ATGL-mediated triglyceride turnover and the regulation of mitochondrial capacity in skeletal muscle

Ruth C. R. Meex,1,2,* Andrew J. Hoy,2,3# Rachael M. Mason,1 Sheree D. Martin,4 Sean L. McGee,4 Clinton R. Bruce,5 and Matthew J. Watt1
1Biology of Lipid Metabolism Laboratory, Department of Physiology, Monash University, Clayton, Victoria, Australia; 2Discipline of Physiology, School of Medical Sciences & Bosch Institute, University of Sydney, New South Wales, Australia; 3Boden Institute of Obesity, Nutrition, Exercise & Eating Disorders, University of Sydney, Sydney, New South Wales, Australia; 4Metabolic Remodelling Laboratory, Metabolic Research Unit, School of Medicine, Deakin University, Burwood, Victoria, Australia; and 5Centre for Physical Activity and Nutrition Research, School of Exercise and Exercise Sciences, Deakin University, Burwood, Victoria, Australia

Submitted 18 December 2014; accepted in final form 2 April 2015

Meex RC, Hoy AJ, Mason RM, Martin SD, McGee SL, Bruce CR, Watt MJ. ATGL-mediated triglyceride turnover and the regulation of mitochondrial capacity in skeletal muscle. Am J Physiol Endocrinol Metab 308: E960–E970, 2015. First published April 7, 2015; doi:10.1152/ajpendo.00598.2014.—Emerging evidence indicates that skeletal muscle lipid droplets are an important control point for intracellular lipid homeostasis and that regulating fatty acid fluxes from lipid droplets might influence mitochondrial capacity. We used pharmacological blockers of the major triglyceride lipases, adipose triglyceride lipase (ATGL) and hormone-sensitive lipase, to show that a large proportion of the fatty acids that are transported into myotubes are trafficked through the intramyocellular triglyceride pool. We next tested whether increasing lipolysis from intramyocellular lipid droplets could activate transcriptional responses to enhance mitochondrial and fatty acid oxidative capacity. ATGL was overexpressed by adenoviral and adenoassociated viral infection in C2C12 myotubes and the tibialis anterior muscle of C57Bl/6 mice, respectively. ATGL overexpression in C2C12 myotubes increased lipolysis, which was associated with increased peroxisome proliferator-activated receptor (PPAR)-α activity, transcriptional upregulation of some PPARα target genes, and enhanced mitochondrial capacity. The transcriptional responses were specific to ATGL actions and not a generalized increase in fatty acid flux in the myotubes. Marked ATGL overexpression (20-fold) induced modest molecular changes in the skeletal muscle of mice, but these effects were not sufficient to alter fatty acid oxidation. Together, these data demonstrate the importance of lipid droplets for mitochondrial capacity by enhancing lipid droplet lipolysis in vitro; however, this adaptive program is of minor importance when superimposing the normal metabolic stresses encountered in free-moving animals.

adipose triglyceride lipase; lipolysis; fatty acid metabolism

LIPOLYSIS INVOLVES THE BREAKDOWN of triglycerides (TAG) stored within intracellular lipid droplets and results in the production of glycerol and fatty acids (FA). In contrast to adipose tissue, where FA liberated during lipolysis can be released into the circulation to provide metabolic substrate for other tissues, there is minimal FA release from skeletal muscle (18, 37). Instead the FA derived from intramyocellular lipolysis serve as an important local metabolic substrate, particularly during exercise (30, 38, 40). It has been known for some time that a large proportion of the FA transported from the plasma into skeletal muscle are first trafficked into TAG before they are released for oxidative metabolism (5, 15, 29). Hence, TAG stored within intracellular lipid droplets form a central pool that regulates the delivery of FA to the intramyocellular environment (15). Aside from their role as a metabolic substrate, FA are involved in a myriad of processes that ensure cellular growth and survival. FA also generate “lipid signals” that alter the cell’s phenotype in response to extracellular stimuli. One such example is activation of nuclear transcriptional factors, such as peroxisome proliferator-activated receptors (PPARs), which increase the expression of proteins involved in fat metabolism in the face of enhanced FA delivery. Taken together, these observations indicate that the control of FA flux from intracellular lipid droplets is critical to maintain normal cell function (41).

