Hormone-sensitive lipase knockout mice have increased hepatic insulin sensitivity and are protected from short-term diet-induced insulin resistance in skeletal muscle and heart

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Park, So-Young, Hyo-Jeong Kim, Shupei Wang, Takamasa Higashimori, Jianying Dong, Yoon-Jung Kim, Gary Cline, Hong Li, Marc Prentki, Gerald I. Shulman, Grant A. Mitchell, and Jason K. Kim. Hormone-sensitive lipase knockout mice have increased hepatic insulin sensitivity and are protected from short-term diet-induced insulin resistance in skeletal muscle and heart. Am J Physiol Endocrinol Metab 289: E30–E39, 2005. First published February 8, 2005; doi:10.1152/ajpendo.00251.2004.—Insulin resistance in skeletal muscle and heart plays a major role in the development of type 2 diabetes and diabetic heart failure and may be causally associated with altered lipid metabolism. Hormone-sensitive lipase (HSL) is a rate-determining enzyme in the hydrolysis of triglyceride in adipocytes, and HSL-deficient mice have reduced circulating fatty acids and are resistant to diet-induced obesity. To determine the metabolic role of HSL, we examined the changes in tissue-specific insulin action and glucose metabolism in vivo during hyperinsulinemic euglycemic clamps after 3 wk of high-fat or normal chow diet in awake, HSL-deficient (HSL-KO) mice. On normal diet, HSL-KO mice showed a twofold increase in hepatic insulin action but a 40% decrease in insulin-stimulated cardiac glucose uptake compared with wild-type littermates. High-fat feeding caused a similar increase in whole body fat mass in both groups of mice. Insulin-stimulated glucose uptake was reduced by 50–80% in skeletal muscle and heart of wild-type mice after high-fat feeding. In contrast, HSL-KO mice were protected from diet-induced insulin resistance in skeletal muscle and heart, and these effects were associated with reduced intramuscular triglyceride and fatty acyl-CoA levels in the fat-fed HSL-KO mice. Overall, these findings demonstrate the important role of HSL on skeletal muscle, heart, and liver glucose metabolism.

Insulin resistance plays a major role in the pathogenesis of type 2 diabetes and diabetes-associated cardiovascular events and may be causally associated with alteration in lipid metabolism (3, 10, 24, 32, 40, 41). Adipose tissue is an important endocrine organ, capable of producing various metabolic hormones/ cytokines, including resistin, adiponectin, leptin, tumor necrosis factor (TNF)-α, and interleukin (IL)-6, and dysregulated production of adipocyte-derived cytokines has been shown to alter whole body glucose metabolism (23, 25, 26, 49, 55). Additionally, adipocytes affect glucose homeostasis by releasing fatty acids into circulation, and elevated levels of circulating fatty acids and/or lipolysis have been shown to cause insulin resistance (4, 27, 42). Our previous studies (28) demonstrated that muscle-specific and liver-specific increases in fat levels, mediated by tissue-specific overexpression of lipoprotein lipase, caused insulin resistance in skeletal muscle and liver, respectively. Additionally, mice with heart-specific overexpression of peroxisome proliferator-activated receptor (PPAR)α were characterized by increases in cardiac lipid oxidation and decreases in cardiac glucose metabolism (12). In contrast, Hajri et al. (19) showed that mice deficient in CD36/FAT, a fatty acid transporter, were characterized by reduced fatty acid uptake and enhanced insulin sensitivity. All together, these findings demonstrated the important relationship between fatty acids and glucose metabolism. The underlying mechanism by which fatty acids cause insulin resistance may involve intracellular accumulation of fatty acid-derived metabolites (e.g., fatty acyl-CoA) and activation of serine kinase cascade leading to defects in insulin signaling and glucose metabolism (48).

