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


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ABSTRACT

Adipose triglyceride lipase (ATGL) is the rate-limiting enzyme mediating triacylglycerol hydrolysis in virtually all cells including adipocytes and skeletal myocytes and, hence, plays a critical role mobilizing fatty acids. Global ATGL deficiency promotes skeletal myopathy and exercise intolerance in mice and humans, and yet the tissue-specific contributions to these phenotypes remain unknown. The goal of this study was to determine the relative contribution of ATGL-mediated triacylglycerol hydrolysis in adipocytes versus skeletal myocytes to acute exercise performance. To achieve this goal, we generated murine models with adipocyte- and skeletal myocyte-specific targeted deletion of ATGL. We then subjected untrained mice to acute peak and submaximal exercise interventions and assessed exercise performance and energy substrate metabolism. Impaired ATGL-mediated lipolysis within adipocytes reduced peak and submaximal exercise performance, reduced peripheral energy substrate availability, shifted energy substrate preference towards carbohydrate oxidation, and decreased HSL Ser\textsuperscript{660} phosphorylation and mitochondrial respiration within skeletal muscle. In contrast, impaired ATGL-mediated lipolysis within skeletal myocytes was not sufficient to reduce peak and submaximal exercise performance or peripheral energy substrate availability and instead tended to enhance metabolic flexibility during peak exercise. Furthermore, the expanded intramyocellular triacylglycerol pool in these mice was reduced following exercise in association with preserved HSL phosphorylation, suggesting that HSL may compensate for impaired ATGL action in skeletal muscle during exercise. These data suggest that adipocyte rather than skeletal myocyte ATGL-mediated lipolysis plays a greater role during acute exercise, in part, due to compensatory mechanisms that maintain lipolysis in muscle, but not adipose tissue, when ATGL is absent.
Keywords: Adipose triglyceride lipase, exercise, lipolysis
INTRODUCTION

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% VO_{2max}), carbohydrates account for only 10-30% of total energy production and are derived primarily from circulating glucose (50). At higher exercise intensities (85%-100% VO_{2max}), 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 (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 influence 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 due to 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 re-expressing ATGL in cardiac muscle
(52), indicating that skeletal muscle dysfunction cannot be entirely explained by cardiac
dysfunction. These data implicate either adipose tissue and/or skeletal muscle ATGL action in
exercise performance. We previously 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 versus adipocytes influence acute
exercise performance remains unknown.

The primary goal of this study was to determine the relative contribution of adipocyte
versus skeletal myocyte ATGL-mediated TAG hydrolysis to acute exercise performance in mice.
To achieve this goal, we generated murine models with both 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^tm1eek^ (Atgl-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^{flox/flox} Cre/+ mice were mated to female Atgl^{flox/flox} +/+ mice to generate Atgl^{flox/flox} Cre/+ (SMAKO or AAKO) and Atgl^{flox/flox} +/+ (control) experimental mice. All mice were congenic (n>10) on C57BL/6NTac. Animal experiments were approved by the IACUC and conducted in conformity with PHS Policy for Care and Use of Laboratory Animals.

**Peak exercise protocol.** At 12 weeks of age, mice were subjected to a 3-day acclimation protocol with progressively increasing 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 4h 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 (VO2) and carbon dioxide production (VCO2). Mice were fasted for 4h with testing being completed between 10am-1pm. 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 warm up 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 sec. 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 (non-terminal) 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 weeks of age. Tests were conducted between 10am-3pm following an ~3h 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 measures were collected for 1 min, and blood was collected via the tail vein. A second (terminal) submaximal endurance exercise challenge was performed in 20 week-old mice to evaluate for potential differences in tissue substrates, mitochondria, and/or gene/protein expression/phosphorylation. Mice were re-acclimated to the treadmill and run for 45 min (representing ~1/2 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 CO₂ inhalation and blood/tissue collected for analysis (17).

