Adipose triglyceride lipase deletion from adipocytes, but not skeletal myocytes, impairs acute exercise performance in mice

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PHYSICAL ACTIVITY REQUIRES A CONSTANT SUPPLY of energy. The source and type of energy substrates are influenced not only by their availability but also by the intensity and duration of physical activity. In humans, at low exercise intensities (≤30% \( V_\text{O}_2\text{max} \)), carbohydrates account for only 10–30% of total energy production and are derived primarily from circulating glucose (50). At higher exercise intensities (85–100% \( V_\text{O}_2\text{max} \)), carbohydrates become the predominant energy substrate (50) and are derived primarily from skeletal muscle glycogen (26). In contrast, during more prolonged low- to moderate-intensity exercise, fatty acids (FAs) become the primary energy substrate and are derived from intracellular triacylglycerol (TAG) stores within adipocytes (reviewed in Ref. 30) and/or skeletal muscle (60). Although up to 70% of these FAs are derived from adipocyte lipolysis (35, 65), intramyocellular triacylglycerols (IMTGs) can decrease more than 50% in response to moderate intensity exercise (12, 65), suggesting that IMTGs also play an important role in exercising muscle. Since alterations in extra- and/or intramyocellular lipid metabolism occur frequently in both normal physiology (i.e., fasting/feeding, physical activity) and disease (i.e., diabetes, insulin resistance), understanding how FA mobilization from adipose tissue and skeletal muscle influences muscle metabolism and function is of considerable biomedical relevance.

Adipose triglyceride lipase (ATGL) is the rate-limiting enzyme mediating TAG hydrolysis (69) and a major contributor to lipolysis in both adipose tissue (19, 62, 66) and skeletal muscle (54). Indeed, humans (20, 53) and mice (24) with ATGL deficiency exhibit dramatic accumulation of IMTGs in cardiac and skeletal muscle despite markedly reduced adipocyte lipolysis and serum lipids, indicating a critical role for ATGL action in muscle. These effects contribute to early morbidity and mortality in part because of lipotrophic cardiomyopathy. However, although impaired ATGL action within cardiomyocytes clearly contributes to cardiac muscle dysfunction (25, 33), the tissue-specific contribution of ATGL action to skeletal muscle dysfunction remains poorly understood. In mice, global ATGL deletion impairs exercise performance (31, 52). This impairment persists even after reexpressing ATGL in cardiac muscle (52), indicating that skeletal muscle dysfunction cannot be explained entirely by cardiac dysfunction. These data implicate either adipose tissue and/or skeletal muscle ATGL action in exercise performance. Previously, we reported that skeletal muscle-specific targeted deletion or overexpression of ATGL dramatically alters IMTG content but is not sufficient to alter metabolic phenotypes at baseline or in response to nutritional stress (i.e., diet-induced obesity) (54). Whether alterations in ATGL action in skeletal myocytes vs. adipocytes influence acute exercise performance remains unknown.

The primary goal of this study was to determine the relative contribution of adipocyte vs. skeletal myocyte ATGL-mediated TAG hydrolysis to acute exercise performance in mice. To achieve this goal, we generated murine models with adipocyte- and skeletal myocyte-specific targeted deletion of ATGL. We then subjected these mice to peak and submaximal endurance exercise interventions and assessed their exercise performance and energy substrate...
metabolism. We found that ATGL deletion from adipocytes, but not skeletal myocytes, alters energy substrate metabolism and impairs acute exercise performance in mice.

MATERIALS AND METHODS

Animals. B6.129-Pnpla2<sup>tm1/leek</sup> (Atgl<sup>-</sup>flox) mice were generated as described (54). ATGL-flox mice were crossed to either MyoCre (40) or AdipoQCre (18) mice to generate skeletal myocyte-specific (SMAKO) (54) or adipocyte-specific (AAKO) ATGL knockout mice. For both models, male Atgl<sup>fl</sup>flox/Cre/+ mice were mated to female Atgl<sup>fl</sup>flox/+/+ mice to generate male Atgl<sup>fl</sup>flox/Cre/+ (SMAKO or AAKO) and Atgl<sup>fl</sup>flox/+/+ (control) experimental mice. All mice were congenic (n > 10) on C57BL/6NTac. Animal experiments were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and conducted in conformity with Public Health Service Policy for Care and Use of Laboratory Animals.

