Adipose triacylglycerol lipase deletion alters whole body energy metabolism and impairs exercise performance in mice

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Fatty acids derived from the hydrolysis of adipose tissue and skeletal muscle triacylglycerol (TG) are an important energy substrate at rest and during prolonged moderate-intensity exercise (28, 31). Hormone-sensitive lipase (HSL) was long considered to be the rate-limiting enzyme for adipocyte (4) and skeletal muscle TG (35) lipolysis. However, the understanding of TG lipolysis regulation was recently challenged with the finding that adipose triacylglycerol lipase (ATGL) is the predominant TG lipase in adipose tissue (5, 11, 39) and an important regulator of TG degradation in skeletal muscle (36).

Thus, it is now proposed that ATGL and HSL regulate lipolysis in a serial manner (34, 37). Under basal conditions, ATGL catalyzes the degradation of TG to diacylglycerol (DG), and HSL catalyzes the conversion of DG to monoacylglycerol. Monoacylglycerol lipase then cleaves the final fatty acid from the glycerol backbone. Although it is generally agreed that ATGL is an important mediator of basal lipolysis, its role in β-adrenergic (stimulated) lipolysis remains controversial. Studies employing ATGL siRNA in murine adipocytes (11, 39) and genetic inactivation of ATGL in mice (5) show markedly reduced FFA release upon β-adrenergic stimulation, while chemical inhibition of HSL indicates that HSL is the major regulator of stimulated lipolysis in human fat cells (12).

Thus, the hierarchical importance of ATGL and HSL for lipolytic control and the involvement of ATGL during adrenergic stimulation and other physiological settings remains unresolved (14, 20).

The metabolic phenotypes of ATGL−/− and HSL−/− mice have been evaluated under resting conditions. ATGL−/− mice are mildly obese, store more TG in ectopic tissues, and develop heart failure due to myocardial fibrosis and a defect in contractile performance that ultimately reduces their life span (5). Circulating FFA are reduced in ATGL−/− mice, which is thought to contribute to their impaired capacity for thermogenesis and greater glucose utilization, perhaps via a reverse Randle cycle, although definitive biochemical evidence supporting this mechanism is lacking. Muscle-specific ATGL overexpression increases TG hydrolysis and alters lipid partitioning in resting skeletal muscle, which results in reduced intramyocellular TG storage (36). HSL−/− mice have slightly reduced fasted plasma FFA (3, 7, 17) and blunted β-adrenergic stimulated lipolysis (6), maintain normal body mass and adiposity (16, 30, 38), and are resistant to diet-induced (7) and genetic obesity (24). This surprising resistance to obesity may be explained by increased energy expenditure (7, 24), perhaps via increased uncoupling in brown adipose tissue (7) and decreased reesterification of FFA in white adipose tissue (7). The absence of ATGL or HSL clearly results in varied phenotypes, which is further supported by gene array studies demonstrating differential transcriptional regulation within critical metabolic tissues of ATGL and HSL−/− mice (18).

Despite the importance of these TG lipases in the regulation of whole body energy metabolism, several important questions remain unresolved. First, the impact of ATGL and HSL deletion on fatty acid homeostasis in skeletal muscle is unknown. Skeletal muscle is a major site of fatty acid metabolism, which underscores the requirement to define the contribution of TG

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lipases in this tissue. Second, very little is known about the impact of ATGL and HSL deletion on exercise performance. Recently, Fernandez et al. (3) showed that exercise performance was compromised in HSL−/− mice. This is consistent with heavy posttranslational modification and activation of HSL during exercise (19, 32). The lack of compensation in HSL−/− mice also indicates that ATGL might not play an important role in skeletal muscle lipid metabolism during exercise.

Accordingly, we investigated the role of ATGL and HSL deletion in metabolic regulation in resting and contracting skeletal muscle and examined muscle energetics and exercise performance. We hypothesized that deletion of either ATGL or HSL would compromise the capacity to enhance fat turnover during exercise and thereby compromise exercise performance.

