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Review

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Mitochondrial pathways in sarcopenia of aging and disuse muscle atrophy

Abstract: Muscle loss during aging and disuse is a highly prevalent and disabling condition, but knowledge about cellular pathways mediating muscle atrophy is still limited. Given the postmitotic nature of skeletal myocytes, the maintenance of cellular homeostasis relies on the efficiency of cellular quality control mechanisms. In this scenario, alterations in mitochondrial function are considered a major factor underlying sarcopenia and muscle atrophy. Damaged mitochondria are not only less bioenergetically efficient, but also generate increased amounts of reactive oxygen species, interfere with cellular quality control mechanisms, and display a greater propensity to trigger apoptosis. Thus, mitochondria stand at the crossroad of signaling pathways that regulate skeletal myocyte function and viability. Studies on these pathways have sometimes provided unexpected and counterintuitive results, which suggests that they are organized into a complex, heterarchical network that is currently insufficiently understood. Untangling the complexity of such a network will likely provide clinicians with novel and highly effective therapeutics to counter the muscle loss associated with aging and disuse. In this review, we summarize the current knowledge on the mechanisms whereby mitochondrial dysfunction intervenes in the pathogenesis of sarcopenia and disuse atrophy, and highlight the prospect of targeting specific processes to treat these conditions.

Keywords: apoptosis; autophagy; fission; fusion; mitophagy; oxidative stress.

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Introduction

Adult skeletal fibers are postmitotic cells that possess an extraordinary capacity to adapt their size in response to the level of load and utilization. Such plasticity resides in their unique morphological and biological properties. Skeletal myofibers are the largest animal cells. As such, they cannot be supported by only one nucleus. Indeed, each nucleus has a limited synthetic capacity (Cavalier-Smith, 1978). Moreover, the number of nuclear pores through which RNA is transported into the cytosol could further limit the volume of cytoplasm served by a single nucleus (Cavalier-Smith, 1978). For these reasons, muscle fibers rely on syncitia of hundreds or thousands of nuclei, where each nucleus regulates gene expression within the surrounding domain of sarcoplasm (myonuclear domain or DNA unit; Hall and Ralston, 1989). Although the validity of the myonuclear domain paradigm has recently been questioned (Gundersen and Bruusgaard, 2008), a wealth of experimental evidence supports the notion that the volume and number of DNA units modulate muscle fiber remodeling in response to load conditions, aging and diseases (Allen et al., 1999). Simplistically, myonuclear domains shrink during atrophy, when entire DNA units can be removed via an apoptosis-like process (myonuclear apoptosis; Allen et al., 1999). This may result in either atrophy or removal of the fiber (Dupont-Versteegden, 2005). In some sense, the reverse occurs during hypertrophy, which is attained through and preceded by the incorporation into the fiber of nuclei provided by stem cells (satellite cells) situated under the basal lamina. Eventually, this process results in the formation of new DNA units (Schiaffino et al., 1972; Bruusgaard et al., 2010).

Muscle atrophy can develop acutely (e.g., due to disuse) or chronically (e.g., sarcopenia of aging). These two conceptually- and clinically-distinct types of atrophy occur through partly different mechanisms and have an impact on specific fiber types, with preferential involvement of slow-twitch, postural fibers in acute atrophy (Tomanek and Lund, 1974), and selective degeneration of fast-twitch fibers during aging (Larsson, 1983). Mitochondria are central in the regulation of the myonuclear domain, both in aging and disuse atrophy (Marzetti et al., 2010a; Min et al., 2011). These organelles, besides providing the ATP necessary for cell viability and muscle contraction, are also involved in the regulation of redox homeostasis and cellular quality control (QC) mechanisms as well as in the integration of cell death signaling (Marzetti et al., 2010a). Notably, skeletal muscle, similar to the heart, contains two bioenergetically- (Palmer et al., 1977) and structurally- (Riva et al., 2006) distinct mitochondrial subpopulations: subsarcolemmal mitochondria (SSM), located directly underneath the sarcolemma, and intermyofibrillar mitochondria (IFM), arranged in parallel rows between the myofibrils. These two subpopulations possess a distinct repertoire of enzymatic and non-enzymatic antioxidant defenses (Servais et al., 2003), show different rates of fragmentation and autophagic degradation (Riley et al., 1990; Wagatsuma et al., 2011), display diverse susceptibility towards apoptotic stimuli (Adhihetty et al., 2005), and may therefore be differentially involved in the pathogenesis of sarcopenia of aging and other muscle atrophying conditions.

Given the central role of mitochondria in energy production, redox homeostasis, and the regulation of cellular QC and cell death pathways, it is not surprising that alterations in mitochondrial function are considered primary instigators of myocyte atrophy during aging and disuse muscle wasting. In this review, we discuss prominent findings and controversial issues concerning the involvement of mitochondria in the pathogenesis of sarcopenia and disuse atrophy. We also highlight the prospect of targeting specific mitochondrial pathways to obtain therapeutic gain in these conditions.

Mitochondria as a source of oxidants

Role of mitochondria-derived oxidants in muscle physiology

Reactive oxygen species (ROS) are constantly generated within cells by several enzymatic reactions, including those catalyzed by cyclooxygenases, NAD(P)H oxidase, and xanthine oxidase; however, the bulk of ROS production (~90%) occurs as a byproduct of mitochondrial oxidative phosphorylation (OXPHOS). In physiological conditions, 0.2–2.0% of biatomic oxygen is incompletely reduced to superoxide anion ($O_2^{\bullet-}$) by electrons leaking at complex I and III of the electron transport chain (ETC; Chance et al., 1979). To cope with this physiological ROS production, mitochondria have evolved a multileveled defense network comprising detoxifying enzymes and non-enzymatic antioxidants. Within the mitochondrial matrix, manganese-dependent superoxide dismutase (MnSOD or SOD2) converts $O_2^{\bullet-}$ to hydrogen peroxide (H_2O_2), which is further detoxified into O_2 and H_2O by glutathione peroxidase 1 (Gpx-1) and peroxiredoxine III. Alternatively, $O_2^{\bullet-}$ can be released in the intermembrane space (IMS) where it is converted to H_2O_2 by copper-zinc-dependent SOD (SOD1). In addition, $O_2^{\bullet-}$ leaked into the IMS can be scavenged by cytochrome *c* (Pasdois et al., 2011). If mitochondrial antioxidant defenses are fully functioning and electron leakage occurs within the physiological range, oxidative damage is almost completely prevented. In such circumstances, the small amounts of ROS generated function as second messenger molecules that modulate the expression of several genes involved in metabolic regulation and stress resistance (mitochondrial hormesis or mitohormesis; Handy and Loscalzo, 2012). In contrast, excessive ROS generation, defective oxidant scavenging, or both, have been implicated in the aging process and in the pathogenesis of several conditions, including acute muscle atrophy and sarcopenia (Fulle et al., 2004; Powers et al., 2011b; Handy and Loscalzo, 2012).

The dual nature of ROS is epitomized by their effects on muscle physiology. Under physiological conditions, the small quantities of H_2O_2 and $O_2^{\bullet-}$ generated by the ETC (and by other cellular sources) are essential for force production (Reid et al., 1993). The highly toxic hydroxyl radical ($^{\bullet}OH$), which is formed via the Fenton and Haber-Weiss reactions in the presence of metal ions (e.g., iron-sulfur clusters in complex I, complex II, or the mitochondrial aconitase), is essentially undetectable under basal conditions (Kondo et al., 1994). In contrast, during aging or protracted disuse, extensive oxidative damage occurs due to the excessive generation of ROS (including $^{\bullet}OH$) and/or defective oxidant scavenging, which can contribute to muscle wasting (Kondo et al., 1994; Reid and Durham, 2002). The following subsections illustrate relevant mechanisms that are responsible for altered mitochondrial redox homeostasis in sarcopenia and acute atrophy and describe the consequences of abnormal ROS emission on muscle trophism.

Mechanisms and consequences of mitochondrial dysfunction and oxidative stress in the aging muscle

According to the widely-accepted mitochondrial free radical theory of aging (MFRTA), mitochondrial dysfunction arising from oxidative damage to mitochondria DNA (mtDNA) is the central mechanism driving the aging process (Miquel et al., 1980). MtDNA is especially prone to oxidative damage due to its proximity to the ETC, the absence of protective histones and a less efficient repair system compared with nuclear DNA (Yakes and Van Houten, 1997; Herrero and Barja, 1999). Moreover, the mitochondrial genome lacks introns, which makes each mutation likely to affect gene integrity and, hence, protein function. This implies that mtDNA mutations can lead to the synthesis of defective ETC components, resulting in OXPHOS impairment, decreased ATP production and further ROS generation (Harman, 1972). It is worth mentioning that mitochondrial iron content increases with aging in rodent postmitotic tissues, including skeletal muscle, which may amplify the extent of oxidative damage by enhancing the generation of highly reactive species, such as $^{\bullet}OH$ (Xu et al., 2010).

As predicted by the MFRTA, increased ROS production, extensive damage to mtDNA, and mitochondrial dysfunction have been detected in muscles from aged rodents and non-human primates (Lee et al., 1998; Wanagat et al., 2001; Figueiredo et al., 2009; Lee et al., 2010). In the human *vastus lateralis* muscle, the number of fibers harboring ETC abnormalities increases from 6% at 49 years of age to 31% at 92 years (Bua et al., 2006). ETC alterations co-localize with the clonal expansion of somatically-derived mtDNA deletion mutations, such that the mtDNA deletion mutation load approaches 99% in fiber regions with ETC abnormalities (Bua et al., 2006). High levels of mtDNA deletion mutations and ETC alterations co-localize with morphological aberrations, including segmental atrophy, fiber splitting and breakage. Conversely, mtDNA mutations and ETC abnormalities are not detectable in phenotypically normal regions within individual fibers. In addition to this, Short et al. (2005) found that advanced age was associated with declines in mtDNA abundance, transcript levels of several mtDNA-encoded proteins, and mitochondrial ATP production in the *vastus lateralis* muscle of healthy men and women. The authors also showed that impairments in mitochondrial bioenergetics were associated with increases in DNA oxidative damage and diminished aerobic capacity.

