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Autophagy-Dependent Beneficial Effects of Exercise

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Exercise has long been recognized as a powerful physiological stimulus for a wide variety of metabolic adaptations with implications for health and performance. The metabolic effects of exercise occur during and after each exercise bout and manifest as cumulative adaptive responses to successive exercise bouts. Studies on the beneficial effects of exercise have traditionally focused on the biosynthesis of metabolic proteins and organelles. However, the recycling of cellular components by autophagy has recently emerged as an important process involved in the adaptive responses to exercise. This review covers the regulation of autophagy by exercise, with emphasis on the potential autophagy-dependent beneficial effects of exercise.

ADAPTATIONS TO EXERCISE TRAINING

Exercise training can induce multiple adaptations in skeletal muscle, the type of which depends on the mode of exercise. Hence, resistance exercise training is characterized by increased muscle fiber cross-sectional area (hypertrophy), whereas endurance exercise training is characterized by metabolic adaptations in skeletal muscle.

Resistance exercise training-induced increases in muscle mass contribute to increased muscle strength and muscle performance (Chesley et al. 1992; Biolo et al. 1995) with importance for both athletic ability and everyday physical function. This is, in part, facilitated by a net increase in myofilament proteins. In accordance, a single resistance exercise bout has been shown to increase protein synthesis (Chesley et al. 1992; Biolo et al. 1995; Phillips et al. 1997) for up to 48 hours involving increased translational initiation. Of notice is, however, that resistance exercise also increases protein degradation for up to 24 hours (Biolo et al. 1995; Phillips et al. 1997) underlining the importance of a well-regulated balance between protein synthesis and degradation in the regulation of muscle mass.

Endurance exercise training-induced metabolic adaptations in skeletal muscle contribute to increasing the maximal oxidative capacity as well as the metabolic efficiency of skeletal muscle with concomitant improvements in endurance performance and health. Endurance exercise training-induced increases in content and activity of proteins involved in oxidative metabolism in human and rodent skeletal muscle are well established (Holloszy 1967; Gollnick et al. 1972; Henriksson and Reitman 1977). These

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adaptations have, in general, been reported as a result of increased synthesis of metabolic proteins and investigations of the underlying mechanisms behind metabolic adaptations with exercise training have primarily focused on the regulation of protein synthesis. However, the original study by Henriksson and Reitman showing that the activity of oxidative enzymes in human skeletal muscle increased over the course of several weeks of endurance exercise training also showed that the gained enzyme activity was rapidly lost within 1–2 weeks of detraining (Henriksson and Reitman 1977). This underlines the high turnover of metabolic proteins in skeletal muscle. Furthermore, more recent evidence suggests that the regulation of protein removal by autophagy is of equal importance as increased synthesis of metabolic proteins for obtaining the full beneficial metabolic effects of endurance exercise training.

MECHANISMS OF AUTOPHAGY

The term autophagy is derived from the ancient Greek words "auto" (self) and "phagein" (to eat). Autophagy is a conserved mechanism in which targeted cellular components are engulfed in maturing autophagosomes that subsequently fuse with lysosomes for hydrolytic degradation and recycling of proteins, membranes, and other cellular components.

Important knowledge has been gathered regarding key molecular mechanisms involved in initiation and execution of cellular recycling through autophagy, which have recently been comprehensively reviewed (Ktistakis and Tooze 2016). In brief, a central set of autophagy-related (ATG) proteins controlling autophagosome formation and elongation has been identified in yeast, most of which have been found to have one or more mammalian homologs. An essential step in autophagosome maturation is the recruitment of the ULK1 kinase complex (including ATG13, ATG101, and FIP200) and the class III PI 3-kinase (PI3K) complex (including VPS34, Beclin-1, ATG14, and p150) to the maturing autophagosome membrane (Fig. 1). The interaction between the ULK1 complex, LC3/GABARAP, and ATG5-12-16L1 at the autophagosome membrane increases the activity of ULK1 and sustains PI3K complex activity, which drives phosphatidylinositol 3-phosphate (PI3P) formation and promotes autophagosome maturation. Furthermore, ATG5-12-16L1-dependent phosphatidylethanolamine (PE) lipidation of LC3-I to LC3-II on the autophagosome membrane is crucial for autophagosome maturation (Fig. 1) (Mizushima and Komatsu 2011; Ktistakis and Tooze 2016). LC3-II and the LC3-II/LC3-I ratio are therefore widely used as markers for autophagosome content and autophagy flux, although interpretations of LC3-II as a general autophagy marker should be made cautiously (Mizushima and Yoshimori 2007; Rubinsztein et al. 2009; Klionsky et al. 2016). Although elevated LC3-II content can be interpreted as a marker of increased autophagosome content and therefore induction of autophagy, a reduction in LC3-II can, conversely, also be the result of increased autophagic flux, because LC3-II itself is degraded in the lysosome. Tracking of other proteins known to be degraded during autophagy can therefore be useful in interpreting alterations in autophagy (Klionsky et al. 2016).