Peak exercise protocol. At 12 wk of age, mice were subjected to a 3-day acclimation protocol with progressively increased intensity and duration of treadmill exposure (RM Exer-3/6 Open Treadmill with Manual Incline; Columbus Instruments). On day 1 of the acclimation protocol, mice were fasted for 4 h, and blood was collected through the tail vein for assessment of baseline circulating substrates. For the peak test, mice were run on an enclosed single-lane treadmill (Modular Enclosed Metabolic Treadmill for Mice; Columbus Instruments) attached to an Oxymax/Comprehensive Laboratory Animal Monitoring System (CLAMS; Columbus Instruments) that allowed for real-time measurements of oxygen consumption (V<sub>O</sub>2) and carbon dioxide production (V<sub>CO</sub>2). Mice were fasted for 4 h, with testing being completed between 10 AM and 1 PM. Mice were placed in the treadmill and allowed a 2-min acclimation period prior to the initiation of the peak test. The running protocol was as follows: an initial warmup run was conducted at 6 m/min for 5 min followed by 10 m/min for 2 min. Thereafter, speed was subsequently increased by 1 m/min every minute until fatigue, which was defined as the inability to return to treadmill running after 10 s. Once fatigue was reached, the treadmill was stopped, and respiratory values were collected for 1 min. Mice were then removed from the treadmill, and blood was collected via tail vein for analysis.

Submaximal endurance exercise challenge protocols. For the initial (nonterminal) submaximal endurance exercise challenge, mice were rested for 7 days following the peak exercise challenge and then subjected to a submaximal endurance exercise challenge at 13 wk of age. Tests were conducted between 10 AM and 3 PM following a ~3-h fast. The submaximal running velocity was calculated as 55% of the peak running speed obtained in the control mice, an intensity shown to maximize substrate usage, specifically intramyocellular lipids (58). Thus, all mice ran at the same absolute exercise intensity during the submaximal challenge. As with the peak test, mice were run until fatigue, at which point the treadmill was stopped, respiratory measurements were collected for 1 min, and blood was collected via the tail vein. A second (terminal) submaximal endurance exercise challenge was performed in 20-wk-old mice to evaluate for potential differences in tissue substrates, mitochondria, and/or gene/protein expression/phosphorylation. Mice were reacclimated to the treadmill and run for 45 min (representing approximately one-half the total endurance time of the control mice) rather than to fatigue. Following the experiment, blood glucose was determined, and mice were immediately euthanized by CO<sub>2</sub> inhalation and blood/tissue collected for analysis (17).

Energy expenditure and metabolic measurements. V<sub>O</sub>2, V<sub>CO</sub>2, and respiratory exchange ratio (RER) were determined using the CLAMS. Respiratory data from the final 30 s of peak and submaximal exercise performance were used in calculations. Respiratory data were normalized to body weight raised to the power of 0.75 (56). Rates of carbohydrate (c) and fat (f) oxidation were calculated using the equations of Frayn: c = (4.55 × V<sub>O</sub>2) − (3.21 × V<sub>CO</sub>2) and f = (1.67 × V<sub>O</sub>2) − (1.67 × V<sub>CO</sub>2) (protein oxidation ignored due to minimal role during exercise) (21). Results of all respiratory measurements and associated calculations were unchanged following adjustment for fat or lean mass. Whole blood glucose was determined using a One-touch Fast-Take glucometer (LifeScan). Serum TAGs (Infinity Triglycerides Liquid Stable Reagent; Thermo Scientific) and nonesterified fatty acids [NEFAs; HR Series NEFA-HR(2) Reagents, Wako Diagnostics] were determined by colorimetric assay as described (54).

Tissue and mitochondrial analyses. Muscle glycogen and TAG were determined in liquid nitrogen-crushed quadriceps samples using a commercially available kit (Glycogen Assay Kit; Cayman Chemical) and Infinity Triglycerides in Liquid Stable Reagent (9). For tissue imaging, muscle samples were cut into 10-μm sections and stained for neutral lipids (54) or glycogen (27). Mitochondrial respiration was assessed in permeabilized muscle fibers in the basal state, as described previously (54). Protein expression in skeletal muscle was determined by Western blot analysis using the following antibodies as described (54): MitoProfile antibody cocktail (MS604; Mitosciences), anti-hormone-sensitive lipase (HSL) (4017s; Cell Signaling Technology), and anti-phosphorylated (p)-HSL (Ser565 and Ser660) (4137S and 4126S; Cell Signaling Technology).

Statistical analysis. Data are expressed as means ± SE and analyzed using the Statistical Package for the Social Sciences for MAC version 22. Baseline between-group comparisons were determined by one-way analysis of variance (ANOVA). No differences were observed between control littermates for AAKO or SMAKO mice, so data were combined into a single control group for subsequent analyses. Repeated-measures ANOVA was used to evaluate pre- and postexercise test outcome variables and to assess between-group interactions. Univariate analysis addressed the question of genotype and activity (sedentary vs. exercise) for specific outcome variables. When an interaction was identified, a one-way ANOVA on the relative percent change was used to determine group differences. Data were log transformed and reanalyzed if the assumption of homogeneity of variance was invalid. Presentation of the data is nontransformed. Statistical significance was assumed at P ≤ 0.05.

