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Regulation of Exercise-Induced Autophagy in Skeletal Muscle

Altea Rocchi and Congcong He

Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Abstract

Purpose of Review—Physical exercise is a highly effective method to prevent several pathogenic conditions, such as obesity, type 2 diabetes and cardiovascular diseases, largely due to metabolic adaptations induced by exercise in skeletal muscle. Yet how exercise induces the beneficial effects in muscle remains to be fully elucidated. Autophagy is a lysosomal degradation pathway that regulates nutrient recycling, energy production and organelle quality control. The autophagy pathway is upregulated in response to stress during exercise and muscle contraction, and may be an important mechanism mediating exercise-induced health benefits.

Recent Findings—A number of studies have indicated that physical exercise induces non-selective autophagy and selective mitophagy in skeletal muscle in animal models and humans. The AMPK-ULK1 and the FoxO3 signaling pathways play an essential role in the activation of the upstream autophagy machinery in skeletal muscle during exercise. The autophagy activity is required for health benefits of exercise, as in different autophagy-deficient mouse lines exercise-induced effects are abolished.

Summary—This review aims to summarize and highlight the most recent findings on the role of autophagy in muscle maintenance, the molecular pathways that upregulate autophagy during exercise, and the potential functions of exercise-induced autophagy and mitophagy in skeletal muscle.

Keywords

Autophagy; Mitophagy; Skeletal muscle; Physical exercise; AMP-activated protein kinase; Forkhead box protein transcription factor 3

Introduction

Autophagy is an evolutionarily conserved degradative pathway, in which intracellular cargos are broken down inside lysosomal compartments (1). Under normal conditions, autophagy occurs at a low basal level, which is essential for the maintenance of cellular homeostasis

Corresponding Author: congcong.he@northwestern.edu.

Co-author: altea.rocchi@northwestern.edu

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through recycling of cytoplasmic molecules and removal of damaged organelles or toxic proteins; whereas in the presence of stressors such as starvation, hypoxia, oxidative stress or physical exercise, the autophagy activity is significantly induced (2). Autophagy is not only important for cell survival under stress conditions, but also key in several physiological processes including metabolism, development and differentiation. In fact, impaired autophagy has been implicated in several diseases such as neurodegeneration, metabolic syndrome, muscular atrophy and cancer (3–5). Thus, it is necessary to elucidate the functions and mechanisms of autophagy activation under physiological and pathological conditions. As an effective physiological autophagy inducer, physical exercise has been widely recognized to exert beneficial effects against a number of chronic diseases (6). Especially in skeletal muscle, exercise induces metabolic adaptations, including improved glucose metabolism, enhanced mitochondrial function, and better physical performance and growth of muscle mass (7–9). Therefore, autophagy activation by exercise may be a key mechanism underlying exercise-induced health benefits in skeletal muscle. In addition, in order to enable contraction, muscle requires a high level of ATP provided by mitochondria (10, 11). Thus, maintenance of healthy mitochondria is important for endurance performance and exercise-induced muscle remodeling. A selective form of autophagy, known as mitophagy, removes damaged mitochondria and is also suggested to play a role in mediating the beneficial effects of exercise.

Here we will review the current advances on the evidence linking the signaling pathways activated by muscle contraction to autophagy induction in skeletal muscle, and the role of autophagy and mitophagy in skeletal muscle adaptation after physical exercise. We will also describe the different effects of specific exercise models on autophagy levels in muscle, and discuss the future directions and challenges in better understanding how exercise regulates autophagy to maintain muscle homeostasis and combat muscular diseases.

Molecular Mechanisms Regulating Autophagy

Various stressors, such as hypoxia and starvation, initiate the formation of phagophores, which incorporate cellular components, elongate and complete into double-membrane vesicles, known as autophagosomes. The autophagosome then fuses with a lysosome, generating an autolysosome, where the autophagosomal cargos are digested by the lysosomal resident enzymes, such as proteases, lipases and nucleases (Figure 1A).