**Energy expenditure and metabolic measurements.** VO₂, VCO₂, and respiratory exchange ratio (RER) were determined using the CLAMS. Respiratory data from the final 30 seconds 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: 
\[ c = (4.55*\text{VCO}_2) - (3.21*\text{VO}_2) \]
\[ f = (1.67*\text{VO}_2) - (1.67*\text{VCO}_2) \] (21) (protein oxidation ignored due to minimal role during
exercise). Results of all respiratory measures and associated calculations were unchanged after adjusting for fat or lean mass. Whole-blood glucose was determined using a One-touch FastTake glucometer (Lifescan). Serum TAGs (Infinity Triglycerides Liquid Stable Reagent, Thermo Scientific) and non-esterified 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 triglyceride was determined in liquid nitrogen crushed quadriiceps samples using a commercially available kit (Caymen Chemical) and infinity triglycerides Liquid Stable Reagent (Thermo Scientific), respectively (9). For tissue imaging, muscle samples were cut into 10 micron sections and stained for neutral lipids (54) or glycogen (27). Mitochondrial respiration was assessed in permeabilized muscle fibers in the basal state as previously described (54). Protein content in skeletal muscle was determined by Western Blot analysis using the following antibodies: MitoProfile Antibody Cocktail (MS604; Mitosciences), anti-HSL (4017s; Cell Signaling Technology), anti-pHSL (Ser565 and Ser660) (4137S and 4126S; Cell Signaling Technology) as described (54).

**Statistical analysis.** Data are expressed as means ± SEM and analyzed using the Statistical Package for the Social Sciences for MAC v22. Baseline between groups 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 post-exercise test outcome variables and to assess between group interactions. Univariate analysis addressed the question of genotype and activity (sedentary versus 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 non-transformed. 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 (non-exercised) state. To determine the relative impact of adipocyte versus 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, Figure 1A). Likewise, dropout rates during a peak exercise challenge were similar for control and SMAKO mice but greater for AAKO mice (Figure 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 – increased serum glucose and reduced serum TAGs. In contrast, AAKO mice demonstrated the opposite response – decreased serum glucose and increased serum TAGs. Serum NEFAs tended to respond differently between the genotypes (p=0.06 for group interaction) with decreased NEFAs in SMAKO (post hoc 2-tailed pair-wise comparison, p=0.05) but not control or in AAKO mice.
Thus, inhibition of ATGL action in adipocytes, but not skeletal myocytes, alters circulating energy substrate availability and impairs peak exercise performance in mice.

Maximal aerobic capacity and metabolic flexibility in response to peak exercise are impaired by inhibition of adipocyte but not intramyocellular ATGL-mediated lipolysis. Peak exercise represents maximal aerobic capacity and is regulated by neuronal, cardiopulmonary, and metabolic factors (63). Among these, substrate availability and oxidation play key roles in exercise performance. To determine the relative effects of adipocyte versus skeletal myocyte ATGL action on maximal aerobic capacity and relative substrate oxidation, we measured whole-body gas exchange in response to a peak exercise challenge. At baseline, O₂ consumption (VO₂, Figure 2A) and CO₂ production (VCO₂, Figure 2B) were similar between control and SMAKO mice but lower in AAKO mice. In response to a peak exercise challenge, O₂ consumption and CO₂ production increased for all genotypes but remained lower in AAKO mice compared to the other groups. The baseline respiratory exchange ratio (RER=VCO₂/VO₂) in AAKO mice was higher than control and SMAKO mice, consistent with a baseline preference for carbohydrate over FA oxidation (Figure 2C). In response to peak exercise, the RER increased for all genotypes (p<0.01), however the magnitude of this increase in RER relative to baseline was smaller for AAKO (0.03±0.01 au, p<0.01) compared to control (0.07±0.01 au) or SMAKO (0.10±0.01 au) mice. In contrast, the RER at peak exercise was higher in SMAKO (0.88±0.01, p<0.05) compared to control (0.84±0.01) mice, as was the magnitude of the increase in RER relative to baseline (p<0.05). Thus, inhibition of ATGL action in adipocytes impairs maximal aerobic capacity and metabolic flexibility (i.e. the ability to switch between carbohydrate and FA
oxidation) at peak exercise, whereas inhibition of ATGL action in skeletal myocytes does not alter maximal aerobic capacity and may enhance metabolic flexibility at peak exercise.

