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Exercise Training-Induced Regulation of Mitochondrial Quality

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YAN, Z., V.A. LIRA, and N.P. GREENE. Exercise training-induced regulation of mitochondrial quality. *Exerc. Sport Sci. Rev.*, Vol. 40, No. 3, pp. 159–164, 2012. *Mitochondria are dynamic organelles in skeletal muscle critical in physical performance and disease. The mitochondrial life cycle spans biogenesis, maintenance, and clearance. Exercise training may promote each of these processes, conferring positive impacts on skeletal muscle contractile and metabolic functions. This review focuses on the regulation of these processes by endurance exercise and discusses potential benefits in health and disease.* **Key Words:** skeletal muscle, exercise-induced adaptation, mitochondrial biogenesis, mitochondrial fission, mitochondrial fusion, autophagy, mitophagy

INTRODUCTION

Endurance exercise long has been recognized to not only enhance physical performance but also bring about beneficial health outcomes. The most studied phenotypic adaptations in skeletal muscle to chronic exercise are increased vascularization (angiogenesis), fiber type transformation toward oxidative myofibers, and increased mitochondrial content/function. Extensive effort has been afforded to the pursuit of understanding the underlying molecular mechanisms that regulate mitochondrial biogenesis, the process by which new mitochondria are formed. These efforts have culminated with the discovery of peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator-1α (PGC-1α) (38) and the unveiling of its function in exercise-induced mitochondrial biogenesis in skeletal muscle. Interestingly, data from Krieger *et al.* (20) suggest that exercise improves the function of the subsarcolemmal portion of mitochondria. Therefore, exercise seems to not only increase the number of organelles but also improve the function/efficiency of the mitochondrial pool/network. The resulting functional improvement in the mitochondrial network most likely results from increased rates of mitochondrial biogenesis and efficient removal of dysfunctional/damaged mitochondria. In other words, mitochondrial biogenesis is critical but may not be the only regulatory event that leads to the improved function of the mitochondrial network in skeletal muscle. Although a discussion of the many facets of mitochondrial function is beyond the scope of this review, it is known that this organelle is not only responsible for the aerobic synthesis of adenosine triphosphate (ATP) but also may affect calcium (Ca^{2+}) homeostasis and redox state in muscle cells. In fact, exercise training seems to positively impact all of these aspects in skeletal muscle. However, mitochondria, like other organelles, are subject to damage, and the mitochondrial DNA is especially susceptible to deletions caused by oxidative stress and aging compared with nuclear DNA (26). Therefore, it is imperative that muscle cells have means not only to generate new mitochondria but also maintain the healthy ones and remove the damaged/dysfunctional ones. The regulation of this mitochondrial life cycle, from the biogenesis of new mitochondria to the removal of damaged/dysfunctional mitochondria, ultimately determines the overall quantity and, most importantly, quality and function of mitochondria in skeletal muscle, which are the determinants of metabolic function and physical performance (Figure). These processes allow for a program to replace old unhealthy mitochondria with new healthy mitochondria; analogous to the idea of replacing old cars of low fuel efficiency for new cars of high fuel efficiency to clean the environment. In this review, we present evidence suggesting that exercise training stimulates not only the biogenesis of mitochondria but also the removal of old and

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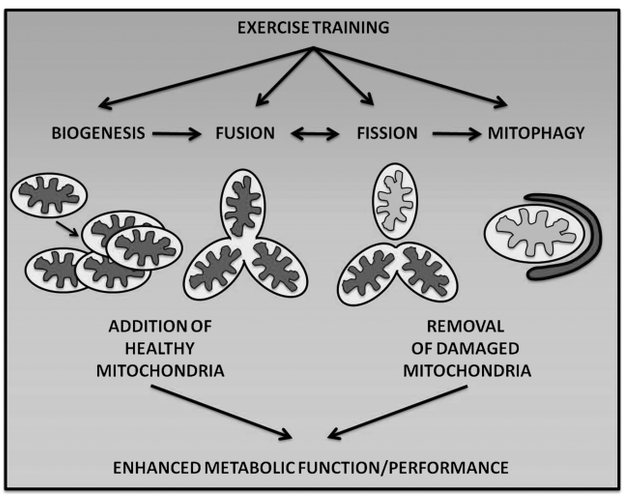

Figure. Schematic representation of exercise-induced mitochondrial maintenance. Exercise is proposed to promote biogenesis, fusion, fission, and mitophagy in an effort to promote the formation of new mitochondria and the identification and removal of damaged and dysfunctional mitochondria to improve metabolic function.

unhealthy mitochondria through mitochondrial dynamics and autophagy.