Autophagy is regulated by the autophagy-related (ATG) genes, which are highly conserved from yeast to mammals (12, 13). Briefly, autophagy is activated by the upstream AMPK-mTOR-ULK1 signaling axis (14). The ULK1/Atg1 kinase complex, which comprises of the serine/threonine kinase ULK1 and the subunits Atg13, Atg101 and FIP200, positively regulates autophagosome formation (15, 16). Under normal conditions, the ULK1 complex is phosphorylated and sequestered by the major autophagy inhibitor mTOR complex; upon autophagy induction, ULK1 is activated by dephosphorylation and consequent dissociation from mTOR (17). A second level of ULK1 regulation is mediated by AMPK (AMP-activated protein kinase), which is activated by autophagy-inducing stimuli. Once activated, AMPK induces the ULK1 kinase complex by both inhibiting mTOR and directly

phosphorylating residues on ULK1 that are different from the mTOR phosphorylation sites (18).

The downstream substrates of the ULK1 kinase have been recently studied, one of which is the class III phosphatidylinositol (PtdIns) 3-kinase (PtdIns3K) complex (19). The class III PtdIns3K complex consists of the PtdIns3 kinase Vps34 and subunits Becn1/Atg6, Atg14, Vps15 and Ambra1, and regulates the autophagosome nucleation step. Once free, ULK1 phosphorylates Vps34 at Ser249, and up-regulates the PtdIns3K kinase activity (20). The Vps34 kinase complex generates the lipid phosphatidylinositol 3-phosphate (PI3P), which in turn recruits additional PI3P-binding autophagy proteins, including WIPI1 and DFCP1, and promotes autophagosome formation (21–23).

The expansion of the autophagosome membrane requires two ubiquitin-like conjugation systems, Atg12-Atg5-Atg16 and Atg8-PE (phosphatidylethanolamine) (24–26). LC3 is the mammalian homolog of yeast Atg8, and is widely used as a marker for autophagosomes (27). Upon autophagy induction, LC3 is converted from cytosolic non-lipidated form (LC3-I) to autophagosome-associated PE-conjugated form (LC3-II), which can be resolved by western blot or visualized as fluorescent puncta (27, 28). The Atg12-Atg5-Atg16 complex functions as an ubiquitin E3-like ligase for the conjugation of Atg8/LC3-PE, allowing the latter to be incorporated into the autophagosomal membrane.

Function of Autophagy in Skeletal Muscle

A number of studies have suggested that autophagy plays an essential role in the maintenance of muscle homeostasis, and autophagy dysregulation has been associated with muscle atrophy and myopathy. Basal autophagy is suggested to play an important role in preserving muscle mass and myofiber integrity, from studies on mouse models of muscle-specific deletion of autophagy genes. In particular, muscle-specific Atg5- or Atg7-null mice accumulated abnormal mitochondria, membranous inclusions and ubiquitinated protein aggregates, and manifested aggravated muscle weakness during denervation or fasting (29, 30). Similar phenotypes have also been observed upon conditional muscle deletion of components of the autophagy-inducing class III PtdIns3K complex, including Vps15 and Vps34, which leads to massive accumulation of autophagosomal and lysosomal structures and development of severe myopathies (31, 32). These findings indicate an essential role of autophagy in muscle functionality. However, it should be noted that deletion of Vps34 in muscle also resulted in aberrant accumulation of plasma membrane proteins (such as dystrophin and caveolin-3), indicating that besides autophagy, impairments in endocytic trafficking may also contribute to muscular dystrophy in the absence of Vps34. In addition, in myoblasts derived from patients of Danon disease, a lysosomal storage disorder caused by the mutated lysosomal protein LAMP2B and characterized by glycogen accumulation in lysosomes, overexpression of Vps15 seems to be able to ameliorate glycogen accumulation (31), suggesting that in contrast to the common view, increased autophagosome formation mediated by Vps15 does not overburden the lysosomal degradation defects in Danon disease. Thus, further studies are needed to better understand the molecular mechanisms of autophagy in the regulation of myopathies.

