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Review Article

Recent advances in mitochondrial turnover during chronic muscle disuse

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ABSTRACT

Chronic muscle disuse, such as that resulting from immobilization, denervation, or prolonged physical inactivity, produces atrophy and a loss of mitochondria, yet the molecular relationship between these events is not fully understood. In this review we attempt to identify the key regulatory steps mediating the loss of muscle mass and the decline in mitochondrial content and function. An understanding of common intracellular signaling pathways may provide much-needed insight into the possible therapeutic targets for treatments that will maintain aerobic energy metabolism and preserve muscle mass during disuse conditions.

1. Introduction

Skeletal muscle is the largest tissue in the body, representing approximately 40% of the total mass of a healthy adult. This tissue is well characterized as exceptionally malleable, and a diversity of stimuli, such as prolonged inactivity,[1] denervation,[2] starvation,[3] aging,[4] or chronic disease,[3,5] can negatively impact muscle mass. Each unique stimulus yields similar, yet characteristic, molecular, functional, and phenotypic alterations in skeletal muscle. The resulting muscle atrophy is defined by an overall loss of proteins, organelles, and cytoplasm. Mitochondria are the main source of energy in skeletal muscle and they provide adenosine triphosphate by means of oxidative phosphorylation.[6] It is not surprising then that mitochondria are also quite adaptable, as their cellular content can be fine-tuned to the tissue's energy requirements. Mitochondrial content is regulated by two opposing processes, mitochondrial synthesis (biogenesis)[7] and mitochondrial degradation (mitophagy).[8] In the context of muscle disuse, a decrement in mitochondrial abundance is one of the major adaptations observed,[9] as the demand for energy is diminished. Understanding the interaction between mitochondrial biogenesis and mitophagy and their relationship to muscle atrophy during disuse is therefore invaluable for our comprehension of cellular homeostasis. The following review provides a concise summary of the mechanisms moderating muscle mass and mitochondrial content during conditions of chronic muscle disuse.

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2. Atrophy signaling during muscle disuse

Skeletal muscle mass is determined by the balance between protein synthesis and degradation. One of the most significant alterations associated with prolonged inactivity of skeletal muscle is the net loss of muscle protein, which results in myofiber atrophy.[10–14] This loss of muscle mass occurs as a consequence of enhanced activation of the cell's major proteolytic executors, the ubiquitin–proteasome system (UPS), and the autophagy–lysosome pathway (ALP), which together regulate the half-life of a majority of cellular proteins.[15] The study of the molecular pathways controlling this balance is an important area of sustained research.

Muscle mass maintenance and myofiber hypertrophy are governed, in part, by insulin-like growth factor-1 signaling. This protein can promote muscle growth partially through phosphatidylinositol 3-kinase–Akt signaling,[16–18] which stimulates myofibrillar protein synthesis via mammalian target of rapamycin complex 1 (mTORC1).[19] Thus, the direct activation of Akt is associated with muscle hypertrophy, and interestingly is sufficient to block atrophy during muscle disuse.[16] During muscle disuse, a reduction in phosphatidylinositol 3-kinase–Akt signaling is evident and could, in theory, result in reduced mammalian target of rapamycin (mTOR) activity. Notably, mTORC1 has been implicated in stimulating mitochondrial biogenesis[20–22] and in the inhibition of autophagy.[23] Thus, a decrease in mTOR activity would result in reduced protein synthesis and mitochondrial biogenesis, while stimulating autophagy. Both decreases in mitochondrial function and marked increases in autophagy are known to trigger cellular signaling events that contribute to muscle atrophy. However, several studies have noted a counter intuitive hyperactivation of mTOR during chronic muscle disuse, likely as a result of enhanced amino acid influx from the cellular degradative pathways, the UPS and ALP.[24,25] Therefore, although the exact role of mTOR-related signaling during muscle wasting is still contentious,[24–29] this complex appears to represent a critical rheostat in the control of an abundance of cellular processes during muscle growth and atrophy.