Hormone-sensitive lipase (HSL) is a multifunctional cytoplasmic enzyme that catalyzes the hydrolysis of triglyceride stored in adipocytes and is regulated by catecholamines and insulin under various physiological states (21, 22, 46, 56). Catecholamines, such as epinephrine, promote adipose-derived lipolysis by activating HSL, whereas insulin suppresses lipolysis by inhibiting HSL activity (1, 11, 15). Previous studies indicated that insulin’s ability to suppress HSL was blunted in diabetic and obese states, and this defect was partly responsible for elevated circulating fatty acids levels in these subjects (9, 30, 34). The important role of HSL on whole body lipid metabolism was further evident in HSL-deficient mice that showed reduced circulating fatty acids and altered triglyceride stores in tissues (14, 18, 20, 35, 53, 60). Additionally, HSL-deficient mice developed dysregulated production of adipocyte-derived cytokines and were resistant to diet-induced obesity and adipogenesis (35, 51). Furthermore, whole body insulin sensitivity and pancreatic insulin secretion were shown to be altered in mice lacking HSL, whereas the variation in the promoter segment of the human HSL gene has been associated with alteration in insulin sensitivity (33, 44, 51, 52). Although the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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it is clear that HSL deletion impacts whole body lipid and glucose metabolism, the specific tissues responsible for altered glucose homeostasis are controversial. Thus, to determine the role of HSL on whole body glucose metabolism, we examined the changes in tissue-specific insulin action and glucose metabolism in HSL knockout mice during hyperinsulinemic euglycemic clamps following 3 wk of high-fat diet or normal chow diet.

MATERIALS AND METHODS

Animals and diet. Male HSL knockout (HSL-KO) and wild-type littermates (54) weighing ~25 g (~12 wk of age) were housed under controlled temperature (23°C) and lighting (12 h of light, 0700–1900; 12 h of dark, 1900–0700) with free access to water and standard mouse chow diet (6% fat by calories; Harlan Teklad, Madison, WI). Mice used in this study were back-crossed to C57BL/6 background for five generations (14). To examine the diet-induced changes in glucose metabolism, a high-fat diet (55% fat by calories; Harlan Teklad) or normal diet was fed ad libitum for 3 wk in the HSL-KO and wild-type mice (n = 8–10 per group).

Body composition analysis and surgery. At least 4 days before in vivo experiments, whole body fat and lean mass were measured in awake mice using 1H MRS (Bruker Minispec Analyzer; Echo Medical Systems, Houston, TX). After the body composition measurement, mice were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg body wt) and xylazine (10 mg/kg body wt), and an indwelling catheter was inserted in the right internal jugular vein as previously described (26). On the day of the clamp experiment, a three-way connector was attached to the jugular vein catheter to intravenously deliver solutions (e.g., glucose and insulin), and the blood samples were obtained from the tail vessels (26). All procedures were approved by the Yale University Animal Care and Use Committee.

Hyperinsulinemic euglycemic clamps to assess insulin action in vivo. After an overnight fast (~15 h), a 2-h hyperinsulinemic euglycemic clamp was conducted with a primed continuous infusion of human insulin (Humulin; Eli Lilly, Indianapolis, IN) at a rate of 15 pmol·kg⁻¹·min⁻¹ to raise plasma insulin within a physiological range (400–500 pM). Blood samples (20 μl) were collected at 20-min intervals for the immediate measurement of plasma glucose concentration, and 20% glucose was infused at variable rates to maintain glucose at basal concentrations (~7 mM). Basal and insulin-stimulated rates of whole body glucose uptake were estimated with a continuous infusion of [3-¹³C]glucose (PerkinElmer Life and Analytical Sciences, Boston, MA) for 2 h before the clamps (0.05 μCi/min) and throughout the clamps (0.1 μCi/min), respectively (26). To estimate insulin-stimulated glucose uptake in individual tissues, 2-deoxy-[¹-¹⁴C]glucose (2-[¹⁴C]DG; PerkinElmer Life and Analytical Sciences) was administered as a bolus (10 μCi) at 75 min after the start of clamps, as previously described (26). Blood samples were taken before, during, and at the end of clamps for measurement of plasma [¹⁴C]glucose, [¹³C]H₂O, 2-[¹⁴C]DG, and insulin and/or fatty acid concentrations. At the end of the clamps, mice were anesthetized with pentobarbital sodium injection. Within 5 min, three muscles (gastrocnemius, tibialis anterior, and quadriceps) from both hindlimbs, epididymal white adipose tissue, intrascapular brown adipose tissue, and heart were taken for biochemical and molecular analysis.

Biochemical assays and calculation. Glucose concentration during clamps was analyzed using 10 μl plasma by a glucose oxidase method on a Beckman Glucose Analyzer 2 (Beckman, Fullerton, CA). Plasma insulin concentration was measured by radioimmunoassay (RIA) using kits from Linco Research (St. Charles, MO). Plasma fatty acid concentration was determined using an acyl-CoA oxidase-based colorimetric kit.