On the other hand, in skeletal muscle, overactivation of the Forkhead box protein transcription factor 3 (FoxO3) regulates both lysosomal and proteasomal degradation pathways, and leads to muscle wasting (33, 34). Autophagy seems to contribute to FoxO3-mediated muscle protein proteolysis and muscle atrophy synergistically with the ubiquitin-proteasome system, as knockdown of LC3 expression partially prevents FoxO3-mediated muscle loss. FoxO3 induces the expression of several autophagy-related genes, including LC3B, GABARAPL1, and ATG12, BNIP3L (also known as NIX) and BNIP3, by directly binding to the FoxO response element in their promoters (35, 36). Among the FoxO3-targeting genes, BNIP3 is one of the most induced atrogenes during muscle atrophy and is required for autophagy induced by FoxO3 (35). Regarding the upstream regulation of FoxO3a, it seems to be controlled by AKT and AMPK signaling pathways in opposite directions. While AKT inhibits its transcriptional activity (37), AMPK activates FoxO3 through the phosphorylation on the Ser588 residue (38, 39). Thus, AMPK promotes autophagy in muscle via activating two routes, the kinase activity of ULK1 or the transcriptional activity of FoxO3 (Figure 1C).

Overall, autophagy functions as a double-edged sword in skeletal muscle, where both inhibition and overactivation of autophagy exert detrimental effects. Thus, a deeper understanding of autophagy in muscle is necessary for the development of potential therapeutic strategies involving autophagy modulation.

Effects of Exercise on Autophagy in Skeletal Muscle

The first evidence connecting exercise with the autophagy pathway is demonstrated by ultrastructural analysis of muscle fibers in a study back in 1984, which revealed an accumulation of autophagic vacuoles in mouse skeletal muscle during recovery from injury caused by strenuous prolonged running (9 h) (40). Yet the molecular evidence and functional importance of exercise-induced autophagy have only been investigated recently (41, 42). We, and others, have elucidated that 80-min treadmill running effectively stimulates autophagosomes formation (measured by LC3 lipidation and GFP-LC3 puncta) and autophagy flux (measured by LC3 flux and p62 degradation) in mouse skeletal muscle (41, 42). Importantly, both studies suggest that exercise induces beneficial effects in muscle of WT mice, but not of autophagy-deficient mice. For instance, failure to induce autophagy in Collagen IV null (*Col6a1*^{-/-}) mice, which are characterized by low muscle strength and impaired autophagy flux in muscle, exacerbates muscle wasting after 3-month voluntary exercise, suggesting that autophagy activation may be important for muscle adaptation in response to physical training (41), although in this study many autophagy-independent pathways may also be affected by collagen depletion and contribute to accelerated myofiber degeneration. Using a more specific autophagy-deficient mouse model containing non-phosphorylatable mutations in the autophagy inhibitor BCL-2 (*BCL-2*^{AAA}), which constitutively inhibits the autophagy protein Becn1 and impairs induction of autophagy by exercise, we demonstrated that exercise mediates metabolic benefits through autophagy stimulation. In contrast to WT mice, in the *BCL-2*^{AAA} mice 8 weeks of treadmill exercise were not able to reverse glucose intolerance induced by high-fat diet (HFD) (42). These mice also showed reduced exercise endurance compared to WT mice. Similarly, autophagy-deficient *Becn1*^{+/-} KO mice, which are defective in exercise-induced autophagy, failed to

show improvement in endurance capacity after long-term voluntary running as WT mice (43).