Central to the regulation of muscle mass during disuse are members of the forkhead box class O (FoxO) proteins. Although this family of transcription factors is involved in numerous intracellular processes,[30,31] these proteins also operate as critical mediators of myofiber atrophy during muscle disuse, as they orchestrate the induction of an atrophic gene program that is implicated in both UPS- and ALP-mediated catabolism.[29,32,33] In this scenario Akt is able to prevent muscle atrophy by inhibitory phosphorylation of FoxO3 on multiple residues, effectively prohibiting its nuclear entry by facilitating its sequestration in the cytoplasm though interactions with 14-3-3 proteins.[34] Indeed, Akt activity is reduced during muscle disuse,[16] allowing FoxO3 to enter the nucleus and stimulate a gene expression program that promotes an atrophic phenotype.[33] In particular, FoxO nuclear translocation has been shown to upregulate the expression of E3 ubiquitin ligases muscle RING finger 1 (MuRF1) and atrogin-1/MAFbx.[33] These E3 ligases are major effectors of the UPS, as they mediate muscle loss by targeting muscle structural proteins and components related to protein translation for degradation.[1,35–38]

Apart from Akt-mediated phosphorylation, the activity and subcellular localization of FoxO transcription factors can be fine-tuned via post-translational modifications by other upstream factors. AMP-activated protein kinase (AMPK), a metabolic sensor, has the capacity to phosphorylate FoxO on numerous residues, which positively influences its transcriptional activity.[39,40] The activation of this AMPK–FoxO axis was documented to occur with muscle disuse[41] and appears to contribute to muscle protein degradation. The increase in AMPK activation with muscle inactivity also likely inhibits protein synthesis,[42–44] further accelerating muscle protein loss. AMPK activation has also been shown to promote mitochondrial biogenesis,[45–49] however, a process that is downregulated during muscle disuse. Thus, AMPK activation at the onset of muscle disuse could serve as an early signal to mitigate energy stress, by enhancing energy substrate availability through protein breakdown and attenuating the loss of mitochondria at the early stages of muscle disuse.

FoxO3 activity can also be modulated by acetylation.[50–52] In response to muscle disuse, FoxO3 can be deacetylated by Histone deacetylase 1 (HDAC1),[50] promoting its nuclear translocation and activity. Conversely, recent research has also drawn attention to the role of SirT1, an NAD+-dependent deacetylase, in the inhibition of denervation-induced myofiber atrophy through the deacetylation of FoxO3.[53] It appears possible that the differential acetylation of lysine residues could account for the contrasting results observed between these studies. Nonetheless, the capacity of FoxO3 to be post-translationally regulated during muscle disuse represents a critical inflection point in the control of the atrophy gene program.

Another critical system contributing to disuse-induced muscle atrophy is the nuclear factor-κB (NF-κB) signaling pathway (Fig. 1). Extracellular factors, such as tumor necrosis factor α (TNFα), stimulate this pathway, activating the inhibitor of κB kinases (IKKα and IKKβ), which prompts the nuclear localization of NF-κB transcription factors. These transcription factors bind NF-κB response elements on atrophy genes, such as *MuRF1*, *FoxO3*, and *Runx1* among others,[54] and promote their transcription. Overexpression of a negative regulator of this system (inhibitor of NF-κBα) is sufficient to block disuse-induced atrophy,[55,56] and muscle-specific abolition of IKKα and IKKβ via genetic knockout (KO), or expression of a dominant negative form, is also protective of muscle mass.[57–59] Attenuation of myofiber atrophy in these models appears to result from a decrease in the activity of the NF-κB transcription factors and coactivators,[55,58,60] the most important of which are p50 and Bcl-3, which have notable roles in the expression of many genes associated with muscle atrophy.[54,61] Moreover, NF-κB signaling has also been demonstrated to impair mitochondrial biogenesis in skeletal muscle (Fig. 1).[62–64] Thus, NF-κB signaling also contributes to muscle atrophy by promoting a decline in mitochondrial content and function, increasing mitochondrial reactive oxygen species (ROS) production and stimulating nuclear apoptosis.