Fig. 1. Metabolic parameters in the hormone-sensitive lipase (HSL) knockout (KO) and wild-type (WT) mice fed normal or high-fat diet. A: whole body fat mass, as assessed by 1H MRS, in awake mice. B: overnight-fasted levels of plasma fatty acids. Values are means ± SE for 8–10 experiments. *P < 0.05 vs. WT mice fed normal diet. †P < 0.05 vs. WT mice fed high-fat diet.
Plasma concentrations of [3-3H]glucose, 2-[14C]DG, and 3H2O were determined after deproteinization of plasma samples, as previously described (26). The radioactivity of 3H in tissue glycogen was determined by digesting tissue samples in KOH and precipitating glycogen with ethanol. For the determination of tissue 2-[14C]DG-6-phosphate (2-[14C]DG-6-P) content, tissue samples were homogenized, and the supernatants were subjected to an ion exchange column to separate 2-DG-6-P from 2-DG (26).

Rates of basal hepatic glucose production (HGP) and insulin-stimulated whole body glucose uptake were determined as the ratio of the [3H]glucose infusion rate [disintegrations per minute (dpm/min)] to the specific activity of plasma glucose (dpm/H\text{mol}) at the end of basal period and during the final 30 min of clamps, respectively. HGP during the clamps was determined by subtracting the glucose infusion rate from the whole body glucose uptake rate. Whole body glycolysis was calculated from the rate of increase in plasma 3H2O concentration, determined by linear regression of the measurements at 80, 90, 100, 110, and 120 min of clamps. Whole body glycolysis plus lipid synthesis was estimated by subtracting whole body glycolysis from whole body glucose uptake, assuming that glycolysis and glycogen plus lipid synthesis account for the majority of insulin-stimulated glucose uptake. Because 2-DG is a glucose analog that is phosphorylated but not metabolized, insulin-stimulated glucose uptake in individual tissues can be estimated by subtracting whole body glycolysis from whole body glucose uptake, assuming that glycolysis and glycogen plus lipid synthesis account for the majority of insulin-stimulated glucose uptake. Because 2-DG is a glucose analog that is phosphorylated but not metabolized, insulin-stimulated glucose uptake in individual tissues can be estimated by subtracting whole body glycolysis from whole body glucose uptake, assuming that glycolysis and glycogen plus lipid synthesis account for the majority of insulin-stimulated glucose uptake. Because 2-DG is a glucose analog that is phosphorylated but not metabolized, insulin-stimulated glucose uptake in individual tissues can be estimated by subtracting whole body glycolysis from whole body glucose uptake, assuming that glycolysis and glycogen plus lipid synthesis account for the majority of insulin-stimulated glucose uptake. Because 2-DG is a glucose analog that is phosphorylated but not metabolized, insulin-stimulated glucose uptake in individual tissues can be estimated by subtracting whole body glycolysis from whole body glucose uptake, assuming that glycolysis and glycogen plus lipid synthesis account for the majority of insulin-stimulated glucose uptake.

Skeletal muscle glycolysis was estimated as the difference between muscle glucose uptake and muscle glycogen synthesis. Measurements of triglyceride and fatty acyl-CoA in muscle, liver, and heart. For the measurement of tissue-specific triglyceride concentration, skeletal muscle (quadriceps), liver, and heart were homogenized in chloroform-methanol (26), the organic layer was separated using H2SO4, and concentrations were determined using a triglyceride assay kit (Sigma Diagnostics) and spectrophotometry. To determine the intramuscular concentrations of fatty acyl-CoAs by use of liquid-chromatography tandem mass spectrometry, skeletal muscle (quadriceps) samples were homogenized and prepared as previously described (2, 57). A PerkinElmer Sciex API 3000 tandem mass spectrometer (Applied Biosystems, Foster City, CA) interfaced with TurbolonSpray ionization source was used for the analysis. The intracellular concentrations of long-chain fatty acyl-CoAs (C16:0, C16:1, C18:0, C18:1, C18:2, and C18:3) were detected in negative electrospray mode, and C17 CoA ester was used as an internal standard. The doubly charged ions of these compounds were transmitted, and singly charged product ions were quantified in multiple reaction mode.