These discoveries have raised further interests and attention in the field, and many subsequent studies have been published to characterize the autophagy process during exercise. Both endurance exercise and resistance exercise training (RET) stimulate the expression of autophagy proteins and the autophagy flux in muscle (43, 44). Moreover, besides forced exercise, voluntary exercise also effectively induces autophagy (45, 46). Yet the levels of autophagy induction in skeletal muscle depend on the intensity and duration of exercise. A single bout of high intensity exercise has been reported to be more effective in inducing the autophagy flux than prolonged exercise with moderate intensity, measured by increase in the LC3-II/LC3-I ratio and reduction of p62 levels (47, 48) (Table 1). Similar results are also found in human. In well-trained athletes, high-intensity, but not low-intensity, exercise activates the autophagy flux and the AMPK pathway, and increases the mRNA level of LC3B, p62/SQSTM1, Gabarap, and Cathepsin L (49). Thus, exercise intensity, rather than duration, seems to determine the level of autophagy induction. In addition, autophagy induction is detected immediately after exercise(45); yet it is unclear whether it persists during the recovery phase (e.g., 3, 6, 12 or 24 hours after exercise), when many autophagy-related genes seem to be downregulated in both rodents and humans (50, 51). Therefore, altogether, we conclude that exercise induces autophagy and autophagy gene expression in mice, which exerts either acute or adaptive responses according to the chosen exercise protocol.

Only limited data are available on exercise-induced autophagy in human skeletal muscle (Table 2). Endurance exercise, which increases oxygen consumption such as running or swimming, appear to be more effective in inducing protein and mRNA levels of autophagy genes (such as Atg4, Atg12, Gabarap and LC3b) than resistance exercise, such as one-legged knee extension exercise (49, 51–54). However, most human studies used the ratio of LC3-II/LC3-I as a sole readout, which may not accurately reflect the autophagy flux, because the flux is determined by a combined outcome of autophagosome formation and degradation. This may be a limitation in human studies, where the use of lysosomal inhibitors is not suitable. Moreover, another important factor to consider with human subjects is that the effects of acute exercise on autophagy may be influenced by prior experience of exercise training, which may lead to adaptive changes in autophagy activity. Therefore, the effect of aerobic and resistance exercise training on autophagy in human skeletal muscle needs to be further elucidated.

Studies on both rodents and humans demonstrate that exercise activates phosphorylation of AMPK at Thr172 and AMPK-dependent downstream autophagy kinase ULK1 at Ser317 and Ser555. Meanwhile, exercise inhibits the Akt/mTOR signaling, the Akt-mediated phosphorylation of FoxO3a at Thr32 and Ser253, and mTOR-dependent phosphorylation of ULK1 at Ser757. These findings suggest that acute exercise stimulates autophagy through upregulation of ULK1 kinase activity by inhibiting the Akt/MTOR pathway and activating AMPK signaling (47, 55). Intriguingly, pharmacological activation of AMPK by the AMPK agonist AICAR does not change the LC3-II/LC3-I ratio in mouse skeletal muscle or cultured human myotubes, indicating that activating AMPK alone is not sufficient to induce

autophagy in skeletal muscle during exercise (52). Along this line, no changes in the LC3-II/LC3-I ratio or p62 levels are detected after supraphysiological *in situ* electrical stimulation (48). Thus, we speculate that systematic circulating factors may also play an important role in autophagy induction by exercise.

Potential Role of Mitophagy during Exercise

It remains unclear how autophagy mediates the health benefits induced by exercise. One study using inducible Atg7-null mice shows that autophagy deficiency leads to an accumulation of muscle fibers with depolarized mitochondria, which are potential sources of toxic reactive oxygen species (ROS) generated during muscle contraction (56). ROS production contributes to insulin resistance; thus, removal of dysfunctional mitochondria by mitophagy may be a key mechanism of autophagy in exercise-mediated insulin sensitization.