Substrate kinetics during peak exercise are impaired by inhibition of adipocyte but not intramyocellular ATGL-mediated lipolysis. To more specifically characterize energy substrates during peak exercise, we next determined the relative rates of carbohydrate and fat oxidation by respirometry at baseline and in response to a peak exercise challenge (21). For carbohydrate oxidation (Figure 3A-C), absolute rates of carbohydrate oxidation (Figure 3A) as well as percent carbohydrate relative to total oxidation (Figure 3B) were similar between control and SMAKO mice but higher in AAKO mice at baseline. In response to a peak exercise challenge, carbohydrate oxidation (both absolute and percent total) increased in all genotype groups (Figure 3A-B), but the magnitude of this increase was much smaller in AAKO (9.03±1.26 mg/kg^{0.75}/min) compared to control (15.35±1.33 mg/kg^{0.75}/min) and SMAKO (19.69±2.24, mg/kg^{0.75}/min) mice (Figure 3A). Likewise, the percent change in carbohydrate oxidation relative to baseline increased for all genotype groups, but the magnitude of this response was attenuated in AAKO mice (Figure 3C). For fat oxidation (Figure 3D-F), both absolute rates of fat oxidation (Figure 3D) as well as percent fat to total oxidation (Figure 3E) were similar between control and SMAKO mice but lower in AAKO mice at baseline. In response to a peak exercise challenge, fat oxidation (both absolute and percent total) decreased for all genotype groups (Figure 3D-E), but the magnitude of this decrease was much lower in AAKO (-0.45±0.61 mg/kg^{0.75}/min, absolute) compared to control (-2.87±0.65 mg/kg^{0.75}/min, absolute) and SMAKO (-4.08±1.11 mg/kg^{0.75}/min, absolute) mice (Figure 3D). Likewise, the percent change in fat oxidation relative to baseline decreased for all genotype groups with the greatest reduction
in SMAKO mice and the least reduction in AAKO mice (Figure 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 versus 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 (6289.61±483.48 sec) and SMAKO (7528.56±660.10 sec) mice but lower in AAKO mice (2558.79±562.59 sec, p<0.01, Figure 4A). Likewise, dropout rates during the submaximal endurance exercise challenge were similar for control and SMAKO mice but greater for AAKO mice (Figure 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 versus 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, O₂ consumption (Figure 5A) and CO₂ production (Figure 5B) were similar between control and SMAKO mice but lower in AAKO mice. In response to a submaximal endurance exercise challenge, neither O₂ consumption nor CO₂ 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 to control and SMAKO mice and remained higher at fatigue (Figure 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 are 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 (Figure 6A-C), the rates of carbohydrate oxidation were similar between control and SMAKO mice baseline (Figure 6A and 6B) but higher in AAKO mice when expressed as percent carbohydrate to total oxidation (Figure 6B). In response to a submaximal endurance exercise challenge, carbohydrate oxidation (both absolute and percent of total) decreased in all genotype groups (Figure 6A-B), but the magnitude of this decrease was much smaller in AAKO (-2.85±2.74 mg/kg^{0.75}/min) compared to control (-7.98±1.64 mg/kg^{0.75}/min) and SMAKO (-12.04±2.62 mg/kg^{0.75}/min) mice (Figure 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 to AAKO mice (Figure 6C). For FA oxidation (Figure 6D-F), both absolute rates of FA oxidation (Figure 6D) as well as percent FA to total oxidation (Figure 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 percent of total) increased for all genotype groups (Figure 6D-E). Although the percent changes in FA oxidation relative to baseline were similar between groups (Figure 6F), the absolute magnitude of this decrease was lower in AAKO (1.51±0.77 mg/kg^{0.75}/min) compared to control (3.23±0.52 mg/kg^{0.75}/min) and SMAKO (4.90±0.84 mg/kg^{0.75}/min) mice (Figure 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 versus 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 (Figure 7A-C), mice were exercised as for the non-terminal submaximal exercise challenge except that they were euthanized after 45 minutes of running so that all mice could be examined after the same run duration. Sedentary (non-exercised) age-, weight-, and genotype-matched mice were used for comparison. Consistent with results of the non-terminal submaximal exercise study, the terminal submaximal exercise study (Table 3) revealed that circulating energy substrates (glucose, TAGs, 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 to 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 (Figure 7A-B). Skeletal muscle glycogen content was comparable in control and SMAKO mice but lower in both sedentary and exercised AAKO mice (Figure 7A). Skeletal muscle glycogen did not decrease significantly with submaximal exercise in any of the groups. As we have previously shown (54), IMTG content was markedly higher in sedentary SMAKO mice compared to control and AAKO mice (Figure 7B). In response to exercise, IMTG content decreased in SMAKO mice but not in control or AAKO mice. To explore the possibility that hormone sensitive lipase (HSL) might be contributing to IMTG hydrolysis in SMAKO mice, we next assessed skeletal
muscle HSL phosphorylation at Ser\textsuperscript{565} and Ser\textsuperscript{660} (sites phosphorylated by AMP-activated protein kinase and PKA, respectively, during acute exercise resulting in increased HSL lipolytic activity (64)) (Figure 7C). Indeed, phosphorylation of HSL at both Ser\textsuperscript{565} and Ser\textsuperscript{660} were increased in skeletal muscle of exercised compared to sedentary mice for all genotype groups, suggesting that HSL might be sufficient to maintain IMTG hydrolysis during exercise despite loss of ATGL in SMAKO mice. Notably, both basal and exercise-stimulated phosphorylation of HSL at Ser\textsuperscript{660} 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 (Figure 7 D and E). As we previously demonstrated (54), mitochondrial respiration in isolated skeletal muscle fibers of control and SMAKO mice were similar under basal, substrate-stimulated, and uncoupled conditions (Figure 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 (Figure 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 (Figure 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 versus skeletal myocytes differentially influence intramyocellular factors that regulate substrate metabolism during submaximal endurance exercise.
The overall goal of this study was to determine the relative contribution of adipocyte versus 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 in comparison 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 (i) reduced systemic energy substrate availability (FAs and glucose), (ii) reduced metabolic flexibility (decreased ability to switch between FA and glucose substrates depending on energy availability and/or energy requirements), (iii) reduced phosphorylation of HSL at Ser660 in skeletal muscle, and/or (iv) 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 (i) sustained/adequate peripheral energy substrate delivery, (ii) maintenance (or even enhancement) of metabolic flexibility, and/or (iii) 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 intensively studied, 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 decreased O₂ consumption and CO₂ production and enhanced rates of whole-body carbohydrate oxidation at rest. Our data parallel those from Huijsman et al. (31) who demonstrate higher RERs during the light cycle in global ATGL, as well as HSL, knockout mice compared to 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, similar 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 counter-regulatory 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 intramyocellular 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), 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. We likewise 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. While the exact mechanism for this result is unclear, one possible explanation is that the lipolytic action of HSL, which is non-fiber 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 fat oxidation and a tendency for greater relative increases in fat oxidation with skeletal myocyte-specific ATGL deletion. In contrast, and in agreement
with our peak exercise data, inhibition of adipocyte ATGL action attenuates exercise performance due to a greater reliance on carbohydrate oxidation and 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 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.