Lipolysis is controlled by the co-ordinate actions of several lipases and their interactions with lipid droplet-associated proteins (44). Adipose triglyceride lipase (ATGL) is the major regulator of TAG lipolysis in most tissues, including skeletal muscle (11, 32, 39, 46). The physiological importance of ATGL is highlighted by studies in Atgl−/− mice demonstrating reduced lipolysis, intracellular TAG accumulation, reduced FA oxidation, impaired exercise capacity, and cardiac failure leading to premature death (11, 14). A growing body of evidence indicates that ATGL is also important in modulating mitochondrial function. Most prominently, ATGL generates essential lipid ligands for PPARα activation in the heart, which is essential for maintaining mitochondrial FA oxidation, preventing myocardial lipid accumulation, and avoiding lethal cardiomyopathy (12). Similarly, overexpressing ATGL in white adipose tissue promotes an “oxidative phenotype” in adipocytes, which is associated with increased FA oxidation, increased whole body energy expenditure, and resistance to diet-induced obesity (3). Consistent with this notion, ATGL-mediated lipolysis is required for maintaining normal brown adipose tissue (BAT) function, and ATGL deletion converts BAT, which is rich in mitochondria, to a tissue that more closely resembles white adipose tissue characterized by a low oxidative capacity (2). ATGL deletion in the liver has a less pronounced impact on transcripts for mitochondrial biogenesis but resulted in decreased FA oxidation (36). In skeletal muscle, deletion (14) or modest overexpression of ATGL (32) has no effect on the expression of key metabolic genes, mitochondrial

* R. C. R. Meex and A. J. Hoy contributed equally to this work.
Address for reprint requests and other correspondence: M. J. Watt, Dept. of Physiology, Monash Univ., Clayton, Victoria, Australia; 3800 (e-mail: matthew.watt@monash.edu).
content, or respiration. These tissue-specific effects of ATGL on cellular adaptation are perplexing, and this uncertainty is a major hurdle in moving forward with pharmacological strategies that manipulate ATGL function for metabolic disease (22).

Emerging evidence indicates that skeletal muscle lipid droplet biology and mitochondrial metabolism are intimately linked (17), although there is some debate in the field (21, 32). We hypothesized that increasing lipolysis from intramyocellular lipid droplets would act as a molecular signal to increase mitochondrial biogenesis and FA oxidative capacity. To test the hypothesis, we overexpressed ATGL in cultured myotubes and murine skeletal muscle and examined molecular adaptations and FA metabolism.

METHODS

Cell culture studies. C2C12 myoblasts were grown in Dulbecco’s Modified Eagle Medium (DMEM; GibcoLife Technologies, Mulgrave, VIC, Australia) (5 mmol/l glucose, 10% fetal bovine serum, 1% penicillin-streptomycin) until confluent and were differentiated into myotubes by changing the medium to DMEM with 2% horse serum. For overexpression studies, cells were infected on day 2 of differentiation with recombinant adenoviral vectors Ad-GFP [1.24 × 10^9 plaque-forming units (pfu)/well] expressing enhanced green fluorescent protein (GFP) or Ad-ATGL (1.56 × 10^9 pfu/well) expressing wild-type ATGL with GFP (fusion protein). The adenoviral vectors were produced and purified by Vector Biolabs (Philadelphia, PA). Experiments were performed 5 days after differentiation. For some experiments, myotubes were treated for 24 h with 125 μM FA mixture (2:1 oleate-palmitate) conjugated to 0.5% FA-free bovine serum albumin (BSA) (Bovogen, Essendon, Victoria, Australia) or 1 μM of the PPARα agonist CAY-10592 (Sapphire Bioscience, Waterloo, NSW, Australia) before lysing.