Selective Autophagy

Although autophagy can function in a nonselective manner, for example to provide substrates during energy deprivation, mechanisms for selective clearance of specific organelles and proteins have been identified. The autophagy adaptor protein p62 contains a carboxy-terminal ubiquitin-associated domain and directly interacts with LC3-II, thereby facilitating the incorporation of ubiquitinated substrates into autophagosomes (Fig. 1). Because p62 is degraded during autophagy, changes in p62 protein content have together with LC3 lipidation been used as a surrogate marker for autophagy flux (Klionsky et al. 2016).

An emerging autophagy-dependent mechanism with potential implications for exercise training adaptations is the autophagic clearance of damaged mitochondria termed mitophagy and a molecular machinery that serves to target mitochondria for degradation has been discov-

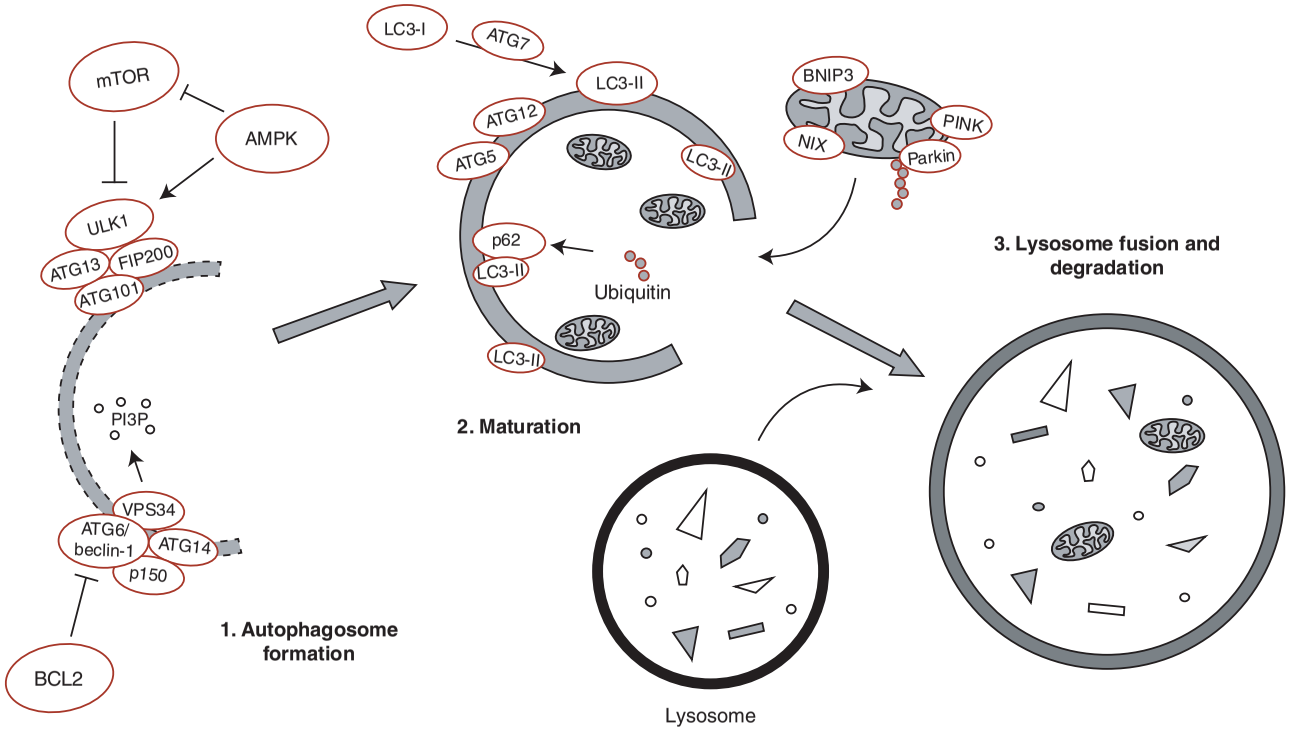

Figure 1. Overview of basic molecular mechanisms involved in initiation of autophagy. The UNC-51-like kinase 1 (ULK1) kinase complex (including autophagy-related genes (ATG1)3, ATG101, and FAK family kinase-interacting protein of 200 kDa (FIP200)) and the class III PI3-kinase complex (including the catalytic vacuolar protein sorting mutant 34 (VPS34) and the regulatory ATG6/Beclin-1, p150, and ATG14) are stabilized on a phagophore membrane and generate a pool of phosphatidylinositol-3-phosphate (PI3P), which is essential for autophagosome formation. The 5′ AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) act as opposing regulators of ULK1 complex activity through stimulatory and inhibitory phosphorylation of ULK1, respectively, whereas AMPK inhibits mTOR activity. Dissociation of ATG6/Beclin-1 and B-cell lymphoma 2 (BCL2) is required for the assembly of the class III PI3-kinase complex. ATG7 regulates the recruitment of cytosolic LC3-I to the maturing autophagosome membrane, where ATG5-ATG12-dependent lipidation forms membrane-bound LC3-II. (*Legend continues on following page.*)

ered. The kinase PTEN-induced putative kinase 1 (PINK1) is stabilized on depolarized mitochondrial membranes, for example, in response to oxidative stress, and recruits the E3 ubiquitin ligase parkin to mitochondria. On activation, parkin ubiquitinates several mitochondrial membrane proteins, thereby targeting mitochondria for degradation. Furthermore, specific mitophagy receptors such as BCL2/adenovirus E1B 19-kDa protein-interacting protein 3 (BNIP3) and NIP3-like protein X (NIX) present on mitochondrial membranes can directly interact with LC3-II to induce mitophagy (Fig. 1) (Youle and Narendra 2011).

Acute Exercise-Induced Autophagy