We demonstrate that impaired adipocyte ATGL action dramatically reduced both mitochondrial performance and content. Similarly, global and cardiomyocyte-specific ATGL deficiency markedly reduces PPAR 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 versus adipocyte ATGL action may only manifest certain physiological situations in which sustained high energy demand is required (i.e. chronic exercise training). Also, in contrast to cardiac muscle, global (47) but not skeletal muscle-specific (54) ATGL deficiency downregulates genes associated with electron transport and ATP synthesis in skeletal muscle. Interestingly, mitochondrial capacity, as measured by $^{31}$P-MRS in the mixed fiber type calf complex, does not seem to be impaired with global ATGL deficiency under conditions of maximal tetanic electrical stimulation (45). Since exercise training markedly promotes mitochondrial biogenesis in rodents (23, 51), additional studies are required to define the role of adipocyte and skeletal myocyte ATGL action on skeletal muscle metabolism, mitochondrial function, and exercise performance in response to chronic exercise training. Our study suggests that, in the setting of acute exercise, adipocyte- but not skeletal myocyte-specific ATGL-mediated lipolysis is essential for effect on mitochondrial function in skeletal muscle.
It is important to reiterate that, although skeletal muscle-specific ATGL deletion was not sufficient to impair acute peak and submaximal endurance exercise performance in this study, these data do not exclude an important role for ATGL action in skeletal muscle lipolysis. To the contrary, a recent study by Alsted et al. demonstrated that ATGL and HSL, together, account for greater than 98% of contraction-stimulated lipolysis in skeletal muscle (5). Furthermore, the absence of HSL is not sufficient to impair contraction-stimulated skeletal muscle lipolysis (5) or exercise performance (31). These data suggest that ATGL not only plays an important role skeletal muscle lipolysis but that it is sufficient to compensate for loss of HSL. Likewise, in our study, absence of ATGL action in skeletal muscle is not sufficient to impair acute exercise performance, suggesting that other factors, such as HSL action or systemic delivery of energy substrates, are sufficient to compensate for loss of ATGL in skeletal muscle in the context of acute peak and submaximal exercise. In addition, our data do not exclude an important role for ATGL action in skeletal muscle lipolysis in humans. IMTGs accumulate in both mice (54) and humans (20, 53) with ATGL deficiency. Substantial evidence supports the importance of ATGL action in human skeletal muscle lipid 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 contribute 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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DISCLOSURES

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

J.J.D., M.T.S., and E.E.K were project leaders and contributed to all aspects of this work. All authors performed experiments, contributed intellectually, and reviewed/edit ed the manuscript. E.E.K is the guarantor of this work.