Mice studies. All experimental procedures were approved by the Monash University School of Biomedical Science Animal Ethics Committee and were conducted in accordance with the National Health and Medical Research Council of Australia’s guidelines on animal experimentation. Male C57BL/6J mice aged 8 wk were purchased from Monash Animal Services (Clayton, Victoria, Australia). Mice were housed under controlled temperature (22°C) and lighting (12:12-h light-dark cycle) and had free access to water and standard rodent chow (Specialty Feeds irradiated rat and mouse pellets; 14.3 MJ/kg digestible energy). ATGL adenoassociated virus (AAV6-CMV-m-PNPLA2, 30 μl, 5 × 10^10 gene copies/ml) was directly injected into the right tibialis anterior (TA) muscle. An empty AAV vector (AAV6-CMV-Null, 30 μl, 5 × 10^10 gene copies/ml) was injected into the left tibialis anterior muscle and used as a control. Five weeks later, mice were fasted from 0700 to 1100 and killed by cervical dislocation, and tissues were rapidly dissected for subsequent analysis. A portion of the TA was embedded in mounting media and frozen in liquid nitrogen. Because the AAV technique also results in vector expression in the extensor digitorum longus (EDL) muscle, which lies in close proximity to the TA muscle, the EDL was excised from tendon to tendon and used for FA metabolism experiments.

FA metabolism. To measure metabolism of exogenous FA, myotubes were incubated for 4 h with [1-14C]palmitate (0.5 μCi/ml; PerkinElmer, Boston, MA) and cold palmitate (0.5 mM), with 2% FA-free BSA in low-glucose DMEM. To determine the specific activity of the TAG pool, some cells were harvested at the end of the pulse (i.e., 0 time point, Fig. 1B). 14C-labeled FA incorporation in the TAG pool was assessed after thin-layer chromatography (TLC, see below), and the total TAG content was determined by biochemical analysis (GPO-PAP reagent; Roche Diagnostics). Following the pulse, myotubes were chased for 4 h in DMEM containing 2% FA-free BSA. The ATGL inhibitor R-Bel (40 μM) and HSL inhibitor NNC (2 μM) were added during the final hour of the pulse and during the chase periods. The production of 14CO2 was measured in independent experiments that assessed TAG-derived (endogenous FA oxidation) and extracellular-derived FA oxidation.

In all experiments, myotubes were harvested in 0.6 ml PBS at the end of the pulse and chase periods to determine 14C-FA incorporation into TAG and diglyceride and for assessment of protein content. The lipid extract was separated by TLC using heptane-isopropanol-acetic acid (60:40:3, vol/vol/vol) as developing solvent. All assays were performed in triplicate, and data were normalized to cell protein content.

Lipolysis. Myotubes were incubated in low-glucose DMEM for 3 h in the presence or absence of 1 μM isoproterenol. Lipolysis was assessed by measuring glycerol release in the culture medium (Free Glycerol Reagent, F6428; Sigma).

