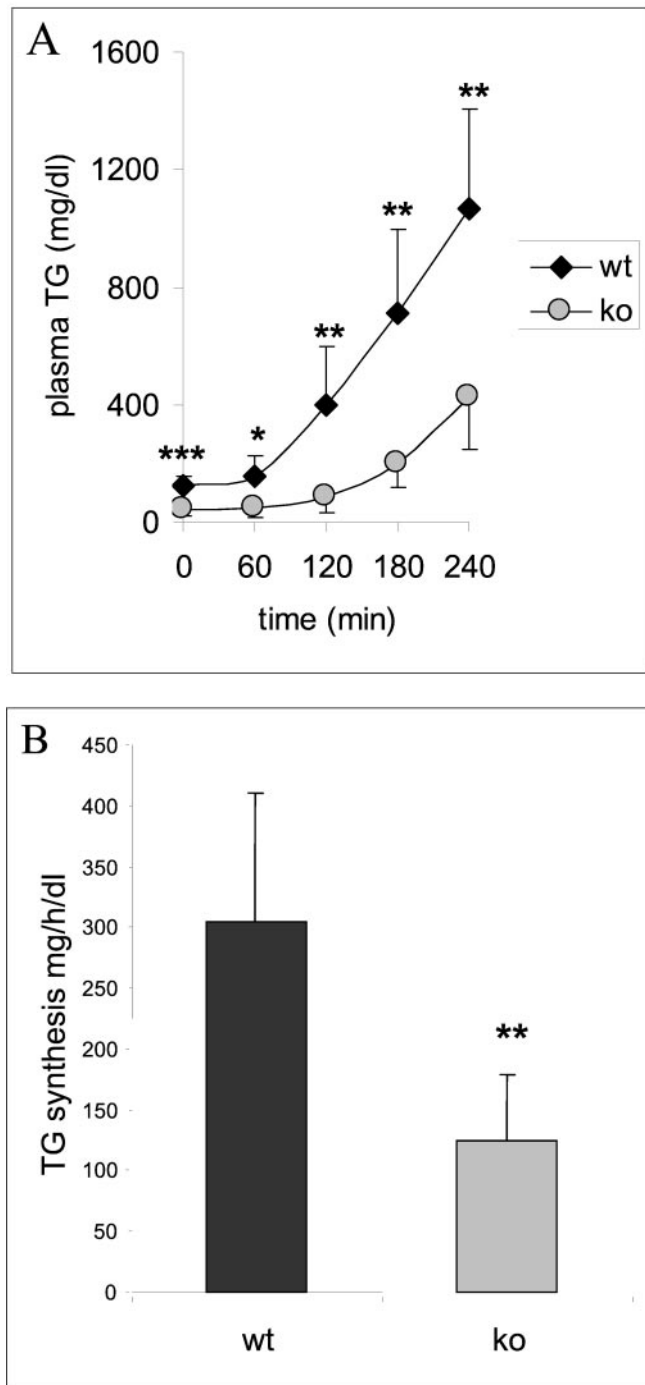


FIG. 2. Triglyceride production in Triton WR1339-treated mice. Anesthetized fasted mice were injected intravenously with 500 mg of Triton WR1339/kg of body weight as a 15 g/dl solution in 0.9% NaCl. Plasma TG were determined before and after Triton WR1339 injection at a time course of 4 h (A). TG synthesis rate was calculated by linear regression from the increase in plasma TG between 1 and 4 h after injection (B). Data are shown as the mean \pm S.D. of 6 wt- and 6 ko mice. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

availability suggested that HSL-ko mice are insufficiently supplied with NEFA. Therefore, the storage pool of neutral lipids in cardiac muscle was determined by measuring the tissue-associated TG levels of animals in the fed and fasted state. As shown in Fig. 8 the TG content was decreased by 45% in the fed and 70% in the fasted state in the hearts of HSL-ko mice compared with controls.