RESULTS

Peak exercise capacity and serum substrate kinetics are impaired by inhibition of adipocyte but not intramyocellular ATGL-mediated lipolysis. In humans (10) and rodents (6), peak exercise performance is highly dependent on circulating energy substrates. Previous studies have reported that adipocyte- (2, 66) but not skeletal myocyte-specific (54) deletion of ATGL in mice reduces circulating glucose and FA substrates in the basal (nonexercised) state. To determine the relative impact of adipocyte vs. skeletal myocyte ATGL action on energy substrates in response to peak exercise, we first assessed these parameters in age- and weight-matched (26.27 ± 0.22 g) control, SMAKO, and AAKO mice subjected to a peak exercise challenge. Peak running speeds were similar in control (28.98 ± 0.89 m/min) and SMAKO (31.68 ± 0.97 m/min) mice but lower in AAKO mice (22.04 ± 1.05 m/min, P < 0.01) (Fig. 1A). Likewise, dropout rates during a peak exercise challenge were similar for control and SMAKO mice but greater for AAKO mice (Fig. 1B). Baseline circulating energy substrates, including serum glucose, TAGs, and NEFAs, were comparable between control and SMAKO mice but lower in AAKO mice (Table 1). In response to a peak exercise challenge, control and SMAKO mice demonstrated similar changes in both circulating glucose and TAGs, i.e., increased serum glucose and reduced serum TAGs. In contrast, AAKO mice demonstrated the opposite response, i.e., decreased serum glucose and increased serum TAGs. Serum NEFAs tended to respond differ-
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Fig. 1. Peak exercise performance. A: peak exercise speed. B: dropout rates during peak exercise. Interval, 1–10 s. For each group/variable (i.e. all data), male mice were used (12 wk, fasted 4 h; n = 26 control, n = 14 skeletal myocyte-specific adipose triglyceride lipase (ATGL) knockout (SMAKO) mice, and n = 15 adipocyte-specific ATGL knockout (AAKO) mice). *P ≤ 0.05 for group difference; noncorresponding letters are different.

Table 1. Substrate kinetics before and after a peak exercise challenge in untrained mice

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SMAKO</th>
<th>AAKO</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Peak</td>
<td>Basal</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>26.17 ± 0.30</td>
<td>26.21 ± 0.58</td>
<td>27.42 ± 0.22</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>173.27 ± 5.66</td>
<td>246.26 ± 9.37</td>
<td>181.29 ± 5.93</td>
</tr>
<tr>
<td>TAG, μg/μl</td>
<td>0.72 ± 0.05</td>
<td>0.50 ± 0.03</td>
<td>0.86 ± 0.09</td>
</tr>
<tr>
<td>NEFA, mEq/l</td>
<td>0.45 ± 0.04</td>
<td>0.54 ± 0.06</td>
<td>0.56 ± 0.08</td>
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Data are means ± SE; 12 wk, fasted 4 h; n = 26 control, 14 skeletal muscle adipose triglyceride lipase (ATGL) knockout (SMAKO) mice, and 15 adipocyte-specific ATGL knockout (AAKO) mice. TAG, triacylglycerol; NEFA, nonesterified fatty acids. *P ≤ 0.05 for exercise effect. **P ≤ 0.05 for baseline group effect; noncorresponding letters are different. *P ≤ 0.05 for group difference; noncorresponding letters are different.
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Fig. 2. Whole body gas exchange and respiratory exchange ratio (RER) during peak exercise. A and B: whole body oxygen consumption (\(\dot{V}O_2\); A) and carbon dioxide production (\(\dot{V}CO_2\); B) normalized to body weight (kg) raised to the 0.75 power at baseline (black bars) and at peak exercise (open bars) by indirect calorimetry. C: RER (\(\dot{V}CO_2/\dot{V}O_2\)) at baseline and 25, 50, 75, and 100% of peak exercise run duration. For each group/variable (i.e. all data), male mice were used (12 wk, fasted 4 h; n = 26 control, 14 SMAKO, and 15 AAKO). \(^{*}P < 0.05\) for baseline group difference; noncorresponding letters are different. \(^{a+c}P < 0.05\) for peak exercise group difference; noncorresponding letters are different. \(^{a}P < 0.05\), effect of exercise; \(^{G}P < 0.05\) for effect of genotype, AAKO > control and SMAKO; \(^{T}P < 0.05\) for effect of time (i.e., baseline compared with peak), all groups.

magnitude of this decrease was much lower in AAKO (-0.45 ± 0.61 mg·kg\(^{-0.75}\)·min\(^{-1}\), absolute) compared with control (-2.87 ± 0.65 mg·kg\(^{-0.75}\)·min\(^{-1}\), absolute) and SMAKO (-4.08 ± 1.11 mg·kg\(^{-0.75}\)·min\(^{-1}\), absolute) mice (Fig. 3D). Likewise, the percent change in fat oxidation relative to baseline decreased for all genotype groups, with the greatest reduction in SNAKO mice and the smallest reduction in AAKO mice (Fig. 3F). Thus, inhibition of ATGL action in adipocytes, but not skeletal myocytes, reduces the ability to adjust energy substrate oxidation in response to peak exercise.