METHODS

Animals. All procedures were approved by the Monash University School of Biomedical Sciences Animal Ethics Committee and conformed to National Health and Medical Research Council (Australia) guidelines regarding the care and use of experimental animals. Male ATGL−/− or HSL−/− and wild-type (WT) littermate mice (C57Bl/6 background) were used for all exercise experiments. Female mice were used in isolated muscle contraction studies. Mice were generated by targeted homologous recombination as described (5, 6). Knockout and WT mice for each genotype were generated by breeding heterozygous mice for the deleted allele. Mice were bred and housed under controlled temperature (22°C) and lighting (12:12-h light-dark cycle) and had free access to standard mouse chow and water.

Fatty acid metabolism in isolated skeletal muscle. Mice were fasted for 2–4 h and then anesthetized with an intraperitoneal injection of pentobarbital sodium. The soleus muscle was excised tendon to tendon and placed in a glass vial containing 2 ml of warmed (30°C), preagassed (95% O2-5% CO2, pH 7.4), modified Krebs-Henseleit buffer containing 2% fatty acid-free BSA, 6 mM glucose, and 0.5 mM palmitate. This was the base buffer used in all ex vivo experiments. After 30 min, fresh Krebs buffer containing 1 μCi/ml of [1-14C]palmitate (Amersham Life Science) was added to vials, and muscles were incubated for an additional 90 min. Exogenous palmitate oxidation was monitored by the production of 14CO2 (complete oxidation) and accumulation of 14C in an acid-soluble metabolite fraction (ASM, incomplete oxidation). Incorporation of [1-14C]palmitate into endogenous lipids were isolated by thin-layer chromatography.

Muscle contraction protocol. Mice were anesthetized with pentobarbital sodium (Nembutal, 60 mg/kg ip; Rhone Merieux, Pinkenba, Queensland, Australia) with supplemental doses administered to maintain a depth of anesthesia that prevented all responses to tactile stimuli. The extensor digitorum longus (EDL) and the soleus muscles of both legs were tied at their proximal and distal tendons with braided surgical silk and were then surgically removed. Muscles from both limbs were excised and transferred to organ baths (Radnoti, Monrovia, CA) filled with Krebs-Henseleit buffer containing 0.01% BSA, 8 mM mannitol, and 2 mM sodium pyruvate. The distal tendon of the muscle was tied to a fixed, immovable hook, and the proximal tendon was attached vertically to a force transducer (model TRI202P, Pan-Muscle, CA). Incubation chambers were oxygenated with 95% O2-5% CO2 and thermostatically maintained at 30°C. The EDL was always tested before the soleus muscle. Square wave electrical pulses generated by a Grass S48 stimulator were amplified using a power amplifier (EP500B; Audio Assemblies, Cambellfield, Victoria, Australia) and delivered to two platinum plate electrodes that flanked but were careful not to touch the muscle. Contractile responses were recorded and analyzed using Chart 5.0 software (AD Instruments, Castle Hill, NSW, Australia). The muscles were first stimulated with a series of isometric twitch contractions, with muscle length adjusted between contractions to determine the muscle’s optimum length (Lo), the length at which twitch force was optimal. Muscles were stimulated at 60 Hz for either 350 ms (EDL) or 600 ms (soleus) duration. EDL and soleus muscles received 10 or 12 contractions/min, respectively, for 10 min.

Running protocol. Mice were subjected to a 3-day familiarization protocol that consisted of progressively increasing the intensity and duration of treadmill running (Eco 3/6 treadmill; Columbus Instruments, Columbus, OH) before experimental testing. All experiments were performed at 1000, and food was withdrawn from mice 4 h prior to running. To determine maximal running speed, mice ran at 10 m/min at a 5% grade for 2 min. The velocity was increased by 2 m/min every 2 min until fatigue. This was defined as spending >10 s at the base of the treadmill despite manual encouragement. Two days later, endurance capacity was assessed by running mice at 16 m/min at a 5% grade until they reached fatigue. Based on these results, the experimental trial consisted of running at 16 m/min (5% grade) for 30 min in an enclosed treadmill (Columbus Instruments) attached to an Oxymax open-circuit calorimeter (Columbus Instruments). Respired gases were evaluated continuously and oxygen uptake (V˙O2) and carbon dioxide production (V˙CO2) were calculated on-line. RER was calculated as V˙CO2/V˙O2. At the conclusion of the experiment, mice were rapidly removed from the enclosed treadmill and euthanized by cervical dislocation. Mice were decapitated, and trunk blood was collected. Tissues were rapidly dissected and frozen in liquid nitrogen.

