The role of autophagy in skeletal muscle homeostasis

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Abstract  Autophagy is a process in which lysosomes participate in the degradation of cellular proteins and organelles, and is essential for cell survival. Recent studies of autophagy have focused on its role in skeletal muscle homeostasis. Autophagic flux in skeletal muscle is induced not only by atrophic stimuli such as fasting and denervation, but also by physical exercise. Excessive activation of autophagy promotes the progression of muscle atrophy. In contrast, impaired or deficient autophagy appears to promote muscle weakness, myopathy, and age-related muscle atrophy due to the accumulation of denatured proteins and damaged organelles. The induction of autophagy during exercise is believed to play a role in physiological adaptations to exercise, such as increased oxidative capacity and insulin sensitivity. Thus, it is important for skeletal muscle homeostasis to appropriately regulate autophagy.

Keywords: autophagy, atrophy, homeostasis, aging, myopathy

Autophagy and proteolysis in skeletal muscle

Autophagy is a crucial survival mechanism during nutrient deprivation and plays a role in the turnover of cell components via self-digestion. Macroautophagy degrades older proteins and eliminates damaged organelles via double-membraned structures called autophagosomes. Levels of autophagy are low under basal conditions, but can be enhanced by stimuli such as fasting or increased levels of denatured proteins.

Skeletal muscle is the most abundant tissue in the human body, accounting for approximately 40% of total body mass, and is the body’s largest protein reservoir. Skeletal muscle protein content is determined by the relative rates of protein synthesis and degradation. Muscle atrophy results when degradation exceeds synthesis. Preferential degradation of muscle proteins has been observed in response to inactivity, denervation, and fasting. Most proteins are degraded by two proteolytic systems in skeletal muscle: the autophagy-lysosome and ubiquitin-proteasome. Induction of autophagy in fasting and in denervation-related muscle atrophy models has been well documented. LC3 is an autophagy-related gene (Atg) homolog that is essential for autophagosome formation, and the phosphatidylethanolamine-conjugated form, LC3-II, is widely used to monitor levels of autophagy.

Under fasting conditions, LC3-II expression increases as skeletal muscle mass decreases. Interestingly, activation of autophagy accompanied by LC3-II expression is seen at higher levels in fast-twitch glycolytic muscles than in slow-twitch oxidative muscles during fasting. This is consistent with the fact that greater muscle atrophy is observed in fast-twitch muscle than in slow-twitch muscle during starvation.

Although the ubiquitin proteasome appears to make a greater contribution to muscle protein degradation than autophagy, high levels of autophagy induction are associated with muscle atrophy. Forkhead box protein class O3a (FOXO3a) is a major regulatory factor for both autophagy and ubiquitin proteasome induction, and genetic activation of FOXO3a results in skeletal muscle atrophy. In an in vivo model of atrophying muscle with constitutive activation of FOXO3a, reduction of LC3 levels by RNA interference partially prevents the loss of muscle mass.

Similarly, Dobrowolny et al. generated transgenic mice expressing mutant SOD1 (SOD1G93A) and observed increased autophagy flux and progression of skeletal muscle atrophy. Inhibition of autophagy using siRNA against LC3 mitigated the reduction in muscle fiber cross-sectional area seen in the skeletal muscle of SOD1G93A mice. These findings suggest that excessive autophagy contributes to loss of skeletal muscle mass.

Autophagy and skeletal muscle maintenance

Proper regulation of autophagy is required for maintenance of skeletal muscle fibers. Deficient autophagy leads to muscle weakness, wasting and myopathy. A muscle-specific knockout of the crucial autophagy gene Atg 7 shows muscle degeneration, including vacuolated and...
centrally nucleated myofibers, and a dramatic decrease in myofiber size and force generation. These autophagy-deficient muscles also show accumulation of abnormal mitochondria, sarcoplasmic reticulum distension, disorganization of sarcomeres, and activation of apoptosis, contributing to loss of muscle mass. This suppression of autophagy via Atg7 gene knockdown additively exacerbates muscle loss during denervation and fasting. Other genetic models have also confirmed the role of autophagy in muscle maintenance. Autophagy null mice with muscle-specific inactivation of the Atg5 gene represent a similar atrophic phenotype, with significant reduction of muscle fiber cross-sectional area and accumulation of ubiquitinylated proteins. These data strongly indicate that suppression of autophagy is harmful, resulting in muscle atrophy, weakness, and several features of myopathy.