Submaximal endurance exercise capacity is impaired by inhibition of adipocyte but not intramyocellular ATGL-mediated lipolysis. In contrast to peak exercise, energy production during submaximal endurance exercise generally relies more heavily on fat oxidation (50), with substrates derived from both adipocyte (65) and skeletal muscle TAG stores (58). To assess the relative impact of adipocyte vs. skeletal myocyte ATGL action on energy substrates in response to submaximal endurance exercise, we next assessed these parameters in age- and weight-matched (27.04 ± 0.19 g) control, SMAKO, and AAKO mice subjected to a submaximal endurance exercise challenge (i.e., mice run ~55% of control group peak run speed until exhaustion). Time to fatigue was similar in control (6,289.61 ± 483.48 s) and SMAKO (7,528.56 ± 660.10 s) mice but lower in AAKO mice (2,558.79 ± 562.59 s, \(P < 0.01\)) (Fig. 4A). Likewise, dropout rates during the submaximal endurance exercise challenge were similar for control and SMAKO mice but greater for AAKO mice (Fig. 4B). Baseline circulating energy substrates, including serum glucose, TAGs, and NEFAs, were comparable between control and SMAKO mice but lower in AAKO mice (Table 2). In response to the submaximal endurance exercise challenge, an interaction effect was observed for circulating energy substrates. Specifically, serum TAGs decreased in control and SMAKO mice but not AAKO mice. Serum NEFAs increased in all genotypes, but the magnitude of this increase was lowest in AAKO mice (Table 2). Conversely, serum glucose decreased in all genotypes, and the magnitude of this decrease was greatest in AAKO mice. These data suggest that reduced circulating substrate availability in response to impaired ATGL action in adipocytes, but not myocytes, limits submaximal endurance exercise performance.

Sustained aerobic capacity and metabolic flexibility in response to submaximal endurance exercise are impaired by inhibition of adipocyte but not intramyocellular ATGL-mediated lipolysis. To determine the effects of adipocyte vs. skeletal myocyte ATGL action on sustained aerobic capacity and substrate oxidation, we measured whole body gas exchange during submaximal endurance exercise to exhaustion. At baseline, \(O_2\) consumption (Fig. 5A) and \(CO_2\) production (Fig. 5B) were similar between control and SMAKO mice but lower in AAKO mice. In response to a submaximal endurance exercise challenge, neither \(O_2\) consumption nor \(CO_2\) production changed significantly in any of the genotype groups. In contrast, \(RER\) decreased in all groups, consistent with a switch to FAs as the preferred energy substrate for oxidation. At baseline, \(RER\) was higher in AAKO mice compared with control and SMAKO mice and remained higher at fatigue (Fig. 5C), suggesting a sustained preference for carbohydrate over FA oxidation. In contrast, \(RER\) was comparable in control and SMAKO mice both at baseline and in response to submaximal endurance exercise. Thus, in contrast to inhibition of ATGL action in adipocytes, inhibition of ATGL action in skeletal myocytes is not sufficient to impair sustained aerobic capacity or the ability to enhance FA oxidation in response to submaximal endurance exercise.

Substrate kinetics with submaximal endurance exercise is impaired by inhibition of adipocyte but not intramyocellular ATGL-mediated lipolysis. To more specifically characterize energy substrate use during submaximal endurance exercise,
we next determined the rates of carbohydrate and FA oxidation by respirometry at baseline and in response to a submaximal endurance exercise challenge. For carbohydrate oxidation (Fig. 6, A–C), the rates of carbohydrate oxidation were similar between control and SMAKO mice baseline (Fig. 6, A and B) but higher in AAKO mice when expressed as percent carbohydrate to total oxidation (Fig. 6B). In response to a submaximal endurance exercise challenge, carbohydrate oxidation (both absolute and %total) decreased in all genotype groups (Fig. 6, A and B), but the magnitude of this decrease was much smaller in AAKO (2.85 ± 2.74 mg·kg⁻⁰·⁷⁵·min⁻¹) compared with control (7.98 ± 1.64 mg·kg⁻⁰·⁷⁵·min⁻¹) and SMAKO (12.04 ± 2.62 mg·kg⁻⁰·⁷⁵·min⁻¹) mice (Fig. 6A). Furthermore, the percent change in carbohydrate oxidation relative to baseline tended (P = 0.06 for interaction effect) to be greater for control and SMAKO mice compared with AAKO mice (Fig. 6C). For FA oxidation (Fig. 6, D–F), both absolute rates of FA oxidation (Fig. 6D) as well as percent FA to total oxidation (Fig. 6E) were similar between control and SMAKO mice but lower in AAKO mice at baseline. In response to a submaximal endurance exercise challenge, FA oxidation (both absolute and %total) increased for all genotype groups (Fig. 6, D and E). Although the percent changes in FA oxidation relative to baseline were similar between groups (Fig. 6F), the absolute magnitude of this decrease was lower in AAKO (1.51 ± 0.77 mg·kg⁻⁰·⁷⁵·min⁻¹) compared with control (3.23 ± 0.52 mg·kg⁻⁰·⁷⁵·min⁻¹) and SMAKO (4.90 ± 0.84 mg·kg⁻⁰·⁷⁵·min⁻¹) mice (Fig. 6A). Thus, inhibition of ATGL-mediated lipolysis in adipocytes, but not skeletal myocytes, reduces the ability to adjust energy substrate oxidation in response to submaximal endurance exercise.