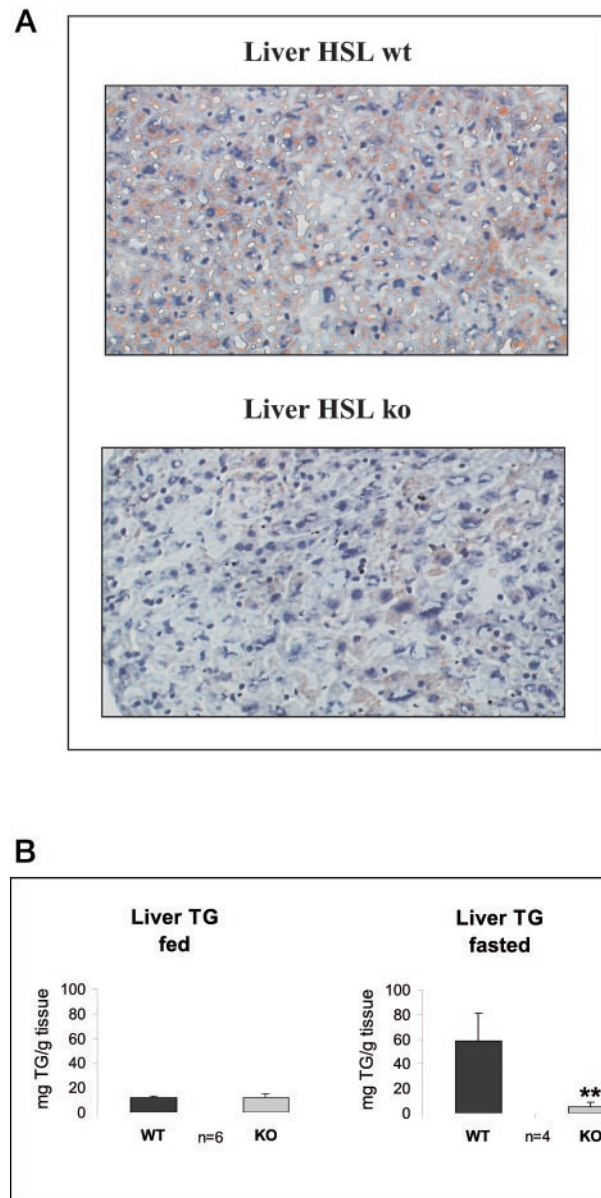


FIG. 3. Liver cryosections and liver TG contents. A, liver cryosections were performed with pieces of livers from mice fasted overnight. Before taking livers, we perfused the mice with 0.9% NaCl to remove any blood lipids. For visualizing neutral lipids, 5 μ m tissue sections were stained with Oil Red. B, liver TG contents of mice in the fed and fasted (16 h) states. Tissues were homogenized, and tissue lipids were extracted by the method of Folch (27). TG contents were determined enzymatically. Values represent means \pm S.D. **, $p < 0.01$.

DISCUSSION

The physiological importance of HSL for the metabolism of AT-associated fat stores is evident from recent studies in HSL-deficient mice (23, 24, 35, 36). In the absence of HSL, the hydrolysis of TG and diglycerides is impaired, leading to a pronounced decrease of plasma NEFA concentrations especially in fasted animals (23). In the present study we show that decreased plasma NEFA concentrations cause marked alterations in the plasma lipoprotein pattern with decreased plasma TG levels and increased total and HDL cholesterol concentrations. In the liver, HSL-ko mice exhibited decreased VLDL synthesis, decreased TG storage droplets, and decreased ketogenesis.

Generally, NEFA that enter hepatocytes are subsequently processed by one of two possible pathways. First, re-esterifica-

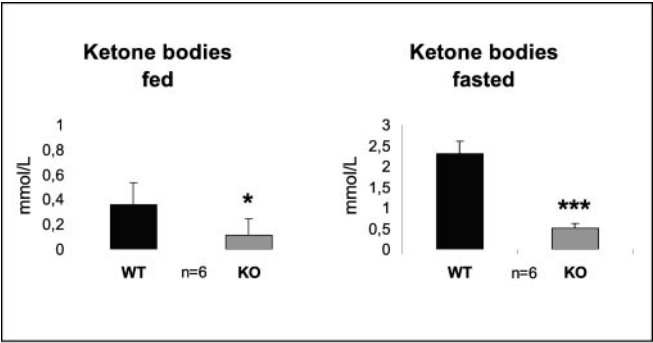
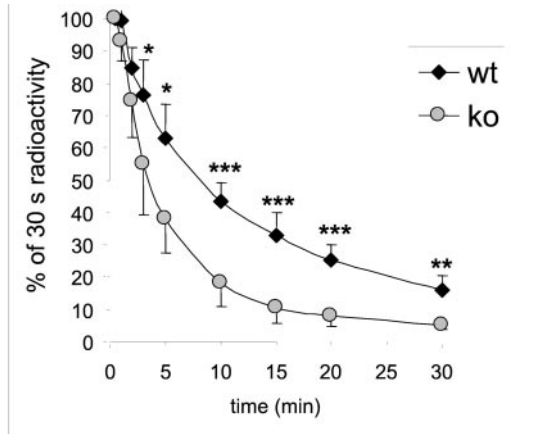


FIG. 4. **Plasma ketone bodies.** Ketone bodies (β -hydroxybutyrate) were determined from 16-week-old female mice ($n = 6$) in the fed and fasted states. Values represent means \pm S.D. *, $p < 0.05$; ***, $p < 0.001$.