Recently, the cytokine TNF-like weak inducer of apoptosis (TWEAK) and its receptor Fn14 have emerged as major effectors of disuse-induced atrophy. The expression of Fn14 is upregulated during conditions of muscle disuse,[54,65] as

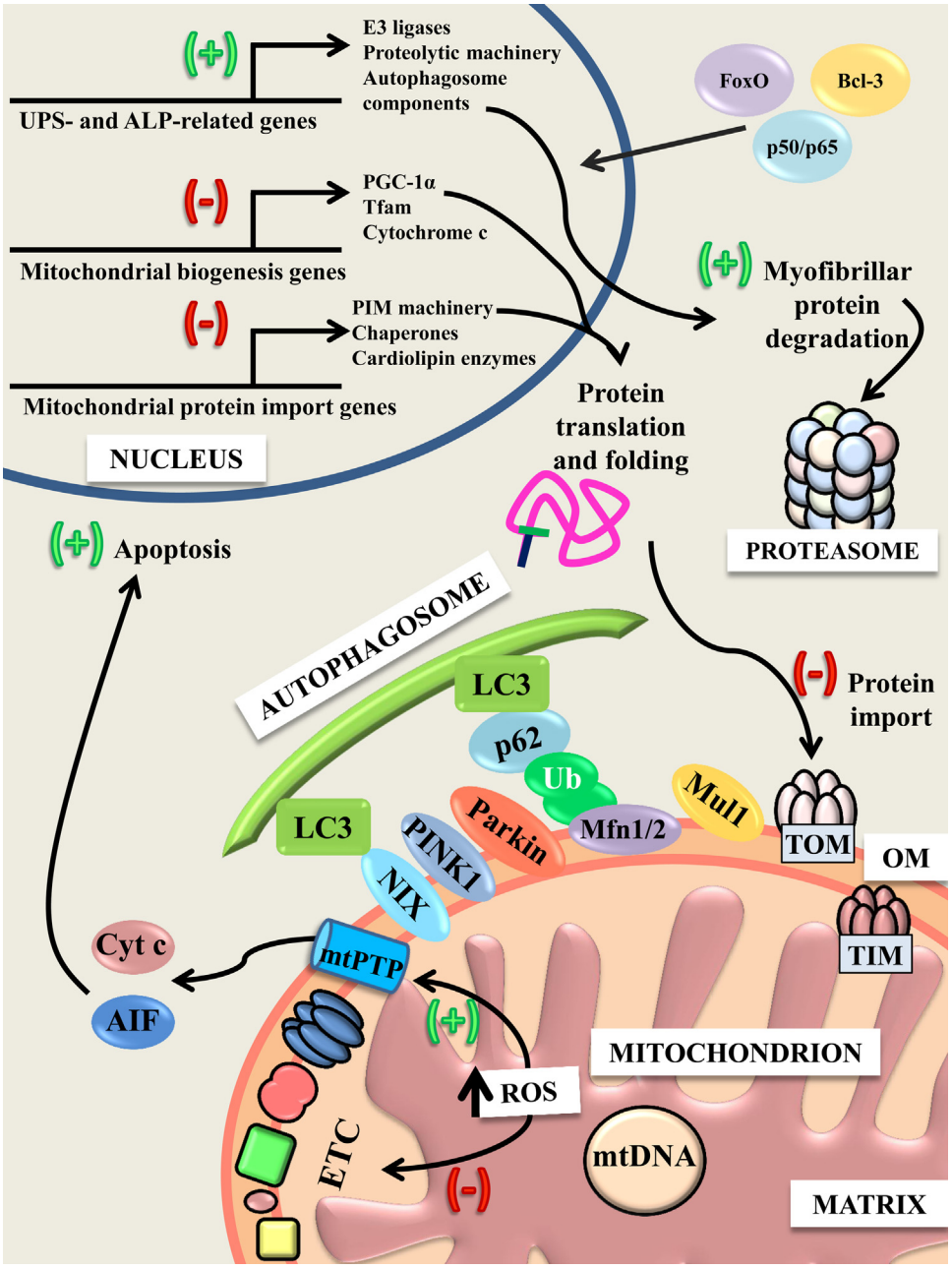

Fig. 1 – Alterations in gene expression and mitochondria during chronic muscle disuse.
During chronic muscle disuse, FoxO and NF-κB transcription factors (p50, p65) and Bcl-3 translocate to the nucleus and upregulate (+) the expression of a host of components related to proteolytic degradation pathways, resulting in increased activity of the autophagy–lysosome pathway (ALP) and the ubiquitin–proteasome system (UPS). Concurrently, the expression of factors associated with mitochondrial biogenesis and protein import are downregulated (-), and this is associated with an impairment in the import of proteins into the organelle. This may be due, in part, to a dysfunction in the electron transport chain, because respiration and mitochondrial membrane potential are impaired whereas reactive oxygen species (ROS) production surges. Dysfunctional mitochondria express PTEN-induced putative kinase 1 (PINK1) on their outer membrane (OM), which recruits Parkin. Along with Mul1, Parkin aids in the ubiquitination of OM proteins, including Mfn1/2. The adaptor factor p62 links ubiquitinated proteins to the main constituent of the autophagosomal membrane, LC3. The mitochondrial protein NIX also serves as a bridge between depolarized mitochondria and LC3. Incorporation of LC3 into the membrane continues until dysfunctional mitochondria are sequestered, at which point they can be transported to the lysosome for degradation. Mitochondrial ROS production can also promote the opening of the mitochondrial permeability transition pore (mtPTP), allowing for the release of proapoptotic factors, cytochrome c (Cyt c), and apoptosis inducing factor (AIF) into the cytosol. These factors promote the fragmentation of nuclear DNA and muscle atrophy.

Fn14 promoter methylation by DNA methyltransferase 3a is decreased.[66] The importance of the TWEAK–Fn14 dyad in disuse atrophy is highlighted by the TWEAK-KO mouse model, which is resistant to denervation atrophy, through impaired NF-κB signaling and MuRF1 expression.[65] Downstream of the TWEAK–Fn14 system is the ubiquitin ligase TNF receptor-associated factor 6 (TRAF6). TRAF6 is a relatively unique ubiquitin ligase, in that it mediates the conjugation of Lys-63-linked polyubiquitin chains, as opposed to the more typical Lys-48-linked chains, to target proteins for degradation.[67,68] Denervation increases the expression of TRAF6, which coordinates the breakdown of muscle-specific proteins as well as mitochondrial factors.[69]