Cellular bioenergetics. The cellular bioenergetics profile of myotubes was assessed using the Seahorse XF24 Flux Analyzer (Seahorse Bioscience). Myotubes were seeded into a 24-well XF24 cell culture microplate (Seahorse Bioscience) and were differentiated and infected with adenovirus as described above. Cells were washed and incubated in 600 μl unbuffered DMEM (containing 5 mM glucose, 1 mM pyruvate, and 1 mM glutamate), pH 7.4, at 37°C in a non-CO2 incubator (1 h before bioenergetics assessment). Three basal oxygen consumption rate measurements were performed using the Seahorse analyzer, and measurements were repeated following injection of oligomycin (1 μM), carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (1 μM), and antimycin A (1 μM). Basal extracellular acidification rate (glycolytic flux) was determined from data collected at basal measurement points. Calculations of respiratory parameters of mitochondrial function were performed as previously described (20), and substrate oxidation was independently assessed. Briefly, for glucose oxidation assays, myotubes were incubated in Krebs buffer containing 25 mM glucose as the sole substrate for 60 min before the start of the assay, at 37°C for 60 min. These myotubes were coincubated with either vehicle (H2O) or 100 mM 2-deoxyglucose throughout this time period, and glucose oxidation was measured as the difference in oxygen consumption between vehicle and 2-deoxyglucose-treated myotubes. The parameters of the oxidation assays were based on the work by Wu et al. (42), which showed that total substrate oxidation is not reduced, and in fact is enhanced, when cells are supplied with multiple substrates following acute exposure to 2-deoxyglucose of concentrations up to 200 mM. For palmitate oxidation assays, myotubes were incubated in Krebs buffer containing 0.25 mM palmitate as the sole substrate and were coincubated with vehicle (H2O) or 40 μM etomoxir, and palmitate oxidation was measured as the difference between vehicle and etomoxir-treated myotubes.

Glycolysis was assessed using an independent, radiometric approach. Differentiated C2C12 myotubes were incubated in DMEM containing 5 mM glucose and 1 μCi/ml N-[3-3H]glucose (PerkinElmer) for 2 h. At the conclusion of the experiment, a portion of the medium was counted for total radioactivity, and another portion was incubated overnight at 60°C to evaporate the 3H2O. Glycolysis was determined from the difference in radioactivity between the total and 3H2O fractions.

Quantitative real-time PCR. Myotubes or TA muscle was homogenized in Qiazol, and total RNA was extracted. Reverse transcription of 1 μg mRNA was performed (iScript cDNA Synthesis Kit; Bio-Rad Laboratories, Hercules, CA), and gene products were determined by
quantitative real-time PCR (Realplex Mastercycler; Eppendorf) using the TaqMan Universal PCR Master Mix and TaqMan Gene Expression Assays for Pnpla2 (ATGL), Pparge1a (PGC1a), Cpt1b (CPT1b), Lpl (LPL), Pdk4 (PDK4), Pdn2 (PLIN2), Ucp2 (UCP2), Dgat1 (DGAT), Gpat (GPAT), and Cd36 (CD36). The relative quantification was calculated using the ∆∆Ct method, using 18S rRNA as the housekeeping gene, and values were normalized to the GFP-treated condition.

**Immunoblot analysis.** Myotubes and TA muscle were lysed in RIP buffer containing 1 mM DTT, protease (complete Protease Inhibitor Cocktail; Roche Diagnostics, Castle Hill, NSW, Australia), and phosphatase (PhosSTOP Phosphatase Inhibitor Cocktail; Roche Diagnostics) inhibitors. Protein concentration was determined (Pierce BCA assay; Thermo Scientific, Rockford, IL), and equal amounts of lysate (20 μg) were loaded into 10% Criterion precast stain-free gels (Bio-Rad Laboratories, NSW, Australia) and transferred to PVDF membranes (Trans-Blot Turbo Transfer System; Bio-Rad Laboratories). Stain-free blot images for total proteome visualization were obtained (Chemidoc MP System; Bio-Rad Laboratories). The membranes were blocked with 5% milk, washed, and probed with antibodies raised against ATGL (no. 2138; Cell Signaling), OXPHOS (Mitoprotein, total OXPHOS rodent WB antibody cocktail, MS604), phospho-acetyl-CoA carboxylase (ACC) Ser79 (no. 3661; Cell Signaling), ACC (C83B10) (no. 3676; Cell Signaling), and AMPK (no. 2532; Cell Signaling). After being washed, membranes were probed with the appropriate secondary antibody (anti-rabbit IgG; GE Healthcare). Bands were detected with enhanced chemiluminescence (Clarity Western ECL Substrate; Bio-Rad Laboratories) and quantified by densitometry (ImageLab, version 4.1; Bio-Rad). The immunoreactive signal was normalized to the density of the total protein loading for each sample, which was obtained by visualization of the stain-free blot image and quantified using ImageLab.

