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Mitochondrial dysfunction and sarcopenia of aging: from signaling pathways to clinical trials

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Abstract

Sarcopenia, the age-related loss of muscle mass and function, imposes a dramatic burden on individuals and society. The development of preventive and therapeutic strategies against sarcopenia is therefore perceived as an urgent need by health professionals and has instigated intensive research on the pathophysiology of this syndrome. The pathogenesis of sarcopenia is multifaceted and encompasses lifestyle habits, systemic factors (e.g., chronic inflammation and hormonal alterations), local environment perturbations (e.g., vascular dysfunction), and intramuscular specific processes. In this scenario, derangements in skeletal myocyte mitochondrial function are recognized as major factors contributing to the age-dependent muscle degeneration. In this review, we summarize prominent findings and controversial issues on the contribution of specific mitochondrial processes – including oxidative stress, quality control mechanisms and apoptotic signaling – on the development of sarcopenia. Extramuscular alterations accompanying the aging process with a potential impact on myocyte mitochondrial function are also discussed. We conclude with presenting methodological and safety considerations for the design of clinical trials targeting mitochondrial dysfunction to treat sarcopenia. Special emphasis is placed on the importance of monitoring the effects of an intervention on muscle mitochondrial function and identifying the optimal target population for the trial.

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Keywords

mitophagy; vascular dysfunction; fusion and fission; apoptosis; biomarkers

1. Introduction

Sarcopenia, the age-associated decline in skeletal muscle mass and function (Roseberg 1989), represents a well-established risk factor for major negative health-related conditions and events, including frailty, disability, institutionalization and mortality (Visser and Schaap 2011). The increasingly recognized clinical and public health relevance of this syndrome has been leading research activities at designing and developing novel preventive and therapeutic strategies (Cesari et al. 2008). The accomplishment of such task requires a thorough understanding of the cellular processes underlying the pathogenesis of sarcopenia, in order to identify selective targets for treatment.

Numerous pathways are proposed to be implicated in the age-dependent muscle degeneration (reviewed by Marzetti et al. 2009 and Buford et al. 2010). The vital functions carried out by mitochondria in the context of energy provision, redox homeostasis, and regulation of several catabolic pathways confer these organelles a central position in the maintenance of myocyte viability. The involvement of mitochondria in the regulation of skeletal myofiber plasticity further highlights the centrality of these organelles in muscle physiology. Adult skeletal myocytes are post-mitotic cells organized in syncytia of hundreds or thousands of nuclei, where each nucleus has jurisdiction over a surrounding volume of sarcoplasm (myonuclear domain or DNA unit) (Cheek 1985). The dynamic behavior of DNA units is believed to govern muscle fiber remodeling in response to load conditions, aging and diseases (Allen et al. 1999).

Although recently called into question (Bruusgaard and Gundersen 2008; Bruusgaard et al. 2012), this concept is supported by numerous studies reporting that modifications in fiber cross-sectional area (CSA) are associated with changes in the number of myonuclei (reviewed by Teixeira and Duarte 2011). Moreover, during muscle accretion, DNA units increase their size, while smaller myonuclear domains are observed during disuse- and aging-associated muscle atrophy (Van der Meer et al. 2011). A wealth of experimental evidence indicates that mitochondria are central in the regulation of myonuclear domain both in physiological and pathological conditions (reviewed by Calvani et al. 2013a). As such, mitochondrial decay is advocated as one of the major factors driving muscle aging (Johnson et al. 2013).

The interpretation of the consequences of mitochondrial dysfunction on muscle physiology is complicated by the existence of two populations of mitochondria in skeletal myocytes: subsarcolemmal mitochondria (SSM), located beneath the plasma membrane and accounting for approximately 20% of total mitochondrial mass, and interfibrillar mitochondria (IFM), arranged between the myofibrils and representing the remaining 80% of myofiber mitochondrial volume (Hoppeler 1986). These two subpopulations possess specific biochemical and functional properties and exhibit a distinct behavior during aging (Koves et al. 2005; Ferreira et al. 2010). For instance, SSM isolated from old muscles produce greater amounts of reactive oxygen species (ROS) and show higher rates of fragmentation and degradation relative to the IFM subfraction (Riley et al. 1990; Chabi et al. 2008; Seo et al. 2008; Wagatsuma et al. 2011). On the other hand, IFM are more prone than SSM to releasing apoptotic mediators under cell death stimuli (Adhihetty et al. 2005). These divergent properties raise the possibility that the two mitochondrial populations could be differentially involved in the pathogenesis of sarcopenia.

A further level of complexity is added by the fact that both mitochondrial function and muscle trophism are influenced by physical activity and anabolic hormones, which both decline with aging (Dela and Helge 2013). Other age-related processes, such as chronic, low-grade inflammation and vascular alterations, can also affect both muscle health and mitochondrial function (Terjung et al. 2002; Jensen 2008). Hence, segregating the impact of age *per se* from that of lifestyle habits and age-associated conditions on muscle pathophysiology poses a relevant challenge (Fig. 1).