Genotype	<i>n</i>	FCR pools/h	ACR pools/h
WT	6	7.2 \pm 1.5	0.36 \pm 0.11
KO	5	12.7 \pm 2.3	0.25 \pm 0.04
<i>P</i> <		0.008	0.097

FIG. 5. **VLDL turnover study.** VLDL was labeled *in vivo* with [3 H]palmitate and injected intravenously into ko- and wt mice, which were fasted for a period of 16 h. At the indicated time points, blood samples were drawn, and the remaining radioactivity in the plasma was determined. The radioactivity obtained after 30 s was set at 100% for each curve. The FCR for VLDL-TG were calculated from the plasma decay curves, assuming a two-pool model by the method of Mathews (30, 31). The ACR was calculated as the product of the FCR \times pool size. Data are shown as mean \pm S.D. of 6 wt and 5 ko mice. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

tion of NEFA at the endoplasmic reticulum leads to the formation of cytoplasmic lipid storage droplets. Second, mitochondrial import feeds into β -oxidation or ketogenesis. According to current models, lipid storage droplets represent the major pool of fatty acids for subsequent VLDL production (37). Following TG hydrolysis by a currently unidentified TG hydrolase and re-esterification at the endoplasmic reticulum (38), TG are incorporated into VLDL by a process that involves microsomal lipid transfer protein (MTP) (39). Our data are consistent with this model and show that in a condition of decreased plasma NEFA level, as observed in fasted HSL-ko mice, both cytoplasmic fat stores and VLDL synthesis are decreased. It is interesting to note that additional potential sources of fatty acids to fuel lipid droplet and VLDL production, such as hepatic *de novo* fatty acid synthesis and/or remnant particle uptake from the vascular system, cannot entirely compensate for the deficiency of fatty acid transport from AT to the liver.