Statistical analysis. Data are expressed as means ± SE. The significance of the difference in mean values between wild-type mice fed the normal diet (control), wild-type mice fed the high-fat diet, HSL-KO mice fed the normal diet, and HSL-KO mice fed the high-fat diet was evaluated using the Duncan’s multiple range test.

RESULTS

Body composition and metabolic parameters. On the normal diet, body weight, whole body fat/lean mass (as measured by

Fig. 2. Whole body glucose metabolism during hyperinsulinemic euglycemic clamps in HSL-KO and WT mice fed normal or high-fat diet. A: steady-state glucose infusion rate, obtained from averaged rates of 90–120 min of clamps. B: insulin-stimulated whole body glucose uptake. C: insulin-stimulated whole body glycogen synthesis. D: insulin-stimulated whole body glycogen plus lipid synthesis. Values are means ± SE for 8–10 experiments. *P < 0.05 vs. WT mice fed normal diet. #P < 0.05 vs WT mice fed high-fat diet.
glucose infusion in both groups of mice (79 ± 15 vs. 110 ± 5 μmol·kg⁻¹·min⁻¹ in the normal diet; Fig. 2C). High-fat feeding or HSL deletion did not significantly alter insulin-stimulated whole body glucose uptake and glycolysis (Fig. 2D).

Hepatic insulin action in HSL-KO mice. Basal HGP was not significantly altered in the HSL-KO mice fed the normal diet (102 ± 14 vs. 79 ± 7 μmol·kg⁻¹·min⁻¹ in wild-type mice). In contrast, hepatic insulin action, as reflected by insulin-mediated suppression of basal HGP, was increased twofold in the HSL-KO mice (Fig. 3A). Increased hepatic insulin sensitivity was associated with an 80% decrease in intrahepatic triglyceride levels in the HSL-KO mice (1.4 ± 0.5 vs. 6.6 ± 2.5 μmol/g in wild-type mice; Fig. 3B). Furthermore, high-fat feeding did not significantly affect basal HGP in the HSL-KO mice (106 ± 11 vs. 78 ± 9 μmol·kg⁻¹·min⁻¹ in wild-type mice). In contrast, although hepatic insulin action showed a tendency to be reduced in the fat-fed wild-type mice, high-fat feeding caused a profound hepatic insulin resistance in the HSL-KO mice (P < 0.01 vs. normal diet-fed HSL-KO mice; Fig. 3A). Consistent with the diet-induced changes in hepatic insulin action, intrahepatic triglyceride levels showed a tendency to be elevated in the fat-fed wild-type mice (P = 0.08 vs. normal diet-fed wild-type mice) and were significantly increased in the fat-fed HSL-KO mice (P < 0.005 vs. normal diet-fed HSL-KO mice; Fig. 3B).

Whole body glucose metabolism in HSL-KO mice. Tissue-specific insulin action and glucose metabolism were examined during a 2-h hyperinsulinemic euglycemic clamp in wild-type and HSL-KO mice fed normal or high-fat diet for 3 wk. During the clamps, plasma glucose levels were maintained at euglycemia, and plasma insulin levels were similarly elevated in all groups of mice (Table 1). The steady-state rates of glucose infusion required to maintain euglycemia during the clamps did not differ between the wild-type mice and HSL-KO mice fed the normal diet (219 ± 28 and 213 ± 25 μmol·kg⁻¹·min⁻¹, respectively; Fig. 2A). Similarly, insulin-stimulated whole body glucose uptake was comparable between the wild-type and HSL-KO mice fed the normal diet (227 ± 31 and 202 ± 23 μmol·kg⁻¹·min⁻¹, respectively), indicating that HSL deletion did not affect peripheral insulin sensitivity (Fig. 2B).

