Role of PGC-1α during acute exercise-induced autophagy and mitophagy in skeletal muscle

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Vainshtein A, Tryon LD, Pauly M, Hood DA. Role of PGC-1α during acute exercise-induced autophagy and mitophagy in skeletal muscle. Am J Physiol Cell Physiol 308: C710–C719, 2015. First published February 11, 2015; doi:10.1152/ajpcell.00380.2014.—Regular exercise leads to systemic metabolic benefits, which require remodeling of energy resources in skeletal muscle. During acute exercise, the increase in energy demands initiate mitochondrial biogenesis, orchestrated by the transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α). Much less is known about the degradation of mitochondria following exercise, although new evidence implicates a cellular recycling mechanism, autophagy/mitophagy, in exercise-induced adaptations. How mitophagy is activated and what role PGC-1α plays in this process during exercise have yet to be evaluated. Thus we investigated autophagy/mitophagy in muscle immediately following an acute bout of exercise or 90 min following exercise in wild-type (WT) and PGC-1α knockout (KO) animals. Deletion of PGC-1α resulted in a 40% decrease in mitochondrial content, as well as a 25% decline in running performance, which was accompanied by severe acidosis in KO animals, indicating metabolic distress. Exercise induced significant increases in gene transcripts of various mitochondrial (e.g., cytochrome oxidase subunit IV and mitochondrial transcription factor A) and autophagy-related (e.g., p62 and light chain 3) genes in WT, but not KO, animals. Exercise also resulted in enhanced targeting of mitochondria for mitophagy, as well as increased autophagy and mitophagy flux, in WT animals. This effect was attenuated in the absence of PGC-1α. We also identified Niemann-Pick C1, a transmembrane protein involved in lysosomal lipid trafficking, as a target of PGC-1α that is induced with exercise. These results suggest that mitochondrial turnover is increased following exercise and that this effect is at least in part coordinated by PGC-1α.

Anna Vainshtein received the AJP-Cell 2015 Paper of the Year Award. Listen to a podcast with Anna Vainshtein and coauthor David A. Hood at http://ajpcell.podbean.com/e/ajp-cell-paper-of-the-year-2015-award-podcast/

Skeletal muscle possesses a remarkable capacity to adapt to alterations in contractile activity, a property referred to as muscle plasticity. This type of cellular remodeling often requires a shift in metabolic profile, with amendments to the structure of the mitochondrial network, as well as changes in mitochondrial content. Organelle density is determined by the balance between its synthesis and degradation. Mitochondrial biogenesis is regulated transcriptionally through the coordinated expression of nuclear and mitochondrial genes, orchestrated largely by the transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) (18, 41). Conversely, organelle degradation is mediated by a selective form of macroautophagy known as mitophagy (13, 15). During mitophagy, dysfunctional mitochondria are first segregated from the healthy network by fission and are then tagged for elimination (14, 37). Mitochondrial targeting can occur via mitophagy-specific receptors such as Bcl-2/adenovirus E1B 19-kDa interacting protein 3-like (Nix/Bnip3) (14, 26, 30) or by ubiquitination of mitochondrial outer membrane proteins by E3 ligases such as Parkin and Mul1 (4, 21, 25). Tagged mitochondria are then recognized by, and engulfed into, double-membrane vesicles termed autophagosomes. Autophagosomes are subsequently delivered to the lysosome for proteolytic degradation.

Several recent studies have indicated that autophagy is activated following an acute bout of endurance exercise (5, 6, 8–10, 20, 22) and may contribute to chronic exercise-induced improvements in muscle health and oxidative capacity. Indeed, deficient autophagy results in lack of exercise-mediated metabolic benefits, as well as progressive degeneration of mitochondrial function and performance (6, 20, 22). Coincidentally, PGC-1α levels have also been demonstrated to increase following an acute bout of endurance exercise (2), and PGC-1α is localized to the nucleus during the postexercise recovery period. The significance of this is that the absence of PGC-1α also results in diminished exercise-induced metabolic benefits (2, 16). However, it is unknown whether PGC-1α plays a role in acute exercise-induced autophagy or in the regulation of mitophagy flux. Thus we set out to investigate the function of PGC-1α in mediating autophagy and mitophagy induction in skeletal muscle following an acute bout of exercise. Our results should shed light on how the mitochondrial biogenesis and degradation pathways may interact to ensure proper mitochondrial remodeling and, thus, contribute to muscle plasticity as a result of exercise.