BIOGENESIS

Our laboratory has published a recent review on the topics of mitochondrial biogenesis, fiber type switching, and angiogenesis in skeletal muscle (50). Here, we will seek to summarize the current understanding of exercise-induced mitochondrial biogenesis and to evaluate new concepts related to the process of mitochondrial maintenance in this field. For decades, investigators have explored the adaptive mechanisms of endurance exercise training and observed increases in mitochondrial density either indirectly assessed by the expression of mitochondrial markers (*e.g.*, cytochrome c oxidase IV (COX IV)) or more directly observed through transmission electron microscopy and mitochondrial function measured by mitochondrial enzyme activity and oxygen consumption. Although it is well known that the process of mitochondrial biogenesis requires a coordinated regulation of both the nuclear and mitochondrial encoded genes, the primary mechanisms of adaptation remains unclear. The discovery of PGC-1α in brown adipose tissue, originally as a cold-inducible regulator of adaptive thermogenesis, has triggered mounting interest in elucidating the molecular and signaling mechanisms underlying exercise-induced mitochondrial biogenesis in skeletal muscle. Biochemically identified as a coactivator of PPAR-γ, PGC-1α is a coactivator of a great number of transcription factors, including the PPAR family transcription factors, nuclear respiratory factors (NRF), transcription factor of activated mitochondria, and others (28,37). PGC-1α is indeed capable of coactivating the required nuclear and mitochondrial genes for the organelle's synthesis. Because in large part of this common theme among the PGC-1α-inducible transcription factors, PGC-1α is now considered a "master regulator" of mitochondrial biogenesis. Recent data from Ugoccioni *et al.* (45) suggest that other factors act in parallel to PGC-1α to improve mitochondrial viability and function. Nevertheless, only more recently, the questions of whether PGC-1α is induced in skeletal muscle by exercise and whether its induction is sufficient and necessary to promote mitochondrial biogenesis have been addressed. The short answer to these questions is now a resounding yes.

Overexpression of PGC-1α Enhances Mitochondrial Biogenesis, and PGC-1α Deficiency Blunts Endurance Exercise-Induced Mitochondrial Biogenesis in Skeletal Muscle

Work by Leick *et al.* (22) demonstrated that mice with global deletion of the PGC-1α gene exhibited normal wheel running activity and were capable of inducing oxidative gene expression in skeletal muscle. However, subsequent work using this same mouse model led to the conclusion that PGC-1α is indeed necessary for exercise training-induced protection from an age-related decline in oxidative function (citrate synthase activity) and antioxidant gene expression (superoxide dismutase 2) (21). It is important to understand that such global gene deletion approaches may directly affect other metabolically active tissues, including the brown adipose and neural tissues. These multiorgan effects, coupled with enhanced adenosine monophosphate-activated protein kinase (AMPK) activity in resting skeletal muscle, may have prevented the delineation of the functional importance of PGC-1α in skeletal muscle, particularly under the condition of exercise training (25). More recently, skeletal muscle-specific PGC-1α knockout mice were generated (15), and data obtained from these mice showed that although exercise training-induced fiber type transformation appeared normal, exercise-induced mitochondrial biogenesis and angiogenesis in skeletal muscle were significantly attenuated (11). These results indicate the necessity of PGC-1α for normal exercise-induced metabolic adaptations, including mitochondrial biogenesis, in skeletal muscle. Considering the previous findings from Lin *et al.* (24) that transgenic mice with a skeletal muscle-specific overexpression of PGC-1α have enhanced expression of mitochondrial genes, fatigue resistance, and fiber type transformation toward oxidative myofibers, we now postulate that enhanced PGC-1α activity is sufficient to induce mitochondrial biogenesis, fiber type transformation, and angiogenesis. However, PGC-1α activity is necessary only for the induction of mitochondrial biogenesis and angiogenesis.

PGC-1α Regulation by Exercise

A relative wealth of data has been published documenting the effects of acute exercise and exercise training on PGC-1α expression. A consensus now exists that exercise training, including both endurance and resistance exercise, enhances PGC-1α expression because of transcriptional regulation (3,7,10). More recent evidence from Perry *et al.* (35) showed that a single bout of exercise was sufficient to induce PGC-1α protein and messenger ribonucleic acid (mRNA) expression in skeletal muscle, whereas increases in markers for mitochondrial biogenesis could be observed only after the third bout. Interestingly, PGC-1α mRNA level returned to baseline

between each bout, and the induction of PGC-1α was lessened with each bout. These findings suggest that the signaling-transcription coupling machinery for the PGC-1α gene can accurately sense the stress induced by altered functional demands of exercise and provide appropriate instructive cues to induce adaptations in skeletal muscle to minimize the exercise-induced stress.