Recent data have also identified the transcription factors p53 and ATF4 as mediators of novel and distinct signaling pathways facilitating atrophy during disuse. ATF4 has previously been implicated in muscle atrophy, because its downstream target Gadd45a induces muscle loss during disuse through enhanced autophagy and caspase-mediated proteolysis.[70] Muscle-specific deletion of either p53 or ATF4 confers partial resistance to disuse atrophy, whereas combined deletion has a synergistic impact on the preservation of myofiber size. These transcription factors have a common downstream effector, p21, which is elevated during muscle disuse and appears to be sufficient to induce muscle loss.[71] Interestingly, loss of p53 also results in reduced mitochondrial content and increased ROS production.[72] Thus the attenuation of muscle mass loss with p53 deletion is unlikely to involve mitochondrial pathways. Indeed, the increase in p53 protein content in muscle observed with denervation[71] might be considered an important transcriptional drive that promotes muscle atrophy. Further work on this pathway is warranted.

ROS are elevated in skeletal muscle during disuse.[2,73–76] Although mitochondria are not the sole site of ROS production in the cell, the increase in mitochondrial-specific ROS during muscle inactivity is notable. In particular, ROS are implicated in the damage of mitochondrial membranes, proteins, and DNA,[77–80] as well as the opening of the mitochondrial permeability transition pore,[81] an event that reduces mitochondrial membrane potential, decreases ATP production, and promotes the release of proapoptotic proteins that induce myonuclear decay. Several studies have noted increases in apoptotic protein expression, DNA fragmentation,[2,82–85] and heightened mitochondrial susceptibility to permeability transition pore opening with denervation.[86] Although controversial,[87] this potential loss of myonuclei could impair the capacity of the cell to maintain a sufficient transcription of genes required for the maintenance of muscle mass and mitochondrial content. In support for a role in mitochondrially-mediated apoptosis, deletion of the proapoptotic protein Bax, alone or in combination with Bak, attenuates apoptotic signaling, oxidative stress, and loss of muscle mass, illustrating at least a partial role for apoptotic cell death in disuse atrophy.[88,89] In addition, the pharmacological inhibition of calpain and caspase-3, two proteases implicated in the activation of the UPS and apoptosis, has been shown to attenuate myofiber atrophy during muscle disuse.[90] These data suggest that an elevated incidence of apoptotic signaling during skeletal muscle disuse is a likely contributor to muscle atrophy.