The proof of principle that the accumulation of mtDNA damage and subsequent mitochondrial dysfunction cause sarcopenia has been provided by the characterization of mice that express an error-prone mtDNA polymerase-γ (PolG; Kujoth et al., 2005). These rodents accumulate a high load of mtDNA mutations and exhibit a premature aging phenotype, including severe muscle atrophy. Mitochondrial respiration and ATP content are greatly reduced in muscles from mtDNA-mutator mice relative to wild-type controls, probably as a consequence of the defective assembly of ETC complexes and subsequent disruption of mitochondrial membrane potential ($\Delta\psi_m$; Hiona et al., 2010). Noticeably, a reduction in $\Delta\psi_m$ also leads to matrix condensation and release of pro-apoptotic factors into the cytosol (Gottlieb et al., 2003). Indeed, in PolG mice, the drop in $\Delta\psi_m$ is accompanied by up-regulation of apoptosis in muscle, as evidenced by increased levels of apoptotic DNA fragmentation (Hiona et al., 2010). Unexpectedly, mitochondrial ROS generation and mtDNA oxidative damage are not increased in muscles from mtDNA-mutator mice, casting doubts on the validity of the MFRTA, at least in this model of sarcopenia (Kujoth et al., 2005; Hiona and Leeuwenburgh, 2008). Indeed, the possibility exists that in PolG mice the excessive load of mtDNA mutations might lead to the apoptotic elimination of myocytes before oxidative damage to mtDNA becomes detectable (Tonska et al., 2009). It should also be considered that, besides oxidized bases, other forms of nucleic acid damage can be inflicted by ROS, including DNA breaks that can result in deletion mutations. Once produced, these mutations do not display any sign revealing their oxidative origin, but retain the ability to cause consequences relevant to the aging process by inducing loss of function or genome instability (Vijg, 2004; Sanz et al., 2006). Furthermore, when PolG mice are crossed with rodents that overexpress catalase targeted to the mitochondrial matrix (mCAT), age-related cardiomyopathy, cardiomyocyte mtDNA mutation load, and the extent of mitochondrial protein oxidation are partially rescued (Dai et al., 2010). These findings imply that the age-dependent cardiomyopathy that develops in mtDNA-mutator mice is mediated in part by mitochondrial oxidative stress.

Overexpression of mCAT also protects against age-associated oxidative damage to muscular mitochondrial proteins and nucleic acids and from decreases in mitochondrial respiration and ATP synthesis (Lee et al., 2010). These effects translate into the prevention of age-dependent declines in mitochondrial biogenesis in skeletal muscle, as evidenced by the maintenance of mitochondrial density, AMP-activated protein kinase (AMPK) activity, and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) expression to levels comparable to those of young mice (Lee et al., 2010). In another recent

study, MnSOD deficiency targeted to type IIB muscle fibers was found to compromise mitochondrial function and to increase oxidative stress in old mice (Lustgarten et al., 2011). Unexpectedly, these alterations were not accompanied by accelerated muscle atrophy. This may be explained by the fact that $O_2^{\bullet-}$ is not reactive enough to cause significant macromolecular damage, unless it becomes protonated into the more reactive perhydroxyl radical ($HO_2^{\bullet}$). In addition, unlike H_2O_2, $O_2^{\bullet-}$ is not freely diffusible across membranes, which limits its leakage from mitochondria and, therefore, its range of damage. The network organization of mitochondria might further explain the reduced damaging capacity of $O_2^{\bullet-}$ relative to H_2O_2. Mitochondrial ROS generation is exacerbated by the ROS themselves via ROS-induced ROS release (RIRR), such that ROS emission by a small number of mitochondria can induce a burst of oxidant production by neighboring mitochondria, eventually propagating the ROS surge to the whole cell (Zorov et al., 2000). Simulation experiments indicate that H_2O_2 might be a more appropriate messenger molecule to propagate ROS and cause RIRR because of its longer lifetime in the cytosol and higher membrane permeability (Park et al., 2011).

Nevertheless, Lustgarten et al. (2009) had previously shown that mitochondrial functional decay secondary to MnSOD deficiency resulted in decreased force generation by the *extensor digitorum longus* and *gastrocnemius* muscles, and diminished running capacity in young mice. This finding suggests that oxidative stress secondary to defective H_2O_2 scavenging may have an impact on muscle quality that is independent of atrophy.

Collectively, studies in mouse models have made a strong argument in favor of the mitochondrial vicious cycle as a contributing factor to muscle aging. However, definitive evidence of the involvement of mitochondrial free radicals in the pathogenesis of sarcopenia requires the reciprocal experiment, i.e., the generation of experimental rodents genetically engineered to experience a reduced rate of mtDNA mutations during aging. If these animals maintained muscle mass and function into late life, the contribution of the mitochondrial vicious cycle to muscle aging would be established conclusively.

It has, however, been suggested that the actual levels of mtDNA mutations observed during normal aging might not substantially impact mitochondrial function in skeletal muscle, since this can occur before critical levels of mtDNA damage are reached (Conley et al., 2007). Besides mtDNA, ROS can also directly damage proteins and lipids in the mitochondrial compartment. Oxidatively-modified proteins within the ETC may actually result in more immediate deleterious effects (i.e., increased uncoupling and decreased OXPHOS efficiency) than would be observed with mtDNA mutations (Conley et al., 2007). In addition, oxidative damage to lipids of the inner mitochondrial membrane (IMM), particularly cardiolipin, can lead to $\Delta\psi_m$ disruption (Gonzalvez and Gottlieb, 2007), reduced activities of ETC complexes (Paradies et al., 2002; Petrosillo et al., 2003), and the release of apoptogenic factors from mitochondria (Shidoji et al., 1999). Finally, derangements in mitochondrial turnover, resulting from reduced biogenesis and/or defective removal of damaged mitochondria, may further contribute to the pathogenesis of sarcopenia (*vide infra*).

Mechanisms and consequences of mitochondrial dysfunction and oxidative stress in disuse muscle atrophy

Prolonged periods of muscle inactivity lead to increased production of ROS, which are important signaling molecules responsible for mediating downstream muscle dysfunction and atrophy. The initial evidence indicating that enhanced oxidative stress plays a role in muscle loss with disuse was provided over 20 years ago by Kondo et al. (1991). This pioneering study demonstrated that the immobilization of rodent muscle was associated with increased levels of oxidative stress, which could be partially rescued by vitamin E supplementation. Subsequent investigations have provided the definitive mechanistic link between oxidative stress and acute muscle loss (Lawler et al., 2003; Arbogast et al., 2007; Servais et al., 2007; Agten et al., 2011; Min et al., 2011). But, what is the source of ROS in acutely atrophying muscles? And, how does increased ROS production affect myocyte protein metabolism and intracellular signaling pathways involved in disuse muscle atrophy?

A large body of evidence indicates that mitochondria are the primary source of ROS during chronic muscle inactivity (reviewed in Powers et al., 2012). Remarkably, administration of the mitochondria-targeted antioxidant SS-31 has recently been shown to attenuate ROS production and myofiber atrophy in hind-limb muscles (Min et al., 2011) and the diaphragm (Powers et al., 2011a) of mice subjected to cast immobilization and mechanical ventilation, respectively. Interestingly, SSM appear to produce greater amounts of ROS than IFM following muscle disuse induced by chronic denervation (Adhihetty et al., 2005, 2007), but more work is required to determine whether the same pattern of oxidant generation occurs under other muscle-atrophying paradigms. Experimental evidence also suggests that chronic muscle

inactivity causes major disturbances in intracellular calcium homeostasis (Soares et al., 1993; Ingalls et al., 1999; Weiss et al., 2010), which might lead to altered mitochondrial calcium handling and increased oxidant production (Brookes et al., 2004). Mitochondrial calcium overload can promote ROS generation through several mechanisms including activation of the citric acid cycle, which results in increased NADH formation, stimulation of nitric oxide synthase, and activation of ROS-generating enzymes such as α-ketoglutarate dehydrogenase (reviewed by Peng and Jou, 2010). Alternatively, chronic muscle inactivity could induce the accumulation of fatty acid hydroperoxides in mitochondria, which may lead to enhanced ROS production by acting as uncouplers, interfering with complex I and III of the ETC, and inhibiting adenine nucleotide translocase (ANT; Bhattacharya et al., 2009, 2011). Another report has indicated that the reduced rate of mitochondrial protein import that occurs with muscle disuse might contribute to increasing ROS production by interfering with mitochondrial function (Singh and Hood, 2011).

Although mitochondria appear to be the primary source of oxidants during muscle inactivity, non-mitochondrial processes may also participate in the overall increase in ROS generation. For instance, xanthine oxidase (Kondo et al., 1993) and/or NAD(P)H oxidase located in the cytosolic fraction (Javesghani et al., 2002) may contribute slightly to oxidative stress associated with chronic muscle disuse.