Congenital muscular dystrophy is a severe type of myopathy characterized by a progressive loss of muscle mass. Mutations in collagen VI, an extracellular matrix protein, can cause muscular dystrophy, including the rare form known as Bethlem myopathy (BM), in humans. Collagen VI null (Col6a1-/-) mice demonstrated muscle fiber degradation, decreased muscle strength, and spontaneous apoptosis. Col6a1-/- mice also displayed accumulation of abnormal mitochondria and sarcoplasmic reticula, and impaired induction of autophagy in skeletal muscle. This impairment of autophagy may explain the accumulation of dysfunctional organelles seen in these mice. Interestingly, it has been reported that forced activation of autophagy by genetic manipulation, energy deprivation, or pharmacological intervention, restored myofiber survival and ameliorated the dystrophic muscle phenotype of Col6a1-/- mice. Specifically, treatment with cyclosporin A or rapamycin, both of which are strong inducers of autophagy, in the skeletal muscle of Col6a1-/- mice, promoted autophagy with a concomitant decrease in apoptotic nuclei and abnormal mitochondria, and recovery of muscle strength.

Thus, if the autophagic machinery is impaired in skeletal muscle, dysfunctional organelles can accumulate, leading to degenerative myopathy. However, if the autophagic machinery is rescued in atrophying muscle, dysfunctional organelles can be eliminated, mitigating the loss of muscle mass. These facts provide evidence for the beneficial role of autophagy in muscle homeostasis.

Relationship between autophagy and physical exercise

Physical exercise has several beneficial effects on health, such as protection against metabolic disorders, and can improve athletic performance. However, during exercise, muscle proteins are damaged by mechanical loading with eccentric muscle contraction, and by oxidative attacks from reactive oxygen species (ROS) produced during ATP generation. An earlier study reported that strenuous physical exercise increased the number of autophagic vesicles in skeletal muscle fibers. Although the role of autophagy during physical exercise has long been unclear, a few recent studies have provided evidence for the beneficial effects of autophagy in skeletal muscle during exercise.

In normal skeletal muscle, long-term exercise training induces physiological adaptations such as muscle hypertrophy and increased oxidative capacity. Interestingly, not only do Col6a1-/- mice fail to show these physiological adaptations in response to exercise, but actually show muscle degradation after exercise training. Because the dystrophic muscles of Col6a1-/- mice are unable to induce autophagy after either acute or prolonged exercise, the lack of exercise adaptation in Col6a1-/- mice may be due to their impaired clearance capacity during muscle contraction.

He et al. generated mutant mice that show normal levels of basal autophagy but are deficient in their response to stimuli such as exercise and starvation, and termed these mice BCL2 AAA mice. BCL2 AAA mice contain knock-in mutations in the BCL2 phosphorylation site (Thr69Ala, Ser70Ala and Ser84Ala). In these mice, impaired exercise-induced autophagy is accompanied by lower endurance exercise capacity: maximal treadmill running distance is lower in BCL2 AAA than in wild-type mice. In addition, BCL2 AAA mice demonstrate a diminished exercise-induced increase in insulin sensitivity: in wild-type mice, plasma glucose levels after acute exercise are reduced in an exercise intensity-dependent manner, but this is not seen in autophagy-deficient BCL2 AAA mice. It is well known that long-term exercise training improves high fat diet-induced glucose tolerance. However, BCL2 AAA mice are not able to acquire these beneficial metabolic effects of exercise training, suggesting that exercise-induced autophagy contributes to improvement of glucose tolerance.

Thus, exercise-induced autophagy in skeletal muscles is not only able to prevent the accumulation of damaged organelles and maintain cellular homeostasis, but also plays an essential role in exercise-induced metabolic adaptation.