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53. Schweiger M, Lass A, Zimmermann R, Eichmann TO, and Zechner R. Neutral lipid storage disease: genetic disorders caused by mutations in adipose triglyceride lipase/PNPLA2 or


FIGURE LEGENDS

Figure 1. Peak exercise performance. A) Peak exercise speed. B) Dropout rates during peak exercise. Interval, 1=10 sec. All data (♂, 12 weeks, fasted 4h; n=26 control; n=14 skeletal muscle ATGL knockout [SMAKO]; n=15 adipocyte ATGL knockout [AAKO]) a,b, p≤0.05 for group difference, non-corresponding letters are different.

Figure 2. Whole-body gas exchange and respiratory exchange ratio during peak exercise. A) Whole-body oxygen consumption (VO₂) and B) carbon dioxide production (VCO₂) normalized to body weight (kg) raised to the 0.75 power at baseline (black bars) and at peak exercise (white bars) by indirect calorimetry. C) Respiratory exchange ratio (RER, VCO₂/VO₂) at baseline, 25%, 50%, and 75%, and 100% of peak exercise run duration. All data (♂, 12 weeks, fasted 4h; n=26 control; n=14 SMAKO; n=15 AAKO). a,b, p≤0.05 for baseline group difference, non-corresponding letters are different. c,d, p<0.05 for peak exercise group difference, non-corresponding letters are different. * 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 to peak), all groups.

Figure 3. Substrate kinetics in response to peak exercise. A) Carbohydrate (CHO) and D) fat oxidation normalized to body weight (kg) raised to the 0.75 power at baseline (black bars) and at peak exercise (white bars) by indirect calorimetry. B) Percent change in CHO and E) fat oxidation relative to baseline. C) CHO and F) fat oxidation as a percent of total oxidation. All data (♂, 12 weeks, fasted 4h; n=26 control; n=14 SMAKO; n=15 AAKO). a,b, p≤0.05 for group
difference, non-corresponding letters are different. c,d, p<0.05 for peak exercise group
difference, non-corresponding letters are different. * p<0.05 effect of exercise.

**Figure 4.** Submaximal endurance exercise performance. **A)** Endurance time until exhaustion. **B)** Dropout rates during submaximal endurance exercise. Interval, 1=10 sec. All data (♂, 13 weeks, fasted 4h; n=23 control; n=11 SMAKO; n=14 AAKO). a,b, p≤0.05 for group difference, non-corresponding letters are different.

**Figure 5.** Whole-body gas exchange and respiratory exchange ratio during submaximal endurance exercise. **A)** Whole-body oxygen consumption and **B)** CO₂ production normalized to body weight (kg) raised to the 0.75 power at baseline (black bars) and at peak exercise (white bars) by indirect calorimetry. **C)** Respiratory exchange ratio (RER, VCO₂/VO₂) at baseline and following the endurance exercise challenge. All data (♂, 13 weeks, fasted 4h; n=23 control; n=11 SMAKO; n=14 AAKO). a,b, p≤0.05 for baseline group difference, non-corresponding letters are different. c,d p<0.05 for peak exercise group difference, non-corresponding letters are different. G, p<0.05 for genotype, AAKO > control and SMAKO. T, p<0.05 for peak compared to baseline.

**Figure 6.** Substrate kinetics in response to submaximal endurance exercise. **A)** Carbohydrate (CHO) and **D)** fat oxidation normalized to body weight (kg) raised to the 0.75 power at baseline (black bars) and at peak exercise (white bars) by indirect calorimetry. Percent change in **B)** CHO and **E)** fat oxidation relative to baseline. **C)** CHO and **F)** fat oxidation as a percent of total oxidation. All data (♂, 13 weeks, fasted 4h; n=26 control; n=14 SMAKO; n=15 AAKO). a,b,
p≤0.05 for group difference, non-corresponding letters are different. c,d, p<0.05 for peak exercise group difference, non-corresponding letters are different. * p<0.05 effect of exercise.