MATERIALS AND METHODS

Animal generation, treatment, and exercise. PGC-1α whole body knockout (KO) and C57BL/6 wild-type (WT) mice were housed in a 12:12-h light-dark cycle and given food and water ad libitum. Generation and characterization of PGC-1α KO mice have been previously described (1, 19). To evaluate autophagy flux, animals were...
Endurance run

Exercise protocol details and outcomes

Blood lactate. Blood lactate was measured prior to exercise, immediately postexercise, and following 90 min of recovery. A 0.2-μl blood sample was obtained by tail nick and immediately analyzed using the Lactate Scout analyzer (EKF Diagnostics, Magdeburg, Germany).

Histology. Cytochrome oxidase (COX) and succinate dehydrogenase (SDH) staining was performed on 10-μm cross sections of extensor digitorum longus muscles as previously described (24). Briefly, frozen muscle sections adhered to glass slides were dried and subsequently incubated with COX or SDH reaction solutions for 30 min in darkness at 30°C. Each slide contained sections from all animals to ensure equal staining across the groups. After they were washed with PBS, glass coverslips were mounted onto the slides with DPX Mountant for histology (catalog no. 44581, Fluka) and sealed. Images of stained muscle sections were captured with a Nikon 90i eclipse upright microscope using a ×20 objective.

COX activity. COX enzyme activity was measured as previously described (38) as the rate of oxidation of fully reduced cytochrome c by isolated enzymatic extract, evaluated as a change in absorbance at 550 nm using a microplate reader (Synergy HT, BioTek).

Gene expression analysis. Quantitative real-time PCR was performed to determine mRNA expression levels. Total RNA was isolated using TRIzol reagent (catalog no. 15596-026, Invitrogen). RNA was reverse-transcribed into cDNA using a SuperScript III First-Strand synthesis kit (catalog no. 18080-044, Invitrogen) according to the manufacturer’s instructions. The primers used for gene expression analysis are listed in Table 1 and were designed on the basis of sequences available in GenBank (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi). Analyses were performed with SYBR Green chemistry (PerfeCta SYBR Green Supermix, ROX, catalog no. 95055-500, Quanta BioSciences) in a StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA). GAPDH (GAPdh) and β-actin (Actb) were used in combination as housekeeping genes.

mRNA array. The gene expression of 84 key autophagic genes was profiled by RT² Profiler autophagy PCR arrays (catalog no. PAMM-084, SABioscience) as recommended by the manufacturer. RT-PCRs were performed in 96-well-plate format using the StepOnePlus real-time PCR system. Fold changes in autophagic gene expression from KO samples relative to WT control samples were calculated using the comparative threshold (ΔΔCt) method with the integrated software package for PCR array systems provided by the manufacturer (RT² Profiler PCR Array Data Analysis Template v3.3). ΔΔCt values from

Fig. 1. Lack of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) results in diminished mitochondrial content and reduced exercise performance. A: representative images of cytochrome oxidase (COX) and succinate dehydrogenase (SDH) staining of extensor digitorum longus muscle from control [wild-type (WT)] and PGC-1α knockout (KO) animals. Scale bars = 100 μm. B: COX activity as a surrogate measure of mitochondrial content in WT and KO animals. C: after 2 days of habituation to the treadmill, animals were run to failure utilizing an incremental exercise protocol on a 0% incline. Animals began with a warm-up period of 5 min at 5 m/min and 10 min at 10 m/min followed by 45 min of endurance running at 15 m/min. Finally, running speed was increased by 1 m/min every 2 min until the animals refused to continue. D: running performance (i.e., total distance run) in WT and KO animals injected with water [vehicle (Veh)] or 0.4 mg/kg colchicine (Col). E: blood lactate levels in WT and KO animals prior to exercise (Con), immediately following exercise (Ex), and following 90 min of recovery (Ex + R). Values are means ± SE; n = 4–12 for all groups. *P < 0.05, significant effect of exercise. †P < 0.05, significant effect of genotype.
Membranes were incubated overnight at 4°C with the appropriate which were blocked with 5% skim milk or 5% BSA solution. rated by SDS-PAGE and transferred to nitrocellulose membranes, sections (22), isolated mitochondria, or nuclear extracts were sepa-

mM MgCl2, 1 mM EDTA, and 1 mM EGTA) supplemented with isolation buffer (250 mM sucrose, 20 mM HEPES, 10 mM KCl, 1.5 using a Teflon pestle and mortar and suspended in mitochondrial subfractions were isolated by differential centrifugation, as previously normalized to the appropriate loading control.