Regardless of the mechanism(s) responsible for excessive ROS generation during disuse atrophy, oxidative stress activates transcription factors that regulate the expression of mediators involved in catabolic pathways (Li et al., 2003; Dodd et al., 2010; McClung et al., 2010). In addition, ROS can directly activate key intracellular proteolytic enzymes such as calpains and promote the initiation of the apoptotic cascade via the release of mitochondrial pro-apoptotic factors (Adhihetty et al., 2005; Smuder et al., 2010). Lastly, oxidative stress can modify contractile elements, thereby making them targets for proteolysis (Grune et al., 2003).

Emerging evidence also suggests that oxidative stress can decrease protein synthesis to further promote muscle atrophy (Wu et al., 2010). Indeed, ROS suppress the rate to which mRNA products are processed by the translational machinery (Wu et al., 2010). Specifically, oxidative stress inhibits the phosphorylation of mammalian target of rapamycin (mTOR)-associated factors (e.g., eukaryotic initiation factor 4E-binding protein and p70 S6 kinase) to ultimately reduce the rate of protein synthesis (Wu et al., 2010).

In summary, current evidence suggests that oxidative stress secondary to mitochondrial dysfunction plays an important role in disuse muscle atrophy through the activation of multiple catabolic pathways and the inhibition of protein synthesis. Although the exact mechanisms have yet to be fully established, excessive mitochondrial ROS generation during inactivity-induced muscle wasting may be due to calcium dyshomeostasis and/or derangements in bioenergetic pathways. The extent to which each of these mechanisms contributes to overall mitochondria-mediated oxidative stress, however, remains to be elucidated. This knowledge may reveal pharmacological targets that can reduce ROS emission and attenuate atrophy during chronic muscle inactivity.

Role of altered mitochondrial quality control in sarcopenia and disuse muscle atrophy

Mitochondrial QC is essential for the maintenance of a healthy mitochondrial pool within post-mitotic skeletal myofibers. This task is accomplished through the complex coordination of several processes (reviewed by Fischer et al., 2012). The first line of defense is provided by ROS-scavenging systems that act to prevent oxidative damage to mitochondrial molecules. A second battery of QC pathways is engaged after molecular damage has occurred, and includes mechanisms devoted to repairing specific modifications to mitochondrial constituents. Such pathways include mtDNA-repair systems, reductase systems that repair oxidized proteins, and chaperones that assist in the restoration of misfolded proteins to their native three-dimensional structures. Irreversibly-damaged mitochondrial proteins are selectively removed by an intramitochondrial proteolytic system and replaced by *de novo* synthesized proteins (Luce et al., 2010). When molecular QC processes are overwhelmed, pathways acting at the whole organellar level are activated. For instance, the functionality of damaged mitochondria can be complemented, and possibly restored by their fusion with neighboring intact mitochondria (Twig et al., 2008b). Finally, severely damaged mitochondria are segregated from the vital mitochondrial network through fission and eventually eliminated by a specialized form of autophagy (Twig et al., 2008b). The following sections illustrate the mechanisms whereby alterations in mitochondrial dynamics and autophagy may intervene in the pathogenesis of sarcopenia and disuse atrophy.

Contribution of altered mitochondrial dynamics to sarcopenia and disuse muscle atrophy

The metabolic plasticity of skeletal myofibers is largely dependent on the dynamic nature of mitochondria and on the ability of these organelles to alter their organization (i.e., shape and size) and position within a cell in response to both intracellular and extracellular signals (Jood et al., 2006; Liesa et al., 2009). Mitochondrial morphology is regulated by continuous fusion and fission events that are not only crucial for determining organellar shape, but also for transmitting redox-sensitive signals, maintaining mtDNA integrity, and regulating cell death pathways (reviewed by Schafer and Reichert, 2009; Figure 1). The balance between fusion and fission is dependent upon a complex mitochondrial dynamics machinery. Among the best characterized mitochondrial fusion factors in mammals are the dynamin-related GTPases mitofusin 1 and 2 (Mfn1 and Mfn2), that are responsible for the tethering and fusion of outer mitochondrial membranes (OMMs) between two organelles (Eura et al., 2003), and optic atrophy protein 1 (OPA1), that controls IMM fusion (Misaka et al., 2002; Figure 1).

In mammalian cells, mitochondrial fission is regulated by the dynamin-related protein 1 (Drp1; Smirnova et al., 2001) and fission protein 1 (Fis1; Mozdy et al., 2000; Figure 1). Several accessory morphology proteins have been identified, including the mitochondrial distribution and morphology protein 10 (Mdm10; Sogo and Yaffe, 1994), mitochondrial protein 18 kDa (Tondera et al., 2004), and ganglioside-induced differentiation-associated protein 1 (Niemann et al., 2005), which are required for the proper coordination of mitochondrial morphogenesis.

Mitochondrial dynamics are centrally involved in the maintenance of cell homeostasis. Indeed, the fusion of isolated mitochondria results in the formation of a network, which enables mitochondria to mix their contents, redistribute metabolites, proteins and mtDNA, and prevent the local accumulation of abnormal mitochondria (Ono et al., 2001). Fission segregates components of the mitochondrial network that are irreversibly damaged or unnecessary, allowing for their autophagic removal (Twig et al., 2008a). A functional link therefore exists between mitochondrial dynamics and autophagy, which is essential for mitochondrial QC (Twig et al., 2008b). In fact, Parkin and PTEN-induced putative kinase 1 (PINK1), both involved in the regulation of mitochondrial autophagy (Narendra et al., 2008; Matsuda et al., 2010), promote mitochondrial fission and inhibit fusion (Deng et al., 2008). The segregation of damaged mitochondria by fission and subsequent inhibition of their fusion machinery are hence prerequisites for their autophagic degradation (Twig et al., 2008b). According to this view, autophagy functions as a receiver

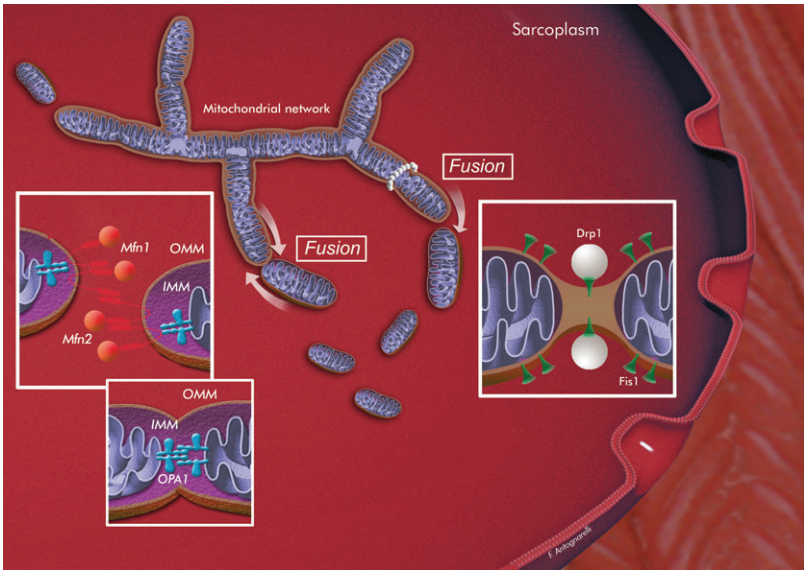

Figure 1 The dynamic nature of the mitochondrial network in skeletal muscle. Mitochondria are organized in a complex and dynamic network regulated by highly coordinated fusion and fission processes. Outer mitochondrial membrane (OMM) fusion is performed by Mfn1 and Mfn2, while optic atrophy protein 1 (OPA1) connects the inner mitochondrial membranes (IMM) of joining mitochondria. Fission is mediated by Drp1, which interacts with other proteins such as Fis1, to form a collar-like structure on the mitochondrial surface. The constriction of this structure eventually splits mitochondrial membranes.

of the segregation output and is therefore placed at the end of the mitochondrial QC axis (Twig et al., 2008b).

An imbalance in mitochondrial fusion-fission events, associated with altered mitochondrial degradation, contributes to the mitochondrial dyshomeostasis observed in atrophying muscles (Figure 2). For instance, enlarged mitochondria are frequently encountered in aging muscles (Tandler and Hoppel, 1986). These mitochondria are characterized by highly interconnected networks, aberrant morphology and reduced bioenergetic efficiency, the causes of which could be ascribed to increased fusion and/or diminished fission and lower autophagic rates (Yoon et al., 2006). This would suggest that a shift in mitochondrial dynamics toward fusion might play a prominent role in the mitochondrial–lysosomal vicious cycle (*vide infra*), by hindering the autophagic disposal of dysfunctional organelles (Figure 2). The up-regulation of mitochondrial fusion in the aging muscle, however, could be interpreted as an attempt to cope with increased levels of mtDNA mutations (Nakada et al., 2001). In this regard, Chen et al. (2010) showed that muscle-specific conditional deletion of Mfn1 and Mfn2 resulted in severe mtDNA depletion, the accumulation of mtDNA point mutations and deletions, mitochondrial dysfunction, and muscle atrophy. Furthermore, disruption of mitochondrial fusion via Mfn1 deletion was found to increase mitochondrial dysfunction and lethality in PolG mice (Chen et al., 2010). Based on these observations, it may be hypothesized that fusion rates are heightened in aging myocytes to withstand the increasing load of mtDNA mutations. This would allow cell viability to be preserved until a critical threshold of pathogenic mtDNA is breached and the degree of mitochondrial interconnection becomes incompatible with the autophagic removal of damaged mitochondria.