The ability of a single endurance exercise bout to induce an autophagic response in skeletal muscle was first described in 1984. Thus, using electron microscopy (EM), the formation of autophagic vacuoles was detected in mouse muscle fibers 2 to 7 days after an extremely strenuous (9 h) treadmill running bout (Salminen and Vihko 1984). However, the notion that exercise may stimulate autophagic removal of cellular components has only recently received widespread attention following reports that LC3-II as well as autophagosome content detected by EM was increased in mouse muscle after a single bout of treadmill running (Grumati et al. 2011). This finding has since been supported by several other studies in rodents (He et al. 2012; Jamart et al. 2012b; Pagano et al. 2014; Saleem et al. 2014; Fritzen et al. 2015; Liu et al. 2015; Vainshtein et al. 2015b; Halling et al. 2016). However, the autophagic response in human muscle to acute endurance exercise seems to be more complex. Hence, ultra-endurance running has been shown to potently increase LC3-II content (Jamart et al. 2012b), whereas exercise bouts of lower duration have been reported to lower the content of LC3-II (Fritzen et al. 2015; Moller et al. 2015; Schwalm et al. 2015). Moreover, some studies have observed unchanged skeletal muscle LC3-II content in humans following a single cycling exercise bout (Masschelein et al. 2014; Tachtsis et al. 2016). In addition, reduced p62 content following endurance exercise has been reported in both mouse (He et al. 2012; Pagano et al. 2014) and human (Schwalm et al. 2015) skeletal muscle, although others have observed unchanged p62 content after a single endurance exercise bout (Jamart et al. 2012b; Fritzen et al. 2015; Moller et al. 2015; Halling et al. 2016; Tachtsis et al. 2016). Altogether, there is robust evidence suggesting that autophagy is stimulated in skeletal muscle in response to acute endurance exercise. However, the exact mechanism behind induction of autophagy may depend on factors such as intensity/duration of exercise (Schwalm et al. 2015), feeding (Jamart et al. 2013), and timing of muscle sampling (Halling et al. 2016), which may contribute to the variability of the observed autophagic responses to acute endurance exercise.

Several studies have also examined the effects of strength/resistance exercise on regulation of autophagy. It has been shown that LC3-II protein and GABARAP mRNA were reduced from 3 to 24 hours after a single session of resistance exercise in both young and aged human muscle, which was interpreted as decreased activation of autophagy (Fry et al. 2013). Furthermore, it has been shown that the decreased LC-II content following resistance exercise was dependent on post-exercise intake of essential amino acids (Glynn et al. 2010), suggesting that resistance exercise-induced regulation of au-

Figure 1. (*Continued*) The autophagy adaptor protein p62 links ubiquitinated substrates with LC3-II. Selective autophagic clearance of mitochondria (mitophagy) is regulated by the kinase PTEN-induced putative kinase 1 (PINK1), which is stabilized on depolarized mitochondrial membranes and recruits the E3 ubiquitin ligase parkin to mitochondria. Parkin regulates ubiquitination of mitochondrial membrane proteins, thereby targeting mitochondria for degradation. The mitophagy receptors BCL2/adenovirus E1B 19-kDa protein-interacting protein 3 (BNIP3) and NIP3-like protein X (NIX) present on mitochondrial membranes can directly interact with LC3-II to induce mitophagy. Finally, the mature autophagosomes fuse with lysosomes causing hydrolytic degradation of autophagosomal cargo.