Mitophagy is the selective degradation of mitochondria by the autophagy machinery (57). Studies in cultured cell lines demonstrate that the regulation of mitophagy can be categorized into two pathways: the first mechanism is dependent on the PTEN-induced kinase 1 (PINK1) and the E3 ubiquitin-protein ligase Parkin (58), and the second is dependent on receptor proteins localized in the mitochondrial outer membrane (59–61). In the PINK/Parkin-dependent pathway, PINK1 is continuously imported and degraded in the mitochondrial inner membrane in healthy mitochondria; whereas upon loss of the mitochondrial membrane potential, PINK1 is recruited to and accumulated on the mitochondrial outer membrane, where it phosphorylates and activates Parkin. Parkin can subsequently build ubiquitin chains on the mitochondria, which amplifies the signal for the recognition and degradation of mitochondria by the selective autophagy machinery (Figure 1B). Several cytosolic receptors have been identified from cell culture studies to facilitate the sequestration of ubiquitinated mitochondria into autophagosomes (62, 63), including p62/SQSTM1, NDP52 (nuclear dot protein 52 kD), optineurin, TAX1BP1 and NBR1 (64–69). What they have in common is that all of the receptors contain an ubiquitin-associated (UBA) domain that specifically interacts with ubiquitinated proteins and an LIR (LC3-interacting region) motif that binds to the autophagosomal protein LC3. Among the cytosolic receptors, NDP52 and optineurin seem to be two primary mitophagy receptors on the mitochondrial membrane recruited by PINK in HeLa cells. These two receptors are able to recruit the upstream autophagy machinery (such as ULK1, WIPI1 and DFCP1) to mitochondria and activate mitophagy independently of Parkin (70, 71).

On the other hand, mitophagy mediated by mitochondrial membrane receptors can occur without an abnormal mitochondrial membrane potential. Receptor-based mitophagy seems to be important in controlling mitochondrial quality and quantity in response to changing energy needs or other cellular cues. It is regulated by mitochondrial receptor proteins localized in the outer membrane, such as BNIP3 (Bcl2/adenovirus E1B 19kD interacting protein 3), BNIP3L and FUNDC1 (FUN14 domain-containing protein 1) (60, 61, 72). Once activated, these receptors bind LC3 and mediate the degradation of the mitochondria inside the lysosomal compartment (Figure 1B). BNIP3 is induced by voluntary exercise in muscle groups consisting of mixed fiber types, such as plantaris, but not in oxidative muscles such as soleus (43), indicating that the occurrence of mitophagy during exercise may depend on

the oxidative type of muscle. However, how the mitophagy proteins are activated by upstream cues or signaling cascades is not yet clear, and how PINK1/Parkin- and mitochondrial receptor-mediated mitophagy crosstalk with each other remains to be fully elucidated.

The importance of understanding the role and mechanism of exercise-induced mitophagy has only recently been realized, and this research field is at a very early stage and is developing. It is still unclear whether the same mechanisms identified in cell culture systems regulate mitophagy after exercise in vivo. One recent study showed that acute exercise increased mitochondrial ubiquitination and mitochondria-localized Parkin, p62 and LC3-II levels in skeletal muscle of WT mice (73), suggesting that the PINK/Parkin-dependent pathway may be involved in exercise-mediated mitophagy. Notably, these changes on mitochondria were abolished in PGC-1 α KO animals, indicating a role of PGC-1 α in the regulation of muscle mitophagy during physical exercise. However, direct imaging evidence of mitophagy in vivo during or after exercise is still lacking; the recent development of a number of mitophagy reporters (such as MitoTimer) will be of help to investigate the function and mechanism of mitophagy in vivo (74). Further metabolic analyses using autophagy or mitophagy mutant mice are also necessary to clarify the molecular mechanisms and consequences of autophagy/mitophagy stimulation in skeletal muscle during physical activity.