In this review, we illustrate relevant mechanisms that are proposed to underlie the relationship among aging, muscle mitochondrial dysfunction and sarcopenia. First, we present a brief overview on the evidence linking mitochondrial dysfunction to human muscle aging. We then discuss prominent mitochondrial pathways that have been implicated in the pathogenesis of sarcopenia. Subsequently, we summarize extramuscular factors that can have an impact on myocyte mitochondrial function, thus potentially serving as targets for anti-sarcopenic interventions. Finally, methodological considerations for the design of clinical trials on mitochondrial dysfunction and sarcopenia are presented.

2. Involvement of mitochondrial dysfunction in muscle aging: evidence from human studies

One major consequence of the age-associated mitochondrial dysfunction is a decline in bioenergetics. Both resting and maximal oxygen (O₂) consumption decreases with advancing age (Short et al. 2004). This decline is independent of fat-free mass, indicating that either muscle mitochondrial function or content (or both) is reduced as a function of age.

Studies on muscle specimens from healthy individuals have revealed age-related declines in mitochondrial mass (Welle et al. 2003), activities of tricarboxylic acid cycle enzymes (Crane et al. 2010), O₂ consumption (Joseph et al. 2012; Coen et al. 2013), and ATP synthesis (Short et al. 2005). Moreover, a 40–50% decrease in oxidative phosphorylation (OXPHOS) activity has been detected *in vivo* via ³¹P nuclear magnetic resonance (³¹P-NMR) spectroscopy in older persons compared with younger controls (Conley et al. 2000; Petersen et al. 2003).

The impact of mitochondrial bioenergetic decline on muscle aging is witnessed by the existence of a correlation between ATP synthesis/O₂ consumption and preferred walking speed in healthy elderly (Coen et al. 2013). In this regard, it is noteworthy that slow walking speed has been adopted as a defining criterion for sarcopenia (Cruz-Jentoft et al. 2010) and physical frailty (Fried et al. 2001). Another mechanism linking mitochondrial dysfunction to sarcopenia is the possible impact of ATP shortage on protein synthesis, which is reflected by the concomitant decrease in whole-body bioenergetics and muscle protein anabolism over the course of aging (Short et al. 2004). As discussed further on, the bioenergetic failure of the aged muscle is likely the result of a vicious cycle involving oxidant production, damage and depletion of mitochondrial DNA (mtDNA), and defective mitochondrial quality control (MQC) (Brunk and Terman 2002) (Fig. 2).

A relevant consequence of mitochondrial dysfunction is the activation of apoptosis, a mechanism believed to represent a final common pathway through which sarcopenia and physical frailty ensue (Marzetti and Leeuwenburgh 2006) (Fig. 2). This idea is supported by the observation that mitochondrial apoptotic signaling correlates with slow walking speed and reduced muscle volume in older persons (Marzetti et al. 2012d).

3. Mechanisms and consequences of mitochondrial dysfunction in the sarcopenic muscle

3.1. The vicious cycle between oxidative stress and mitochondrial dysfunction in the aged muscle

Mitochondria are at the same time the major source of oxidants within cells and a primary target of oxidative stress. The mtDNA is intrinsically vulnerable to oxidative damage due to its proximity to the source of oxidants, the absence of histones and introns, and a less robust repair system compared with nuclear DNA (Yakes and Van Houten 1997). According to the widely accepted mitochondrial free radical theory of aging (MFRTA), mitochondrial dysfunction arising from oxidative damage to mtDNA would trigger a vicious cycle in which the synthesis of defective ETC subunits, results in OXPHOS impairment, decreased ATP production and further ROS generation (Harman 1972; Miquel et al. 1980).

The involvement of the mitochondrial free radical vicious cycle in muscle aging is supported by findings in preclinical models (Lee et al. 1998; Wanagat et al. 2001; Figueiredo et al. 2009; Lee et al. 2010) and humans (Bua et al. 2006). In an elegant study, Bua et al. (2006) analyzed the occurrence of ETC abnormalities and the abundance of mtDNA deletion-mutations along the length of laser-captured microdissected fibers isolated from human *vastus lateralis* (VL) muscle specimens. The number of fibers harboring ETC abnormalities, identified via histochemical staining for cytochrome *c* oxidase (COX) and succinate dehydrogenase (SDH) activities, increased from 6% at 49 years of age to 31% at 92 years. Fibers displaying a COX negative and SDH hyper-reactive (COX⁻/SDH⁺⁺) phenotype co-localized with clonal expansions of somatically-derived mtDNA deletion-mutations and were more frequent in fiber segments with morphological aberrations. Conversely, mtDNA mutations and ETC abnormalities were absent in phenotypically normal regions within individual fibers.