Western blotting. Muscle and adipose tissue were lysed, and protein concentration was determined (Pierce Kit no. 23225; Quantum Scientific, QLD, Australia). Lysates were solubilized, resolved by SDS-PAGE on polyacrylamide gels, transferred to membranes, and blocked with 5% milk. Immunoblotting was performed using the following primary antibodies: anti-actin (Sigma-Aldrich, St. Louis, MO), anti-Perilipin A (Sigma), anti-HSL (AbNova), anti-ATGL (36), and anti-CGI-58 (comparative gene identification 58; AbNova). After incubation with protein G-HRP secondary antibody (Bio-Rad, Hercules, CA), the immunoreactive proteins were detected with enhanced chemiluminescence and quantified by densitometry (Image J, NIH).

qRT-PCR. RNA was extracted from mixed gastrocnemius muscle and reverse transcribed, and quantitative real-time PCR was performed as described previously (36). Primers for carnitine palmitoyltransferase Ib (Cpt1b), uncoupling protein-2 (Ucp2), acyl-Coenzyme A dehydrogenase (Acadm), and cytochrome c oxidase subunit II (COX2) were purchased from Applied Biosystems.

Tissue metabolite analysis. Muscle and liver glycogen was degraded to glucosyl units by boiling in 2 M HCl that was neutralized with 0.667 M NaOH. Glucosyl units were assessed by the glucose oxidase method (Kit GAGO-20, Sigma). Muscle triacylglycerols were extracted as described previously (31), and glycerol was determined by an enzymatic colorimetric method (glycerol reagent F6428, Sigma).

Plasma metabolite analysis. Whole blood was centrifuged at 8,000 g for 5 min, and plasma was analyzed for glucose by the glucose oxidase method (Sigma) and free fatty acids (NEFA-C, Wako) and glycerol (Sigma) by enzymatic colorimetric methods. β-Hydroxybutyrate was analyzed by a spectrophotometric method (BioVision, Mountain View, CA).

Statistics. Data were analyzed by two-way analysis of variance and Tukey’s post hoc tests. All data are presented as means ± SE. Statistical significance was set a priori at P < 0.05.

RESULTS

Fatty acid metabolism in ATGL and HSL mice at rest. We first assessed fuel metabolism by indirect calorimetry in the Comprehensive Laboratory Animal Monitoring System (CLAMS). Whole body V˙O2 (Fig. 1A) and activity (not shown) were not affected in ATGL−/− mice over the course of the day. In contrast, the respiratory exchange ratio (RER) was increased.
during the light cycle in ATGL−/− mice, indicating an inability to adequately switch from carbohydrate to fat metabolism in the fasted state (Fig. 1B). The percentage of total energy generated from fatty acid oxidation over 24 h was 44.9 and 28.1 for WT and ATGL−/− mice, respectively. VO2 (Fig. 1C) and activity were unaffected in HSL−/− mice, whereas the RER was markedly elevated in HSL−/− mice (main effect, \( P = 0.001 \)), demonstrating enhanced whole body carbohydrate oxidation and reduced fat oxidation (Fig. 1D). Over the course of 24 h, the calculated percentage of energy generated from fatty acids was 71.7 and 47.8 for WT and ATGL−/− mice, respectively. The subtle differences in RER diurnal responses within the WT mice from the HSL and ATGL lines is difficult to explain given that mice were the same sex and age and were housed in the same environment. This highlights the need to use control mice from heterozygous breeding pairs when metabolic analysis is performed in genetically modified mice.