Minescence. Quantification was performed with ImageJ software for that room temperature and visualized with enhanced chemilu-

Sciences). Membranes were subsequently washed and incubated with p62 (Sigma-Aldrich), PGC-1 dynamin-related protein 1 (Drp-1; BD Transduction Laboratories), dependent anion channel, and Niemann-Pick C1 (NPC1; Abcam), (LC3), and Parkin (Cell Signaling Technology), GAPDH, voltage-

RESULTS

PGC-1α KO animals exhibit diminished mitochondrial content, reduced endurance capacity, and metabolic stress with exercise. Deletion of PGC-1α resulted in significantly lower mitochondrial content in skeletal muscle, as demonstrated by reduced SDH, as well as COX, staining and a ~40% decrease in COX activity (Fig. 1, A and B). To examine the involvement of PGC-1α in exercise-induced autophagy, 3-mo-old PGC-1α KO and WT animals were subjected to an acute bout of incremental treadmill running (Fig. 1, C–E). The lack of PGC-1α resulted in diminished endurance performance, as the KO animals ran significantly less than their WT counterparts (Fig. 1D). Moreover, KO animals exhibited a ~40% higher blood lactate basally, which increased by 3.8-fold with exer-

Table 1. Primer sequences based on gene transcripts available in GenBank

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Fig. 2. Signaling kinases are activated with exercise. A–C: blots and quantifi-

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cise, compared with a 2.8-fold increase in WT animals (Fig. 1E). We also noted that the blood lactate of KO animals did not return to baseline as effectively as that of WT animals during the 90-min recovery period. This indicates increased metabolic stress and a greater reliance on glycolysis in the KO animals. Metabolic stress was evident from changes in the phosphorylation of the metabolically sensitive p38 MAPK and AMPPK. Exercise resulted in a ~2.5-fold increase in phosphorylation of AMPPK in WT animals, while KO animals exhibited a ~4-fold induction (Fig. 2, A and B). Phosphorylation of p38 MAPK was elevated by ~50% with exercise in WT and KO animals. However, KO animals expressed higher levels of phosphorylated p38 overall, which did not return to baseline during recovery (Fig. 2, A and C).

PGC-1α localization to the nucleus and gene expression are elevated in response to exercise, resulting in induction of mitochondrial biogenesis in WT animals. Exercise induced localization of PGC-1α to the nucleus (Fig. 3A), which was accompanied by an 8.5-fold increase in PGC-1α (Ppargc1a) mRNA expression following recovery (Fig. 3B). The increase in PGC-1α with exercise resulted in ~60% and ~50% inductions in expression of the downstream targets COX subunit IV (Coxiv) and mitochondrial transcription factor A (Tfam), which were not evident in the KO animals (Fig. 3, C and D). Thus an acute bout of exercise was sufficient to induce the onset of mitochondrial biogenesis in a PGC-1α-dependent manner, an effect that was not observed in KO animals.

Acute exercise results in induction of autophagy, which is differentially regulated in PGC-1α KO animals. During autophagy, p62 and LC3 are consumed within the lysosome while accompanying their respective organelle targets. A continuous transcriptional contribution is, therefore, required to avoid exhaustion of these important autophagy factors. We observed ~80% and ~94% inductions of microtubule-associated protein 1 light chain 3 [Maplc3b (LC3)] and sequestosome 1 [Sqstm1 (p62)] transcript levels with exercise, respectively. However, this effect was abolished in KO animals (Fig. 4, A and B). In contrast, the transcript levels of additional autophagy [autophagy related 7 (Atg7) and beclin 1 (Becn1)] and lysosomal [cathepsin D (Catsd) and lysosome-associated membrane protein 2 (Lamp2)] markers were not different between WT and KO animals and did not change with exercise or recovery (data not shown). Our acute exercise protocol was sufficient to induce LC3 lipidation in the muscle of WT animals; however, we did not observe this increase in the KO mice until after the recovery period, indicating a delayed response to exercise in KO animals (Fig. 4, C and D). The levels of p62 did not change immediately following exercise in WT animals but increased with recovery (Fig. 4, C and E). Conversely, p62 levels were elevated immediately after exercise and following recovery in KO animals (Fig. 4, C and E). This is indicative of impaired autophagosome degradation of p62 in KO animals.