tophagy is dependent on nutrient availability. However, the apparent modulation of autophagy by resistance exercise in combination with amino acid intake was not associated with changes in either overall protein synthesis or protein breakdown at 2 hours post-exercise (Glynn et al. 2010). This indicates that autophagy-mediated release of amino acids is not involved in anabolic/catabolic processes in the early recovery period. The reported lowering of LC3-II and LC3-II/I content in human skeletal muscle during the early recovery period after a resistance exercise bout has been recapitulated in several recent studies (Dickinson et al. 2016; Francaux et al. 2016; Smiles et al. 2016). This may suggest a deactivation of autophagy during recovery from resistance exercise, assuming that LC3-II/I is a valid marker for autophagic flux. In contrast, others have reported no change (Smiles et al. 2015) or increase (Ogborn et al. 2015) in autophagy markers at various timepoints after resistance exercise. However, as previously described, LC3-II itself is degraded when autophagosomes fuse with lysosomes suggesting that, in certain cases, a reduction in LC3-II content can actually be the result of increased autophagic flux (Mizushima and Yoshimori 2007; Rubinsztein et al. 2009). It is, therefore, noteworthy that a recent study showed resistance exercise-induced activation of chaperone-assisted selective autophagy and LC3 puncta formation in human skeletal muscle up to 24 hours into recovery (Ulbricht et al. 2015), similar to that observed after treadmill running in mice (Grumati et al. 2011; He et al. 2012). Therefore, it is uncertain how resistance exercise affects autophagic flux. An approach that may be used to address this is colchicine-induced blockade of lysosomal degradation as previously used to determine autophagic flux in mice in response to endurance exercise (Vainshtein et al. 2015b). However, rodent models of resistance exercise may not be comparable with humans, underlined by the observation that "resistance exercise" did not affect LC3-II levels in rat skeletal muscle (Ogasawara et al. 2016). On the other hand, the activity of VPS34, a component of the PI3K complex, has been shown to be increased following high-resistance electrically stimulated contraction of rat skeletal muscle (MacKenzie et al. 2009). This suggests that autophagy is activated during recovery from resistance exercise similarly to endurance exercise, although further studies are required to directly assess the effects of resistance exercise on autophagic flux in human muscle.

MOLECULAR MEDIATORS OF EXERCISE-INDUCED AUTOPHAGY

AMPK

Cell culture studies have indicated that the 5′ AMP-activated protein kinase (AMPK) regulates autophagy through ULK1 Ser-317 and Ser-555 phosphorylation (Meley et al. 2006; Egan et al. 2011) during conditions of cellular energy demand. Accordingly, ULK1 Ser-317, and Ser-555 phosphorylation has been shown to increase in mouse muscle in response to a single exercise bout (Pagano et al. 2014). Furthermore, it has been shown that the catalytic AMPK α2 subunit is required for exercise-induced LC3 lipidation and ULK1 Ser-777 phosphorylation in mouse skeletal muscle (Liu et al. 2015). The link between AMPK and autophagy is also supported by the observation that BCL2 AAA mutated (Thr69Ala, Ser70Ala, and Ser84-Ala) mice with impaired dissociation of the Beclin-1/BCL2 complex, causing inhibition of PI3K complex formation and blunted exercise-induced activation of autophagy, showed lower AMPK phosphorylation levels (He et al. 2012). On the other hand, inducible muscle-specific knockout (KO) of ATG7 resulted in impaired exercise-induced autophagy, but did not affect AMPK phosphorylation in mouse skeletal muscle (Lo et al. 2014). Moreover, an association between exercise-induced AMPK activation, ULK1 phosphorylation and modulation of autophagy is supported by results from some human studies (Moller et al. 2015; Schwalm et al. 2015). However, others have reported that there was no correlation between exercise-induced AMPK activation and LC3 lipidation in human muscle as well as no apparent effects on LC3 lipidation with pharmacological activation of AMPK in incubated mouse skeletal muscle or

human myotubes (Fritzen et al. 2015). Still, AMPK seems to be persistently required for ULK1 Ser-555 phosphorylation (Egan et al. 2011; Fritzen et al. 2015). Taken together, evidence suggests that AMPK-dependent activation of autophagy can occur in skeletal muscle in response to acute exercise. Because AMPK is known as an intracellular energy sensor, this may suggest that exercise-induced autophagy activation occurs in response to lowered intracellular energy charge. However, a recent study showed that exercise-induced activation of autophagy was independent of continuous systemic glucose infusion during exercise (Moller et al. 2015), suggesting that activation of autophagy in skeletal muscle during exercise is not caused by insufficient substrate supply.