Exercise-induced mitochondrial biogenesis also may be controlled by targeted activation of PGC-1α, indicated by its translocation to the nucleus (43), and this adaptive process may begin before increased PGC-1α expression (48). Intriguingly, Safdar *et al.* (41) showed that a single bout of exercise increases PGC-1α level not only in the nucleus but also in the mitochondria. Furthermore, the increased PGC-1α expression also was associated with enhanced interaction between PGC-1α and transcription factor of activated mitochondria at the mitochondrial DNA D-loop, as well as increased binding of PGC-1α to the NRF-1 promoter in the nucleus. Therefore, exercise is not only capable of inducing PGC-1α protein expression but also the translocation of PGC-1α to necessary compartments to stimulate the transcription of the nuclear and mitochondrial encoded mitochondrial genes, as well as the replication of mitochondrial DNA.

PGC-1α also is regulated by posttranslational modification, such as phosphorylation and deacetylation. For example, PGC-1α can be deacetylated by sirtuin (silent mating type information regulation 2 homolog) 1 (SIRT1). Although there is no correlation between the total muscle content of SIRT1 and PGC-1α or mitochondrial biogenesis, SIRT1 activation is associated with increases in PGC-1α target gene expression, consistent with SIRT1 activation of PGC-1α through deacetylation (14). Interestingly, this SIRT1-mediated PGC-1α activation requires phosphorylation of PGC-1α by AMPK (4). The fact that PGC-1α activity is controlled both transcriptionally and posttranscriptionally reveals an elegant regulatory system with great fidelity in integrating both contractile and metabolic cues in exercise-induced skeletal muscle adaptation.

PGC-1α expression and activity are largely regulated by upstream signaling pathways of protein kinases. The two primary protein kinases involved in the regulation of PGC-1α in skeletal muscle are AMPK (18) and p38γ mitogen-activated protein kinase (p38γ MAPK) (1,36). At least two AMPK phosphorylation sites have been identified on PGC-1α (18). AMPK not only activates PGC-1α but also promotes the transcription of the PGC-1α gene (17), controlling both PGC-1α expression and activity. It also has been shown that AMPK induces mitochondrial gene expression through PGC-1α (18). In fact, AMPK has been referred to as a "master metabolic switch" for acute regulation of energy metabolism and exercise training-induced adaptations. Acute exercise appears to activate AMPK by phosphorylation at Thr172 (22). Although a single bout of exercise results in increased AMPK activity in skeletal muscle, long-term exercise training leads to an increase in AMPK protein content (6,43). However, neither acute exercise nor long-term exercise training is capable of increasing AMPK-α protein content in skeletal muscle of PGC-1α-deficient mice (22). This suggests that not only is PGC-1α reliant on AMPK but also, at least, the α subunit of AMPK is reliant on PGC-1α.

We have previously shown that a single bout of voluntary running in mice is sufficient to activate the p38 MAPK pathway (1). We further showed that p38 MAPK activity is sufficient to promote the PGC-1α promoter activity through myocyte enhancer factor 2 and activating transcription factor 2 (1). In subsequent studies, we demonstrated that muscle-specific deletion of either p38α or p38β MAPK does not affect exercise-induced mitochondrial biogenesis and angiogenesis in skeletal muscle; however, mice with muscle-specific deletion of the p38γ MAPK or PGC-1α gene exhibit attenuated mitochondrial biogenesis and angiogenesis (11,36). These studies revealed for the first time that p38 MAPK stimulation of PGC-1α is specifically modulated by the γ isoform (36) and led to the conclusion that the p38γ MAPK–PGC-1α regulatory axis is functionally required for the normal exercise-induced metabolic adaptations in skeletal muscle.

MAINTENANCE AND MITOPHAGY

Although exercise-induced addition of new mitochondria is of extreme importance, the maintenance of a healthy population of mitochondria may be of equal or greater value. Mitochondrial damage induced by reactive oxygen species (ROS) (2) can lead to the accumulation of metabolic intermediates (19), which in turn further impair mitochondrial function and trigger a vicious cycle. These pathological changes ultimately hinder the ability of mitochondria to function properly. It is conceivable that efficient removal of damaged mitochondria is critical in maintaining overall mitochondrial function in a tissue/organ like skeletal muscle. Furthermore, an accumulation of damaged mitochondria, associated with sedentary lifestyle and/or high-fat diets, may impair skeletal muscle contractile and metabolic functions. For example, mitochondrial dysfunction has been implicated in the development of insulin resistance (39), likely the result of excessive production of ROS and accumulation of by-products of lipid metabolism. Therefore, it is imperative for the muscle to be able to both recognize and selectively remove damaged mitochondria.