**PPARα/δ activity.** PPARα/δ activity was determined in whole cell lysates from myotubes using a commercially available ELISA.
ATGL and mitochondrial biogenesis...
ATGL overexpression activates a subset of PPARα/δ target genes and promotes Ppargc1a expression. Restoring ATGL function in ATGL null mice activates a subset of PPARα target genes to restore mitochondrial function in the heart (12). While ATGL null mice have normal mitochondrial capacity and function in skeletal muscle (13, 26), we hypothesized that ATGL-mediated FA turnover would provide a ligand(s) to enhance PPAR-mediated signaling and enhance mitochondrial function. ATGL overexpression in myotubes increased a subset of known PPARα/δ genes (6), including Cpt1b, Lpl, Pdk4, and Plin2 (Fig. 3A). ATGL overexpression also increased the expression of Ppargc1a (Fig. 3B), a transcriptional coactivator of mitochondrial biogenesis.

We next tested whether a generalized increase in FA availability, independent of increased ATGL content, would increase the same subset of PPAR target genes. FA uptake rates into myotubes were similar between Ad-GFP and Ad-ATGL (Fig. 3C). FA treatment did not affect PPAR target genes or Plin2 (Fig. 3A). ATGL overexpression also increased the expression of Ppargc1a (Fig. 3B), a transcriptional coactivator of mitochondrial biogenesis.
were not altered by FA treatment, suggesting that increasing FA flux into the myotubes per se is insufficient to modulate the same transcriptional program as the ATGL-mediated lipid flux. Because ATGL overexpression appeared to provide an environment capable of increasing PPAR target genes, we assessed PPARα/δ activity in cell lysates. PPARα activity was not different between treatments (data not shown), whereas PPARδ activity was marginally increased in ATGL- compared with GFP-overexpressing cells (Fig. 3E). We also tested whether the ATGL-mediated gene expression changes overlapped with transcriptional alterations induced by a highly specific PPARδ receptor agonist, CAY10592. There was no consistency in gene expression changes induced by ATGL and CAY10592, suggesting that the changes in gene expression by ATGL overexpression are not exclusively acting through PPARδ (Fig. 3F). Moreover, CAY10592 increased the expression of some genes (Cpt1b, Pdk4) but not others (Lpl, Plin2, Ppargc1a) in ATGL-overexpressing myotubes (data not shown), providing further support for the interpretation that ATGL overexpression induces changes in gene expression independent of PPARδ.

ATGL overexpression enhances mitochondrial capacity. The notion that ATGL overexpression enhances mitochondrial capacity was supported by increased expression of respiratory chain proteins (Fig. 4A). We directly assessed mitochondrial function by measuring cellular respiration in the myotubes. Both basal and maximal respiration was increased in ATGL-compared with GFP-overexpressing cells (Fig. 4, B and C). When using the Seahorse Flux Analyzer, quantitative measures of specific substrates are complicated by the utilization of both extracellular (glucose or FFA added to the culture medium) and intracellular (both TAG-derived FA and glycogen-derived...
glucose) fuels. Therefore, we assessed total glucose oxidation by subtracting total glucose oxidation from 2-deoxyglucose-insensitive oxidation (which measures oxidation from sources other than glucose). Glucose accounted for ~90% of the total substrate oxidized (data not shown), and glucose oxidation was not different between Ad-ATGL vs. Ad-GFP-expressing myotubes (12% difference, \( P = 0.28 \)) (Fig. 4D). Similarly, a small, nonsignificant (7%, \( P = 0.07 \)) increase in FA oxidation was observed in Ad-ATGL myotubes (Fig. 4D). The extracellular acidification rate (i.e., glycolysis) was increased in ATGL-overexpressing myotubes (Fig. 4E). This result was confirmed using an independent radiometric approach (Ad-GFP: 1.01 ± 0.01 vs. Ad-ATGL: 1.15 ± 0.03 nmol·mg protein\(^{-1}\)·h\(^{-1}\), \( P = 0.0001 \)). Collectively, these data demonstrate a mild increase in energy turnover in Ad-ATGL myotubes. The increased substrate oxidation was associated with increased phosphorylation of S\(^{\prime}\)-AMPK at Thr\(^{172} \) (Fig. 4F). Phosphorylation of the AMPK substrate ACC\(\beta\) Ser\(^{221} \) was increased by 275% (Fig. 4, G, H).