Skeletal muscle is a major site for fuel metabolism. We assessed skeletal muscle fatty acid oxidation and storage in isolated soleus muscle. The radiolabeled fatty acids used in these experiments are representative of exogenous or plasma-derived fatty acids. Fatty acid uptake was not different between WT and ATGL−/− mice (Fig. 2A). These exogenous fatty acids were preferentially oxidized in ATGL−/− muscle (Fig. 2B) rather than stored as TG (\( P = 0.08 \), Fig. 2C) or DG (Fig. 2D). This preference for oxidation most likely reflects the inability to mobilize intracellular fatty acids stored in TGs for oxidation. Indeed, overexpressing ATGL in culture myotubes enhances TG-derived fatty acid oxidation and concomitantly reduces exogenous fatty acid oxidation (36). The changes in fatty acid metabolism in ATGL−/− mice were not associated with changes in expression of β-oxidation or TCA cycle flux genes (\( CPT1b \): WT, 1.20 ± 0.32 vs. ATGL−/−, 0.72 ± 0.67; \( MCAD \): WT, 1.34 ± 0.52 vs. ATGL−/−, 0.93 ± 0.53; \( UCP2 \): WT, 1.08 ± 0.14 vs. ATGL−/−, 0.94 ± 0.19; \( COX2 \): WT, 1.00 ± 0.19 ± ATGL−/−, 2.62 ± 0.79 arbitrary units, \( n = 7 \) for each group).

In HSL−/− mice, total fatty acid uptake (Fig. 2E) and the incorporation of exogenous fatty acids into TGs (Fig. 2G) was not different from WT mice. Incorporation of fatty acid into DG was increased in HSL−/− mice (Fig. 2H). Fatty acid oxidation was increased by 20% in HSL−/− mice (Fig. 2F).

Endurance exercise training does not affect ATGL and HSL protein expression. A hallmark of endurance exercise training is an enhanced capacity for fatty acid mobilization and oxidation (10). We examined the impact of endurance exercise training on TG lipase expression in adipose tissue and skeletal muscle of C57Bl/6 mice, with the expectation that ATGL and HSL would be upregulated in accordance with enhanced fat oxidative capacity. The exercise training involved treadmill running 5 days a week for 5 wk. Both exercise duration and intensity were progressively increased such that by the final week mice were running at 16 m/min (5% incline) for 45 min. A maximal running capacity test confirmed the success of the endurance training program, with trained mice running more than twice as long as untrained mice (Fig. 3A). Surprisingly, skeletal muscle ATGL was mildly reduced with endurance training while adipose ATGL was unchanged (Fig. 3B). Protein expression of HSL and the ATGL activator protein CGI-58 were not altered by training in either muscle or adipose tissue (data not shown).

Exercise performance in ATGL and HSL mice. We next tested the maximal running capacity (equivalent to a \( V_{\text{O}2\max} \) test in humans) and the endurance capacity of ATGL−/−, HSL−/− and WT mice. Maximal running capacity was compromised in the ATGL−/− mice (Fig. 3C). Only the ATGL−/− mice capable of running at >18 m/min during the maximal speed test (673 ± 82 s, \( n = 5 \)) were selected for the endurance running capacity test. Endurance capacity was reduced by 46% in ATGL−/− mice but was not compromised in ATGL+/− mice (Fig. 3D).
Fatty acid metabolism was examined, and fatty acid uptake (Fig. 2. Skeletal muscle fatty acid metabolism in ATGL \( \text{E}^{508} \) ATGL DELETION, ENERGY METABOLISM, AND EXERCISE PERFORMANCE). Lipolysis at rest and during exercise in ATGL\(^{-/-}\) and HSL\(^{-/-}\) mice. Fatty acids are released into the circulation from adipose tissue during lipolysis to provide substrate for the working muscle. To test whether ATGL or HSL ablation reduce lipolysis and thereby fatty acid availability, we assessed plasma glycerol and FFA before and after exercise. Western blot confirmed that the key regulators of adipose tissue lipolysis, HSL, perilipin A, and CGI-58, were normally expressed in ATGL\(^{-/-}\) mice (Fig. 4A). Plasma FFA was decreased in ATGL\(^{-/-}\) mice at rest and during exercise compared with WT (main effect, \( P = 0.0008 \); Fig. 4B). Plasma glycerol was not reduced in ATGL\(^{-/-}\) mice (Fig. 4C). The exercise-induced increases above preexercise for plasma FFA and glycerol were 42 and 31% in WT mice, whereas no increases were observed in ATGL\(^{-/-}\) mice (Fig. 4, B and C). The inability of ATGL\(^{-/-}\) mice to increase lipolysis was also observed at lower exercise intensities (60 min at 10 and 14 m/min; data not shown). Plasma \( \beta \)-hydroxybutyrate was not affected by genotype or exercise (Fig. 4D).