Autophagy in age-related muscle atrophy

A common feature of aging is the accumulation of denatured proteins and abnormal organelles, such as swollen nuclei and mitochondria, leading to the decreased survival capacity of the organism. Age-related muscle atrophy, known as sarcopenia, appears to partially involve the appearance of abnormal organelles. It is well known that mitochondrial dysfunction is associated with the aging process and that oxidative damage to mitochondrial DNA and proteins accumulates over time due to ROS produced by the electron transport chain. Segmental damaged mitochondria and mitochondrial DNA deletion mutations have been observed in aged skeletal muscle. Muscle fibers...
harboring mutated mitochondria often display sectional atrophy, splitting, and increased steady-state levels of oxidative nucleic acid damage. Thus, age-related mitochondrial mutations are suspected to play a role in muscle fiber disruption and atrophy through ROS generation. Therefore, elimination of mutated mitochondria in aged muscle could attenuate the progression of sarcopenia.

Both caloric restriction (CR) and physical exercise are well known to ameliorate sarcopenia by decreasing myofiber atrophy and loss. Given the role of autophagy in the clearance of damaged organelles, as described above, the beneficial effects of these interventions may be associated with activation of autophagy, because the autophagy-lysosomal system is known to deteriorate with age. Wohlgemuth et al. observed that either lifelong mild CR (8% restriction) alone or voluntary exercise combined with CR, reduced levels of 4-hydroxy-nonenal (4-HNE)-modified protein, a marker of lipid peroxidation, in the mitochondria of aged skeletal muscle, and increased lysosome-associated membrane protein (LAMP)-2 gene expression. In their study, there was a negative correlation between 4-HNE-modified mitochondrial protein and LAMP-2 gene expression. We also observed that intermittent fasting (48 h per week) for half a year preserved a higher muscle weight than ad libitum feeding in 32-month-old senescent rats (unpublished data).

AMP-activated protein kinase (AMPK) is a sensor of cellular energy status and is activated by stimuli resulting in an increase in the cellular AMP:ATP ratio (such as energy deprivation or exercise). It is known that increased AMPK activity leads to clearance of denatured proteins and damaged cellular structures through autophagy. Moreover, activation of AMPK can reduce ROS and endoplasmic reticulum (ER) stress. Because increased ROS and ER stress are common characteristics of aging, decreased AMPK activation capacity may cause impaired autophagy in aged skeletal muscle. Reznick et al. demonstrated that acute administration of 5′-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) and exercise increased AMPK activity in the muscles of young rats; but this effect was blunted in the muscles of aged rats. Therefore, the amelioration of sarcopenia by CR and physical exercise training may partially be explained by the induction of autophagy though improved AMPK activation capacity. However, at this time, there is no direct evidence linking CR and exercise training to improved autophagic machinery via AMPK, and further study is needed to identify the activators of autophagy in aged skeletal muscle.

Conclusions

Under many types of stress, including nutrient deprivation, mitochondrial ROS generation, and accumulation of damaged organelles, autophagy appears to mediate a stress adaptation pathway that promotes cell survival. Either reduced or excessive autophagy is detrimental for muscle health, and proper regulation of autophagic flux is essential for skeletal muscle homeostasis (Fig. 1). Therefore, autophagy represents a potential therapeutic target for treatment of degenerative myopathy and sarcopenia. However, little is currently known about the regulation and function of autophagy in skeletal muscle. Further studies are expected to clarify the therapeutic potential of treatments targeting autophagy and to identify unknown functions of autophagy in skeletal muscle.

![Autophagy](image)

**Fig. 1** Autophagy must be regulated in response to physiological conditions. Excessive autophagy triggers muscle atrophy through increased protein degradation. Insufficient autophagy can also result in muscle damage, such as dystrophic myopathy and sarcopenia, and maladaptation to physical exercise.

**If excessive,**
- Increased protein degradation rate
  - loss of muscle mass

**If optimal,**
- Maintenance of muscle integrity and mass

**If defective,**
- Accumulation of damaged organelles and denatured proteins
  - muscle weakness
  - myopathy
  - maladaptation to physical exercise
  - progression of age-related atrophy
References