Maintenance and Mitochondrial Dynamics

Mitochondria form tubular networks in mammalian cells, which extend through the cytosol and exist in close proximity to other important organelles and structures, such as the nucleus, endoplasmic reticulum, and cytoskeleton. Interestingly, skeletal muscle fibers contain two distinct mitochondrial populations (*i.e.*, subsarcolemmal and intermyofibrillar). Although the intermyofibrillar mitochondria maintain a relatively high respiratory capacity, the subsarcolemmal mitochondria more readily adapt to exercise training (20). Mitochondria are dynamic organelles that move about the cell, joining and separating as necessary. However, because of the physical limitation posed by the dense contractile apparatus in skeletal muscle, it is unlikely that such processes occur frequently across different mitochondrial populations in skeletal muscle. This joining and separating of mitochondria from the network are referred to as fusion and fission, respectively, and allow healthy, metabolically active cells to

form a large interconnected network of mitochondria for sharing of components (proteins, substrates, mitochondrial DNA) and removal of dysfunctional regions. Therefore, the balance between these processes and the signals responsible for their regulation are of extreme importance to the maintenance of the mitochondrial network.

The machinery involved in mitochondrial dynamics requires the participation of several proteins. Mitochondrial fusion involves mitofusins 1 and 2 (MFN1 and MFN2), which control fusion of the mitochondrial outer membrane, and optic atrophy type 1 (Opa1), which controls fusion of the mitochondrial inner membrane. Fission of the mitochondrial outer membrane in mammalian species is largely controlled by dynamin-related protein 1, which may be recruited by Fission 1 (Fis1) or mitochondrial fission factor. Westermann has previously published a more complete review on the machinery of mitochondrial dynamics (46).

Genetic manipulation of the genes encoding the machinery of mitochondrial dynamics reveals the importance of a balance between the fusion and fission processes. For example, knockdown of Opa1 produced a fragmented mitochondrial structure as the cells might be capable of performing fission but incapable of fusion (12). In contrast, animals with cardiac-specific deletion of MFN2 demonstrated abnormally enlarged subsarcolemmal mitochondria, but not intermyofibrillar mitochondria in cardiomyocytes (32). These findings demonstrate the importance of mitochondrial dynamics in maintaining a normal healthy mitochondrial network. However, certain conditions favor one process over the other for the overall maintenance of this network. For example, mitochondrial fusion appears to be shut down in depolarized mitochondria, whereas mitochondria undergoing a fission event are often depolarized (44). The depolarization and fission events appear to precede the removal of those mitochondria by mitophagy (44), providing a possible link from the regulation of mitochondrial dynamics to the removal of damaged portions of the mitochondrial network.

Exercise training appears to regulate both mitochondrial fusion and fission processes. For example, seven sessions of high-intensity interval training have been shown to progressively increase the protein content of MFN1 and Fis1 (35). Ding *et al.* (8) further showed that a single bout of treadmill running in rats induced increased MFN1 and MFN2 mRNA 24 h after exercise while both MFN1 and MFN2 proteins remained at baseline. Cartoni *et al.* (5) demonstrated that MFN1 and MFN2 mRNA content was enhanced in human skeletal muscle at 24 h after a single bout of cycling exercise, concurrent with an increase in COX IV mRNA. It is of note that cycling exercise induced increases in PGC-1α and estrogen-related receptor-α (ERR-α) mRNA at 2 h after exercise, before the changes in MFN1, MFN2, and COX IV. Interestingly, subsequent *in-vitro* studies showed that MFN1 and MFN2 transcription was regulated by PGC-1α through ERR-α (5). Together, these findings suggest that PGC-1α plays an important function in regulating the expression of the machinery for at least the mitochondrial fusion process in skeletal muscle under the conditions of exercise. Therefore, not only may mitochondrial biogenesis be regulated by PGC-1α but also the dynamics of mitochondrial fusion and fission.