mTOR

The mammalian target of rapamycin (mTOR) has been identified as a major negative regulator of autophagy through an AMPK-opposing mechanism (Kim et al. 2011). During conditions of high substrate availability, a signaling pathway is activated involving the insulin-stimulated class I PI3K, which recruits Akt leading to activation of mTOR. Consequently, ULK1 is phosphorylated by mTOR at Ser-757, which, contrary to AMPK-mediated phosphorylation, inhibits formation of the ULK1 kinase complex preventing autophagosome maturation (Kim et al. 2011). AMPK activation inhibits mTOR signaling and, indeed, ULK1 Ser-757 phosphorylation has been shown to decrease in mouse skeletal muscle both during exercise (Pagano et al. 2014) and 10 hours into recovery (Halling et al. 2016) from a single treadmill running bout. This suggests that deactivation of mTOR signaling contributes to endurance exercise-induced autophagy. In addition, autophagy activation following high-resistance electrically stimulated contraction of rat skeletal muscle correlated with mTOR activity, while leucine treatment, but not amino acid starvation, of C2C12 cells also increased VPS34 activity (MacKenzie et al. 2009). Furthermore, inhibition of mTOR signaling attenuated protein synthesis rates up to 24 hours after a single resistance exercise bout in rat skeletal muscle (Ogasawara et al. 2016). Together, this suggests that it may be the increased amino acid turnover per se that regulates autophagy through mTOR signaling in response to resistance exercise potentially allowing further stimulation of protein synthesis at later timepoints.

FOXO

Several stress-induced transcription factors have been proposed to regulate the expression of genes involved in autophagy (Pietrocola et al. 2013) of which the best-described example is the forkhead box O (FOXO) family of transcription factors. In particular, FOXO3 has been shown to play an important role in regulating core ATGs, including LC3 and BNIP3 in skeletal muscle (Mammucari et al. 2007; Zhao and Klionsky 2011). FOXOs are responsive to nutrient deprivation and oxidative stress through posttranslational modifications. This includes Akt (PKB)-mediated phosphorylation of FOXO3 on multiple residues leading to inhibition and retention of FOXO3 in the cytosol (Brunet et al. 1999). The observation that Akt 1 and 2 KO in mice increased BNIP3 mRNA in skeletal muscle (Reynolds et al. 2012) supports the relevance of Akt-mediated FOXO3 regulation in autophagy. Furthermore, the findings that the AMPK activator, AICAR, increased FOXO3 phosphorylation with concomitant transient relocalization of FOXO3 to the nuclei and increase in mRNA content of autophagy-related proteins in muscle cells (Sanchez et al. 2012) provide strong evidence that AMPK is involved in posttranslational regulation of FOXO3. In addition, acetylation of FOXO3 has been reported to induce translocation of FOXO3 to the cytosol followed by ubiquitination and degradation of FOXO3 in the proteasome, whereas sirtuin-mediated deacetylation and activation of FOXO3 has been suggested to promote autophagy (Bertaggia et al. 2012). Moreover, AMPK-induced modulation of sirtuin activity is thought to reduce FOXO3 acetylation. Taken together, multiple posttranslational modifications seem to contribute in the

regulation of FOXO3 activity and localization. Interestingly, increased FOXO3 mRNA and protein content as well as stimulatory dephosphorylation of FOXO3 has been observed in human and mouse skeletal muscle following acute endurance exercise (Louis et al. 2007; Jamart et al. 2012a, 2013; Wang et al. 2015). This indicates that FOXO3 regulation, possibly mediated by Akt/AMPK, contributes to transcriptional regulation of autophagy components in response to exercise.

TFEB

The transcription factor EB (TFEB) has been shown to be a key factor in autophagy and lysosome function through transcriptional regulation of genes encoding autophagy and lysosome proteins (Settembre et al. 2011). TFEB localization and activity is regulated by mTORC1-mediated phosphorylation of multiple residues and dephosphorylation by calcineurin. In accordance, exhaustive running exercise in mice has been reported to induce a calcineurin-mediated dephosphorylation of TFEB in skeletal muscle with concomitant nuclear translocation of TFEB and overexpression of a calcineurin inhibitor in muscle by electroporation prevented these effects (Medina et al. 2015). A simultaneous exercise-induced reduction in mTOR-mediated phosphorylation and inhibition of TFEB has been suggested to contribute to ensuring that TFEB translocates to the nucleus in response to muscle contractions (Medina et al. 2015). Together, this shows the potential of TFEB to regulate exercise training induced long-term adaptations in the autophagy and lysosome pathways.