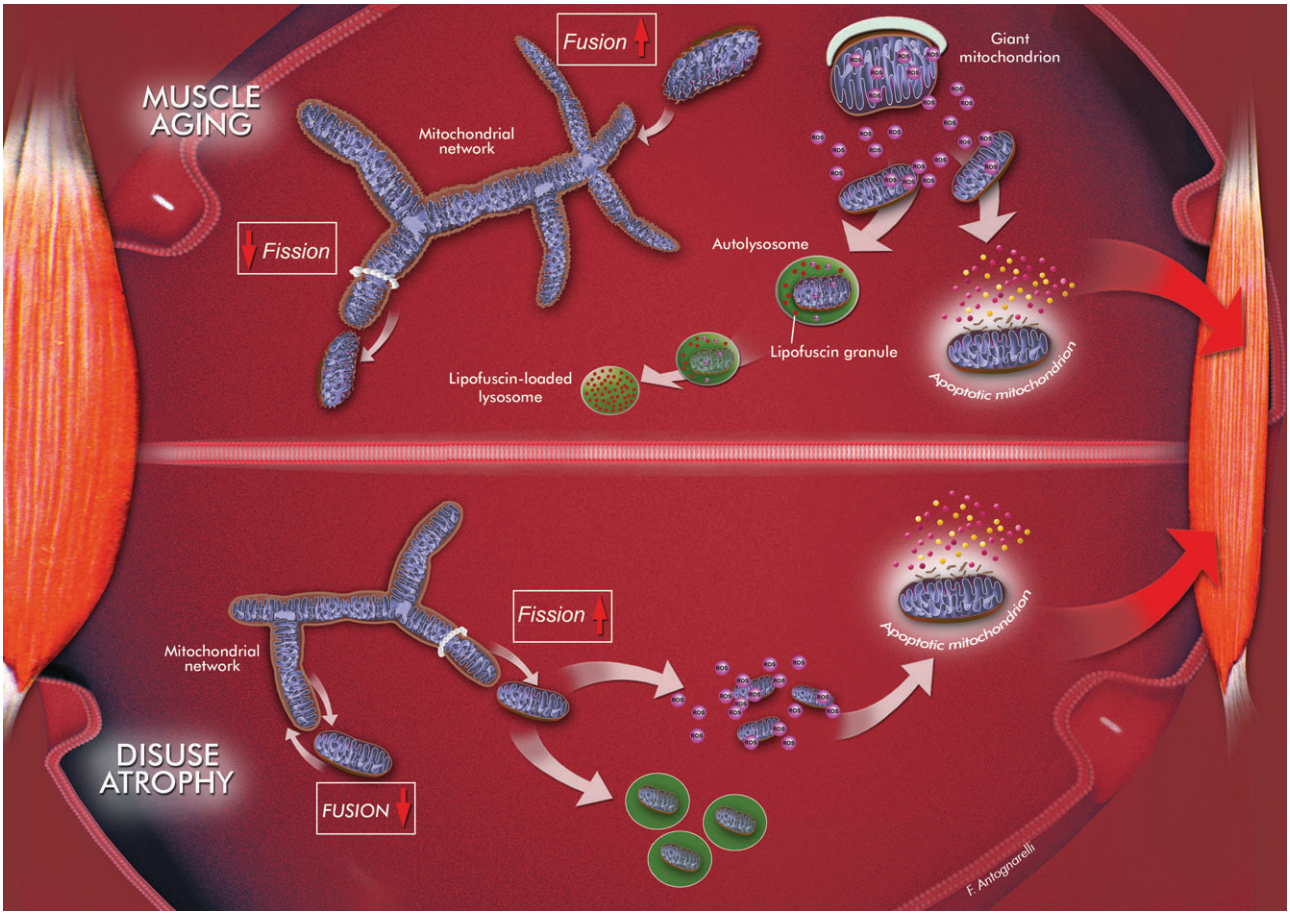

Figure 2 Synoptic representation of the putative mitochondrial pathways contributing to sarcopenia of aging and disuse muscle atrophy. The aging muscle is characterized by an imbalance in mitochondrial fusion–fission events, associated with lower mitochondrial degradation. This leads to the formation of giant mitochondria, characterized by highly interconnected networks, aberrant morphology, reduced bioenergetic efficiency, and increased ROS production. The accumulation of lipofuscin within lysosomes contributes to the age-associated dysfunction of the autophagy–lysosomal pathway, resulting in reduced mitophagic efficiency. Oxidative stress eventually triggers apoptosis. During muscle disuse, mitochondrial dynamics shift toward fission and autophagy is over-activated. ROS generation by mitochondria is increased, which together with the up-regulation of fission stimulates apoptosis.

Contrary to sarcopenia, excessive fission may contribute to the development of acute muscle wasting (Figure 2). Up-regulation of fission and remodeling of the mitochondrial network have been detected in murine muscles atrophied by denervation or fasting (Romanello et al., 2010). It is noteworthy that overexpression of Drp1, Fis1 and Bcl2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) induced mitochondrial dysfunction and fragmentation, the activation of mitochondrial autophagy and fiber atrophy (Romanello et al., 2010). Conversely, the blockade of mitochondrial fission via transfection of murine muscles with vectors producing short hairpin RNAs for Fis1 and BNIP3 resulted in significant protection from atrophy during fasting. Specifically, Fis1 and BNIP3 knockdown reduced the activation of the muscle-specific ubiquitin ligases atrogin-1/muscle atrophy F-box and muscle RING-finger protein-1. Similarly, transfection with a dominant negative mutant of Drp1 attenuated the severity of atrophy and the activation of ubiquitin ligases in muscles co-transfected with constitutively active Forkhead box O3 (FoxO3; Romanello et al., 2010).

Another mechanism whereby mitochondrial dynamics could have an impact on muscle trophism is through the regulation of apoptosis (Karbowski and Youle, 2003). Indeed, overexpression of a dominant-negative mutant of Drp1 inhibits mitochondrial fragmentation, the cytosolic release of cytochrome *c*, and the induction of apoptosis in cell culture systems (Frank et al., 2001). Interestingly, both Drp1 and Mfn2 co-localize with pro-apoptotic members of the B-cell leukemia-2 (Bcl-2) family (i.e., Bax and Bak) in concentrated OMM foci and their activity can be modified by these apoptotic regulators (Karbowski et al., 2006; Cleland et al., 2011). For instance, Mfn2 activity and fusion are inhibited by Bax, whereas the overexpression of an active form of Mfn2 represses Bax activation and downstream apoptotic events (Karbowski et al., 2006). Finally, OPA1 protects from apoptosis by preventing the release of mitochondrial apoptotic mediators (Frezza et al., 2006). These findings indicate that mitochondrial shaping proteins and apoptotic regulators are intimately interconnected and that their interaction is crucial for the modulation of cell death/survival pathways. Nevertheless, whether alterations in mitochondrial dynamics impact myonuclear apoptosis during aging or in the setting of acute atrophying conditions has yet to be explored.

Another critical issue is to establish whether distinct fusion and fission properties exist between IFM and SSM, which may help explain functional differences between these two mitochondrial subpopulations and, more importantly, their divergent response during muscle atrophy. Notably, SSM suffer higher degradation rates than IFM under various atrophying conditions. The greater prevalence of vacuoles and fragmented SSM observed in such circumstances would suggest augmented fission and increased autophagic degradation of this mitochondrial subpopulation (Riley et al., 1990; Wagatsuma et al., 2011).

In conclusion, emerging evidence suggests that alterations in mitochondrial morphogenesis may play a role in muscle atrophy during aging and disuse (Seo et al., 2010). However, a deeper understanding of the mechanisms through which mitochondrial dynamics and remodeling impact myocyte homeostasis is necessary in order to design interventions targeting these cellular pathways for the treatment and/or prevention of muscle atrophy.

Mechanisms of mitochondrial autophagy

Autophagy is a self-eating process through which cells degrade their own components, recycling amino acids and other building blocks that can eventually be reused (reviewed by Kroemer et al., 2010). Macroautophagy (hereby referred to as autophagy) is the best characterized form of autophagy, and consists in a genetically-programmed, evolutionarily-conserved catabolic process that degrades damaged or unnecessary cellular proteins and organelles through their sequestration into a double-membrane structure known as the autophagosome (Kroemer et al., 2010). Autophagosomes then fuse with lysosomes to form autolysosomes, wherein the enveloped content is degraded.

The execution of autophagy is regulated by AuTophaGy-related (Atg) proteins, over 35 of which have been identified. For a detailed description of the function of these proteins, the reader is referred to specialized reviews (for instance, Klionsky et al., 2011). Although initially considered a bulk degradation pathway, it is now widely accepted that autophagy can also be selectively targeted toward the removal of protein aggregates or damaged or superfluous organelles (reviewed by Reggiori et al., 2012). Several cargo-specific autophagic processes have been identified in yeasts and mammals, and each of them has been named based on the nature of the target. Examples include pexophagy for the disposal of peroxisomes, ERphagy for the degradation of endoplasmic reticulum, ribophagy for the elimination of ribosomes, and aggrephagy for the removal of aggregates (Reggiori et al., 2012). In addition, autophagy can be specifically directed toward the degradation of mitochondria (mitophagy; reviewed by Ding and Yin, 2012; Figure 3). This process regulates mitochondrial number to match metabolic demands and also serves as a QC mechanism to remove