HSL deletion did not affect ATGL or CGI-58 protein levels but reduced perilipin A protein expression (WT: 1.00 ± 0.23 vs. HSL\(^{-/-}\): 0.40 ± 0.07; Fig. 4E). Both plasma FFA and glycerol were decreased in HSL\(^{-/-}\) mice at rest and during exercise compared with WT (main effect, \( P = 0.03 \) and \( P = 0.046 \), respectively). Plasma glycerol was increased during exercise in both groups (Fig. 4G). The exercise-induced increases for plasma glycerol were 90 and 24% for WT and HSL\(^{-/-}\) mice, respectively. Plasma FFA tended to increase during exercise in WT but not in HSL\(^{-/-}\) mice (Fig. 4F). The increase in plasma glycerol during exercise, although substantially lower in HSL\(^{-/-}\) mice, indicates that non-HSL lipases can facilitate additional lipolysis during exercise. Plasma \( \beta \)-hydroxybutyrate tended to be decreased in HSL\(^{-/-}\) mice, but this did not reach statistical significance (\( P = 0.14 \)).

Whole body fuel metabolism and tissue metabolite levels before after exercise. It is well known that decreasing plasma FFA availability increases the reliance on carbohydrate as a metabolic substrate (33). We next assessed whole body substrate metabolism by indirect calorimetry during exercise and measured tissue metabolite contents to ascertain whether ATGL or HSL deletion affects the reliance on specific metabolic substrates. \( \dot{V}O_2 \) was increased during exercise in ATGL\(^{-/-}\) and WT mice and was not different between genotypes (data not shown). The ATGL\(^{-/-}\) mice exhibited higher rates of carbohydrate oxidation at 20 and 30 min of the exercise bout (Fig. 5A). A striking phenotype of the ATGL\(^{-/-}\) mice was the reduction in skeletal muscle and liver glycogen storage (Fig. 5, B and C). Despite the lower preexercise contents, the difference between pre- and post-glycogen content for muscle and liver glycogen utilization were further reduced during exercise in ATGL\(^{-/-}\) mice. Plasma glucose was reduced in ATGL mice after exercise (Pre: 6.7 ± 0.4 mM, Post: 5.2 ± 0.2 mM, \( n = 6 \)) but not in WT mice (Pre: 7.1 ± 0.3 mM, Post: 6.6 ± 0.5 mM, \( n = 5 \)). As expected (5), muscle TG was increased in ATGL\(^{-/-}\) mice, and there was no degradation of muscle TG in either WT or ATGL\(^{-/-}\) mice after exercise (Fig. 5D). Collectively, these data indicate that the reduction in plasma FFA availability is driving greater carbohydrate oxidation during.

\[ \text{Fig. 2. Skeletal muscle fatty acid metabolism in ATGL}\(^{-/-}\) and HSL\(^{-/-}\) mice. Fatty acid metabolism was examined, and fatty acid uptake (A and E) and oxidation (B and F) and triacylglycerol (TG) (C and G), and diglyceride (DG) esterification (D and H) were assessed in isolated soleus muscles from ATGL\(^{-/-}\) and HSL\(^{-/-}\) mice and WT littermates. \( *P < 0.05 \) vs. WT. ATGL\(^{-/-}\), \( n = 6 \); WT, \( n = 6 \). HSL\(^{-/-}\), \( n = 10 \); WT, \( n = 10 \). \]
exercise despite the fact that endogenous carbohydrate storage is compromised in ATGL−/− mice.

\( \dot{V}O_2 \) was increased during exercise in HSL−/− and WT mice and was not different between genotypes (data not shown). The RER was increased in HSL−/− compared with WT mouse throughout exercise (Fig. 5E) and was due to enhanced muscle and liver glycogen utilization (Fig. 5, Post: 6.4 ± 0.5 mM, n = 4) or HSL−/− mice (Pre: 7.3 ± 0.2 mM, Post: 6.8 ± 0.2 mM, n = 6). Intramuscular TG were not different between genotypes at rest and were actually increased in both HSL−/− and WT mice after exercise (Fig. 5H).