High-fat feeding markedly reduced the steady-state rates of glucose infusion in both groups of mice (79 ± 17 and 90 ± 22 μmol·kg⁻¹·min⁻¹ in the fat-fed wild-type and HSL-KO mice, respectively; Fig. 2A). Tracer analysis further confirmed the onset of diet-induced insulin resistance, as reflected by a 40% decrease in insulin-stimulated whole body glucose uptake in the wild-type mice (136 ± 15 μmol·kg⁻¹·min⁻¹; Fig. 2B). Interestingly, insulin-stimulated whole body glucose uptake was significantly increased in the fat-fed HSL-KO mice (182 ± 12 μmol·kg⁻¹·min⁻¹) compared with the fat-fed wild-type mice and did not differ from the normal diet-fed HSL-KO mice (Fig. 2B). Furthermore, insulin-stimulated whole body glycolysis showed a similar pattern of changes to the whole body glucose uptake. High-fat feeding decreased insulin-stimulated whole body glycolysis by ~40% in the wild-type mice (82 ± 13 vs. 137 ± 27 μmol·kg⁻¹·min⁻¹ in the normal diet) but did not affect the rate of glycolysis in the HSL-KO mice (125 ± 10 vs. 110 ± 5 μmol·kg⁻¹·min⁻¹ in the normal diet; Fig. 2C).
Skeletal muscle insulin action and glucose metabolism in HSL-KO mice. On the normal diet, insulin-stimulated glucose uptake in skeletal muscle (gastrocnemius) did not significantly differ between the wild-type and HSL-KO mice (246 ± 35 and 196 ± 23 nmol·g⁻¹·min⁻¹, respectively; Fig. 4A), and this was consistent with comparable rates of insulin-stimulated whole body glucose metabolism in both groups of mice (Fig. 2B). Insulin-stimulated glucose metabolic fluxes (i.e., glycolysis and glycogen synthesis) and intramuscular (quadriiceps) triglyceride levels were also unaltered in the HSL-KO mice (Fig. 4, B, C, and D).

Diet-induced whole body insulin resistance in the wild-type mice was mostly due to ~40% decreases in insulin-stimulated glucose uptake and glycolysis in skeletal muscle (150 ± 6 and 137 ± 9 nmol·g⁻¹·min⁻¹, respectively; Fig. 4, A and B). In contrast, HSL-KO mice were completely protected from diet-induced insulin resistance in skeletal muscle, as reflected by significantly elevated skeletal muscle glucose uptake in the fat-fed HSL-KO mice (251 ± 36 nmol·g⁻¹·min⁻¹) compared with the fat-fed wild-type mice (Fig. 4A). Insulin-stimulated skeletal muscle glycolysis synthesis was not significantly altered by high-fat feeding or HSL deletion (Fig. 4C). Furthermore, diet-induced skeletal muscle insulin resistance in the wild-type mice was associated with a twofold increase in intramuscular triglyceride level following high-fat feeding (8.4 ± 1.9 vs. 4.3 ± 0.4 μmol/g in normal diet; Fig. 4D). In contrast, HSL-KO mice were protected from diet-induced increases in intramuscular triglyceride level (5.5 ± 0.7 μmol/g; Fig. 4D). Additionally, intramuscular (quadriiceps) levels of individual and total fatty acyl-CoAs (sum of C16:0, C16:1, C18:0, C18:1, C18:2, and C18:3) were unaltered in both groups of mice fed a normal diet (Fig. 5A) but showed a tendency to be reduced in the fat-fed HSL-KO mice compared with the fat-fed wild-type mice (P = 0.08 for C16:1, P = 0.07 for C18:2, P = 0.08 for C18:3, and P = 0.1 for total fatty acyl-CoAs; Fig. 5B). We have previously shown that fatty acid-mediated insulin resistance was due to blunted insulin signaling associated with insulin receptor substrate (IRS)-1 in skeletal muscle (26–28). In this regard, insulin-stimulated tyrosine phosphorylation of IRS-1 in skeletal muscle (gastrocnemius) was significantly increased in the fat-fed HSL-KO mice compared with the fat-fed wild-type mice (679 ± 84 vs. 426 ± 47 arbitrary units in fat-fed wild-type mice).

Insulin-stimulated glucose uptake in heart and adipose tissues. On the normal diet, insulin-stimulated glucose uptake in heart was decreased by ~30% in the HSL-KO mice (951 ± 115 vs. 1,452 ± 168 nmol·g⁻¹·min⁻¹ in wild-type mice; Fig. 6A), whereas myocardial triglyceride levels did not differ between the wild-type and HSL-KO mice (5.0 ± 1.8 and 5.6 ± 2.0 μmol/g, respectively; Fig. 6B). Insulin-stimulated glucose uptake in brown (intrascapular) and white (epididymal) adipose tissues were not significantly altered in the HSL-KO mice (Fig. 6, C and D).