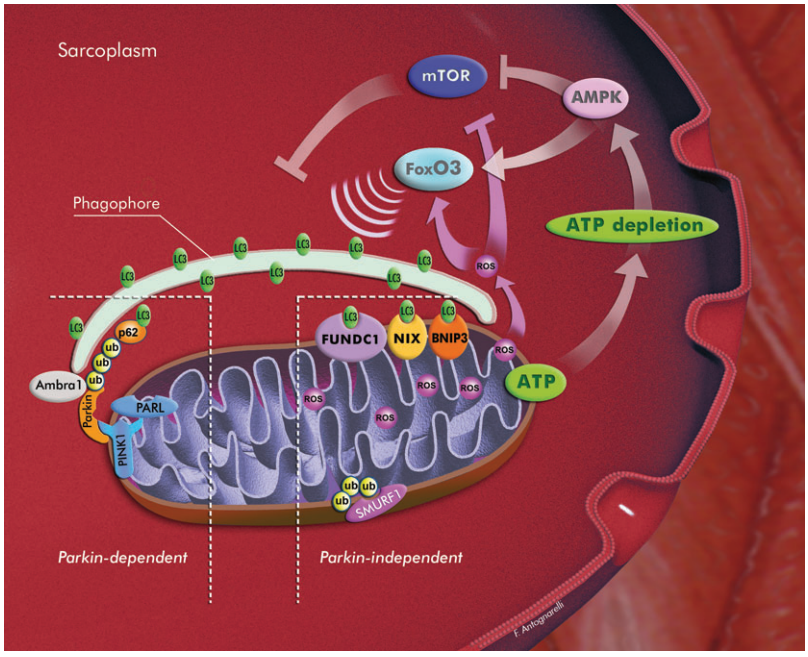

Figure 3 Molecular regulation of mitophagy in skeletal muscle. The priming of mitochondria to degradation can occur via Parkin-dependent or -independent pathways. Following mitochondrial depolarization, PTEN-induced putative kinase 1 (PINK1) accumulates on the mitochondrial surface, leading to the recruitment of Parkin, which ubiquitinates proteins located in the outer mitochondrial membrane. Ubiquitination of the presenilin-associated rhomboid-like (PARL) protease promotes the execution of mitophagy by preventing PINK1 degradation. Ubiquitin-tagged mitochondria bind to p62, which assists in the recruitment of autophagosomal membranes to mitochondria. Parkin can also interact with activating molecule in Beclin1-regulated autophagy (Ambra1), which stimulates the activity of the class III phosphatidylinositol 3-kinase (PI3K) complex required for phagophore formation. In the Parkin-independent pathway, FUN14 domain-containing protein 1 (FUNDC1) is engaged, followed by the recruitment of microtubule-associated protein 1 light chain 3 (LC3) to mitochondria. In addition, upon mitochondrial depolarization, SMAD-specific E3 ubiquitin protein ligase 1 (SMURF1) targets mitochondria to mitophagy via the ubiquitination of mitochondrial proteins. Finally, Bcl2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) and Nip3-like protein X (NIX) can trigger mitophagy by mitochondrial depolarization, competitive disruption of the inhibitory interaction between Bcl-2 and Beclin1, and direct interaction with LC3. Mitochondria-derived ROS and AMP-activated protein kinase (AMPK) activation by ATP depletion converge on Forkhead box O3 (FoxO3) to induce mitophagy. AMPK and FoxO3 also relieve autophagy inhibition by mammalian target of rapamycin (mTOR). For a detailed description of the molecular mechanisms and regulation of mitophagy, the reader is referred to specialized reviews on the subject (for instance, Youle and Narendra, 2011; Ding and Yin, 2012).

damaged mitochondria (Ding and Yin, 2012). Mitophagy is preceded by mitochondrial fission, which divides mitochondria into pieces of manageable size for engulfment and mediates the segregation of damaged material for subsequent disposal (Twig et al., 2008a). The loss of $\Delta\psi_m$ represents a major trigger of mitophagy (Twig et al., 2008a). Recent evidence also suggests that the opening of the mitochondrial permeability transition pore (mPTP) may be required for the selective removal of damaged mitochondria by inducing $\Delta\psi_m$ dissipation (Carreira et al., 2010).

In addition to the relevance of autophagy to mitochondrial QC, several lines of evidence also suggest that mitochondria can substantially influence the autophagic process. Indeed, mitochondria have been linked to virtually every step of autophagy through the localization of numerous autophagic regulators on mitochondrial membranes, the integration of these organelles in several signaling networks, and their ability to modulate these pathways (reviewed by Rambold and Lippincott-Schwartz, 2011). Hence, the crosstalk between mitochondria and autophagy places both these elements in a unique position, where defects in one or the other system can increase the risk for various disease conditions.

The execution of mitophagy is performed by a complex machinery and involves the coordinated actions of mediators responsible for the recognition of depolarized mitochondria and their subsequent disposal (Ding and Yin, 2012; Figure 3). The mTOR/AMPK pathway is proposed to be a major checkpoint for the induction of mitophagy (Hirota et al., 2012). Importantly, AMPK, in addition to stimulating mitochondrial removal through autophagy, enhances the activity of sirtuin-1 and its downstream target PGC-1α, resulting in the stimulation of mitochondrial

biogenesis (Canto et al., 2009). Hence, through the activity of AMPK, mitophagy and mitochondrial biogenesis are coordinately regulated to maintain a healthy and functional pool of mitochondria in the cell.

In skeletal muscle, the regulation of autophagy presents some interesting peculiarities (Figure 3). Two distinct mechanisms have been shown to activate autophagy in myocytes: a rapid, but transient, transcription-independent mechanism mediated by mTOR, and a slower but more robust pathway that is independent of mTOR and requires gene expression induced by FoxO3 (Zhao et al., 2007). Short-term exposure of myotubes to rapamycin induces lysosomal proteolysis to a much lesser extent than observed when protein kinase B (PKB/Akt) is inhibited (Zhao et al., 2007). Moreover, in leucine-restricted myotubes, autophagy is induced independent of mTOR. Lastly, prolonged treatment with rapamycin has marginal effects on autophagosome formation in adult skeletal muscle *in vivo* (Mammucari et al., 2007; Zhao et al., 2007). These and other observations have led to the proposition that the Akt-FoxO axis, rather than the Akt-mTOR pathway, is the major regulator of autophagic flux in adult skeletal myocytes (Mammucari et al., 2008). It is worth noting that FoxO3 controls the transcription of genes related to both autophagy and the ubiquitin proteasome system (UPS; Mammucari et al., 2007; Zhao et al., 2007). Thus, FoxO3 represents a critical checkpoint during the development of muscle atrophy by controlling two major cellular degradation pathways. Notably, the activity of FoxO3 is greatly stimulated by oxidative stress, which links mitochondrial dysfunction and the activation of autophagy with muscle atrophy (Dobrowolny et al., 2008). The following subsections illustrate the relevance of alterations in mitochondrial autophagy to sarcopenia of aging and disuse muscle atrophy.

Role of impaired mitochondrial autophagy in sarcopenia

QC and renewal pathways are crucial for the homeostasis of long-lived cells, such as skeletal myocytes, which are poorly replaced through division and differentiation of stem cells.

In this context, failure of mitochondrial turnover resulting from insufficient biogenesis and/or defective autophagic removal of dysfunctional mitochondria has been implicated in the pathogenesis of muscle wasting during aging as well as in disuse atrophy (reviewed by Sandri, 2010). Indeed, the various mechanisms whereby mitochondria are thought to contribute to muscle loss (i.e., increased ROS production, bioenergetic failure, and induction of apoptosis) converge on autophagy (Lee et al., 2012), highlighting the central position of this cellular pathway in the control of myocyte homeostasis and viability. However, while the impaired autophagic clearance of damaged mitochondria is intuitively associated with the accumulation of dysfunctional organelles and, hence, muscle damage, excessive activation of autophagy can also be responsible for muscle atrophy, at least under certain circumstances (Sandri, 2010).

A decline in autophagy during normal aging has been described for invertebrates and higher organisms (Rajawat et al., 2009). This phenomenon, in conjunction with the age-related accumulation of macromolecular and organellar damage, has led to the proposition that inefficiency of the autophagosomal-lysosomal pathway could be centrally involved in the aging process (Rajawat et al., 2009). Advanced age is associated with the accumulation within postmitotic cells, including skeletal myocytes (Orlander et al., 1978), of a nondegradable, polymeric, toxic pigment called lipofuscin (Jung et al., 2007). Lipofuscinogenesis results from peroxide-induced Fenton reactions elicited by intralysosomal materials that produce highly reactive $^{\bullet}OH$, causing crosslinking of proteins and lipids. Peroxides involved in these Fenton reactions can diffuse into the lysosomes from cytosolic-damaged mitochondria or can originate from autophagocytosed yet undegraded mitochondria (reviewed by Terman et al., 2010). The accumulation of such intracellular garbage eventually overburdens the autophagosomal degradative capacity by acting as a sink for lysosomal hydrolases (Terman et al., 2010). In fact, lyosomes, although enriched with hydrolases, cannot digest lipofuscin, which is a nondegradable material. At the same time, a smaller quantity of lysosomal enzymes remains available for autophagic degradation, including mitophagy. Hence, autophagic failure and the accumulation of dysfunctional mitochondria perpetuate each other, leading to extensive oxidative damage, increased lipofuscinogenesis, and eventually collapse of the catabolic machinery (Terman et al., 2010; Figure 2). This assumption is the main tenet of the so-called mitochondrial-lysosomal axis theory of aging (Brunk and Terman, 2002).