Conclusions and Future Perspectives

In summary, exercise induces autophagy in skeletal muscle in both rodents and humans, and the level of autophagy is affected by the intensity, duration and modality of the exercise protocols. Several signaling pathways that are stimulated by physical exercise regulate the induction of autophagy in skeletal muscle. Both non-selective autophagy and selective mitophagy are essential for skeletal muscle adaptation to physical exercise, yet many questions remain unanswered in this emerging field. For example, the molecular mechanisms by which exercise modulates autophagy needs to be further investigated, in order to understand how exercise promotes health benefits and improves physical performance. Future studies will also focus on characterizing the roles of exercise-induced autophagy in metabolic diseases including obesity and type 2 diabetes, and muscular disorders as Pompe disease and Danon disease. In addition, the function of autophagy has been suggested as a double-edged sword. Long-term exhaustive exercise causes high expression of autophagy-related proteins such as Atg7, Becn1, and FoxO3, as well as the muscle atrophy markers atrogin-1 and MuRF1 (muscle specific ring finger protein 1) (75); thus, it is important to further investigate whether over-activation of autophagy may occur after extreme exercise and lead to detrimental effects in skeletal muscle. Finally, there is a lack of understanding on the mitophagy machinery induced by acute or chronic exercise. Since abnormal mitochondrial contents and oxidative capacity is associated with a number of chronic diseases, such as muscular atrophy (76), it is necessary to elucidate whether exercise-induced mitophagy can serve as a mitochondrial quality and quantity control mechanism to help meet energy demand and maintain muscle integrity under pathogenic conditions.

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Figure 1.

A. Schematic representation of the non-selective autophagy pathway. After the nucleation and elongation step, the mature autophagosome containing a portion of cytoplasm fuses with the lysosome, in which cellular contents are degraded. **B.** Schematic representation of mitophagy, in which the autophagy machinery selectively recognizes either cytosolic receptors (such as p62 recruited by the PINK1/Parkin pathway) that bind to ubiquitinated mitochondria, or mitochondrial outer-membrane receptor proteins, such as BNIP3 and FUNDC1. Mitochondria are then degraded in the autolysosome. **C.** Major upstream signaling

pathways that induce autophagy and mitophagy during exercise. Activation of AMPK stimulates autophagy by inhibiting mTOR and inducing the ULK1/2 kinase. AMPK also promotes the expression of the FoxO3 transcription factor, which upregulates multiple autophagy genes at the transcriptional level. Unknown circulating factors may as well contribute to autophagy induction after exercise.

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Table 1

Summary of exercise-induced autophagy marker changes in animal models (upper, single-bout exercise/ bottom, long-term exercise)

References	Exercise Modality	Autophagy regulation	Tissues
(41)	60 min treadmill increasing speed (10m/min to 40 m/min-slope 5°)	WT mice: ↑ LC3II/LC3I ↑ LC3 puncta Col6a1^{-/-} mice: Muscle wasting ↓ LC3II/LC3I	Tibialis anterior and diaphragm
(42)	80 min treadmill 900 m at 75% of their maximal running capacity (slope 10°)	WT mice: ↑ LC3 puncta ↑ LC3II/LC3I ↑ p62 degradation GLUT4 localization in skeletal muscle Bcl2AAA mice: ↓ LC3 puncta ↓ LC3II/LC3I ↓ p62 degradation ↓ maximal exercise capacity ↓ exercise-induced increase in insulin sensitivity ↓ GLUT4 localization in skeletal muscle	Skeletal muscle (vastus lateralis, tibialis anterior, extensor digitorum longus and soleus)
(50)	50 min treadmill Speed 12.5 m/min	WT mice: ↓ LC3II ↓ Beclin 1, Atg7, Atg12+5, LAMP2A (from 0 to 12 h after exercise)	Gastrocnemius
(48)	30 or 120 min treadmill at 50% of their maximal running capacity (slope 0°) 25 min stimulation in situ muscle contractions	WT mice: No change in LC3II/LC3I No change in p62 degradation AMPK KO mice: No change in LC3II/LC3I No change in p62 degradation	Quadriceps
(47)	30, 60, 90, and 120 min and exhaustion time (Te) of treadmill increasing speed (starting at 10m/min)	WT mice: ↑ pAMPK ↑ FoxO3a prot. level (3h after exe) ↑ LC3II/LC3I at 120 min and Te ↑ p62 degradation at Te	Soleus and quadriceps
(52)	4h of swimming (8 bouts of 30min)	WT rats: ↑ LC3II/LC3I ↑ LC3II	Tibialis anterior
(45)	90 min treadmill increasing speed (10m/min to 17 m/min-slope 10°)	WT mice: ↑ LC3 puncta ↑ LC3II ↑ p62 degradation	Vastus lateralis
(56)	Treadmill running increasing speed until exhaustion (slope 10°)	cKOAtg7 mice: ↓ running distance Accumulation of dysfunctional mitochondria	Tibialis anterior
(73)	Treadmill running increasing speed (from 5 m/min to 30m/min until exhaustion)	WT mice: ↑ pAMPK ↑ PGC-1 α and downstream Coxiv ↑ LC3II ↑ mitochondrial LC3II PGC-1α KO mice: ↓ endurance performance ↑ p38 MAPK No significant change for LC3II and mitochondrial LC3II	Skeletal muscle
(77)	Treadmill 40 min at 16.3 m/min (slope of 5°) 5 days/week for 8 weeks	Young (4 months) WT mice: ↓ Atg7, Beclin1 No change LC3II Old (12 months) WT mice: ↑ Atg7, LC3II	Gastrocnemius and EDL