Figure 7. Skeletal muscle substrates, mitochondrial performance and content, and lipase phosphorylation with exercise. A) Skeletal muscle glycogen and B) triacylglycerol content under sedentary (black bars) and submaximal endurance exercise (white bars) conditions (♂, 20 weeks, fasted 3h, 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 content of phosphorylated HSL (Ser565, Ser660) normalized to total HSL and representative immunoblots (right) under sedentary (S) and submaximal endurance exercise (E) conditions for control (C), SMAKO (S), and AAKO (A) (♂, 20 weeks, tibialis anterior; n=4-6/group). a,b, p≤0.05 for group difference, non-corresponding letters are different. c,d, p<0.05 for submaximal endurance exercise group difference, non-corresponding letters are different. *p<0.05 effect of exercise. D) Mitochondrial respiration in permeabilized muscle fibers (♂, 20 weeks, fasted 12h, soleus; n=6/group). Oxygen consumption was measured following the sequential addition of the following substrates: palmitoylcarnitine (P), malate (M), ADP (D), glutamate (G), succinate (S), and carbonylcyanide-p-trifluoromethoxyphenylhydrazone (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. E) Oxidative phosphorylation protein content of complexes I–V (NDUFB8 [complex I], SDHB [complex II], UQCRC2 [complex III], MTCO1 [complex IV], and ATP5A [complex V]) (♂, 20
weeks, fasted 3h, tibialis anterior; n=6/group). Data are normalized to protein content of β-actin. 

a,b,c, p<0.05 effect of genotype. Non-corresponding letters are different. Representative immunoblots (right) for control (C), SMAKO (S), and AAKO (A).
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>
</tr>
</thead>
<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\textsuperscript{a}</td>
<td>246.26 ± 9.37\textsuperscript{c}</td>
<td>181.29 ± 5.93\textsuperscript{a}</td>
</tr>
<tr>
<td>Triacylglycerol (μg/μl)</td>
<td>0.72 ± 0.05\textsuperscript{a}</td>
<td>0.50 ± 0.03\textsuperscript{d}</td>
<td>0.86 ± 0.09\textsuperscript{a}</td>
</tr>
<tr>
<td>NEFA (mEq/L)</td>
<td>0.45 ± 0.04\textsuperscript{a}</td>
<td>0.54 ± 0.06</td>
<td>0.56 ± 0.08\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Data are mean±SEM. SMAKO, skeletal muscle ATGL knockout. AAKO, adipocyte tissue ATGL knockout. NEFA, non-esterified fatty acids. ♂, 12 weeks, fasted 4h; n=26 Control; n=14 SMAKO; n=15 (AAKO). \textsuperscript{a,b} p≤0.05 for baseline group effect, non-corresponding letters are different. \textsuperscript{a} p≤0.05 for exercise effect. \textsuperscript{c,d} p≤0.05 for group effect (interaction), non-corresponding letters are different.
Table 2. Substrate kinetics before and after an exhaustive submaximal endurance challenge in untrained mice

<table>
<thead>
<tr>
<th></th>
<th>Control Basal</th>
<th>Control Post</th>
<th>SMAKO Basal</th>
<th>SMAKO Post</th>
<th>AAKO Basal</th>
<th>AAKO Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>26.87 ± 0.27</td>
<td>27.27 ± 0.54</td>
<td>27.25 ± 0.23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>165.58 ± 6.82a</td>
<td>161.73 ± 13.32c</td>
<td>169.96 ± 11.3a</td>
<td>139.21 ± 18.04c</td>
<td>146.11 ± 14.31b</td>
<td>85.64 ± 10.06d</td>
</tr>
<tr>
<td>Triacylglycerol (μg/μl)</td>
<td>0.76 ± 0.07a</td>
<td>0.46 ± 0.07a</td>
<td>0.78 ± 0.06a</td>
<td>0.42 ± 0.03a</td>
<td>0.21 ± 0.02b</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>NEFA (mEq/L)</td>
<td>0.46 ± 0.05a</td>
<td>1.24 ± 0.08c</td>
<td>0.53 ± 0.09a</td>
<td>1.38 ± 0.19c</td>
<td>0.23 ± 0.04b</td>
<td>0.35 ± 0.04d</td>
</tr>
</tbody>
</table>