Studies into the role of autophagy in muscle aging have yielded mixed results. For instance, Wenz et al., (2009) found that the ratio of microtubule-associated protein 1 light chain 3 (LC3)-II to LC3-I, a marker of ongoing autophagy (Kadowaki and Karim, 2009), was significantly increased in the *biceps femoris* muscle of aged mice compared with younger controls. This adaptation can be interpreted as an attempt by old myofibers to cope

with enhanced oxidative damage due to declines in mitochondrial function. Intriguingly, mitochondrial damage and elevation of autophagic markers were not detected in the muscles of old mice with PGC-1α overexpression (Wenz et al., 2009). These findings suggest that PGC-1α could intervene in the mitochondrial-lysosomal vicious cycle by promoting mitochondrial biogenesis and improving antioxidant defenses. This, in turn, could result in reduced lipofuscinogenesis and optimization of mitochondrial turnover. Conversely, our laboratory showed that advanced age was associated with reduced mRNA abundance of lysosomal-associated membrane protein 2 (LAMP-2) in rat *plantaris* muscle (Wohlgemuth et al., 2010), suggestive of reduced autophagy. LAMP-2 is needed for the efficient fusion of autophagosomes and lysosomes (Klionsky et al., 2011). Life-long mild calorie restriction with or without endurance training prevented the age-related decline in LAMP-2 expression (Wohlgemuth et al., 2010). This effect was paralleled by the attenuation of oxidative stress and apoptotic DNA fragmentation, suggesting that the preservation of autophagy may mitigate age-related myocyte degeneration. Moreover, we recently found that a 6-month weight-loss program combined with moderate intensity exercise increased the transcript levels of the autophagy regulators LC3B, Atg7, and LAMP-2 in the *vastus lateralis* muscle of overweight older women compared with controls (Wohlgemuth et al., 2011). The intervention also increased the gene expression levels of the mitochondrial biogenesis markers PGC-1α and mitochondrial transcription factor A. The differential response in autophagy signaling elicited by dietary restriction and exercise in rats and humans, besides possibly reflecting species-specific peculiarities in the regulation of this cellular pathway, could also be attributed to methodological differences between the two studies (protein vs. gene expression analysis) as well as to the type and duration of the interventions, and their effect on body weight (prevention of age-related adiposity *vs*. weight loss).

As a whole, the available evidence suggests that preservation of autophagy may be important for the maintenance of skeletal myocyte homeostasis and optimal mitochondrial turnover in the aged muscle. It should, however, be considered that the gene or protein expression levels of a limited number of autophagic mediators are usually determined, while direct measurements of the actual autophagic flux have not yet been performed. This limitation precludes definite interpretations about the effects of aging and behavioral interventions on autophagy. Future studies adopting standardized methods for monitoring this cellular pathway, such as those proposed by Klionsky et al. (2012), are required to conclusively establish whether interventions that ameliorate mitochondrial QC through the fine tuning of autophagy may represent effective means by which to delay muscle aging and treat sarcopenia.

Role of altered autophagy in disuse muscle atrophy

Different from sarcopenia, activation of autophagy can aggravate muscle loss in the setting of acute atrophying conditions (reviewed by Sandri, 2010; Figure 2). Denervation is associated with the induction of autophagy in skeletal muscle, an effect that is antagonized by Runt-related transcription factor 1 (Wang et al., 2005). Oxidative stress is a key signaling event in the initiation of autophagy in denervated muscles (Lee et al., 2012). In mice with muscle-specific expression of mutated SOD1 ($SOD1^{G93A}$), a defect found in one-fifth of familial amyotrophic lateral sclerosis, progressive muscle atrophy develops in association with reduced muscle strength, sarcomere disorganization, aberrations in mitochondria morphology and disposition, and derangements of the sarcotubular system (Dobrowolny et al., 2008). Interestingly, a reduction in autophagic flux via small interfering RNA-mediated LC3 inactivation is sufficient to rescue the muscle phenotype in $SOD1^{G93A}$ mice, indicating that the signaling pathways activated by ROS converge on autophagy to induce atrophy (Dobrowolny et al., 2008). Altered mitochondrial bioenergetics, increased levels of mitochondrial ROS generation and the up-regulation of several autophagic proteins (LC3, ULK1, Beclin1, and Atg7) have been detected in the early phase of denervation-induced muscle atrophy in mice (O'Leary et al., 2012). Following denervation, the up-regulation of LC3-II was accompanied by increased localization of this autophagic mediator to both IFM (O'Leary and Hood, 2009) and SSM (O'Leary et al., 2012), suggesting that mitophagy may be activated during the development of acute atrophy.

Further evidence linking mitochondrial dysfunction, activation of autophagy and acute muscle atrophy has been provided by the observation that alterations in mitochondrial dynamics and function are required for and precede the induction of an autophagy/UPS-mediated atrophying program in mice subjected to muscle denervation or fasting (Romanello et al., 2010). Conversely, prevention of mitochondrial fragmentation via the inhibition of fission proteins results in reduced activation of both autophagy and the UPS, with significant protection against starvation- or denervation-induced muscle wasting (Romanello et al., 2010).

Taken together, these findings indicate that alterations in mitochondrial function and disruption of the mitochondrial network induce the activation of a muscle atrophy program executed by autophagy and the UPS under the coordination of FoxO3 (Mammucari et al., 2007; Zhao et al., 2007). These observations also suggest the potential of mitigating acute muscle loss through the inhibition of autophagy. However, abolition of basal autophagy in mice via muscle-specific Atg7 knockout results in severe atrophy, weakness and myofiber degeneration (Masiero et al., 2009). Morphological, biochemical and molecular analyses of autophagy-knockout mice unraveled the presence of protein aggregates, abnormal mitochondria, sarcoplasmic reticulum distension, vacuolization, extensive oxidative damage, and apoptosis. Moreover, inhibition of autophagy does not protect from atrophy during denervation or fasting, but instead promotes greater muscle loss (Masiero et al., 2009).

Thus, myocyte autophagy appears to be a double-edged sword in that its excessive activation contributes to muscle wasting, whereas its defective function causes weakness and degeneration.

Theoretically, there may be crucial differences in the timing during which these opposing events occur (Sandri, 2010). In fact, defective autophagy produces pathological consequences over long periods of time (e.g., aging), due to the progressive accumulation of damaged proteins and dysfunctional organelles within myofibers. In addition, the loss of basal autophagy compromises cellular homeostasis by abrogating the housekeeping functions of this process. Conversely, a brief exacerbation of the autophagic flux can trigger atrophy during muscle disuse. A deeper understanding of the molecular events that regulate basal and stress-induced autophagy in myocytes is imperative for the design of therapeutic strategies that exploit the homeostatic function of this cellular pathway while not initiating the detrimental effects of its excessive activation.

Contribution of mitochondria-driven apoptosis to sarcopenia and disuse muscle atrophy

The mitochondrial apoptotic machinery

One relevant consequence of mitochondrial dysfunction and oxidative stress is the up-regulation of myonuclear apoptosis (reviewed by Dirks et al., 2006). Mitochondria are centrally involved in the regulation of apoptosis, being the principal players in the intrinsic pathway of programmed cell death and housing many mediators that modulate apoptotic signaling (Marzetti et al., 2010a; Figure 4). The critical event for the initiation of mitochondria-mediated apoptosis is OMM permeabilization (OMMP), necessary for the cytosolic release of pro-apoptotic factors residing within the IMS (reviewed by Armstrong, 2006). OMM integrity is regulated by the balance between pro- (e.g., Bax, Bak, and Bid) and anti-apoptotic (e.g., Bcl-2 and Bcl-X_L) members of the Bcl-2 family of proteins (reviewed by Chipuk and Green, 2008). In addition, opening of the mPTP can induce OMMP through disruption of the OMM (reviewed by Kinnally and Antonsson, 2007).

After OMMP has occurred, the execution of apoptosis can be performed through different pathways (Figure 4). The caspase-dependent mitochondrial pathway of apoptosis is triggered by the association of cytochrome *c* with apoptotic protease-activating factor-1 (Apaf-1), dATP, and procaspase-9, forming the apoptosome (Bao and Shi, 2007). Within this macromolecular complex, procaspase-9 is activated via homo-oligomerization and subsequently engages effector caspases (i.e., caspase-3, -6, and -7), which dismantle the cell through proteolytic degradation and by activating a caspase-dependent DNase (CAD).

Mitochondria also participate in an apoptosis pathway that does not involve caspase activation, via the release of apoptosis-inducing factor (AIF; Susin et al., 1999) and endonuclease G (EndoG; Li et al., 2001), which both can directly induce DNA fragmentation. The relevance of mitochondria-mediated apoptosis to sarcopenia and disuse muscle atrophy is illustrated in the following subsections.

Role of mitochondria-mediated apoptosis in sarcopenia

Accumulating evidence indicates that an acceleration of apoptosis takes place in the aging muscle, which may represent a converging mechanism whereby muscle atrophy and physical function decline ensue (Marzetti and Leeuwenburgh, 2006; Figure 2). Notably, the genetic characterization of interleukin 10-deficient mice, a rodent model of frailty, has unveiled the up-regulation of several apoptosis-related genes in skeletal muscle (Walston et al., 2008), further supporting the involvement of accelerated myocyte apoptosis in the pathogenesis of sarcopenia and physical frailty. Given their role in apoptosis regulation, mitochondria are proposed to be the major checkpoint for the execution of myonuclear apoptosis during the development of sarcopenia (Marzetti et al.,