The observation that sarcopenia develops prematurely in mice that express an error-prone mtDNA polymerase- γ has provided the proof of principle linking mtDNA damage, mitochondrial dysfunction and muscle atrophy (Kujoth et al. 2005; Dai et al. 2010; Hiona et al. 2010). Similar findings have recently been obtained in mice with double-strand mtDNA breaks caused by the transient expression of a mitochondrial-targeted endonuclease (*PsfI*) (Wang et al. 2013).

The MFRTA suffers however a major pitfall: within cells, mitochondria fuse continuously with one another forming large syncytia. As a result, a constant mixing of the total cellular mtDNA pool occurs, disrupting the connection between genotype (damaged mtDNA) and phenotype (defective OXPHOS and increased ROS emission) (Sato et al. 2006). Nevertheless, the hypothesis linking mtDNA damage, ETC dysfunction and abnormal ROS generation may still retain validity inasmuch as MQC processes become inefficient in advanced age, as discussed in the next section.

3.2. Defective mitochondrial quality control in the sarcopenic muscle

Mitochondrial structural and functional integrity relies on the efficiency of quality control processes, including intramitochondrial oxidant-scavenging systems, protein repair and degradation pathways, and mitochondrial dynamics and turnover (reviewed by Twig et al. 2008b). MQC is especially relevant to the homeostasis of skeletal myocytes, given their high reliance on OXPHOS for energy provision and their post-mitotic nature, which impedes the dilution of mitochondrial dysfunction via cell division. Due to these vital responsibilities, the disruption of MQC processes is invoked as a mechanism contributing to muscle loss associated with aging and other atrophying conditions (Calvani et al. 2013a) (Fig. 2).

3.2.1 Mechanisms and consequences of altered mitochondrial dynamics in the aging muscle—

Contrary to the traditional view of isolated, ovoidal organelles, mitochondria are indeed dynamic entities that form constantly changing networks within cells (Aon 2010). Mitochondrial dynamics are regulated by coordinated fusion and fission cycles performed by a complex molecular machinery (reviewed by Youle and van der Bliek 2012). Simplified, fusion allows joining mitochondria to mix their contents, thereby redistributing metabolites, proteins and mtDNA, and equilibrating the concentrations of nuclear-encoded proteins across organelles (Ono et al. 2001). Fission segregates components of the network that are irreversibly damaged or unnecessary, for subsequent removal (Twig et al. 2008a).

Derangements in fusion-fission have been proposed as a mechanism underlying the formation of aberrant mitochondria under stress conditions or during senescence (Yoon et al. 2006) (Fig. 2). Giant, dysfunctional mitochondria, characterized by highly interconnected networks and ultrastructural abnormalities, are frequently encountered in aging muscles (Beregi and Regius 1987). Remarkably, the exposure of cultured cells to subcytotoxic doses of hydrogen peroxide (H_2O_2) represses the expression of fission protein 1 (Fis1), thereby promoting the formation of elongated mitochondria with increased oxidant emission (Yoon et al. 2006). This finding, together with the observation that enlarged mitochondria accumulate in old skeletal myocytes, suggests that an imbalance in mitochondrial dynamics towards fusion might occur in the aged muscle (Fig. 2).

It is conceivable that mitochondrial hyperfusion could represent an attempt to cope with increased levels of mtDNA mutations through the dilution of mutant genomes along the network (Sato et al. 2006). In support to this hypothesis, disruption of mitochondrial fusion via mitofusin (Mfn) 1 deletion was found to increase mitochondrial dysfunction and lethality in mtDNA-mutator mice (Chen et al. 2010). Mitochondrial fragmentation and downregulation of fusion have also been detected in muscles from type-2-diabetic and obese subjects, in conjunction with impaired bioenergetics (Bach et al. 2005). It is worth mentioning that both diabetes mellitus and obesity are associated with accelerated development and/or progression of sarcopenia (Buford et al. 2010) (Fig. 1). Interestingly, depletion of the mitochondrial fusion factor optic atrophy protein 1 disintegrates the mitochondrial network and sensitizes cultured cells to apoptosis (Lee et al. 2004). On the contrary, blockade of Fis1 or dynamin-related protein 1 (Drp1) inhibits mitochondrial fragmentation and the execution of apoptosis in cell culture systems (Frank et al. 2001; Lee et al. 2004). Therefore, mitochondrial dynamics and apoptotic signaling appear to be intimately interconnected, which may be crucial for the modulation of cell death/survival pathways.