Data are mean±SEM. SMAKO, skeletal muscle ATGL knockout. AAKO, adipocyte ATGL knockout. NEFA, non-esterified fatty acids. ♂, 12 weeks, fasted 3h; n=23 Control; n=14 SMAKO; n=12 AAKO. a,b p≤0.05 for baseline group effect, non-corresponding letters are different. *p≤0.05 for exercise effect.
### Table 3. Substrate kinetics following a pre-exhaustive submaximal endurance exercise challenge in untrained mice

<table>
<thead>
<tr>
<th></th>
<th>Control Sedentary</th>
<th>Control Exercise</th>
<th>SMAKO Sedentary</th>
<th>SMAKO Exercise</th>
<th>AAKO Sedentary</th>
<th>AAKO Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>31.04 ± 0.78</td>
<td>32.39 ± 0.82</td>
<td>32.50 ± 0.54</td>
<td>32.06 ± 1.42</td>
<td>29.30 ± 0.84</td>
<td>28.95 ± 0.80</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>239.86 ± 8.25a</td>
<td>291.71 ± 19.47*</td>
<td>233.33 ± 3.82a</td>
<td>253.57 ± 13.71</td>
<td>174.00 ± 33.81b</td>
<td>143.33 ± 30.31</td>
</tr>
<tr>
<td>Triacylglycerol (μg/μl)</td>
<td>0.87 ± 0.11a</td>
<td>0.83 ± 0.08</td>
<td>0.91 ± 0.13a</td>
<td>0.80 ± 0.06</td>
<td>0.19 ± 0.02b</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>NEFA (mEq/L)</td>
<td>0.54 ± 0.09</td>
<td>0.80 ± 0.07*</td>
<td>0.68 ± 0.09</td>
<td>0.82 ± 0.06*</td>
<td>0.30 ± 0.06</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td>CHO oxidation (mg/kg·75/min)</td>
<td>9.89 ± 1.05</td>
<td>12.63 ± 0.86</td>
<td>9.87 ± 0.71</td>
<td>11.28 ± 2.57</td>
<td>8.29 ± 1.10</td>
<td>15.22 ± 1.77*</td>
</tr>
<tr>
<td>Fat oxidation (mg/kg·75/min)</td>
<td>13.81 ± 0.44</td>
<td>14.10 ± 0.42</td>
<td>12.35 ± 1.54</td>
<td>15.28 ± 0.37*</td>
<td>7.21 ± 0.65</td>
<td>7.93 ± 0.74</td>
</tr>
</tbody>
</table>

Data are mean±SEM. SMAKO, skeletal muscle ATGL knockout. AAKO, adipocyte tissue ATGL knockout. NEFA, non-esterified fatty acids. CHO, carbohydrate. ♂, 20 weeks, fasted 3h; n=23 Control; n=14 SMAKO; n=12 AAKO. a,b p≤0.05 for baseline group effect, non-corresponding letters are different. *p≤0.05 for exercise effect.
FIGURE 6

A

Baseline □ Submaximal Exercise

CHO ox (ml/kg·min)

Baseline □ Submaximal Exercise

CHO ox (% of total)

% change in CHO ox (relative to baseline)

B

C

D

Baseline □ Submaximal Exercise

fat ox (ml/kg·min)

Baseline □ Submaximal Exercise

fat ox (% of total)

% change in fat ox (relative to baseline)

E

F

Baseline □ Submaximal Exercise

Baseline □ Submaximal Exercise

Control SMAKO AAKO

Control SMAKO AAKO

Control SMAKO AAKO

Control SMAKO AAKO

Control SMAKO AAKO
FIGURE 7

A

Muscle glycogen (µg/mg)

Sedentary | Exercise

Control | SMAKO | AAKO

Muscle triglyceride (mg/mg)

Sedentary | Exercise

Control | SMAKO | AAKO

B

C

pHSL₁/₆₄/HSL

Sedentary | Exercise

Control | SMAKO | AAKO

D

C₂ flux (pMol/mg/sec)

Control | SMAKO | AAKO

Substrate:

PM  | PMD  | PMGD  | PMGSD  | FCCP

State:

4  | 3  | U

E

Protein (au)

Control | SMAKO | AAKO

Mitochondrial Complex:

I  | II  | III  | IV  | V  | Total

a  | b  | c  | d  | e  | f

Note: Letters a, b, c, d, and e indicate statistically significant differences at p<0.05.