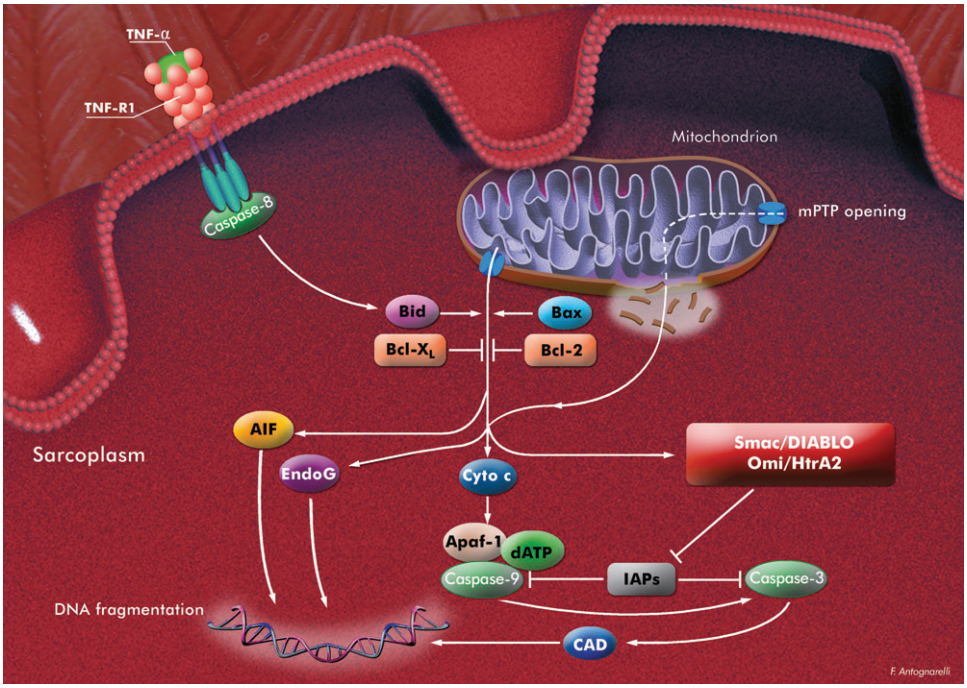

Figure 4 The mitochondrial apoptotic machinery in skeletal muscle. Release of pro-apoptotic factors from the mitochondrial intermembrane space occurs as a result of an imbalance between pro- (e.g., Bax and Bid) and anti-apoptotic (e.g., Bcl-2 and Bcl-X_L) members of the Bcl-2 family of proteins and/or following mitochondrial permeability transition pore (mPTP) opening. Mitochondrial caspase-dependent apoptosis is initiated by the cytosolic release of cytochrome *c* (Cyto c), which associates with apoptotic protease-activating factor-1 (Apaf-1), dATP and procaspase-9. The resulting apoptosome activates caspase-9, followed by the engagement of caspase-3, which performs protein breakdown and DNA fragmentation via a caspase-activated DNase (CAD). Second mitochondria-derived activator of caspases/direct inhibitor of apoptosis-binding protein with low pI (Smac/DIABLO) and heat requirement A2 protein (Omi/HtrA2), block the activity of inhibitor of apoptosis proteins (IAPs). Mitochondrial caspase-independent apoptosis is executed by apoptosis-inducing factor (AIF) and endonuclease G (EndoG). Crosstalk between tumor necrosis factor-α (TNF-α)-mediated and mitochondria-driven apoptosis can occur via cleavage and activation of Bid by caspase-8, which is recruited by the death-inducing signaling complex associated with tumor necrosis factor receptor 1 (TNF-R1).

2010a). As previously discussed, mitochondria-driven apoptosis can proceed with or without the participation of caspases. Studies indicate that both mechanisms are operative in the aging muscle (Marzetti et al., 2010b). OMMP, the event required for the release of apoptogenic factors from the IMS, is triggered by the imbalance between pro- and anti-apoptotic members of the Bcl-2 family proteins and by the opening of the mPTP (reviewed by Adhihetty et al., 2008). Increased expression of Bax and reduced levels of Bcl-2 have been detected in the skeletal muscle of old rodents (Alway et al., 2002; Pistilli et al., 2006; Song et al., 2006). However, up-regulation of both pro- and anti-apoptotic Bcl-2 family proteins has also been found in muscles from old rats, with no changes in the Bax-to-Bcl-2 ratio (Siu et al., 2005; Marzetti et al., 2008). A specific pattern of Bcl-2 and Bax expression in aged rats has been reported depending on the muscle type (Rice and Blough, 2006). Bax content was increased in the fast-twitch *extensor digitorum longus*, and unchanged in the slow-twitch *soleus*. In contrast, both muscles exhibited increased expression of Bcl-2. The elevation of Bcl-2 detected in aged muscles could be interpreted as compensatory, yet imperfect action aimed at limiting myonuclear loss in the presence of strong pro-apoptotic pressure. In fact, Braga et al. (2008) demonstrated that increased expression of Bcl-2 in the *gastrocnemius* muscle of old mice was paralleled by enhanced serine-phosphorylation and subsequent inactivation of Bcl-2, which prevented its anti-apoptotic actions in spite of elevated expression.

Enhanced susceptibility toward mPTP opening has also been demonstrated in aged skeletal muscles (Chabi et al., 2008; Seo et al., 2008; Picard et al., 2011). Furthermore, mitochondrial levels of cyclophilin D were found to be elevated relative to ANT and voltage-dependent anion channel (VDAC) in the *gastrocnemius* muscle of senescent rats (Marzetti et al., 2008). This finding supports the involvement of mPTP opening in age-related OMMP, given the central role postulated for cyclophilin D in the formation of the mPTP (Baines et al., 2005). In a recent study, a fiber-type differential

susceptibility toward mPTP opening has been described in old rats, with impaired mPTP function observed in fast-twitch muscles (i.e., *extensor digitorum longus* and *gastrocnemius*), but not in the slow-twitch *soleus* (Picard et al., 2011). This observation may provide a further explanation for the preferential atrophy of fast-twitch fibers that occurs with aging.

Following OMMP, apoptogenic factors housed in the mitochondrial IMS are released and initiate the apoptotic cascade. Elevated cytosolic levels of cytochrome *c* (Siu et al., 2005), Apaf-1 (Dirks and Leeuwenburgh, 2004; Siu et al., 2005; Chung and Ng, 2006) and active caspase-9 (Chung and Ng, 2006; Tamilselvan et al., 2007; Braga et al., 2008) have been documented in the skeletal muscle of old rodents. However, other studies did not observe increases in cytosolic cytochrome *c* or active caspase-9 levels in aged rat muscles in spite of elevated levels of apoptotic DNA fragmentation (Dirks and Leeuwenburgh, 2002, 2004; Pistilli et al., 2006).

These observations suggest that alternative apoptotic pathways may operate in the aging muscle. In this context, caspase-independent mitochondrial apoptotic signaling may be particularly important, as it could allow for the elimination of individual myonuclei without subsequent dismantling of the entire fiber by caspases (Dupont-Versteegden, 2005). This idea is supported by several reports where mitochondrial caspase-independent apoptotic pathways were activated in muscles from old rodents. For instance, EndoG was found to co-localize with myonuclei in the *soleus* muscle of old rats, indicating that nuclear translocation of EndoG had occurred during aging (Leeuwenburgh et al., 2005). In addition, AIF gene expression increased progressively over the course of aging in rat *plantaris* muscle and paralleled the development of sarcopenia (Baker and Hepple, 2006). While this finding may reflect changes in mitochondrial mass and/or mitochondrial AIF levels in old muscles, an age-related increase in both the cytosolic and nuclear levels of AIF and EndoG was indeed observed in rat *gastrocnemius* muscle (Marzetti et al., 2008). In addition, Park et al. (2010) found increased expression of AIF in the *semitendinosus* muscle of middle-aged men relative to younger controls, whereas no age-related changes in caspase-3 levels were detected. However, given the fact that only mRNA levels of apoptotic regulators were determined, the findings by Park and coworkers (2010) do not allow us to make inferences about the activation of caspase-3 and the nuclear translocation of AIF. The involvement of caspase-independent apoptosis in human muscle aging is not supported by a recent study from our group, in which we report that caspase-dependent, but not caspase-independent, mitochondrial apoptotic signaling is correlated with sarcopenia indices (i.e., muscle volume and gait speed) in *vastus lateralis* muscle samples obtained from a cohort of community-dwelling older persons (Marzetti et al., 2012b). While the discrepancies between our findings and those by Park et al. (2010) may be related to methodological differences between the two studies as well as to the different ages of the participants, they also highlight the complexity of mitochondria-mediated apoptosis regulation in the context of sarcopenia. Finally, species-specific differences in myonuclear apoptosis regulation may explain the lack of concordance between our recent findings in humans and previous work on rodents.

In summary, the available evidence supports the hypothesis that excessive myonuclear elimination triggered by mitochondrial apoptotic signaling may contribute to the development of sarcopenia. Furthermore, results from studies employing behavioral, pharmacological or genetic interventions to rescue muscle mass in old age indicate that the preservation of muscle integrity is accompanied, and perhaps mediated, by decreases in the extent of myocyte apoptosis (Marzetti et al., 2012a). Further research is required to conclusively establish whether down-regulating myonuclear apoptosis is effective in maintaining muscle mass and function in late life, to identify the most relevant apoptotic pathway(s) to target, and to determine the optimal timing for intervention.

Role of mitochondria-mediated apoptosis in disuse muscle atrophy

During muscle atrophy induced by unloading, immobilization or denervation, a reduction in the number of myonuclei per fiber and an increased incidence of apoptotic DNA fragmentation become evident (Allen et al., 1997; Smith et al., 2000; Siu and Alway, 2006). Similar to sarcopenia, mitochondria-mediated apoptotic signaling is considered to be centrally involved in the pathogenesis of disuse muscle atrophy (Marzetti et al., 2010a; Figure 2). Distinct pathways of apoptosis may be activated depending on age, muscle type and the disuse model investigated. For instance, two studies provoked muscle atrophy in the *soleus* (Leeuwenburgh et al., 2005) and *gastrocnemius* (Siu et al., 2005) muscles of young and aged rats by 14 days of hind-limb suspension (HS). Both studies reported decreased muscle mass following HS in the two age groups. However, the degree of atrophy in the *soleus* muscle was highest in young animals (Leeuwenburgh et al., 2005), in contrast to the *gastrocnemius*, where atrophy was more severe in old rats (Siu et al., 2005). The

activation of apoptosis was confirmed by elevated levels of DNA fragmentation in both muscle types in all of the HS animals. Interestingly, higher levels of apoptosis were observed in the *gastrocnemius* of both age groups (Siu et al., 2005) and the *soleus* of aged rats (Leeuwenburgh et al., 2005), suggesting that apoptosis has a marginal role in *soleus* muscle atrophy in young animals.