ATGL-mediated lipolysis in adipocytes vs. skeletal myocytes differentially influences skeletal muscle substrates, lipolytic enzyme, and mitochondria in response to submaximal endurance exercise. To better understand intramyocellular factors contributing to differences in acute exercise performance in mice lacking ATGL in either adipocyte or skeletal myocytes, we next evaluated skeletal muscle substrates, mitochondria, and lipolytic enzymes in age- and weight-matched control, AAKO, and SMAKO mice. For muscle substrate and protein analysis (Fig. 7, A–C), mice were exercised just as they were for the nonterminal submaximal exercise challenge, except...
they were euthanized after 45 min of running so that all mice could be examined after the same run duration. Sedentary (nonexercised) age-, weight-, and genotype-matched mice were used for comparison. Consistent with results of the nonterminal submaximal exercise study, the terminal submaximal exercise study (Table 3) revealed that circulating energy substrates (glucose, TAGs, and NEFAs) were comparable between control and SMAKO mice but lower in both sedentary and exercised AAKO mice. Likewise, both sedentary and exercise AAKO mice demonstrated a greater preference for carbohydrate relative to FA oxidation than control or SMAKO mice.

To better understand the role of intramyocellular energy substrates in the above metabolic and functional phenotypes during exercise, we next measured glycogen and TAG content within several different skeletal muscle types using both biochemical and histological methods (Fig. 7, A and B). Skeletal muscle glycogen content was comparable in control and SMAKO mice but lower in both sedentary and exercised AAKO mice (Fig. 7A). Skeletal muscle glycogen did not decrease significantly with submaximal exercise in any of the groups. As we have shown previously (54), IMTG content was markedly higher in sedentary SMAKO mice compared with control and AAKO mice (Fig. 7B). In response to exercise, IMTG content decreased in SMAKO mice but not in control or AAKO mice. To explore the possibility that HSL might be contributing to IMTG hydrolysis in SMAKO mice, we next assessed skeletal muscle HSL phosphorylation at Ser565 and Ser660 [sites phosphorylated by AMP-activated protein kinase and PKA, respectively, during acute exercise, resulting in increased HSL lipolytic activity (64)] (Fig. 7C). Indeed, phosphorylation of HSL at both Ser565 and Ser660 was increased in skeletal muscle of exercised compared with sedentary mice for all genotype groups, suggesting that HSL might be sufficient to maintain IMTG hydrolysis during exercise despite the loss of ATGL in SMAKO mice. Notably, both basal and exercise-stimulated phosphorylation of HSL at Ser660 was markedly reduced in AAKO mice, a finding consistent with decreased PKA-stimulated lipolysis in skeletal muscle (37). Finally, given the differences in muscle substrate profiles and lower rates of gas exchange in AAKO mice, we next assessed mitochondrial function in skeletal muscle of sedentary control, SMAKO, and AAKO mice (Fig. 7, D and E). As we demonstrated previously (54), mitochondrial respiration in isolated skeletal muscle fibers of control and SMAKO mice was similar under basal, substrate-stimulated, and uncoupled conditions (Fig. 7D). In contrast, mitochondrial respiration was dramatically lower in AAKO mice during all of the above conditions. Consistent with these results, expression of mitochondrial oxidative phosphorylation proteins was similar between control and SMAKO mice (Fig. 7E), with the exception of slightly higher complex III in SMAKO mice. In contrast, AAKO mice had lower expression of complex I, ATP synthase, and total OXPHOS proteins (Fig. 7D). Normalization of the respirometry data to expression of OXPHOS proteins suggested that AAKO mice had intrinsic mitochondrial dysfunction that was not solely due to decreased mitochondrial content. Thus, ATGL-mediated lipolysis in adipocytes vs. skeletal myocytes differentially influences intramyocellular factors that regulate substrate metabolism during submaximal endurance exercise.