Fig. 4. ATGL overexpression in myotubes increases mitochondrial protein content and oxidative capacity. Myotubes were infected with adenovirus containing Pnpla2 (ATGL) or GFP. A: contents of respiratory chain proteins were assessed by immunoblot; \( n = 4 \) lane from 2 separate experiments. B–D: mitochondrial respiration was assessed in a Seahorse XF analyzer. B: basal respiration. C: maximal respiratory capacity was assessed after addition of carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone. D: oxygen consumption rate in response to glucose or FA substrates. E: glycolysis was assessed by measuring the extracellular acidification rate; \( n = 18 \) from 4 independent experiments. F and G: AMP-activated protein kinase (AMPK) Thr\(^{172} \) phosphorylation (F) and acetyl-CoA carboxylase (ACC)-\(\beta\) Ser\(^{221} \) phosphorylation (G) determined by immunoblot analysis; \( n = 3 \) Ad-GFP and \( n = 6 \) Ad-ATGL from 2 independent experiments. H: representative immunoblot for AMPK Thr\(^{172} \), AMPK, ACC\(\beta\) Ser\(^{221} \), and ACC. *\( P < 0.05 \) vs. Ad-GFP.
In vivo overexpression of ATGL promotes moderate changes in skeletal muscle and does not enhance FA oxidation. To explore the role of ATGL on molecular adaptations in skeletal muscle in vivo, we injected an AAV-containing Pnpla2 (ATGL) into the TA muscle of one leg and an AAV-containing enhanced GFP in the contralateral TA of mice. The AAV increased ATGL mRNA (Fig. 5A) and protein content (Fig. 5, B and C) by ~20-fold above the AAV- and GFP-injected leg. ATGL protein content was also increased by ~15-fold in the EDL muscle (data not shown). Immunohistochemistry showed that the AAV targeted the vast majority of myofibers (Fig. 5D). ATGL overexpression induced modest effects in skeletal muscle. Expression of genes encoding proteins involved in lipid metabolism tended to increase (Fig. 5E), there was a tendency for several respiratory complex proteins to increase (Fig. 5F), and succinate dehydrogenase content was increased (Fig. 5G). The maximal activity of citrate synthase, a regulator of tricarboxylic acid cycle flux, was increased with ATGL overexpression (Fig. 5H), whereas the maximal activity of the β-oxidation enzyme β-hydroxyacyl CoA-dehydrogenase was not changed (Fig. 5I). Functionally, ATGL overexpression resulted in a reduction of radiolabeled FA incorporation into the TAG pool (Fig. 5J), with no change in FA oxidation (Fig. 5K). Consistent with our results in cultured myotubes, ATGL overexpression increased AMPK and ACCβ protein contents (Fig. 5, L and M). Altogether, marked ATGL overexpression induced modest molecular changes in skeletal muscle, but these were not sufficient to alter FA oxidation.