Muscle bioenergetics are not compromised in ATGL−/− and HSL−/− mice. The ATGL−/− mice were originally described to have severe cardiac TG deposition, cardiac dysfunction, and premature death (5). We confirmed the marked cardiac TG deposition in ATGL−/− mice aged 10 ± 1 wk (WT: 20 ± 2, ATGL−/−: 17 ± 4, ATGL−/−: 81 ± 15 μmol/g). Subtle changes in substrate and/or hormone delivery and blood flow, which could result from impaired cardiac function, can influence muscle metabolism and exercise performance. Accordingly, we performed two experiments. We first assessed exercise endurance in young mice (6 wk) that did not display cardiac lipid deposition. When running at 12 m/min, ATGL−/− mice exhibited reduced endurance capacity compared with WT littermates (WT: 4,060 ± 562 vs. ATGL−/−: 2,220 ± 272 sec, P < 0.05). These data indicate that defective cardiac performance does not underpin the reduced exercise capacity in ATGL−/− mice. However, the heart relies on local TG stores as a fuel source, and we cannot discount the possibility that cardiac performance during exercise might be somewhat compromised in the young animals by the inability to draw on local lipids.

Next, we isolated EDL (predominantly type II fiber) and soleus (type I fiber) muscles from ATGL−/− mice and examined muscle energetics by contracting muscles in an organ bath. The initial force produced by ATGL−/− muscles did not differ from that of WT mice, and the rate of fatigue in both EDL and soleus muscles was identical between ATGL−/− mice and their littermate controls (Fig. 6). These data indicate that muscle function was not compromised in skeletal muscles of ATGL−/− mice during submaximal contractions.

DISCUSSION

TG lipases are important proteins that regulate lipid metabolism and thereby whole body energy homeostasis (34). The two predominant TG lipases are ATGL and HSL, and together these proteins account for ~95% of TG hydrolase activity in adipose tissue (22). While HSL is relatively well studied, the role and regulation of ATGL remains largely unresolved. ATGL belongs to the patatin-like phospholipase family, specifically hydrolyzes TG, and is predominantly expressed in adipose tissue, with less, yet significant, expression in skeletal and cardiac muscle (11, 39). ATGL’s association with the protein CGI-58 enhances its lipolytic activity (13), while PKA-mediated phosphorylation of the lipid-droplet associated protein perilipin A at Ser517 regulates ATGL-dependent lipolysis in adipocytes (15). The precise nature of these interactions remains to be elucidated. Functional experiments show that inhibition of ATGL reduces TG breakdown in cell culture (11, 26, 39) and mice (5) and that ATGL overexpression increases TG breakdown and alters lipid metabolism (36). To further explore the metabolic role of TG lipases, we examined substrate metabolism in ATGL−/− and HSL−/− mice at rest and during exercise.