p53

The tumor suppressor p53 has also been shown to be involved in regulation of autophagy. However, whether p53 functions as an activator or suppressor of autophagy seems to be dependent on the context and subcellular localization, because cytosolic p53 has been shown to inhibit autophagy (Tasdemir et al. 2008), whereas p53 induces transcription of key stimulatory autophagy genes in the nucleus (Maiuri et al. 2009; Kenzelmann et al. 2013). Furthermore, the findings that the nuclear abundance of p53 is increased in human muscle (Tachtsis et al. 2016) and decreased in mouse muscle (Saleem and Hood 2013) during recovery from exercise underline the complexity of p53 regulation. Together, the possible role of p53 in the regulation of exercise-induced autophagy remains to be fully elucidated. However, the finding that p53 is dispensable for metabolic adaptations to exercise training in mice (Saleem et al. 2009) suggests that p53-mediated exercise-induced autophagy may not be required for the beneficial metabolic effects of exercise.

PGC-1α

The exercise responsive transcriptional co-activator peroxisome proliferator-activated receptor γ coactivator (PGC)-1α has been identified as a key regulator of mitochondrial biogenesis (Wu et al. 1999; Pilegaard et al. 2003; Lin et al. 2005). Moreover, several recent studies have suggested that PGC-1α plays a role in exercise-induced autophagy. Hence, the finding that muscle-specific PGC-1α overexpression (MCK) was associated with increased BNIP3 protein content and increased basal autophagy (Lira et al. 2013) indicates that PGC-1α contributes to the regulation of autophagy in resting skeletal muscle. This was accompanied by results showing that autophagy levels are higher in oxidative than glycolytic muscles (Lira et al. 2013). The finding that exercise increased LC3-II protein content during recovery from a single treadmill running bout in PGC-1α MCK mice, but not in wild-type (WT) mice, suggests that elevated levels of PGC-1α also contributes in inducing autophagy in response to acute exercise. This was supported by a blunted treadmill exercise-induced increase in skeletal muscle LC3-II protein in muscle-specific PGC-1α KO mice (Halling et al. 2016). In addition, an attenuated treadmill exercise-induced increase in mitochondrial LC3-II flux in skeletal muscle from whole-body PGC-1α KO mice determined by monitoring LC3-II accumulation after treatment with the autophagy inhibitor colchicine

further underlines the impact of PGC-1α in exercise-induced autophagy (Vainshtein et al. 2015b). Together, this may reflect that the metabolic profile of PGC-1α overexpression and KO mice affects the exercise-induced autophagy response. Thus, PGC-1α may influence autophagy indirectly through regulation of muscle oxidative capacity. However, it is also possible that PGC-1α affects exercise-induced autophagy through transcriptional regulation of autophagy proteins. Hence, the observations that PGC-1α KO mice had lower TFEB protein in skeletal muscle than WT mice (Vainshtein et al. 2015a) and did not increase the mRNA content of LC3, p62, and Niemann–Pick C1 in response to treadmill running (Vainshtein et al. 2015b) indicate that PGC-1α also plays a role in the endurance-exercise-induced adaptive gene responses of autophagy proteins in skeletal muscle. Taken together, this suggests that PGC-1α influences exercise training-mediated adaptations in key autophagy markers resulting in increased capacity of the autophagy machinery with exercise training.

AUTOPHAGY-DEPENDENT BENEFICIAL EFFECTS OF EXERCISE

Exercise Performance and Oxidative Capacity

The possibility that exercise-induced autophagy influences exercise performance is supported by the finding that BCL2 AAA mutated mice that are incapable of exercise-induced BCL2–Beclin-1 disassociation and autophagy activation have lower maximal exercise capacity than control mice (He et al. 2012). Of notice is that the lower maximal exercise capacity was not because of differences in basal muscle properties, but the inability to induce autophagy during running. Maximal running distance was also shown to be lower in ATG6 (Beclin-1)$^{+/-}$ mice with reduced ATG6 protein content in skeletal muscle, but normal basal autophagy (He et al. 2012), although others did not observe changes in time to exhaustion during treadmill running in ATG6$^{+/-}$ mice (Lira et al. 2013). This suggests that the impact of the level of ATG6 on exercise performance may depend on exercise type, intensity, and/or duration. In accordance, exercise performance during regular treadmill running was not impaired in inducible skeletal-muscle-specific ATG7 KO mice, despite a blunted exercise-induced autophagy response (Lo et al. 2014), supporting that acute autophagy activation is not universally required for sustaining muscle contractions. However, the inducible deletion of ATG7 in skeletal muscle reduced performance and caused profound mitochondrial membrane depolarization in skeletal muscle during downhill running (eccentric muscle contractions) in female mice (Lo et al. 2014), suggesting that autophagy influences exercise performance during more damaging muscle contractions (Fig. 2).