Taken together, it is evident that the regulation of muscle mass during muscle inactivity largely depends on the interplay between the regulation of protein turnover, the activity of apoptotic signaling, and the balance between cellular energy supply and demand mediated by mitochondrial form and function.

3. Expression of nuclear genes encoding mitochondrial proteins during muscle disuse

During muscle disuse, the loss of muscle mass is accompanied, or preceded, by decrements in mitochondrial content and function. This is likely due to a reduced drive for mitochondrial biogenesis, and an increased removal of dysfunctional organelles. Research delving into the alterations in the pathways governing mitochondrial biogenesis and degradation in the context of muscle disuse has gained significant ground in recent years, and the resolution of these mechanisms will provide formidable insight into the maintenance of skeletal muscle health during periods of prolonged inactivity.

Mitochondrial biogenesis is a product of the integrated contributions of both the nuclear and mitochondrial genomes, and the coordinated expression of these genomes is required for optimal mitochondrial function. Although the nuclear genome encodes >99% of the proteins within mitochondria, the mitochondrial genome contributes 13 proteins critical to electron transport chain (ETC) function, which is embedded within the inner mitochondrial membrane (IMM).[91,92] Mitochondrial biogenesis is a complex process that synchronizes the transcription, translation, and subsequent import of nuclear encoded-proteins into the mitochondrion, along with the replication and expression of mitochondrial DNA .[7,92] Newly-synthesized mitochondrial proteins and lipids are incorporated into the existing mitochondrial reticulum and contribute to its expansion. The peroxisome proliferator activated receptor γ coactivator-1 (PGC-1) family of transcriptional coactivators is critical for the coordinated expression of these genomes.[93,94] A member of this family, PGC-1α1, is well established as an orchestrator of mitochondrial biogenesis, as it can bind a number of nuclear transcription factors, including nuclear respiratory factors (NRF)-1 and -2, thereby promoting the expression of nuclear genes encoding mitochondrial proteins.[92,95] One of these is mitochondrial transcription factor A (Tfam), a vital factor for mitochondrial DNA replication and transcription.[96–99]

Because PGC-1α1 plays a fundamental role in the regulation of mitochondrial biogenesis, the study of this coactivator and its downstream effectors is of interest in the context of muscle disuse. The reduced expression of this family of coactivators with denervation-induced disuse occurs early after the cessation of muscle activity.[32,100] This is followed by decreased expression of estrogen-related receptor α (ERR α), NRF-1/2, and Tfam, in addition to the mitochondrial markers cytochrome c and COX IV at the mRNA and protein level.[2,100–103] Interestingly, an overexpression of either PGC-1α1 or -β attenuates protein degradation and muscle atrophy in denervated muscle,[104] implying a role for these coactivators in the maintenance of muscle mass. The attenuation of muscle atrophy by PGC-1α during disuse appears to be

mediated via a suppression of FoxO3-mediated transcription of E3 ligases MuRF1 and atrogin-1.[32] The protection conferred by PGC-1α1 overabundance is also observed in the sarcopenic muscle of aged mice, because overexpression of this coactivator mitigates the age-associated reductions in muscular function, mitochondrial content, antioxidant response, and protein synthesis.[105] Because muscle inactivity promotes a strong reduction in PGC-1α expression, it is likely that the PGC-1α-induced suppression of FoxO3 signaling is released, promoting myofiber atrophy.

In addition to a dampening of FoxO activity, a recent study has demonstrated a role for PGC-1α in the neutralization of TWEAK-mediated muscle atrophy.[62] PGC-1α overexpression is sufficient to attenuate the loss of muscle mass during denervation by restraining Fn14 expression and TWEAK-mediated NF-κB signaling, further highlighting the importance of this transcriptional coactivator in the protection of muscle mass.

The PGC-1 family of coactivators also facilitates the expression of genes involved in mitochondrial morphology. In particular, the interaction between PGC-1α/β and ERRα stimulates the transcription of mitofusin (Mfn) 2,[106,107] which regulates outer mitochondrial membrane (OMM) fusion.[108,109] Because these factors are downregulated during muscle disuse, it is not surprising that the integrity of the mitochondrial reticulum is compromised. Muscle inactivity induces a substantial decrease in the expression of the OMM fusion proteins Mfn2 and Opa1, presumably shifting the cellular balance in favor of mitochondrial fission.[9,103,110] An increase in fission facilitates mitochondrial-specific degradation processes, such as mitophagy,[111,112] resulting in a net loss of mitochondria from muscle.