Lipopysis at rest and during exercise. For many years, HSL was thought to be the rate-limiting enzyme for adipose tissue lipolysis both at rest and during exercise (9). Here, we show that fasting plasma FFA levels are reduced in ATGL−/− mice,
thereby demonstrating that ATGL is important for efficient basal lipolysis in vivo (5) and that HSL or other TG lipases cannot compensate for ATGL deficiency. Interestingly, plasma FFA are mildly reduced in fasted HSL/−/− mice, demonstrating that HSL also contributes to basal lipolysis, which is consistent with its activity against both TG and DG substrates. This premise is consistent with previous studies demonstrating suppressed basal lipolysis in isolated human adipocytes (12) and residual TG hydrolase activity in ATGL/−/− adipose lysates (23) treated with a pharmacological HSL inhibitor. We also show that both ATGL and HSL play an important lipolytic role during exercise. This conclusion is based on the observation that the normal exercise-induced increase in plasma FFA and glycerol was severely attenuated during exercise in both ATGL/−/− and HSL/−/− mice. The small increase in plasma FFA during running in HSL/−/− mice is consistent with the notion that ATGL-mediated lipolysis is subject to acute regulation. Regulation by activating protein kinases is a possibility given the heavy phosphorylation of ATGL reported in mammalian cells (39), and this warrants further examination. Alternatively, there are 23 adipocyte proteins that exhibit TG lipase activity in vitro (1), suggesting that TG lipases other than ATGL can induce small changes in lipolysis. Finally, the marked blunting of complete lipolysis (i.e., glycerol release) in HSL/−/− mice during exercise supports a primary role of HSL as a DG lipase (6). Collectively, these results demonstrate a critical role for ATGL in basal and exercise-stimulated lipolysis, whereas HSL appears to play a permissive role for basal lipolysis but is essential for complete lipolysis during exercise.
Energy metabolism at rest. We used whole body indirect calorimetry and isolated skeletal muscle preparations to reveal novel insights into the role of TG lipases in resting energy metabolism. ATGL deletion impaired the ability of mice to adequately switch from carbohydrate to fatty acid metabolism during fasting, which is consistent with a previous report in ATGL$^{-/-}$ mice (5). Thus, ATGL is essential for the maintenance of basal lipolysis and thereby FFA delivery to key energy-consuming tissues, such as skeletal muscle. The small increase in carbohydrate oxidation in ATGL$^{-/-}$ mice at rest was associated with reduced glycogen storage in skeletal muscle and liver. It is possible that ATGL$^{-/-}$ mice possess a primary defect in carbohydrate synthesis enzymes, because their nonstimulated glucose uptake is actually enhanced (5). Indeed, HSL$^{-/-}$ mice oxidized more carbohydrate yet maintained normal glycogen stores, indicating a genotype-specific defect in ATGL$^{-/-}$ mice. Our studies in noncontracting isolated muscles revealed that fat oxidative capacity is not limiting in ATGL$^{-/-}$ mice under resting conditions. In these experiments, exogenous FFA availability was maintained at 500 μM, thereby circumventing the effects of substrate availability on substrate competition (25, 27). In these experiments, the skeletal muscle from ATGL$^{-/-}$ mice oxidized more exogenous fatty acids compared with muscle from control littermates. The increased reliance on exogenous fatty acids by ATGL$^{-/-}$ muscle most likely reflects an inability to generate ATP from intramyocellular TG-derived fatty acids. ATGL$^{-/-}$ mice express more FAT-CD36, the rate-limiting enzyme for sarcolemmal fatty acid transport, in muscle (WT: 1.0 ± 0.1 vs. ATGL$^{-/-}$: 1.9 ± 0.2 arbitrary units, P < 0.02) and take up 28% more fatty acid (P = 0.15) suggesting that an adaptation to long-term lipolytic suppression is an enhanced capacity to transport FFA into metabolically active tissues. Thus, when the whole body and isolated muscle experiments are considered together, the primary metabolic defect of ATGL$^{-/-}$ mice is defective lipolysis that in turn drives carbohydrate metabolism to maintain metabolic homeostasis.

Energy metabolism during exercise. At the onset of exercise, hormonal and intracellular signals activate key metabolic steps to increase the provision and metabolism of carbohydrate and fatty acids to meet the increased demand for energy. Our studies reveal that both ATGL and HSL deletion result in increased muscle and liver glycogen depletion and carbohydrate oxidation during exercise. Previous maneuvers designed to limit TG lipolysis in adipose tissue and other ectopic TG stores, such as nicotinic acid administration (29, 33), have reported reduced fatty acid oxidation and concomitant increases in whole body carbohydrate metabolism during exercise. Previous maneuvers designed to limit TG lipolysis in adipose tissue and other ectopic TG stores, such as nicotinic acid administration (29, 33), have reported reduced fatty acid oxidation and concomitant increases in whole body carbohydrate metabolism during exercise. Our findings extend on previous work and provide a new understanding of the role of TG lipases in energy metabolism during exercise.