Several studies suggest that autophagy is essential for cellular adaptations to exercise training. A potential link between exercise-induced autophagy and exercise training-mediated adaptations was examined using ATG6$^{+/-}$ mice. Exercise training did not improve endurance exercise performance and did not increase the protein content of the oxidative markers cytochrome *c* and COXIV, or LC3 and BNIP3 protein in skeletal muscle of ATG6$^{+/-}$ mice (Lira et al. 2013). This suggests that exercise-induced autophagy is required for exercise training-mediated adaptations in skeletal muscle oxidative capacity and exercise performance (Fig. 2). This is supported by the finding that there was a positive correlation between LC3-II content and the conversion of glycolytic type IIX muscle fibers toward the more oxidative type IIA fibers in response to endurance exercise training in rats (Tam et al. 2015).

Muscle Mass

Given the reported effects of resistance exercise on regulation of autophagy, it can be speculated that autophagy-induced amino acid replenishment contributes to the hypertrophic response to resistance exercise training. In addition, the ability of resistance exercise training to preserve muscle mass during aging has been associated with regulation of autophagy (Luo et al. 2013). Thus, aged rats subjected to 9 weeks of weight-bearing exercise had higher muscle cross-sec-

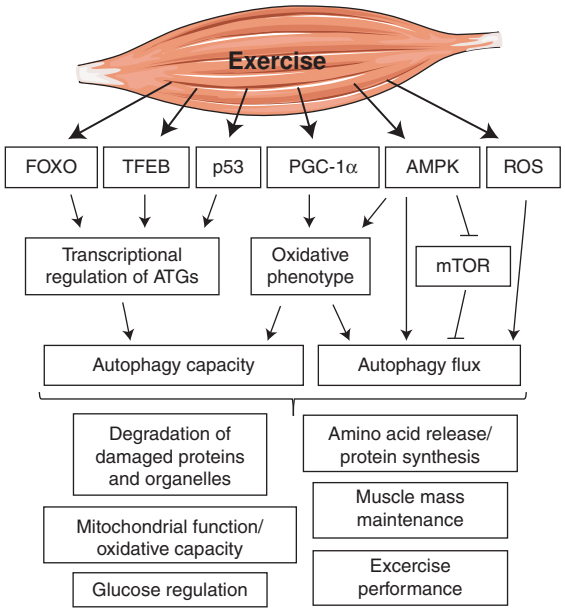

Figure 2. Overview of molecular mediators of exercise-induced autophagy and the autophagy-dependent beneficial effects of exercise. Exercise-induced activation of autophagy in skeletal muscle has been shown to be influenced by transcriptional regulation of autophagy-related genes (ATGs) mediated by forkhead box O (FOXO) family of transcription factors, transcription factor EB (TFEB), p53, and peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) to increase the autophagic capacity. The oxidative phenotype of skeletal muscle is linked to autophagy capacity/flux and is positively regulated by the exercise responsive 5′ AMP-activated protein kinase (AMPK) and PGC-1α. AMPK and mammalian target of rapamycin (mTOR) act as an activator and suppressor of exercise-induced autophagy, respectively. Exercise-induced generation of reactive oxygen species (ROS) seems to serve as a signal for autophagy activation. Autophagy-dependent beneficial effects of exercise include degradation of oxidatively damaged proteins and organelles, improved mitochondrial oxidative capacity, improved glucose regulation, protein synthesis, preservation of muscle strength and mass, and improved endurance exercise performance.

tional area and strength as well as higher cathepsin L activity, Beclin-1, ATG5 and ATG7 protein content, whereas LC3-II and p62 protein content was lower than in aged untrained rats, altogether indicating increased basal autophagic flux with resistance exercise training (Luo et al. 2013). Furthermore, mice lacking collagen-type VI α (COL6A KO) with impaired autophagy have been shown to display a blunted exercise-induced increase in LC3II/LC3I with concomitant accumulation of dysfunctional mitochondria, altered mitochondrial network (based on SDH staining), muscle fiber degeneration, and decreased muscle strength (Grumati et al. 2010, 2011). These observations showing that an exercise bout elicited extensive muscle damage in COL6A KO mice, but not WT mice, indicate that exercise-induced autophagy is an important myoprotective process. Accordingly, 3 months of exercise training, increasing SDH staining in WT skeletal muscle, reduced skeletal muscle LC3-II/I and exacerbated the dystrophic phenotype with extensive muscle degradation in COL6A KO mice (Grumati et al. 2011). Taken together, this suggests that exercise-induced autophagy is crucial for maintaining muscle mass, ultrastructure, and function (Fig. 2).