PGC-1α also plays a regulatory role in the biosynthesis and remodeling of cardiolipin (CL), a mitochondria-specific phospholipid implicated in the physical stabilization of the ETC, and in the assembly of the protein import machinery (PIM).[113,114] Chronic muscle disuse is associated with a decrease in CL content within muscle,[115,116] which is linked with mitochondrial dysfunction. This impairment in mitochondrial function drives a vicious cycle, because the production of mitochondrial ROS is enhanced, likely driving the oxidation of CL. This facilitates the involvement of CL in proapoptotic events,[117–119] which mediate a portion of the muscle atrophy observed with muscle disuse.

4. Protein import into the mitochondrion

Mitochondria rely heavily on nuclear transcription for a majority of the genes necessary for their proper function.[120] Since mitochondria are not formed *de novo*, proteins that are translated from nuclear-derived transcripts must translocate into the mitochondria via the PIM.[121] The mechanisms of this protein import process are highly specialized and complex,[122,123] and the process represents a critical step at which mitochondrial content can be regulated.

It is well known that chronic exercise or contractile activity is accompanied by mitochondrial biogenesis that is closely associated with the elevated expression of PIM components[124–126] as well as mitochondrial CL content.[115,127] Chronic contractile activity also augments the expression of factors important for the assembly of the translocase of the outer mitochondrial membrane complex, which serves as the main channel for protein import.[124] Furthermore, contractile activity elicits increases in the expression of the cytosolic and mitochondrial chaperone proteins Hsp60, mtHsp70, and Hsp70,[128] which are integral to the stabilization, import, and refolding of precursor proteins into mitochondria. Indeed, increases in the PIM following contractile activity correlate well with the accelerated rates of import of proteins such as mitochondrial Tfam into the mitochondrial matrix.[125,129] Taken together, the increase in mitochondrial protein import with contractile activity is critical for mitochondrial biogenesis, and it promotes the expansion of the mitochondrial reticulum.

In direct contrast to contractile activity, chronic muscle disuse results in substantial reductions in mitochondrial content,[2,76,116] which correlate well with diminished protein import into the mitochondrion.[76] This suggests that the reduction in mitochondrial content is mediated, at least in part, by reductions in protein import. This is attributable to a number of factors, including changes in the content and distribution of CL, reductions in the expression of PIM components, and an increase in the production of ROS.[115,116] With respect to CL, reductions in the content of this phospholipid can result in destabilization of ETC complexes III and IV[130–132] and a loss of membrane potential, which impairs the import of proteins into the organelle.[133] Furthermore, muscle disuse may alter the membrane localization of CL. A majority of mitochondrial CL typically resides in the IMM.[134,135] However, denervation has been shown to increase the expression of phospholipid scramblase-3, which serves to transport CL from the IMM to the OMM.[115] Since the location of CL can have an impact on its cellular role in the context of apoptosis,[117,136,137] the probable increase in OMM CL with denervation may contribute to the increased mitochondrial apoptosis observed with denervation.[2]

In addition to CL, several PIM components have been shown to be significantly reduced in response to muscle disuse, including Tim23, Tom20, and mtHsp70. These effects were observed to occur as early as 3 days following denervation, and likely precede the loss in mitochondria and muscle mass observed with denervation.[76] In addition to the reduction in the expression of PIM components, the increase in mitochondrial ROS noted with denervation also appears to underlie deficits in mitochondrial matrix-destined import during muscle disuse. Elevated ROS levels inhibit the import of matrix-destined proteins and increase their susceptibility to proteasome-mediated degradation.[76,138] The reduction in import is likely related to the redox modulation of the cysteine- or thiol-rich residues of the import components. In the future, investigations should be conducted to identify the principal mechanisms that enable ROS to inhibit protein import.