**Fig. 5.** Whole body substrate metabolism and tissue metabolite levels in ATGL$^{-/-}$ and HSL$^{-/-}$ mice at rest and after exercise. A and E: RER before and during exercise. *P < 0.05 vs. WT at the same time point. Muscle glycogen (B and F), liver glycogen (C and G), and intramuscular TG (IMTG; D and H) were assessed in one cohort of mice at rest and in a second cohort immediately after 30-min exercise. **P < 0.05, main effect genotype. #P < 0.05, main effect for time. ATGL$^{-/-}$, n = 8; WT, n = 8. HSL$^{-/-}$, n = 8; WT, n = 8.

**Fig. 6.** Muscle force production in ATGL$^{-/-}$ mice during isolated contractions. EDL and soleus muscles were surgically excised from anesthetized mice and contracted for 10 min. Fatigue curves are shown for EDL (top) and soleus (bottom) muscles from ATGL$^{-/-}$ mice and their WT littermates.
mechanistic basis for the reciprocal regulation of carbohydrate and fat metabolism during exercise; that is, dysregulation of either ATGL or HSL can limit fatty acid availability and promote carbohydrate oxidation. Interestingly, glycogen depletion was similar in ATGL−/− and WT mice despite lower preexercise glycogen levels, which is unusual, as preexercise muscle glycogen availability is positively related to glycojenolysis during an exercise bout (8). The absence of normal glucoregulation further demonstrates the energy stress induced by reduced fatty acid availability in ATGL−/− mice.

TG lipases and exercise performance. Exercise performance was compromised in ATGL−/− mice but was unaffected in HSL−/− mice. The reduction in high-intensity exercise tolerance and endurance capacity at a moderate exercise intensity is consistent with human studies showing that homzygous mutations in PNPLA2 (ATGL gene) that produces a truncated ATGL protein confers susceptibility to exercise intolerance and early fatigue (2). The diminished exercise performance in older ATGL−/− mice was most likely related to cardiac lipid deposition, aberrant cardiac function, and impaired blood flow (5). However, further experiments in young mice that did not display cardiac lipid accumulation revealed that endurance treadmill running was compromised with ATGL deletion, suggesting that potential cardiovascular responses do not fully explain reduced exercise tolerance. Intrinsic defects in muscle metabolism are unlikely to explain the impaired exercise capacity because contractile function was normal in isolated EDL and soleus muscles from ATGL−/− mice. Thus, these data indicate that small reductions in fatty acid availability and lower preexercise carbohydrate stores impair endurance exercise performance in ATGL−/− mice, although other factors are likely to be involved given the dramatic impairment in exercise tolerance. The finding that maximal exercise capacity and endurance performance were not compromised in HSL−/− mice contrasts with a recent study showing impaired exercise performance in HSL−/− mice (3). The reasons for these differences may be related to the genetic background of the mice (C57Bl/6 vs. mixed background) or the greater exercise intensity employed in the previous study. In that study, mice were run at a steeper grade (5 vs. 20°) and greater velocity (16 vs. 18 m/min) that resulted in complete liver glycogen depletion (3). Although liver and muscle glycogen was not measured at fatigue in our study, the rate of glycogen depletion recorded in the 30-min trial and maintenance of carbohydrate metabolism suggest that factors other than muscle glycogen depletion are mediating fatigue with our protocol. The reduction of blood glucose in ATGL−/− mice, and not HSL−/− mice, implies that exercise fatigue may be centrally mediated. Thus, we concluded that, although HSL−/− mice have reduced fatty acid availability, neither contractile function nor exercise performance is compromised. Finally, it is possible that partial lipolysis of adipose and muscle TG in HSL−/− mice is sufficient to sustain exercise in the HSL−/− mice and better maintain muscle glycogen stores compared with ATGL−/− mice.

In summary, ATGL plays a critical role in energy metabolism in mice. We have shown that defects in TG metabolism mediated by ATGL deletion severely disrupts whole body substrate partitioning at rest, specifically resulting in reduced lipolysis and fatty acid availability, which in turn enhances carbohydrate oxidation during fasting and is associated with depleted muscle and liver glycogen storage and reduced blood glucose. We also show that both ATGL and HSL exert an important lipolytic role during exercise. In light of the genetic associations of ATGL with free fatty acids, TG and type 2 diabetes (21), and fatigue susceptibility (2), future studies examining the relevance of ATGL for human metabolism are warranted.

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