Glucose Regulation

A few studies have examined the role of exercise-mediated autophagy on metabolic adjustments in response to exercise. The observation that

plasma lactate, glucose, and free fatty acid concentrations after exercise were unaffected by inducible muscle-specific KO of ATG7 with impaired exercise-induced autophagy (Lo et al. 2014) suggests that autophagy is not required for exercise-induced adjustments in circulating substrates and metabolites. In contrast, pharmacological inhibition of autophagy reduced insulin-stimulated glucose uptake in C2C12 myotubes (Liu et al. 2015), supporting a role of autophagy in glucose regulation. In accordance, the impaired exercise-induced activation of autophagy in BCL AAA and ATG6$^{+/-}$ mice was associated with reduced GLUT4 translocation to the sarcolemma in response to exercise and lower radiolabeled glucose uptake in the isolated soleus muscle. This suggests an association between exercise-regulated autophagy and glucose uptake in skeletal muscle. BCL2 AAA mutated mice also showed impaired exercise training–mediated protection against high fat diet–induced glucose intolerance (He et al. 2012), further supporting that exercise-induced activation of autophagy is required for the beneficial effects of exercise on glucose metabolism (Fig. 2).

ROS Protection

The findings that COL6A KO mice had impaired exercise-induced activation of autophagy and accumulated damaged mitochondria (Grumati et al. 2011) suggest that exercise-induced autophagy serves to remove dysfunctional organelles. In accordance, skeletal muscle protein carbonylation (used as marker of oxidative damage) was shown to be reduced in the recovery period from a single exercise bout coinciding with an increase in LC3-II (Halling et al. 2016). This indicates that exercise-induced autophagy plays a role in removing oxidized proteins to prevent the accumulation of dysfunctional proteins. Furthermore, muscle-specific ATG7 KO mice have been shown to exhibit higher ROS accumulation and mitochondrial membrane depolarization in skeletal muscle than WT mice after eccentric contractions (Lo et al. 2014). This supports that the level of reactive oxygen species may play a role in the regulation of autophagy (Fig. 2). It should be noted that antioxidant treatment did not prevent exercise-induced activation of mitophagy and the ability of exercise to restore the mitochondrial membrane potential (Lo et al. 2014), showing that additional factors must also be involved. However, *N*-acetylcysteine (NAC) antioxidant treatment reduced basal mitophagy in mice (Lo et al. 2014; Qi et al. 2014). This may suggest a mechanism explaining the observation that antioxidant supplementation can have adverse effects on exercise performance as well as metabolic adaptations to exercise training (Gomez-Cabrera et al. 2008; Ristow et al. 2009; Gliemann et al. 2013; Olesen et al. 2014).

Disease States

The impact of exercise-induced autophagy has also been studied through inhibition of autophagy by treatment with the lysosomal inhibitor chloroquine 5 days/week for 16 weeks resulting in sporadic inclusion body myositis, a condition causing muscle weakness and wasting (Kwon et al. 2015). Resistance exercise training was shown to improve muscle strength in chloroquine-treated rats and to prevent chloroquine-induced increases in ATG6 (Beclin-1) and p62 (Kwon et al. 2015). This suggests that resistance exercise training modulates autophagy in atrophying skeletal muscle with potential protective effects on muscle function. Although exercise-induced autophagy normally seems to exert beneficial effects, uncontrolled enhancement of autophagy may lead to muscle wasting and oxidative stress. Hence, treating rats with the autophagy-stimulating antitumor agent doxorubicin was shown to increase LC3-II/I, oxidative stress, and muscle degradation. However, exercise training prevented doxorubicin-induced apoptosis, and elevated the mRNA and protein content of autophagy genes in skeletal muscle (Smuder et al. 2011). This suggests that, in addition to the bona fide autophagy-dependent beneficial effects of exercise, exercise training can contribute in adjusting the level of autophagy in disease states, which may serve to reduce the disease burden.

CONCLUDING REMARKS

Numerous studies have provided evidence that autophagy is activated in skeletal muscle in response to exercise. The exercise-induced regulation of autophagy includes both increased autophagy flux as well as transcriptional activation of important autophagy genes potentially resulting in enhanced autophagy capacity. Furthermore, exercise-induced autophagy seems to be involved in mediating many of the beneficial effects of exercise. However, much more remains to be investigated regarding both the molecular mechanisms involved in acute activation of autophagy and the resulting effects. In particular, the potential impact of autophagy on exercise performance and metabolic adaptations in humans is not clear.

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