Investigations into the pathways contributing to the increased apoptosis observed in acute atrophy revealed remarkable differences depending on the age and muscle type. Indeed, cytosolic levels of AIF were elevated in the *gastrocnemius* muscle of old HS rats, while no AIF release was detected in young HS animals (Siu et al., 2005). The cytosolic levels of cytochrome *c* were elevated in both HS groups. There were no differences, however, in the levels of Apaf-1, caspase-9, or caspase-3 in either young or aged rats following HS. In the case of the *soleus* muscle, caspase-3 activity was increased only in young animals (Leeuwenburgh et al., 2005). In contrast, EndoG was found to co-localize with myonuclei in old HS rats, whereas the amount of nuclear-located EndoG was unchanged in young HS animals. These findings suggest that distinct apoptotic pathways are operative depending on age. More specifically, it appears that the caspase-independent pathway of apoptosis mediated by EndoG or AIF (or both) predominates in aged muscles, whereas a caspase-dependent program is selectively activated at a young age.

To further elucidate the dynamics of mitochondrial apoptotic signaling during acute atrophy, Dupont-Versteegden et al. (2006) investigated the temporal relationship between the occurrence of apoptosis and myonuclear translocation of EndoG in the *soleus* muscle of young rats subjected to short-term HS. A decline in fiber cross-sectional area was observed 2 days following HS, before any measurable decrease in muscle mass could be detected. Evidence of apoptosis was apparent as early as 12 h after HS, reaching a maximum at 2 days, which preceded the elevation in muscle atrophy F-box mRNA. The early increase in apoptosis was specific to myonuclei, whereas apoptotic DNA fragmentation in the interstitial cells did not become significantly elevated until 2 days after suspension. Furthermore, co-localization of EndoG with apoptotic myonuclei occurred 12 h after the initiation of HS, and no such localization was observed in interstitial cells. The activation of caspase-independent apoptotic signaling in the early phases of disuse atrophy has also been proposed by Ferreira et al. (2008). These authors detected maximal AIF expression following 24 h of HS in murine *soleus* muscle, temporally coinciding with the highest degree of apoptotic DNA fragmentation. Although the extent of nuclear localization of AIF was not determined, the temporal relationship between changes in AIF expression and DNA fragmentation further supports the involvement of this caspase-independent mediator in HS-induced myonuclear apoptosis. In contrast, the activity of caspase-8 and caspase-3 was highest at 12 h, concomitant with a decrease in muscle protein concentration. This suggests a role for the caspase cascade in protein degradation rather than apoptosis, at least in this model of disuse atrophy.

Denervation of skeletal muscle results in progressive atrophy similar to that observed with unloading. Early work revealed that the severity of myonuclear apoptosis was increased in patients with spinal muscular atrophies (Migheli et al., 1997). Moreover, high rates of DNA fragmentation have been observed in denervated rat facial muscles, concomitant with greater Bax-to-Bcl-2 ratio relative to denervated and immediately reinnervated counterparts (Tews et al., 1997). Further support to the hypothesis of the involvement of mitochondrial apoptotic signaling in denervation-induced muscle wasting arises from the detection of elevated caspase-9 expression in patients with muscle atrophy due to peripheral neuropathy (Tews et al., 2005). In the same disorder, an increased expression of second mitochondria-derived activator of caspases/direct inhibitor of apoptosis-binding protein with low pI (Smac/DIABLO) limited to atrophied fibers has also been observed (Tews et al., 2008). Smac/DIABLO is housed in the IMS and released following OMMP to block the activity of inhibitor of apoptosis proteins (Du et al., 2000). Altered expression of Bcl-2 family proteins, indicative of the activation of mitochondria-mediated apoptosis, has been reported in rat muscles following denervation (Adhihetty et al., 2007). Specifically, denervation resulted in the up-regulation of Bax and a decrease in Bcl-2, producing a substantial increase in the Bax-to-Bcl-2 ratio. This adaptation was accompanied by a higher incidence of apoptotic myonuclei. An increase in whole-muscle expression of AIF was also detected. Nevertheless, since nuclear levels of AIF were not quantified, it is not possible to establish whether the up-regulation of AIF reflected the activation of a caspase-independent apoptotic program. Similarly, an increased Bax-to-Bcl-2 ratio was found in rat *gastrocnemius* muscle 2 weeks after tibial nerve transection (Siu and Alway, 2005). This coincided with enhanced apoptotic DNA fragmentation sustained by increased cytosolic levels of cytochrome *c*, Smac/DIABLO and AIF. In addition, both the expression and catalytic activity of caspase-9 and -3 were elevated following denervation (Siu and Alway, 2005).

Muscle denervation has also been associated with increased susceptibility toward mPTP opening (Csukly et al., 2006; Adhihetty et al., 2007), indicating that this mechanism may participate in the pro-apoptotic environment that takes place in the atrophying muscle. It is worth noting that Csukly et al. (2006) reported that the enhanced propensity to mPTP opening in rat denervated hindlimb muscles was associated with increased cyclophilin D content relative to ANT and VDAC, suggesting that an altered expression of mPTP components could predispose to mitochondrial permeability transition in denervation-induced muscle atrophy.

Although the involvement of myonuclear apoptosis in disuse muscle atrophy is supported by a vast amount of literature, recent data have led to this paradigm being questioned. Indeed, Bruusgaard and Gundersen (2008) found no apoptotic loss of myonuclei in murine skeletal muscles atrophied for up to 28 days by denervation, nerve impulse block, or mechanical unloading. While this finding seems to rule out apoptosis as a mechanism underlying disuse muscle atrophy, a later study showed that caspase-3 knockout suppressed myonuclear apoptosis and attenuated the severity of denervation-induced atrophy in mice subjected to tibial nerve transection (Plant et al., 2009). Furthermore, mice with Bax and Bak double knockout display reduced activation of apoptosis and suffer less severe muscle wasting following sciatic nerve excision compared with wild-type denervated controls (O'Leary et al., 2012). Similar results were obtained by Siu and Alway (2006), who reported attenuation in pro-apoptotic signaling and in the extent of muscle loss following tibial nerve transection in Bax-deficient mice relative to wild-type controls.

In conclusion, up-regulation of myonuclear apoptosis is likely involved in the pathogenesis of inactivity-induced muscle atrophy. However, further research is required to identify the spectrum of biochemical and morphological events that occur in acutely atrophying muscles and to definitely establish the role played by apoptosis in this process. In addition, a deeper understanding of the apoptotic pathways involved at different ages, in distinct muscle types and specific atrophying conditions is necessary to design effective interventions to rescue skeletal myofibers during periods of prolonged disuse.

Conclusion and future perspectives

The vital functions of mitochondria in the context of energy provision, cellular QC, and integration of cell death/survival pathways place these organelles at the crossroad of signaling pathways that regulate myocyte homeostasis. For the same reasons, loss of mitochondrial functional integrity is centrally involved in muscle degeneration during aging and other atrophying conditions (Figure 2).

Several mitochondrial pathways involved in muscle wasting have been analyzed extensively, but others are still controversial. For instance, short-term antioxidant supplementation may be of benefit in the attenuation of mitochondria-mediated oxidative stress and muscle loss during protracted immobilization (Servais et al., 2007). However, chronic antioxidant administration may not necessarily be protective due to the abrogation of signaling pathways involved in force production and the maintenance of muscle integrity for which mitochondria-derived ROS function as second messengers (Hernandez et al., 2012). In addition, despite the fact that mitochondrial apoptotic signaling is operative in skeletal muscle during aging and disuse atrophy, a direct pathogenetic link between myonuclear apoptosis and muscle loss has yet to be proven. The possibility exists that the apoptotic program may serve to eliminate dysfunctional myonuclei and/or damaged myofibers, the persistence of which would be detrimental for tissue homeostasis. Furthermore, several studies only report changes in mRNA abundance or whole-muscle expression levels of mitochondria-derived apoptotic mediators, which precludes a definite understanding about the involvement of such factors in myonuclear apoptosis and muscle loss. Establishing a mechanistic link between the execution of apoptosis and muscle wasting is a difficult task. In fact, virtually all of the molecules involved in the apoptotic program possess other functions in non-apoptotic conditions. This limits the possibility of genetically manipulating apoptosis without simultaneously affecting other cellular processes.

Mitochondrial QC is based on a heterarchical network of interacting pathways, but the relevance of each of them to sarcopenia and acute atrophy is insufficiently understood. Indeed, impairments in one single pathway secondary to experimental manipulations (e.g., the generation of knockout models) could result in consequences that are too severe to draw conclusions about naturally ongoing processes (Fischer et al., 2012). Furthermore, experimental abrogation of such pathways could elicit compensatory effects, impeding a clear distinction between primary and secondary outcomes and cause–effect relationships.

Further research is needed to untangle the complexity of cellular pathways governed by mitochondria and the role played by each of these processes in the pathogenesis of sarcopenia and disuse muscle atrophy. In addition,

studies are required to establish the extent to which mitochondrial dysfunction is involved in the pathogenesis of muscle loss during aging and acute atrophying conditions in humans. Elucidation of these critical research issues will likely provide clinicians with novel and more effective therapeutics to counter muscle wasting associated with aging and disuse.

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