Mitophagy

Autophagy is an evolutionarily conserved process for lysosome-dependent degradation of organelles and macromolecules. Two major forms of autophagy are known: nonselective autophagy, which is often stimulated by starvation, and selective autophagy, which may be triggered by and functions to remove aggregate/misfolded proteins and damaged organelles (*e.g.*, mitochondria) (27). Autophagy begins when a preautophagosomal structure of unknown origin gives rise to a phagophore, which elongates and engulfs the target, forming a double-membrane structure known as autophagosome. The autophagosome then fuses with lysosome to form autophagolysosome for execution of degradation (33). The whole process requires a family of proteins termed "*a*utophagy-related genes" (Atgs), including Atg1 (also known as Ulk1), Atg5, Atg6 (also known in mammalian systems as Beclin 1), Atg7, and Atg14. Specifically, the autophagic removal of mitochondria (mitophagy) may be a critical control step in the maintenance of mitochondrial quality, which presumably occurs after selection of dysfunctional, depolarized mitochondria through the fission/fusion processes. Indeed, inhibition of autophagy (deletion of the *Atg7* gene) resulted in reduced mitochondrial respiration in mouse skeletal muscle and increased oxidative stress in cell culture (49).

The regulation and impact of endurance exercise on autophagy in skeletal muscle only are starting to emerge. Wohlgemuth *et al.* (47) has shown that mild (8%) life-long calorie restriction either alone or in combination with exercise prevented the aging-induced reduction in basal autophagy in skeletal muscle in rats. Smuder *et al.* (42) recently showed that 5 days of treadmill exercise blunted the global induction of autophagy markers in the soleus muscle of rats treated with the antitumor agent doxorubicin, but that it also resulted in moderate increases in Atg6 mRNA and protein and Atg7 mRNA in non–doxorubicin-treated rats. Altogether, these results suggest that exercise training helps maintain the expression of autophagy proteins with aging and may even promote the expression of autophagy proteins in skeletal muscle, which may mediate the beneficial effects of exercise training. In this context, recent studies demonstrate that autophagy is induced by acute exercise in skeletal muscle (13,16,31) and other peripheral tissues (13,16). More importantly, He *et al.* (16) reported that acute exercise-induced increases in autophagy flux was inhibited in mice lacking three conserved phosphorylation residues in B-cell lymphoma 2 (Bcl-2) (mice termed *Bcl-2*^{AAA})), which disables the required dissociation of Atg6 from the Bcl-2 protein for the induction of autophagy. The authors further observed that exercise training–induced improvements in glucose tolerance and blood triglycerides and cholesterol profiles in mice on a high-fat diet were blunted. In addition, deficient activation of autophagy by exercise also prevented normal phosphorylation and activation of AMPK and its downstream target acetyl CoA carboxylase, as well as translocation of glucose transporter 4 to the cell membrane in skeletal muscle, which is a potential mechanism for the lack of metabolic adaptation in *Bcl-2*^{AAA} mice. What regulatory mechanism is responsible for acute exercise-induced autophagy and whether long-term exercise training affects autophagy in skeletal muscle remain to be ascertained.

Mitophagy in skeletal muscle has received growing attention, especially in the context of muscle atrophy (40,49), but very little is known about the key proteins involved and the signals required for the regulation of basal levels of mitophagy. Much of our current understanding of mitophagy comes from studies in neural tissues from animal models of neural diseases, such as Parkinson's disease. We now know that among regulatory factors with key functional roles in the regulation of mitophagy, Bcl-2 anchors to the membranes of both the endoplasmic reticulum (ER) and the mitochondria (34). Bcl-2, a key anti-apoptotic factor that binds to the mitochondrial outer membrane, also binds to and tethers Atg6 to the mitochondrial and ER membranes. Atg6 forms a complex with Atg14 to induce the formation of phagophore (23), playing a critical role in the induction and flux of autophagy. In addition, Parkin and phosphatase and tensin homolog deleted on chromosome 10-induced putative kinase protein 1 (Pink1) have been demonstrated to relay the signals associated with mitochondrial damage to the induction of mitophagy (29).

Other proteins that have been implicated in mitophagy are mitochondrially localized BNIP3 (Bcl-2 and 19 kd interacting protein 37) and BNIP3-like protein (BNIP3L) (30,40). Indeed, forced expression of BNIP3 in adult fibers induces massive mitophagy (40), and BNIP3L seems to be involved in the recruitment of the autophagy machinery to the mitochondria (30).