5. Autophagy and mitophagy during muscle disuse

Macroautophagy (hereafter referred to as autophagy) is a conserved cellular process that is responsible for the elimination

of long-lived proteins, as well as dysfunctional organelles. The ALP is critical for the turnover and maintenance of organelles in skeletal muscle in particular, because this tissue is postmitotic. This process involves the formation of double membrane vesicles, known as autophagosomes, which sequester cytoplasmic materials that are destined for degradation and deliver them to the lysosome. Upon autophagosome–lysosome fusion, the acidic pH and hydrolases native to the lysosome digest the cargo into its constituent parts, which can subsequently be utilized for energy provision. Given its catabolic nature, it is not surprising that autophagy has been implicated in muscle atrophy. The expression of multiple autophagic factors was found to be upregulated under various atrophic conditions, including denervation.[3,29,139] These findings have raised interest in the pharmacological inhibition of autophagy as a potential target during muscle-wasting conditions. Studies involving animals with a deficient ALP, specifically in skeletal muscle, however, have revealed that an intact pathway is actually required for the maintenance of muscle mass and function.[140,141] Moreover, the absence of a functional autophagy program during muscle disuse only exacerbates muscle wasting.[140] Thus, aberrant autophagy underlies several myopathies and muscular dystrophies,[141–145] and interventions restoring ALP functionality ameliorate some of the myopathic phenotypes.[141–144] Conversely, excessive autophagy can also be detrimental by inducing a net catabolic effect, culminating in loss of muscle mass as demonstrated by overactive autophagy.[146] Nonetheless, it appears that the maintenance of a basal level of autophagy is required for the appropriate turnover of long-lived proteins and organelles in skeletal muscle, and that constrained activity of the ALP is essential for muscle mass maintenance and health.

Interestingly, many of the myopathies characterized by deficient autophagy also exhibit the accumulation of dysfunctional and swollen mitochondria, which certainly contribute to disease pathogenesis.[140,142] This is likely due to the insufficient turnover of mitochondria in these conditions, highlighting the integral relationship between rigorous mitochondrial quality control and muscle health. Although mitochondrial content can be regulated in a variety of ways, mitochondria-specific autophagy, known as mitophagy, is currently the only known mechanism for the wholesale removal of mitochondria from postmitotic tissues, such as skeletal muscle. This process is required for the proper progression of the mitochondrial life cycle and is therefore vital for mitochondrial vigor.[147]

Mitophagy is a constitutively active housekeeping mechanism in all cells, and can be further induced under conditions of energetic distress. During an energy deficit, AMPK is activated whereas mTOR is inhibited, leading to the induction of autophagosome formation by the activation and lipidation of microtubule-associated protein light chain 3 (LC3). This is achieved through a coordinated effort by various AuTophaGy (ATG) related genes-dependent conjugation steps. Continuous incorporation of membrane-bound LC3 leads to the formation and elongation of the autophagosome around the tagged cargo until it is completely encapsulated. The specific targeting of mitochondria for elimination by mitophagy is thought to be mediated by two generally distinct pathways: one being indirect and requiring the recruitment of an E3 ubiquitin ligase; and the other necessitating a mitophagy-specific receptor. Current evidence suggests that mitochondrial dysfunction often results in the activation of the indirect pathway, whereas removal of mitochondria under a programed biological event is carried out by the receptor-mediated pathway.[148,149]

With respect to the indirect pathway, loss of mitochondrial membrane potential or a surge in ROS production prompt the stabilization of PTEN-induced putative kinase 1 (PINK1) on the mitochondrial membrane. PINK1 subsequently recruits Parkin, which ubiquitinates various OMM proteins.[148] Interestingly, mice that lack Parkin are partially protected against loss of muscle mass and mitochondria in denervated slow-twitch but not fast-twitch muscle, suggesting a fiber type-specific preference for Parkin-mediated mitophagy.[150] In addition to Parkin, several mitochondria-associated E3 ubiquitin ligases have been implicated in mitophagy, including Mul1 and Gp78.[151–153] These ligases preferentially ubiquitinate the OMM proteins of dysfunctional mitochondria, allowing for their recognition by autophagy adaptor proteins such as p62 and NBR1,[154,155] which effectively link the autophagosome to the malfunctioning mitochondrion, allowing it to be sequestered. Interestingly, Parkin and Mul1 have both been implicated in disuse atrophy. Parkin KO mice showed resistance to denervation in slow-twitch muscle, likely due to reduced denervation-induced proteasomal activation via Nuclear Factor Erythroid 2-Like 1 (NFE2L1/Nrf1).[150] Moreover, Lokireddy et al[152] demonstrated that Mul1 expression is induced by denervation through a mechanism mediated by FoxO1/3 transcription factors, and that Mul1 appears to be both sufficient and required for mitophagy in skeletal muscle.[152] Interestingly, E3 ligases appear to compensate for one another, because Mul1 was recently demonstrated to play a role in maintaining mitochondrial integrity in light of a loss in PINK1/Parkin.[156]