Considering the second possible fate of NEFA that enter

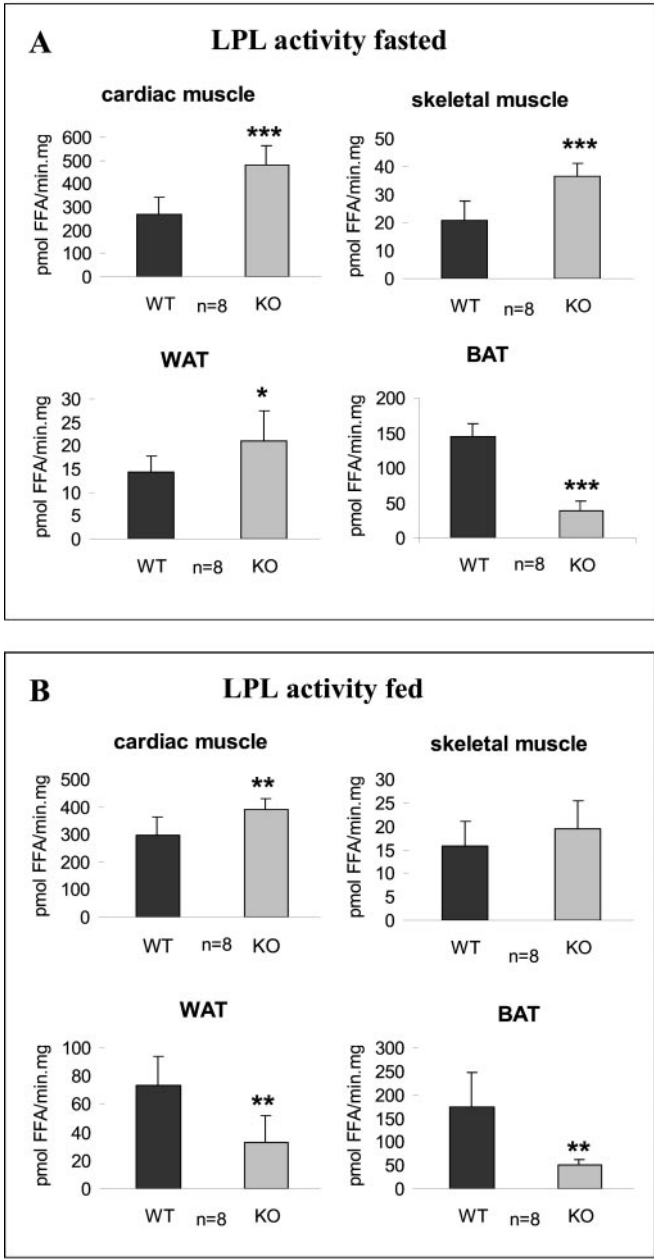


FIG. 6. **Tissue LPL activities in the fasted (A) and fed state (B).** Tissue specimens were surgically removed from mice in the mornings after they had normal access to food (fed samples) and after a fasting period of 16 h. The epididymal fat pads, brown adipose tissue, and cardiac and skeletal muscle specimens were incubated in 1 ml of Dulbecco's modified Eagle's medium with 2% bovine serum albumin and 2 units/ml heparin for 1 h at 37 $^{\circ}$ C. The LPL activity in the supernatants was assayed in duplicate. Values represent means \pm S.D. of tissue samples from 8 mice of each genotype. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

hepatocytes, namely mitochondrial import for β -oxidation or ketogenesis, it was interesting to observe a pronounced decrease in the ketone body concentration in plasma suggesting that this second pathway of fatty acid processing is also less efficient when HSL-mediated production of NEFA from fat stores is absent. Alternatively, increased ketone body utilization by peripheral tissues might also contribute to the drastic decrease in ketone body concentration in plasma of fasted animals. In view of the importance of ketone bodies as an energy substrate, particularly in the brain, low rates of hepatic ketone body production in HSL-ko mice might cause a serious problem during prolonged fasting in HSL-deficient animals.

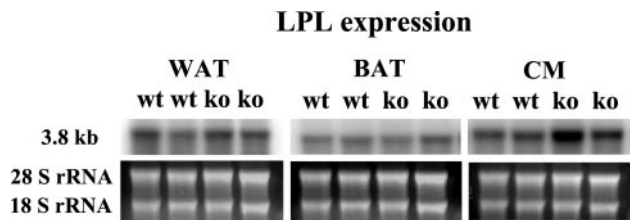


FIG. 7. LPL mRNA levels in WAT, BAT, and cardiac muscle of fasted mice. Northern blotting analysis was performed with RNA tissue samples from WAT, BAT, and cardiac muscle (CM). Total RNA (10 μ g) was subjected to formaldehyde/agarose gel electrophoresis and blotted onto a nylon membrane. After hybridization of the blot with a 32 P-labeled mouse LPL mRNA-specific probe, signals were visualized by exposure to a phosphorimaging screen. The size of LPL mRNA is indicated. For comparison of RNA quantities, the gel was stained with ethidium bromide and photographed before blotting.

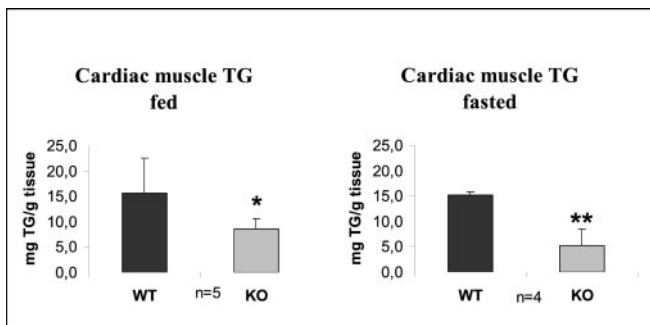


FIG. 8. Cardiac muscle TG contents from mice in the fed state and from mice after a 16-h fasting period. Before surgical removal of the hearts, mice were perfused with 0.9% NaCl to remove any blood lipids. Tissues were homogenized, and tissue lipids were extracted by the method of Folch (27). TG contents were determined enzymatically. Values represent means \pm S.D. *, $p < 0.05$; **, $p < 0.01$.

Decreased hepatic VLDL production as a result of the defective TG lipolysis in AT of HSL-ko mice was also associated with alterations in the catabolic pathways of VLDL. VLDL turnover experiments revealed significantly increased FCR values in HSL-ko mice compared with controls. The increased FCR is explained mainly by the much smaller TG pool size in HSL-deficient animals. In fact, when the ACR was calculated as a product of the FCR \times pool size, a decreased ACR was found in HSL-deficient mice. Because in steady state conditions the ACR is a measure of the production rate, this result confirms our observations of decreased VLDL synthesis in Triton WR-1339 experiments.