High-fat feeding caused ~80% decreases in insulin-stimulated cardiac glucose uptake in the wild-type mice (328 ± 50 nmol·g⁻¹·min⁻¹) but did not significantly affect cardiac glucose uptake.
uptake in the HSL-KO mice (1,090 ± 189 nmol·g⁻¹·min⁻¹; Fig. 6A). Diet-induced cardiac insulin resistance in the wild-type mice was associated with a threefold increase in myocardial triglyceride level (14.4 ± 3.8 μmol/g) compared with the normal diet-fed mice (Fig. 6B). In contrast, HSL-KO mice were protected from diet-induced increases in myocardial triglyceride level (5.6 ± 3.0 μmol/g; Fig. 6B). Furthermore, insulin-stimulated glucose uptake in brown adipose tissue was reduced by 70–80% in both groups of mice following high-fat feeding (720 ± 156 and 503 ± 138 nmol·g⁻¹·min⁻¹ in fat-fed wild-type and HSL-KO mice, respectively, vs. 2,114 ± 350 nmol·g⁻¹·min⁻¹ in normal diet-fed wild-type mice; Fig. 6C). Additionally, insulin-stimulated glucose uptake in white adipose tissue was not significantly altered by high-fat feeding but was significantly increased in the HSL-KO mice compared with the fat-fed wild-type mice (18 ± 3 vs. 6 ± 1 nmol·g⁻¹·min⁻¹ in fat-fed wild-type mice; Fig. 6D).

**DISCUSSION**

We determined the effects of HSL deletion on tissue-specific glucose metabolism by conducting a hyperinsulinemic euglycemic clamp in awake HSL-KO mice and wild-type littermates following 3 wk of high-fat or normal diet. On the normal diet, HSL-KO mice showed increased hepatic insulin action, which was associated with reduced circulating fatty acids and intrahepatic triglyceride levels in the HSL-KO mice compared with the wild-type mice. High-fat feeding caused insulin resistance in skeletal muscle and heart of the wild-type mice, and this was associated with increased intramuscular triglyceride and fatty acyl-CoA levels. In contrast, HSL-KO mice were protected from diet-induced insulin resistance in skeletal muscle and heart, and this was associated with reduced intramuscular and myocardial triglyceride levels in the fat-fed HSL-KO mice compared with the fat-fed wild-type mice. Thus our findings demonstrate the important role of HSL on liver, skeletal muscle, and cardiac glucose metabolism.

To examine the role of HSL on adipocyte biology and whole body lipid metabolism, mice deficient in HSL were recently generated by numerous laboratories (14, 18, 20, 33, 35, 44, 51–54, 60). Wang et al. (54) showed that HSL-deficient mice exhibited reduced abdominal fat mass with hypertrophied white adipocytes, whereas Osuga et al. (35) reported adipocyte hypertrophy of both white and brown adipose tissues with increased brown fat mass. Because HSL is a cytoplasmic protein that catalyzes the hydrolysis of triglyceride (21, 22, 46, 56), adipocyte hypertrophy of HSL-deficient mice might be caused by reduced lipolysis. In this regard, Wang et al. (54) showed blunted β-adrenergic mediated adipocyte lipolysis in the HSL-deficient mice, whereas Haemmerle et al. (18) demonstrated blunted isoproterenol-mediated glycerol release from epididymal white adipocytes of HSL-deficient mice. With reduced lipolysis in adipose tissue, HSL-deficient mice were uniformly characterized by decreases in circulating fatty acid levels compared with their respective wild-type cohorts (18, 20, 53).

Since Randle et al. (40) introduced the concept of substrate competition between glucose and fatty acids more than 40 years ago, numerous studies have examined the relationship between lipid and glucose metabolism and found that increases in circulating fatty acids reduced whole body glucose metabolism (4, 27, 42). Based on this well-established observation, recent studies examined the effects of HSL deficiency on whole body glucose metabolism (14, 18, 20, 33, 35, 44, 51–54, 60). Both Roduit et al. (44) and Mulder et al. (33) found that HSL-deficient mice exhibited a blunted response to glucose tolerance tests, suggesting altered insulin sensitivity in the HSL-deficient mice. A hyperinsulinemic euglycemic clamp further showed that, although insulin-stimulated glucose clearance was normal, insulin-mediated suppression of HGP was impaired in the HSL-deficient mice (33). In contrast, Voshol et al. (53) reported increased hepatic insulin action during a hyperinsulinemic euglycemic clamp in their HSL-deficient mice. The discrepancy between these studies with regard to hepatic insulin sensitivity may be due to different insulin infusion rates used in the clamp experiments [i.e., 20 mU·kg⁻¹·min⁻¹ (33) vs. 3.5 mU·kg⁻¹·min⁻¹ (53)]. In this regard, our clamp experiments using 2.5 mU·kg⁻¹·min⁻¹ of continuous insulin infusion showed increased hepatic insulin action in the HSL-KO mice, similar to the observation of Voshol et al.