**DISCUSSION**

The overall goal of this study was to determine the relative contribution of adipocyte vs. skeletal myocyte ATGL-mediated TAG hydrolysis to acute exercise performance in untrained mice. To achieve this goal, we evaluated exercise performance and energy substrate metabolism in response to peak and submaximal exercise in mice lacking ATGL in either adipocytes or skeletal myocytes compared with control mice. This study revealed several novel findings. First, impaired adipocyte lipolysis due to loss of ATGL action in adipocytes has a greater impact on both peak and submaximal exercise performance in untrained mice than loss of ATGL action in skeletal myocytes. This decrease in exercise performance in AAKO mice is likely to be mediated, at least in part, by 1) reduced systemic energy substrate availability (FAs and glucose), 2) reduced metabolic flexibility (decreased ability to switch between FA and glucose substrates, depending on energy availability and/or energy requirements), 3) reduced phosphorylation of HSL at Ser660 in skeletal muscle, and/or 4) reduced mitochondrial respiration in skeletal muscle. Notably, the latter is profound and may independently be sufficient to reduce exercise performance. Second, impaired intramyocellular lipolysis due to loss of ATGL action in skeletal myocytes is not sufficient to reduce peak or submaximal exercise performance in untrained mice. This lack of an effect on acute exercise performance in SMAKO mice may be due to 1) sustained/adequate peripheral energy substrate delivery, 2) maintenance (or even enhancement) of metabolic flexibility, and/or 3) preserved HSL phosphorylation and HSL-mediated TAG hydrolysis of an expanded IMTG pool. Together, these data suggest that ATGL action in adipocytes plays a greater role than in skeletal myocytes in generating energy for peak and submaximal exercise in untrained mice.

Although IMTG metabolism has been studied extensively, the precise physiological relevance of IMTGs under different physiological and/or pathophysiological conditions remains...
poorly understood (11, 15). In the context of physical activity, adipocyte lipolysis contributes to as much as 75% of FA substrates for moderate-intensity exercise in humans (44). Other studies have suggested that IMTG lipolysis also contributes substantially to fat oxidation for energy during exercise (35, 49, 55). In humans (36) and rodents (31, 52), global inhibition of ATGL-mediated lipolysis impairs exercise performance, and yet the tissue-specific contributions of ATGL action to exercise performance are poorly understood. Using our previously established model of skeletal myocyte-specific ATGL deletion (54) and our newly developed model of adipocyte-specific ATGL deletion, we address this important question. Of particular relevance, inhibition of ATGL-mediated lipolysis in adipocyte but not skeletal myocytes results in reduced peripheral energy substrate availability, specifically circulating NEFAs and TAGs as well as glucose (52). This depletion of circulating glucose in AAKO mice underscores the glucose-sparing effect of adipocyte FA mobilization during exercise. Indeed, impaired adipocyte ATGL action underscores O2 consumption and CO2 production and enhanced rates of whole body carbohydrate oxidation at rest. Our data parallel those of Huijsman et al. (31), who demonstrated higher RERs during the light cycle in global ATGL as well as HSL knockout mice compared with control mice, reflecting an inability to switch from carbohydrate to fat oxidation under fasting conditions. These data indicate that a lack of circulating lipids due to impaired adipocyte lipolysis results in a shift toward carbohydrate oxidation (48). Thus, adipocyte-specific ATGL action is critical for whole body energy substrate availability and oxidation, whereas inhibition of skeletal muscle ATGL action is not sufficient to alter systemic substrate metabolism (54).

Peak exercise performance is the product of cardiovascular dynamics and tissue energy metabolism. Although impaired skeletal myocyte-specific ATGL action is not sufficient to influence energy homeostasis or insulin action at baseline or in response to nutritional stress (i.e., high-fat diet feeding) in mice (54), we hypothesized that it would be required for FA mobilization in the context of a functional stress such as acute exercise. Our data indicate that mice with impaired ATGL action in skeletal myocytes exhibit normal responses to increasing exercise intensity. There are several possible explanations for this observation. First, the inhibition of ATGL-mediated lipolysis in skeletal myocytes results in a compensatory metabolic shift toward greater carbohydrate metabolism as well as total whole body substrate oxidation (i.e., fat + carbohydrate oxidation) during peak exercise. These data suggest a compensatory upregulation of muscle glycolysis (46), presumably through a greater reliance on type II (glycolytic) fibers, which are also more abundant in mice than humans (1). Second, peak exercise does not require a significant contribution of fat metabolism for energy production. As exercise intensity increases, rates of both glycolysis and gluconeogenesis are adjusted to maintain adequate glucose availability (57). Thus, despite the loss of ATGL-mediated intramyocellular lipolysis, peak exercise could remain unaffected. In contrast, reduction of adipocyte ATGL action resulted in a metabolic shift toward whole body carbohydrate oxidation and a reduction in overall substrate availability. Therefore, similarly to global ATGL (31, 52) as well as HSL (31) knockout mice, the increased energy demand of exercise likely resulted in a preferential utilization and depletion of glycogen stores in mice lacking adipocyte lipolysis, coupled with the lack of substrate for gluconeogenesis, thereby contributing to an overall decrease in exercise performance. These observations can be further explained by dysregulation of counterregulatory mechanisms (19) associated with hypoglycemia. Studies in humans have demonstrated that pharmacological inhibition of lipolysis following hypoglycemia results in suppression of endogenous glucose production (42), which is rescued by administration of exogenous lipid. Thus, inhibition of adipocyte but not in-