Finally, two classic exercise-induced pathways, AMPK, the master metabolic switch, and mammalian target of rapamycin (mTOR), perhaps the primary cog of protein synthesis and therefore muscle hypertrophy, have been shown to play important roles in autophagy regulation. In concert with the generally opposed nature of AMPK and mTOR, previous evidence supports that activation of AMPK induces stimulatory phosphorylation of Ulk1 for induction of autophagy, whereas mTOR results in an inhibitory phosphorylation of Ulk1 (9). Because these kinases are differentially regulated depending on the mode and intensity of exercise, they may help to fine tune mitophagy after acute exercise and as an adaptation to exercise training. However, their specific role in skeletal muscle autophagy has yet to be explored. This is an exciting new area of research, and future studies should look into the proteins involved in the mitophagy process, as well as their regulation in the context of exercise.

SUMMARY

Adaptations to exercise training are broad and span multiple organ systems. In skeletal muscle, one of the most important adaptations is enhanced metabolic capacity, which helps improve performance and health. The primary underlying mechanisms involve the regulation of the mitochondrial network. Great attention has been paid in recent years to the mechanisms involved in the generation and addition of new mitochondria, which is now known to be regulated by PGC-1α. Here, we propose that remodeling of the mitochondrial network through fusion and fission and elimination of damaged/dysfunctional mitochondria through mitophagy are all of importance in exercise-induced adaptation. This dynamic process of replacing old unhealthy mitochondria with new healthy mitochondria underscores enhanced quantity and quality of mitochondria in skeletal muscle after exercise training (Figure).

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References

1. Akimoto T, Pohnert SC, Li P, Zhang M, Gumbs C, Rosenberg PB, Williams RS, Yan Z. Exercise stimulates PGC-1α transcription in skeletal muscle through activation of the p38 mapk pathway. *J. Biol. Chem.* 2005; 280:19587–93.
2. Anderson EJ, Lustig ME, Boyle KE, Woodlief TL, Kane DA, Lin CT, Price JW 3rd, Kang L, Rabinovitch PS, Szeto HH, Houmard JA, Cortright RN, Wasserman DH, Neufer PD. Mitochondrial H_2O_2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. *J. Clin. Invest.* 2009; 119:573–81.
3. Baar K, Wende AR, Jones TE, Marison M, Nolte LA, Chen M, Kelly DP, Holloszy JO. Adaptations of skeletal muscle to exercise: Rapid increase in the transcriptional coactivator pgc-1. *FASEB J.* 2002; 16:1879–86.
4. Cantó C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J. AMPK regulates energy expenditure by modulating nad^+ metabolism and sirt1 activity. *Nature*. 2009; 458: 1056–60.
5. Cartoni R, Léger B, Hock MB, Praz M, Crettenand A, Pich S, Ziltener JL, Luthi F, Dériaz O, Zorzano A, Gobelet C, Kralli A, Russell AP. Mitofusins 1/2 and ERRα expression are increased in human skeletal muscle after physical exercise. *J. Physiol.* 2005; 567:349–58.
6. Clark SA, Chen ZP, Murphy KT, Aughey RJ, McKenna MJ, Kemp BE, Hawley JA. Intensified exercise training does not alter AMPK signaling in human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 2004; 286: E737–43.
7. Deldicque L, Atherton P, Patel R, Theisen D, Nielens H, Rennie MJ, Francaux M. Effects of resistance exercise with and without creatine supplementation on gene expression and cell signaling in human skeletal muscle. *J. Appl. Physiol.* 2008; 104:371–8.
8. Ding H, Jiang N, Liu H, Liu X, Liu D, Zhao F, Wen L, Liu S, Ji LL, Zhang Y. Response of mitochondrial fusion and fission protein gene expression to exercise in rat skeletal muscle. *Biochim. Biophys. Acta.* 2010; 1800:250–6.
9. Egan D, Kim J, Shaw RJ, Guan KL. The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR. *Autophagy*. 2011; 7:643–4.
10. Garcia-Roves PM, Huss J, Holloszy JO. Role of calcineurin in exercise-induced mitochondrial biogenesis. *Am. J. Physiol. Endocrinol. Metab.* 2006; 290:E1172–9.
11. Geng T, Li P, Okutsu M, Yin X, Kwek J, Zhang M, Yan Z. PGC-1α plays a functional role in exercise-induced mitochondrial biogenesis and angiogenesis but not fiber-type transformation in mouse skeletal muscle. *Am. J. Physiol. Cell. Physiol.* 2010; 298:C572–9.
12. Griparic L, Van Der Wel NN, Orozco IJ, Peters PJ, Van Der Bliek AM. Loss of the intermembrane space protein MGM1/Opa1 induces swelling and localized constrictions along the lengths of mitochondria. *J. Biol. Chem.* 2004; 279:18792–8.
13. Grumati P, Coletto L, Schiavinato A, Castagnaro S, Bertaggia E, Sandri M, Bonaldo P. Physical exercise stimulates autophagy in normal skeletal muscles but is detrimental for Collagen VI-deficient muscles. *Autophagy*. 2011; 7:1415–1423.
14. Gurd BJ, Yoshida Y, Mcfarlan JT, *et al.* Nuclear Sirt1 activity, but not protein content, regulates mitochondrial biogenesis in rat and human skeletal muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2011; 301:R67–75.