Increases in hepatic insulin sensitivity of HSL-deficient mice may be due to increased hepatic insulin signaling and reduced intrahepatic fat content (53). In this regard, Voshol et al. reported increases in tyrosine phosphorylation of insulin receptor and IRS-1-associated phosphatidylinositol (PI) 3-kinase, which is an important mediator of hepatic insulin signaling and action (39), in HSL-deficient mice (53). Furthermore, previous studies showed a strong inverse relationship between intracel-
lular fat content and insulin sensitivity in both animal models and humans (5, 31, 38). Our recent study (28) demonstrated that liver-specific overexpression of lipoprotein lipase, the rate-controlling enzyme in fatty acid uptake into cells (16), caused defects in hepatic insulin signaling and insulin action, which were associated with elevated levels of intrahepatic triglyceride and fatty acyl-CoAs. In this regard, our present study also showed a profound decrease in intrahepatic triglyceride levels in the HSL-KO mice compared with the wild-type mice. Although altered levels of adipocyte-derived cytokines may also affect hepatic insulin action, the findings of reduced plasma adiponectin levels in the HSL-deficient mice make their role less likely (20). Moreover, Mulder et al. (33) reported reduced insulin-stimulated glucose uptake in isolated soleus muscle of HSL-deficient mice, whereas Voshol et al. (53) found normal insulin-mediated whole body glucose uptake during clamps. Using labeled 2-DG during clamps, we assessed tissue-specific glucose uptake and found that insulin-stimulated skeletal muscle glucose uptake in vivo was unaltered in the HSL-deficient mice. Thus the discrepancy between these findings may be attributed to the in vivo vs. in vitro experimental settings. Overall, our findings indicate that HSL deficiency increased hepatic insulin action without altering skeletal muscle insulin metabolism, and this was associated with decreases in circulating fatty acids and intrahepatic triglyceride levels in the HSL-KO mice.

Recent studies have importantly demonstrated the protective effects of HSL deficiency on diet-induced obesity (14, 18, 20, 35, 47, 51, 53, 60). Previous studies showed that the HSL-deficient mice were completely protected from increased adiposity following chronic feeding of a high-fat diet (20, 35), and Sekiya et al. (47) demonstrated that HSL deficiency ameliorated obesity in leptin-deficient \(ob/ob\) mice. In contrast, high-fat feeding induced a similar gain of body weight in our current study (~3-g increase in whole body fat mass following 3 wk of high-fat feeding). The discrepancy between these results may be due to the duration of chronic high-fat feeding, in which the present study examined a shorter term of fat feeding than other studies. Furthermore, previous findings were associated with an increased number of preadipocytes and reduced expression of PPAR\(_\gamma\) in the white adipose tissue of leptin-deficient or high-fat-fed HSL-deficient mice (20). Harada et al. (20) also showed decreases in circulating adiponectin and leptin levels as well as altered mRNA expression of various adipocytokines in the high-fat-fed HSL-deficient mice. Although these studies examined the effects of long-term fat feeding on adipocyte metabolism in HSL-deficient mice, no study to date has examined the metabolic effects of HSL deficiency on short-term high-fat feeding. High-fat feeding for 3 wk decreased insulin-stimulated glucose uptake and metabolic flux in the skeletal muscle of wild-type mice, and this was associated with marked increases in intramuscular triglyceride and fatty acyl-CoA levels in the fat-fed wild-type mice. In contrast, the HSL-KO mice were completely protected from diet-induced defects in skeletal muscle insulin action, and this protective effect was associated with reduced intramuscular triglyceride and fatty

![Fig. 6. Glucose uptake in heart and adipose tissues of HSL-KO and WT mice fed normal or high-fat diet. A: insulin-stimulated cardiac glucose uptake. B: myocardial triglyceride levels. C: insulin-stimulated glucose uptake in intrascapular brown adipose tissue. D: insulin-stimulated glucose uptake in epididymal white adipose tissue. Values are means ± SE for 8–10 experiments. *P < 0.05 vs. WT mice fed normal diet. #P < 0.05 vs. WT mice fed high-fat diet.](http://www.ajpendo.org)
acetyl-CoA levels compared with the fat-fed wild-type mice. Importantly, the protective effects of HSL deficiency on diet-induced insulin resistance occurred without changes in whole body adiposity, which makes the potential effects of altered adipocytokines less likely to play a role in our study. However, it is possible that altered expression of adipocytokines may play a role in adipocyte metabolism and insulin sensitivity with long-term high-fat feeding.