![Figure 5. Whole body gas exchange and RER during submaximal endurance exercise. A and B: whole body oxygen consumption (A) and CO2 production (B) normalized to body weight (kg) raised to the 0.75 power at baseline (black bars) and at peak exercise (open bars) by indirect calorimetry. C: RER (VCO2/VO2) at baseline and following the endurance exercise challenge. For each group/variable (i.e. all data), male mice were used (13 wk, fasted 3 h; n = 23 control, 11 SMAKO, and 14 AAKO). abP ≤ 0.05 for baseline group difference; noncorresponding letters are different. c-dP < 0.05 for peak exercise group difference; noncorresponding letters are different. GP < 0.05 for genotype. AAKO > control and SMAKO; GP < 0.05 for peak compared with baseline.](http://ajpendo.physiology.org/)

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tramyocellular ATGL action impairs substrate availability necessary for peak exercise performance.

In contrast to the anaerobic energy demand of maximal-intensity exercise, submaximal endurance exercise energy requirements in humans are generally met through the oxidation of FAs (13) derived from adipocyte (28) and/or IMTG stores (22, 58, 60). In mice and humans, IMTG content is highest in type I slow-oxidative fibers (54, 59, 60), which are physically associated with mitochondria (34) and correlated with ATGL content (32, 54). We hypothesized that submaximal endurance exercise performance would be impaired in SMAKO mice due to reduced intramyocellular FA mobilization. Likewise, we hypothesized that submaximal endurance exercise performance would be impaired in AAKO mice due to reduced circulating lipid substrate. Unexpectedly, however, submaximal endurance exercise capacity was not affected by the inhibition of skeletal myocyte-specific ATGL action. To the contrary, SMAKO mice demonstrated a robust switch to greater rates of fat oxidation in the face of moderate-intensity exercise. Although the exact mechanism for this result is unclear, one possible explanation is that the lipolytic action of HSL, which is nonfiber type specific (39) and activated by skeletal muscle contraction (38), is sufficient to compensate for loss of ATGL action in skeletal muscle but not adipocytes during exercise. This is supported by the observation that TAG hydrolase activity is present in ATGL-deficient skeletal muscle (5, 54). Furthermore, the higher IMTG content in SMAKO mice would provide more substrate for HSL-mediated lipolysis, as suggested by the dramatic reduction in IMTG content in exercised SMAKO mice. Indeed, both ATGL and HSL are activated by similar pathways during muscle contraction (43). This would explain our observation of appropriate whole body metabolic inflexibility. Our results are consistent with the body of literature related to substrate competition first proposed by Randle et al. (48) demonstrating that a pharmacological decrease in FA availability in both rodents (14, 67) and humans (8, 29, 41, 68) leads to an overall switch toward carbohydrate oxidation and compensatory reduction in FA oxidation. Together, these data point to metabolic inflexibility within the context of submaximal endurance exercise with the inhibition of adipocyte ATGL-mediated lipolysis.