One major difficulty in evaluating autophagic flux is the extremely dynamic and transient nature of autophagosomes, since the average half-life of this organelle is ~10 min (20). Therefore, we evaluated autophagy flux by measuring the percent change in LC3II and p62 protein in vivo with interperitoneal administration of the microtubule-destabilizing drug colchicine, as previously described (12). Colchicine blocks autophagosome degradation by destabilizing the microtubule tracks on which they travel to the lysosome for degradation, resulting in accumulation of autophagosomes and, thus, a backlog of LC3II and p62. Interestingly, LC3II flux tended to increase immediately following exercise and after recovery in WT animals but was lower overall and did not follow a similar trend in KO animals (Fig. 4F). We also observed a trend for an increase in p62 flux with exercise in WT and KO animals (Fig. 4G); however, this tendency was attenuated in the KO animals. Taken together, these results indicate an induction of au-
ubiquitin ligase Parkin, which has been well documented to geting with exercise, we examined the mitochondrial E3 mitochondrial localization of the protein was induced 3.5-fold, while mitochondrial localization of the protein was induced 3.5-fold, following exercise and remained elevated during the recovery period in WT animals (Fig. 6, A–C). Parkin mRNA tended to increase with exercise in KO animals, but this increase was not statistically significant (Fig. 6A). Importantly, Parkin localization to the mitochondria was both delayed and attenuated in the KO animals, increasing by 2.6-fold only after recovery (Fig. 6, B and C). Elevated Parkin in WT animals translated to a 60% increase in ubiquitination of mitochondrial proteins following exercise, and this effect was absent in KO animals (Fig. 6, B and D). Since an important prerequisite for mitochondrial degradation by autophagy is organelle fragmentation (37), we also examined mitochondrially localized Drp-1, a fission protein that translocates to mitochondria and facilitates their fragmentation. We found that localization of Drp-1 to the mitochondrial subfraction increased 2.2-fold following exercise and returned to baseline during recovery in WT animals (Fig. 6, B and E). No change in mitochondrial Drp-1 levels was observed in KO animals. Thus these results be intimately involved in mitophagy (25). Interestingly, Parkin mRNA levels were elevated 3-fold, while mitochondrial localization of the protein was induced 3.5-fold, following exercise and remained elevated during the recovery period in WT animals (Fig. 6, A–C). Parkin mRNA tended to increase with exercise in KO animals, but this increase was not statistically significant (Fig. 6A). Importantly, Parkin localization to the mitochondria was both delayed and attenuated in the KO animals, increasing by 2.6-fold only after recovery (Fig. 6, B and C). Elevated Parkin in WT animals translated to a 60% increase in ubiquitination of mitochondrial proteins following exercise, and this effect was absent in KO animals (Fig. 6, B and D). Since an important prerequisite for mitochondrial degradation by autophagy is organelle fragmentation (37), we also examined mitochondrially localized Drp-1, a fission protein that translocates to mitochondria and facilitates their fragmentation. We found that localization of Drp-1 to the mitochondrial subfraction increased 2.2-fold following exercise and returned to baseline during recovery in WT animals (Fig. 6, B and E). No change in mitochondrial Drp-1 levels was observed in KO animals. Thus these results
indicate an increased targeting of mitochondria for mitophagy postexercise, which is, at least in part, dependent on PGC-1α.

**Exercise-mediated alterations in transcriptional regulators of autophagy are not different between WT and KO animals.** Transcriptional regulation of autophagy with exercise has not been thoroughly examined. Therefore, we investigated Forkhead box O3 (FoxO3) and transcription factor EB (TFEB), two well-known transcriptional regulators of the autophagy-lysosome system. FoxO3 expression was induced 2.8-fold with exercise in WT animals and was consistently higher in KO animals across all conditions (Fig. 7A). TFEB mRNA expression was not altered during exercise in WT or KO animals but tended to increase after recovery in WT animals only (Fig. 7B). Thus, both TFEB and FoxO3 may play a role in transcriptional regulation of autophagy, but neither appears to be regulated by PGC-1α in this context.