DISCUSSION

The skeletal muscle is subjected to extraordinary metabolic challenges that include pronounced increases in energy turnover during high-intensity activity and the requirement for sustained increases in substrate oxidation and ATP production during prolonged physical exertion. Accordingly, skeletal muscle exhibits remarkable plasticity by responding to mechanical

---

**Fig. 5.** Overexpression of ATGL in skeletal muscle of mice, molecular adaptations and FA metabolism. Mice were injected with adenoassociated virus (AAV) containing ATGL in the tibialis anterior (TA) of one leg and AAV containing GFP in the contralateral leg. A: Pnpla2 expression in TA as assessed by qRT-PCR. B: ATGL content in TA as assessed by immunoblot analysis. C: representative immunoblot of ATGL in TA. D: immunofluorescence images showing GFP expression in TA. Scale bar, 50 μm. E: mRNA levels were measured by RT-qPCR and normalized to 18S RNA. F: contents of respiratory chain proteins in TA were assessed by immunoblot. G: succinate dehydrogenase staining in TA of mice. Nos. in images (1, 2, and 3) denote different mice. H and F: citrate synthase (H) and β-hydroxyacyl CoA-dehydrogenase (I) activities determined in TA lysates. J: incorporation of FA into intracellular TAG in extensor digitorum longus (EDL). K: FA oxidation in EDL. L: AMPK Thr172 content (L) and ACCβ Ser212 content (M) determined by immunoblot analysis in TA; n = 10/group for all analyses except mRNA where n = 8/group. *P < 0.05 vs. Ad-GFP.

---

G and H), but failed to reach statistical significance when expressed relative to ACCβ, which is due to an increase in total ACCβ protein content (+140%).
and metabolic stress signals that in turn activate transcriptional programs to enhance the capacity of the tissue to deal with future metabolic insults (8). FA are ligands for nuclear transcription factors involved in metabolic reprogramming (25), and increasing FA delivery to skeletal muscle through dietary interventions (35) or lipid infusions (7, 23) increases the capacity for FA oxidation. The goal of this study was to determine whether increasing FA flux from intramyocellular TAG by ATGL overexpression is sufficient to induce metabolic adaptations within muscle. Our findings show that ATGL overexpression is sufficient to reprogram skeletal muscle and alter mitochondrial capacity in cultured myotubes in vitro but that ATGL overexpression does not induce marked changes in molecular programming of the skeletal muscle metabolic phenotype in vivo.

A large proportion of the plasma-derived FFA taken up by skeletal muscle is first trafficked into intramyocellular TAG before they are released and enter the long-chain acylcarnitine pools for oxidation (5, 15, 29). These data suggest that the lipid droplet is a central control point for intramyocellular FA flux. A technical limitation of these in vivo approaches using arteriovenous balance techniques in humans is that the specific source of FA draining into the venous circulation cannot be determined because FA can be generated from lipolysis of intramyocellular TAG droplets, intramuscular adipocytes residing within muscle groups, or intramuscular adipocytes residing between muscle groups (10). Examining FA flux in cultured myotubes circumvents these limitations. Hence, the first aim of this study was to determine whether FA first traverse the intramyocellular TAG pool before undergoing lipolysis to be released from the lipid droplet for oxidation or other actions within myocytes. Our pulse-chase studies in cultured myotubes show that pharmacological inhibitors of ATGL and HSL reduce intracellular TAG lipolysis and the oxidation of FA derived from intramyocellular sources. Critically, when radiolabeled FA are added to the culture medium (i.e., representing “plasma”-derived FA), ATGL and HSL inhibition increases radioactivity in the intramyocellular TAG pool and decreases FA oxidation. The reduction in FA oxidation with ATGL and HSL inhibition was proportionally the same whether derived from intracellular or extracellular sources. These data support previous reports in humans showing that ATGL overexpression does not induce marked changes in molecular programming of the skeletal muscle metabolic phenotype in vivo.