The decreased TG pool in fasted HSL-ko animals was not only a result of decreased VLDL production but was also caused by the induction of LPL activities in cardiac and skeletal muscle. The statistically highly significant increase of LPL activities in muscle might reflect an effort to increase the uptake of energy substrate in a situation of extremely low plasma concentrations of NEFA and TG-rich lipoproteins. The regulation of LPL in AT was found to be less uniform. Independent of the feeding/fasting state LPL was markedly reduced in BAT of HSL-ko mice. In WAT, LPL was induced in fasted mice but reduced in fed HSL-deficient mice compared with control mice. Increased LPL activity in the muscle and WAT of fasted mice is expected to effectively lower plasma TG in addition to the decreased VLDL synthesis in HSL-ko mice, because the tissue-specific LPL activity in cardiac muscle has been shown to be particularly powerful in the catabolism of TG-rich lipoproteins (40). Although LPL activity in BAT is decreased, the total tissue LPL activity is essentially unchanged because the tissue mass is increased in HSL-deficient mice (23, 35). Taken together, it is reasonable to assume that the total LPL-

mediated lipolytic activity of the body is determined by the tissue-specific activities in cardiac muscle, skeletal muscle, and WAT, which are all increased in fasted HSL-ko mice.

The molecular mechanisms responsible for the coordinate regulation of LPL in the presence or absence of HSL remain to be elucidated. In human AT, such a coordinated regulation of LPL and HSL was observed, suggesting a control mechanism of fat storage and mobilization (41). A coordinate regulation of the enzymes across tissue boundaries, however, has not been reported. Induction of LPL activity in muscle and WAT and reduction of LPL in BAT is a post-transcriptional process, because LPL mRNA levels were identical in these tissues in HSL-ko and control mice. Apparently, variations in LPL activities are a result of changes in enzyme processing or enzyme translocation to its final destination, the heparan sulfate anchors in the capillary endothelium. Post-transcriptional regulation of LPL has been observed in previous studies in response to hormones and cytokines such as insulin and interleukin-1 (42, 43). The signals that trigger the post-transcriptional induction of LPL in HSL-deficient mice are presently unknown. However, several scenarios are conceivable. First, the lack of induction of LPL in WAT of HSL-ko mice in response to feeding indicates a defect in insulin action, because it is well documented that the postprandial up-regulation of WAT LPL is mediated by insulin. Second, the intracellular lipid stores in peripheral tissues, particularly the heart, might act as a "lipostat" signaling the increased requirement for fatty acids upon depletion. A similar function has been proposed for lipid stores in pancreatic β -cells (36, 44). Third, the decreased availability of fatty acids or a subclass thereof (e.g. essential fatty acids) from plasma or changes in the hormonal status because of HSL deficiency might induce the post-transcriptional processing of LPL. The lipostat hypothesis is also consistent with our observation that, in contrast to all other peripheral tissues, LPL is drastically down-regulated in BAT of HSL-deficient mice. We assume that increased BAT mass and BAT lipid content as observed in HSL-ko mice (23) inhibit LPL expression at the post-transcriptional level.

The induction of LPL was highest in cardiac muscle, in accordance with the current concept that the heart is particularly dependent on NEFA uptake as oxidative fuel. However, despite this induction, the steady state concentration of cardiac muscle TG stores were decreased in HSL-ko mice. The normal heart also exhibits the capacity to store limited amounts of fatty acids as TG droplets (45). This myocardial TG content is kept relatively constant under physiological conditions, suggesting a strictly regulated equilibrium between oxidative fatty acid consumption and fatty acid uptake. In fasted HSL-ko mice, the cardiac TG pool was markedly reduced, indicating an imbalance of fatty acid uptake *versus* consumption, which was not compensated by the up-regulation of the tissue specific LPL activity.

Decreased plasma TG levels as a result of decreased hepatic VLDL synthesis and increased peripheral VLDL catabolism are associated with increased plasma cholesterol and HDL cholesterol concentrations. These data are in agreement with the well established concept that increased catabolism of TG-rich lipoproteins due to the induction of LPL is an important determinant of HDL cholesterol levels. It is generally accepted that the LPL-mediated lipolysis of chylomicrons and VLDL provides "surface remnants" as precursor particles that together with hepatic pre β 1-LpA-I particles are converted to mature α -LpA-I particles by the action of ABC-A1, lipid transfer proteins, and lecithin:cholesterol acyl transferase (46–53). It is reasonable to assume that the increased LPL activities

found in muscle and WAT at least partially account for the observed increase in HDL cholesterol levels.

In summary, we conclude that the phenotypic changes observed in HSL-deficient mice indicate an important function for the enzyme in the regulation of lipid homeostasis and lipoprotein metabolism.

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