NPC1 is a novel autophagy factor regulated by PGC-1α. To identify additional autophagy factors that may be under the control of PGC-1α, we performed an unbiased PCR-based mRNA array and compared the expression of 84 autophagy-related genes (for a complete list of genes see Supplemental Table S1 in Supplemental Material for this article, available online at the Journal website) in WT and PGC-1α KO animals. Several genes, including mammalian target of rapamycin (mTOR), NF-κB (Nfkbi), phosphatidylinositol 4,5-bisphosphate 3-kinase (Pik3cg), and NPC1, were dramatically downregulated in KO compared with WT animals (Fig. 8A). We chose to further investigate NPC1, a novel transmembrane protein responsible for cholesterol trafficking in late endosomes and lysosomes. Mutations in NPC1 result in Niemann-Pick disease type C, an autosomal recessive neurovisceral lipid storage disorder, which is accompanied by impaired autophagy (17, 33). We first confirmed our array findings with real-time PCR and found that NPC1 expression was indeed diminished by the lack of PGC-1α (Fig. 8B). Interestingly, NPC1 expression was induced by ~80% with exercise and returned to basal levels during recovery in WT animals. The exercise-mediated increase in NPC1 was abolished in KO animals. We also found that NPC1 protein levels were significantly lower in KO animals than in their WT counterparts (Fig. 8C). Thus we have identified NPC1 as a novel autophagy factor that is induced with exercise, and this induction appears to be PGC-1α-dependent.

**DISCUSSION**

Skeletal muscle is a malleable tissue that rapidly adapts to its metabolic environment. Much research has focused on establishing the mechanism responsible for this remarkable plasticity, as it has great therapeutic potential for a vast array of muscle and metabolic pathologies. The energy demands stemming from muscle contraction are known to initiate signaling cascades, which lead to increased mitochondrial biogenesis to enhance the energetic potential of muscle. This exercise signaling largely converges on PGC-1α, a transcriptional coactivator responsible for orchestrating the mitochondrial biosynthesis program. However, it is not known how mitochondrial degradation is regulated during exercise, and the role of PGC-1α in this process has not been conclusively determined.

**Fig. 5.** Exercise-induced mitophagy signaling and flux are attenuated in PGC-1α KO animals. A–E: blots and quantification of autophagic proteins and flux in isolated mitochondrial fractions from WT and KO animals in Con, Ex, and Ex + R groups treated with vehicle or colchicine (0.4 mg·kg−1·day−1) for 2 days. Voltage-dependent anion channel (VDAC) was used as loading control. Values are means ± SE; n = 7–9. *p < 0.05, significant effect of exercise.
Thus the purpose of this study was to examine the activation of autophagy and mitophagy during an acute bout of exercise and to evaluate the involvement of PGC-1α in this process.

PGC-1α has been documented to drive mitochondrial biogenesis and expression of oxidative genes (2, 18, 28, 29). It is no surprise, then, that KO animals were found to be profoundly deficient in mitochondria, as observed biochemically by less intense SDH and COX staining, as well as diminished COX activity. This reduction in mitochondrial content translated to a functional deterioration of endurance performance. PGC-1α KO mice exhibited metabolic distress during exercise, evident by elevated lactic acid levels that did not effectively resolve with a 90-min recovery period. This was accompanied by reduced endurance performance compared with WT animals. KO animals also exhibited greater increases in activation of these metabolic sensors AMPK and the cellular stress sensor p38, further supporting metabolic distress in these animals. WT KO animals also exhibited greater increases in activation of the metabolic sensor AMPK and the cellular stress sensor p38, further supporting metabolic distress in these animals. WT animals displayed normal elevations in activation of these sensors in isolated mitochondrial subfractions.

We did not note basal differences in LC3 or p62 levels between WT and KO animals in Con, Ex, and Ex + R groups compared with WT animals in Con group. 