The acute peak and submaximal endurance exercise interventions indicate that impaired ATGL action in adipocytes rather than myocytes has a greater impact on acute exercise performance. To better understand the potential mechanisms associated with this observation, we examined skeletal muscle substrates, mitochondrial performance and content, and markers of lipolysis. Inhibition of available FA substrate through the targeted deletion of ATGL in adipocyte results in decreased basal skeletal muscle glycogen content (31) but does not appear to affect IMTG content. Thus, adipocyte but not skeletal muscle ATGL action is required to maintain circulating and intramyocellular carbohydrates at rest and during physical activity. The lack of an exercise-induced reduction in IMTGs in AAKO mice is somewhat surprising. However, because baseline IMTG content is low in AAKO and control mice (in contrast to SMAKO mice), a further reduction in IMTG content may be below the limit of detection. Alternatively, there may be differences in substrate delivery and/or utilization in AAKO and control mice. Nevertheless, our data from control and AAKO mice are in agreement with previous reports of unchanged IMTG content under acute submaximal exercise conditions in untrained mice (31, 52). These data are in contrast to acute exercise studies in trained humans (60) and under conditions of pharmacological inhibition of adipose tissue lipolysis (61) as well as maximal tetanic stimulation in mice (5). Together, these data emphasize the importance of species (mice and men), training status, and exercise and/or contraction stimulus in the regulation of IMTG.
Fig. 7. Skeletal muscle substrates, mitochondrial performance and content, and lipase phosphorylation with exercise. A and B: skeletal muscle glycogen (A) and triacylglycerol content (B) under sedentary (black bars) and submaximal endurance exercise (open bars) conditions [20 wk, fasted 3 h, biochemical analysis [red quadriceps], histology [gastrocnemius-plantaris-soleus complex (GPS)]; n = 4–6/group] normalized to control sedentary representative images (right) of histological analyses (GPS; n = 3/group). Interaction effect for triacylglycerol (P = 0.04). *P < 0.05 for exercise effect in SMAKO only. C: protein expression of phosphorylated hormone-sensitive lipase (p-HSL; Ser565, Ser660) normalized to total HSL and representative immunoblots (right) under sedentary (SED) and submaximal endurance exercise (E) conditions for control (C), SMAKO (S), and AAKO (A) (20 wk, fasted 3 h, tibialis anterior; n = 4–6/group). a,b P < 0.05 for group difference; noncorresponding letters are different. c,d P < 0.05 for submaximal endurance exercise group difference, noncorresponding letters are different. *P < 0.05, effect of exercise. D: mitochondrial respiration in permeabilized muscle fibers (20 wk, fasted 12 h, soleus; n = 6/group). Oxygen consumption was measured following the sequential addition of the following substrates: palmitoylcarnitine, malate, ADP, glutamate, succinate, and FCCP. The corresponding respiratory states are noted: ADP-driven respiration (state 3), respiration in the absence of ADP (state 4), and uncoupled respiration (state U). G P < 0.05, effect of genotype, AAKO/control and SMAKO for each state. PM, palmitoylcarnitine/malate; PMD, palmitoylcarnitine/malate/ADP; PMGD, palmitoylcarnitine/malate/glutamate/ADP; PMGSD, palmitoylcarnitine/malate/ADP/glutamate/succinate. E: oxidative phosphorylation protein content of complexes I–V [NDUFB8 (complex I), SDHB (complex II), UQRC2 (complex III), MTCO1 (complex IV), and ATP5A (complex V)] (20 wk, fasted 3 h, tibialis anterior; n = 6/group). Data are normalized to protein expression of β-actin. ab,c P < 0.05, effect of genotype; noncorresponding letters are different. Representative immunoblots (right) for control (C), SMAKO (S), and AAKO (A).
We demonstrate that impaired adipocyte ATGL action dramatically reduces both mitochondrial performance and content. Similarly, global and cardiomyocyte-specific ATGL deficiency markedly reduces peroxisome proliferator-activated receptor target gene expression and mitochondrial function in cardiomyocytes, resulting in severe cardiac dysfunction (25). This dramatic phenotype is probably intensified by the constant high energy demand of cardiac muscle. In contrast, skeletal muscle fluctuates between low and high energy demand during rest and exercise, respectively. Thus, the relative importance of skeletal myocyte vs. adipocyte ATGL action may only manifest certain physiological situations in which sustained high energy demand of cardiac muscle. In contrast, skeletal muscle provides critical substrates for energy metabolism (4, 7). Thus, inherent differences between mice and men, such as the percentage of oxidative fibers, may exist. Given the severe myopathy in ATGL-deficient humans, understanding the relative contribution of ATGL action in adipocytes and muscle metabolism function is important. Additional studies are required to more precisely delineate the relative contribution of ATGL and HSL-mediated IMTG hydrolysis to muscle function in both mice and humans.

In summary, the coordinated effects of adipocyte and skeletal muscle lipolysis provide critical substrates for energy homeostasis during rest and physical activity. Dysregulation of lipolysis contributes to insulin resistance and other disease states, whereas modulation of lipolysis can improve health outcomes (3, 16). Understanding the effects of altered lipolysis within the context of physical activity may provide novel insights into these processes that can be therapeutically targeted to improve metabolic disease. Our study provides evidence that inhibition of ATGL-mediated lipolysis in adipocytes, but not skeletal muscle, results in significant impairments in exercise performance. This dramatic phenotype is characterized by robust depletion of circulating and skeletal muscle substrates as well as impaired mitochondrial performance and content. Additional studies are warranted to further characterize the tissue-specific contribution of ATGL action to skeletal muscle function in normal physiology (i.e., in response to exercise training) and disease (myopathy in neutral lipid storage disease). Given the fundamental role of TAG hydrolysis in metabolism, these studies are likely to provide important insights into the critical role of extra- and intramyocellular FA mobilization in overall muscle health and function.

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REFERENCES