15. Handschin C, Chin S, Li P, Liu F, Maratos-Flier E, Lebrasseur NK, Yan Z, Spiegelman BM. Skeletal muscle fiber–type switching, exercise intolerance, and myopathy in PGC-1α muscle-specific knock-out animals. *J. Biol. Chem.* 2007; 282:30014–21.
16. He C, Bassik MC, Moresi V, *et al.* Exercise-induced Bcl2-regulated autophagy is required for muscle glucose homeostasis. *Nature.* 2012; 481: 511–5.
17. Irrcher I, Ljubicic V, Kirwan AF, Hood DA. AMP-activated protein kinase-regulated activation of the PGC-1α promoter in skeletal muscle cells. *PLoS ONE.* 2008; 3:e3614.
18. Jager S, Handschin C, St.-Pierre J, Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1α. *Proc. Natl. Acad. Sci. U. S. A.* 2007; 104:12017–22.
19. Koves TR, Ussher JR, Noland RC, *et al.* Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. *Cell Metab.* 2008; 7:45–56.
20. Krieger DA, Tate CA, Mcmillin-Wood J, Booth FW. Populations of rat skeletal muscle mitochondria after exercise and immobilization. *J. Appl. Physiol.* 1980; 48:23–8.
21. Leick L, Lyngby SS, Wojtasewski JF, Pilegaard H. PGC-1α is required for training-induced prevention of age-associated decline in mitochondrial enzymes in mouse skeletal muscle. *Exp. Gerontol.* 2010; 45:336–42.
22. Leick L, Wojtaszewski JF, Johansen ST, Kiilerich K, Comes G, Hellsten Y, Hidalgo J, Pilegaard H. PGC-1α is not mandatory for exercise- and training-induced adaptive gene responsens in mouse skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 2008; 294(2);E463–74.
23. Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, Levine B. Induction of autophagy and inhibition of tumorigenesis by Beclin 1. *Nature.* 1999; 402:672–6.
24. Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, Spiegelman BM. Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. *Nature.* 2002; 418:797–801.
25. Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, Mootha VK, Jäger S, Vianna CR, Reznick RM, Cui L, Manieri M, Donovan MX, Wu Z, Cooper MP, Fan MC, Rohas LM, Zavacki AM, Cinti S, Shulman GI, Lowell BB, Krainc D, Spiegelman BM. Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1α null mice. *Cell.* 2004; 119:121–35.
26. Melov S, Hinerfeld D, Esposito L, Wallace DC. Multi-organ characterization of mitochondrial genomic rearrangements in ad libitum and caloric restricted mice show striking somatic mitochondrial DNA rearrangements with age. *Nucleic Acids Res.* 1997; 25:974–82.
27. Mizushima N, Levine B, Cuervo Am, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature.* 2008; 451:1069–75.
28. Muoio DM, Koves TR. Skeletal muscle adaptation to fatty acid depends on coordinated actions of the PPARs and PGC1-α: Implications for metabolic disease. *Appl. Physiol. Nutr. Metab.* 2007; 32:874–83.
29. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, Cookson MR, Youle RJ. Pink1 is selectively stabilized on impaired mitochondria to activate parkin. *PLoS Biol.* 2010;8(1):e1000298.
30. Novak I, Kirkin V, McEwan DG, Zhang J, Wild P, Rozenknop A, Rogov V, Löhr F, Popovic D, Occhipinti A, Reichert AS, Terzic J, Dötsch V, Ney PA, Dikic I. Nix is a selective autophagy receptor for mitochondrial clearance. *EMBO Rep.* 2010; 11:45–51.
31. Ogura Y, Iemitsu M, Naito H, *et al.* Single bout of running exercise changes LC3-II expression in rat cardiac muscle. *Biochem. Biophys. Res. Commun.* 2011; 414:756–60.
32. Papanicolaou KN, Khairallah RJ, Ngoh GA, Chikando A, Luptak I, O'Shea KM, Riley DD, Lugus JJ, Colucci WS, Lederer WJ, Stanley WC, Walsh K. Mitofusin-2 maintains mitochondrial structure and contributes to stress-induced permeability transition in cardiac myocytes. *Mol. Cell Biol.* 2011; 31:1309–28.