Fig. 6. Lack of PGC-1α results in attenuated exercise-mediated mitophagy signaling. A: Parkin (Park2) gene expression in WT and KO animals in Con, Ex, and Ex + R groups following an acute bout of exercise (7, 31). We noted an increase in nuclear PGC-1α KO mice exhibited metabolic distress during exercise, evident by elevated lactic acid levels that did not effectively resolve with a 90-min recovery period. This was accompanied by reduced endurance performance compared with WT animals. KO animals also exhibited greater increases in activation of the metabolic sensor AMPK and the cellular stress sensor p38, further supporting metabolic distress in these animals. WT animals displayed normal elevations in activation of these kinases with exercise, which returned to basal levels during the recovery period. Our results confirm that mitochondrial biogenesis is initiated with the first bout of exercise and that recovery period. These increases in PGC-1α transcript and nuclear localization were accompanied by induction of mitochondrial genes encoded by the nuclear genome. This increase was abolished in animals lacking PGC-1α. These findings add further substantive support for the importance of PGC-1α in mediating adaptations in oxidative capacity within muscle as a result of exercise.

Several studies have documented induction of autophagy following an acute bout of exercise (5, 6, 8, 9). Here we demonstrate, for the first time, that the transcript levels of the autophagy factors LC3B and p62 are induced immediately following an acute bout of exercise and that this increase is mediated by PGC-1α. We did not detect alterations in the transcript levels of these factors in KO animals 90 min postexercise. We also noted an increase in LC3 lipidation with exercise in WT animals. This response was delayed in KO animals and did not occur until after the recovery period, when LC3II levels returned to baseline in WT mice. Interestingly, we did not note a decrease in p62 levels in WT animals with exercise, as has been previously described (6, 22). This could be a result of differences in the exercise protocol between the studies and, perhaps, increases in p62 mRNA that we noted with exercise. In KO animals we observed the opposite effect: p62 levels were elevated following exercise. Because of the lack of increase in p62 mRNA levels in KO animals, we conclude that the increase in p62 protein with exercise is likely due to impaired degradation by autophagy in these animals. We did not note basal differences in LC3 or p62 levels between...
the genotypes, indicating that this is an exercise-mediated effect. Moreover, we also observed a trend for increases in LC3II and p62 flux in WT animals as determined with colchicine treatment experiments, but this trend was not found in KO mice. Indeed, LC3II flux was lower in KO animals. These results indicate that autophagy induction, gene expression, and flux are induced by exercise and that this induction is compromised in mice lacking PGC-1α. Thus PGC-1α appears to play a role in the regulation of exercise-induced autophagy.

Activation of mitophagy with exercise has only recently been documented and was deemed to be required for removal of dysfunctional mitochondria following damaging downhill running exercise (22). However, regulation of mitophagy in exercising muscle has not been thoroughly examined. Our findings demonstrate that mitophagy signaling, as well as flux, is induced with an acute bout of exercise and that this effect is diminished in animals deficient in PGC-1α. In our hands, exercise-induced mitophagy localization and flux of LC3II were abolished in KO animals. Interestingly, no alterations in p62 localization to the mitochondria were observed with exercise in either genotype, and p62 flux was similarly elevated in WT and KO animals following recovery only. We also noted that Parkin plays a role in exercise-induced mitophagy, as there was an increase in localization of this E3 ligase to the mitochondria. Enhanced abundance of ubiquitinated proteins within the mitochondrial subfraction with exercise was also observed in the WT animals. In contrast, Parkin localization to mitochondria was delayed in PGC-1α KO animals, occurring following recovery, and no increase in mitochondrial protein ubiquitination was observed. It is interesting to note that the Parkin-PGC-1α axis presents a potential point of communication between mitochondrial biogenesis and degradation follow-

![Diagram](Image)

**Fig. 7.** Transcriptional regulators of autophagy with exercise. A and B: gene expression of transcriptional regulators of autophagy [Forkhead box O3 (FoxO3) and transcription factor EB (TFEB)] in WT and KO animals in Con, Ex, and Ex + R groups compared with WT animals in Con group. Gapdh and Actb were used as housekeeping genes. Values are means ± SE; n = 4–9. *P < 0.05, significant effect of exercise. †P < 0.05, significant effect of genotype.