Consistent with the changes in gene expression, ATGL overexpression also increased PGC1α gene expression, a regulator of mitochondrial biogenesis (28), as well as the protein contents of several components of the respiratory chain. Mitochondrial function was similarly impacted by ATGL overexpression with increased basal and maximal respiration rates. These results demonstrate that an increase in ATGL can increase the oxidative capacity of myotubes, although the importance of ATGL to promote mitochondrial function has been shown to be more prominent in other cell types (1, 34). In macrophages, for example, knock down of ATGL resulted in impaired mitochondrial function and programmed cell death (1). Similarly, in pancreatic β-cells, deletion of ATGL decreased the expression of PPARδ target genes involved in mitochondrial oxidation, and coincided with impaired mitochondrial respiration. Adenoviral overexpression of ATGL restored these mitochondrial defects in pancreatic β-cells and normalized glucose-stimulated insulin secretion (34). Together, these findings confirm the importance of ATGL for maintaining mitochondrial function in skeletal muscle and other cell types in vitro.

Skeletal muscle ATGL content is upregulated ∼2.5-fold by regular endurance exercise training in humans (4), suggesting that FA flux through the lipid droplet may contribute to transcriptional reprogramming. On the other hand, it was previously shown that ATGL transgenic mice harboring a 1.5-fold increase in ATGL protein content above wild-type
mice exhibited a small reduction in intramyocellular TAG and no change in mitochondrial content or function (32). In light of these contrasting observations, we surmised that germ line alterations in ATGL function might induce compensatory molecular regulation in mice and/or a robust increase in ATGL, above the modest 1.5-fold reported previously in transgenic mice (32), is required to drive metabolic reprogramming. To overcome these issues, we increased ATGL in the skeletal muscle of adult mice by AAV-ATGL administration, which increased ATGL content 20-fold. While there was an increase in intramyocellular TAG turnover and the expression of several PPARα target genes, the effect on markers of mitochondrial content and capacity was modest and insufficient to drive changes in FA oxidation. Thus, we conclude that, in contrast to isolated myotubes, marked increases in ATGL expression are not a major factor in regulating mitochondrial function in skeletal muscle in vivo. It is likely that other transcriptional programs that are driven by contraction and energy-sensing signaling pathways or alterations in neural inputs or the endocrine milieu override the effects of ATGL overexpression in vivo.

Our studies add to previous work highlighting the tissue-specific importance of ATGL in metabolic regulation. In adipose tissue, for example, there is convincing evidence to show that ATGL is critical for fasting energy homeostasis. Fasting in combination with a deficiency of ATGL resulted in a progressive reduction of oxygen consumption, reduced FA availability for energy production, lethargy, hypothermia, and a proteolytic stress on many organs, including the heart and skeletal muscle (43). On the other hand, a three- to fivefold overexpression of ATGL in adipose tissue promoted FA oxidation and attenuated diet-induced obesity (3). In brown adipose tissue, ATGL is required to maintain thermogenic capacity by providing FA substrate for uncoupled respiration (2), and, in the heart, ATGL is required to maintain mitochondrial function via PPARα-related programming (12). Thus, while ATGL might be less important in regulating metabolic adaptations that promote mitochondrial function in skeletal muscle (present data and Refs. 14 and 26), it is clearly a major regulator of these events in other tissues.

The implications of this work relate to understanding lipid fluxes in muscle and understanding strategies to combat metabolic complications. The in vitro studies support previous human work showing that a large proportion of FA that enter muscle are first stored in lipid droplets before their eventual oxidation. Furthermore, our findings demonstrate that enhancing FA flux from lipid droplets can remodel myotubes to increase the transcriptional program to drive mitochondrial capacity, which enhances oxidative capacity and reduces the lipid content in vitro. However, this adaptive program is of minor importance when superimposing the normal metabolic stresses encountered in free-moving animals, indicating that, unlike other tissues such as the heart and adipose tissue, ATGL overexpression is of minor importance in the remodeling skeletal muscle to enhance the oxidative disposal of FA.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