33. Pattingre S, Espert L, Biard-Piechaczyk M, Codogno P. Regulation of macroautophagy by mtor and beclin 1 complexes. *Biochimie.* 2008; 90:313–23.
34. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD, Levine B. Bcl-2 antiapoptotic proteins inhibit Beclin 1–dependent autophagy. *Cell.* 2005; 122:927–39.
35. Perry CGR, Lally J, Holloway GP, Heigenhauser GJF, Bonen A, Spriet LL. Repeated transient mrna bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. *J. Physiol.* 2010; 588:4795–810.
36. Pogozelski AR, Geng T, Li P, Yin X, Lira VA, Zhang M, Chi JT, Yan Z. p38γ mitogen–activated protein kinase is a key regulator in skeletal muscle metabolic adaptation in mice. *PLoS One.* 2009; 4:e7934.
37. Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-γcoactivator-1α (PGC-1α): Transcriptional coactivator and metabolic regulator. *Endocr. Rev.* 2003; 24:78–90.
38. Puigserver P, Wu Z, Park C, Graves R, Wright M, Spiegelman B. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell.* 1998;92:829–39.
39. Rector RS, Thyfault JP, Uptergrove GM, Morris EM, Naples SP, Borengasser SJ, Mikus CR, Laye MJ, Laughlin MH, Booth FW, Ibdah JA. Mitochondrial dysfunction precedes insulin resistance and hepatic steatosis and contributes to the natural history of non-alcoholic fatty liver disease in an obese rodent model. *J. Hepatol.* 2010; 52:727–36.
40. Romanello V, Guadagnin E, Gomes L, Roder I, Sandri C, Petersen Y, Milan G, Masiero E, Del Piccolo P, Foretz M, Scorrano L, Rudolf R, Sandri M. Mitochondrial fission and remodelling contributes to muscle atrophy. *EMBO J.* 2010; 29:1774–85.
41. Safdar A, Little JP, Stokl AJ, Hettinga BP, Akhtar M, Tarnopolsky MA. Exercise increases mitochondrial PGC-1α content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis. *J. Biol. Chem.* 2011; 286:10605–17.
42. Smuder AJ, Kavazis AN, Min K, Powers SK. Exercise protects against doxorubicin-induced markers of autophagy signaling in skeletal muscle. *J. Appl. Physiol.* 2011; 111:1190–8.
43. Sriwijitkamol A, Ivy JL, Christ-Roberts C, Defronzo RA, Mandarino LJ, Musi N. LKB1-AMPK signaling in muscle from obese insulin-resistant zucker rats and effects of training. *Am. J. Physiol. Endocrinol. Metab.* 2006; 290:E925–32.
44. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, Shirihai OS. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J.* 2008; 27:433–46.
45. Uguccioni G, Hood DA. The importance of PGC-1α in contractile activity-induced mitochondrial adaptations. *Am. J. Physiol. Endocrinol. Metab.* 2011; 300:E361–71.
46. Westermann B. Mitochondrial fusion and fission in cell life and death. *Nat. Rev. Mol. Cell Biol.* 2010; 11:872–84.
47. Wohlgemuth SE, Seo AY, Marzetti E, Lees HA, Leeuwenburgh C. Skeletal muscle autophagy and apoptosis during aging: Effects of calorie restriction and life-long exercise. *Exp. Gerontol.* 2010; 45:138–48.
48. Wright DC, Han DH, Garcia-Roves PM, Geiger PC, Jones TE, Holloszy JO. Exercise-induced mitochondrial biogenesis begins before the increase in muscle PGC-1α expression. *J. Biol. Chem.* 2007; 282:194–9.
49. Wu JJ, Quijano C, Chen E, Liu H, Cao L, Fergusson MM, Rovira II, Gutkind S, Daniels MP, Komatsu M, Finkel T. Mitochondrial dysfunction and oxidative stress mediate the physiological impairment induced by the disruption of autophagy. *Aging.* 2009; 1:425–37.
50. Yan Z, Okutsu M, Akhtar YN, Lira VA. Regulation of exercise-induced fiber type transformation, mitochondrial biogenesis, and angiogenesis in skeletal muscle. *J. Appl. Physiol.* 2011; 110:264–74.