The mechanism by which increases in tissue fat contents cause skeletal muscle insulin resistance may involve activation of the intracellular serine kinase cascade, of which PKC-θ and/or IkB kinase-β may play a role, by fatty acid-derived metabolites (e.g., fatty acyl-CoA) leading to the serine phosphorylation of IRS-1 (6, 17, 37, 45, 58). Our recent study (57) demonstrated that skeletal muscle insulin resistance induced by a 5-h lipid plus heparin infusion was due to increases in intramuscular fatty acyl-CoA levels, activation of PKC-θ, and subsequent defects in insulin signaling associated with IRS-1. Thus our findings that HSL-deficient mice were protected from diet-induced insulin resistance in skeletal muscle suggest that HSL deficiency prevented diet-induced intramuscular accumulation of fatty acid metabolites and their subsequent deleterious effects on skeletal muscle insulin signaling and insulin action. Decreases in intramuscular fat contents following a high-fat diet in the HSL-KO mice may be due to reduced adipocyte lipolysis and/or activity of lipoprotein lipase in skeletal muscle (20); however, direct effects of HSL deficiency in skeletal muscle cannot be ruled out. Alternatively, HSL deficiency reduced the expression of fatty acid synthase and increased the expression of uncoupling protein-2 in adipocytes of fat-fed mice (20). Also, transcriptional expression of diacylglycerol acyltransferase was reduced in the HSL-KO mice (unpublished findings). Thus altered expression of proteins involved in fatty acid uptake and/or oxidation in skeletal muscle may further contribute to the diet-induced changes in intramuscular fat contents of HSL-deficient mice.

In addition to the protective effects of HSL deficiency against diet-induced insulin resistance in skeletal muscle, HSL-KO mice were also protected from diet-induced cardiac insulin resistance. Cardiac insulin resistance is a major characteristic of diabetic heart and is causally associated with diabetic heart failure (8, 43, 50, 59). Although the mechanism of cardiac insulin resistance is unknown, recent studies have implicated the role of altered cardiac lipid metabolism in the development of cardiac insulin resistance and diabetes-associated cardiac hypertrophy (13, 36). In this regard, our present study showed that a 3-wk high-fat feeding caused cardiac insulin resistance in the wild-type mice, and this was associated with a significant increase in myocardial triglyceride levels. In contrast, HSL-KO mice were protected from diet-induced cardiac insulin resistance, and this was associated with reduced myocardial triglyceride levels in the fat-fed HSL-KO mice. Thus our findings suggest that similar mechanism by which HSL-KO mice are protected from diet-induced insulin resistance may operate in heart and skeletal muscle.

Although HSL-KO mice were protected from diet-induced insulin resistance in skeletal muscle and heart, diet-induced defects in hepatic insulin action were not prevented in the HSL-KO mice. Differential effects on peripheral and hepatic insulin action were recently observed in lipodystrophic mice (A-ZIP/F-1) following treatment of rosiglitazone (29). Our recent study (29) showed that chronic rosiglitzozone treatment ameliorated skeletal muscle insulin resistance but exacerbated hepatic insulin resistance in the lipodystrophic mice. Such differential effects were attributable to increased rosiglitazone-induced PPARγ expression in liver and increased intrahepatic fat contents (7). Whether altered expression of PPARγ is associated with our observation in the fat-fed HSL-KO mice needs to be further examined.

In summary, HSL-KO mice fed a normal diet showed increased hepatic insulin action that was associated with marked reduction in intrahepatic triglyceride levels. Additionally, HSL-KO mice were protected from short-term diet-induced insulin resistance in skeletal muscle and heart, and this was associated with decreases in intramuscular and myocardial fat contents. Taken together, our findings demonstrate the important role of HSL on liver, skeletal muscle, and heart glucose metabolism.

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