References	Exercise Modality	Autophagy regulation	Tissues
(44)	9 weeks RET exercise in climbing apparatus using weights attached to the rat tail	Aged (18–20 months) WT rats: ↑ muscle size ↑ Beclin-1, Atg5/12 and Atg7 ↓ LC3II/LC3I ↑ p62 degradation ↑ AMPK, pAMPK ↓ pAKT, pmTOR ↑ FoxO3a	Gastrocnemius
(41)	Voluntary running 3 months	WT mice: No changes in LC3II/LC3I Col6a1^{-/-} mice: ↑ Inflammation Myofiber degeneration Muscle wasting ↓ running distance ↓ LC3II lipidation ↓ LC3II/LC3I	Tibialis anterior and diaphragm
(43)	Voluntary running 4–5 weeks	WT mice: ↑ LC3, LC3II, Atg6, Bnip3, Pgc1- α in plantaris muscle ↑ p62 degradation Beclin1^{+/-} mice: No difference in LC3II, Bnip3, Pgc1- α and p62 level	Plantaris, soleus and vastus lateralis
(75)	8 weeks treadmill speed of 20 m/min (slope 5°) 1 h per day, 6 days per week	WT rats: Beclin1, LC3II, Atg7, FoxO3a	soleus

Table 2

Summary of exercise-induced autophagy marker changes in human skeletal muscle (upper, single-bout exercise/bottom, long-term exercise)

References	Exercise Modality	Autophagy regulation	Tissues
(49)	Cycling 2 h Low intensity (LI) 55% of VO ₂ peak, High intensity (HI) 70% of VO ₂ peak	In HI group: ↑ pAMPK, pULK1 ↑ mRNA of LC3b, p62, GabarapL1, and Cathepsin L	Vastus lateralis
(54)	24h treadmill	↑ LC3II, Ag5/12, pAMPK ↓ p-AKT, p-FoxO3a, p-mTOR	Vastus lateralis
(53)	28 h marathon (200Km)	↑ mRNA Atg4, Atg12, Gabarap, LC3II, Bnip3, Bnip3l, Ulk1	Vastus lateralis
(51)	45 min of leg extension machine (8 sets of 10 repetition at 70% maximum force) Analyzed 3h, 6h and 24h post exercise	↓ Gabarap mRNA ↓ LC3II ↓ LC3II/LC3I No change in Beclin1 and Atg7	Vastus lateralis
(55)	60 min cycling at 50% VO _{2max}	↑ pAMPK, pULK1 ↓ LC3II/LC3I No change Beclin 1 Gabarap, Atg 5	Vastus lateralis
(52)	60 min bout of one-legged knee extensor exercise at 80% peak work load (PWL)	↓ LC3II/LC3I ↓ LC3II No change p62	Vastus lateralis
(52)	incremental one-legged knee extensor exercise for 3 weeks total	↓ LC3II/LC3I ↑ p62	Tibialis anterior
(78)	Leg resistance exercise for 4 weeks (constant or progressive load)	Progressive load group: ↑ CASA (chaperone-assisted selective autophagy) (BAG3, HSPB) ↑ p62 ↑ CASA target FLNC	Vastus lateralis