Mitochondria can also be directly targeted for degradation by the receptor-mediated pathway involving BNIP3 and the BNIP3-like protein NIX. These factors are regulated by FoxO3 and are able to localize to the mitochondrial membrane, anchoring it to the autophagosome directly via their LC3 interacting region. BNIP3 and NIX were both found to be sufficient to induce muscle wasting, and appear to mediate, in part, FoxO3-induced muscle atrophy.[157] BNIP3 has also been documented to have a role in mitochondrial fragmentation, a prerequisite for mitophagy.[158] Indeed, mitochondrial morphology and dynamics play a key role in sealing mitochondrial fate, and organelle fission alone is sufficient to activate mitophagy.[159] In skeletal muscle, mitochondrial fragmentation induced by Fis1 enhances muscle wasting by leading to energetic stress and the activation of the AMPK–FoxO3 axis. Pharmacological inhibition of mitochondrial fragmentation was sufficient to dampen autophagy, as well as muscle atrophy.[41,160] Of note, the profusion proteins mitofusin1 and 2 are well-characterized targets of both Parkin and Mul1.[152,156,161] Thus, mitochondrial dynamics appear to play a key role in muscle atrophy and this effect may be mediated, at least in part, by the activation of mitophagy.

Increases in autophagy and mitophagy have been noted during various models of muscle atrophy. We have previously demonstrated an upregulation in the expression of numerous

autophagy proteins and transcripts in response to denervation. Furthermore, denervation resulted in the increased localization of LC3-II, p62, and Parkin to mitochondrial membranes, indicating an enhanced targeting of mitochondria for degradation by autophagy.[88,139,162] This may be due to the decrements in mitochondrial oxygen consumption, which prompt an increase in ROS production observed during muscle disuse.[2,76] ROS promote skeletal muscle autophagy and this effect is mediated, in part, through activation of the AMPK and inhibition of Akt, because the scavenging of ROS significantly decreased autophagic flux in mouse skeletal muscles.[163]

Thus, autophagy and mitophagy are activated during a variety of muscle-wasting conditions and participate in muscle atrophy. However, these pathways act as a double-edged sword whereby suppression of mitophagy may temporarily attenuate muscle loss, but if left unchecked can result in mitochondrial dysfunction and cellular oxidative stress, exacerbating myofiber atrophy. It is still unclear whether the activation of autophagy and mitophagy in skeletal muscle during disuse atrophy is a byproduct of the loss of energetic homeostasis, or whether these processes themselves instigate muscle wasting.

6. Conclusion

The regulation of skeletal muscle mass during chronic disuse largely depends on the balance between protein synthesis and protein degradation, with cellular energetic status playing a key role in the signals that fine-tune protein turnover and expression. Mitochondrial quality control is at the epicenter of cellular metabolic regulation and contributes meaningfully to muscle mass maintenance. During periods of muscle disuse, decreases in mitochondrial biogenesis, protein import, and oxidative phosphorylation, coupled with enhanced fragmentation and removal of organelles by mitophagy, lead to a net loss of mitochondrial content and an increase in mitochondrially-mediated nuclear apoptosis. All of these greatly contribute to the observed atrophy. Thus, resolving energetic distress by improving mitochondrial function and enhancing mitochondrial network formation appear to be protective of muscle mass in this scenario. With this in mind, few studies have examined the early signaling events that occur upon the onset of muscle disuse, making it difficult to predict the kinetics or infer causality between mitochondrial alterations and muscle wasting during disuse. Further studies delineating these early signaling events responsible for mitochondrial malfunction and its relation to muscle wasting are warranted.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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