![Image](Image)

**Fig. 8.** Identification of Niemann-Pick C1 (NPC1) as a PGC-1α-regulated autophagy factor through PCR-array analysis. A: heat map showing expression of 84 autophagy-related genes in WT and KO animals. Green indicates reduction, while red indicates increase, in gene expression; the brighter the color, the greater the change in gene expression. (For the full list of fold changes and statistical significance see Supplemental Table S1.) B: gene expression of NPC1 in WT and KO animals in Con, Ex, and Ex + R groups compared with WT animals in Con group. Gapdh and Actb were used as housekeeping genes. C: representative blot and quantification of NPC1 in tibialis anterior muscle extracts. GAPDH was used as loading control. Values are means ± SE; n = 3–4. *P < 0.05, significant effect of exercise. †P < 0.05, significant effect of genotype.
The impairment in mitophagy that we observed in KO animals could also be due, in part, to altered organelle dynamics. Fission is a prerequisite for mitophagy (37), and in our model the localization of the fission protein Drp-1 to the mitochondria was increased after exercise in WT, but not KO, animals. This further supports our findings that mitophagy is activated with exercise and that PGC-1α is involved in this process.

Although several factors have been identified to participate in the transcriptional regulation of autophagy (3, 23, 35, 42), little is known about this process in skeletal muscle, and the regulation of this process with exercise has not been investigated. Therefore, we examined the expression of FoxO3 and TFEB, two transcriptional regulators that have been well documented to induce autophagic gene expression in different cells and tissues. FoxO3 transcript levels were induced with exercise, suggesting a role for this protein in mediating the increased expression of autophagy genes with exercise. However, we also noted an overall higher expression of FoxO3 in KO animals, indicating that FoxO3 is not likely to mediate PGC-1α-induced autophagy. This is in line with previous evidence implicating PGC-1α in the suppression of FoxO3 under atrophic conditions (32). We also investigated TFEB, a master transcriptional regulator of the autophagy-lysosome system (27, 35), previously documented to play a role in the induction of PGC-1α expression in the liver during nutrient deprivation (34). We noted a trend for increased TFEB transcript levels during recovery only in WT animals. The lack of this trend in KO animals suggests a potential role for TFEB in PGC-1α-mediated autophagic induction. Indeed, we previously observed reduced TFEB protein levels in KO animals compared with their WT controls (unpublished observations) under basal conditions. It is possible that PGC-1α may bind and coactivate TFEB on the promoter of autophagy-related genes; however, this warrants further investigation.

In an attempt to further characterize the endogenous role of PGC-1α in autophagy regulation, we performed an unbiased mRNA array to examine 84 genes involved in various aspects of autophagy. Our array data reveal multiple genes that were downregulated in KO animals. We further focused on NPC1, a novel transmembrane protein involved in regulation of cholesterol trafficking from late endosomes and lysosomes. NPC1 protein and mRNA were strongly downregulated in PGC-1α KO animals but were induced with exercise in WT animals, suggesting a role for this protein in exercise-induced adaptations. NPC1 has not been previously studied in this context, but mutations in NPC1 result in Niemann-Pick disease, a devastating lysosomal storage disease characterized by defective autophagy and increased cholesterol load (39). Moreover, polymorphisms or haploinsufficiency in the NPC1 gene have been correlated with obesity and type 2 diabetes (11, 40). Very little is known about NPC1 and its role in skeletal muscle.

Taken together, our findings indicate that exercise-induced metabolic adaptations involve augmented mitochondrial turnover that engages concomitant increases in degradation and biogenesis. Our results also demonstrate that the transcriptional coactivator PGC-1α coordinates mitochondrial biogenesis and mitophagy immediately following exercise and that both of these processes are compromised in the absence of the coactivator. This study sheds light on the mechanisms underpinning mitochondrial turnover induced by exercise, implicating PGC-1α in orchestrating this process.

GRANTS

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

A.V., L.D.T., and M.P. performed the experiments; A.V. analyzed the data; A.V. and D.A.H. interpreted the results of the experiments; A.V. prepared the figures; A.V. and D.A.H. drafted the manuscript; A.V. and D.A.H. edited and revised the manuscript; A.V., L.D.T., M.P., and D.A.H. approved the final version of the manuscript; D.A.H. developed the concept and designed the research.

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