Mitochondrial hyperfusion has an important drawback. Severely damaged organelles within the network may be hard to single out by fission, thereby hindering their disposal. It can therefore be speculated that upregulation of fusion and/or downregulation of fission could allow maintaining myocyte viability until a critical threshold of pathogenic mtDNA is breached and the degree of mitochondrial interconnection becomes incompatible with the removal of damaged mitochondria (Calvani et al. 2013a) (Fig. 2). On a clinical level, this course of events could translate into a progressively reduced muscular performance and, eventually, the development of muscle atrophy.

In overt contrast to the mitochondrial hyperfusion hypothesis, recent findings show that fission signaling may indeed be upregulated in muscles from old rats, as evidenced by the higher expression of Fis1 and Drp1 relative to younger controls (Iqbal et al. 2013). A concomitant downregulation of Mfn2 is also reported, suggestive of decreased fusion. In addition, O'Leary et al. (2013) found that the expression of both fission and fusion proteins

was upregulated in muscles from old rats. It should however be noted that in both of the aforementioned studies experiments were restricted to the SSM population, which does not allow extending those results to the more abundant IFM fraction.

An excessive activation of fission can also induce mitochondrial dysfunction and muscle atrophy (Fig. 2), at least under certain experimental paradigms. Using a transgenic mouse model, Romanello et al. (2010) showed that the expression of the fission machinery was sufficient to induce mitochondrial dysfunction, remodeling of the mitochondrial network, protein breakdown and fiber atrophy. Blockade of mitochondrial fission, in contrast, resulted in significant protection from atrophy during fasting as well as in mice with constitutively active Forkhead box O3 (FoxO3), a transcription factor that controls major catabolic pathways in skeletal myocytes (Sandri 2013).

In conclusion, the essential functions of mitochondrial morphogenesis in the context of MQC and the intersection with proteolytic and apoptotic pathways candidate mitochondrial shaping proteins as potential targets for interventions against sarcopenia. Further research is necessary to address several critical issues. First, it must be established whether distinct fusion and fission properties exist between IFM and SSM, which may help explain their divergent behavior during muscle aging. Second, the complex relationship among mitochondrial dynamics, muscle protein metabolism and apoptotic signaling needs to be fully deciphered. Lastly, studies are warranted to characterize the age-related changes in mitochondrial dynamics and their impact on muscle trophism in humans.

3.2.2. Contribution of impaired mitochondrial turnover to muscle aging—

Mitochondrial renewal is performed by two opposing, yet coordinated processes: the degradation of dysfunctional or unnecessary mitochondria through a specialized form of autophagy (mitophagy) and the generation of new organelles via biogenesis. The description of the molecular pathways involved in mitophagy and mitochondrial biogenesis is beyond the scope of the present review, and the reader is referred elsewhere (for instance, Jornayvaz and Shulman 2010 and Bhatia-Kissova and Camougrand 2013).

Accumulating evidence indicates that mitochondrial turnover is altered during muscle aging, potentially affecting mitochondrial function and myocyte homeostasis (reviewed by Calvani et al. 2013a and Vina et al. 2009). A reduced expression of peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α), indicative of decreased mitochondriogenesis, has been detected in muscles of old experimental animals (Koltai et al. 2012) and elderly persons (Safdar et al. 2010). Disruption of PGC-1 α signaling also influences protein metabolism. Indeed, PGC-1 α attenuates muscle protein degradation by the ubiquitin-proteasome system (UPS) through blocking the activities of nuclear factor κ B (NF- κ B) and FoxO3 (Sandri et al. 2006; Brault et al. 2010). Furthermore, a splice variant of PGC-1 α , PGC-1 α 4, stimulates protein synthesis and suppresses myostatin signaling and UPS activity in cultured myotubes and the murine *tibialis anterior* (TA) muscle (Ruas et al. 2012). The expression of both PGC-1 α and PGC-1 α 4 is upregulated by physical exercise (Ruas et al. 2012), suggesting that, in older individuals, the beneficial effects of exercise training on muscle mitochondria biogenesis and protein metabolism could be mediated, at least in part, by the restoration of PGC-1 α signaling. This hypothesis deserves further investigation.

A decline in autophagic function is a common trait of the aging process (Rajawat et al. 2009). This phenomenon, together with the progressive accumulation of macromolecular and organellar damage over the life course, has led to the proposition that impairments in the autophagosomal-lysosomal pathway could be centrally involved in the aging process (Brunk and Terman 2002). The autophagic failure experienced by old skeletal myocytes could be

linked to the intralysosomal buildup of a non-degradable pigment called lipofuscin (Orlander et al. 1978; Terman and Brunk 2004) (Fig. 2). Lipofuscin-induced lysosomal clogging and mitochondrial decay concur to a self-perpetuating process in which mitochondrial ROS promote lipofuscinogenesis, while the accumulation of lipofuscin granules compromises mitochondrial autophagy (Terman et al. 2010). This vicious cycle forms the basis for the garbage catastrophe theory of aging, also known as the mitochondrial-lysosomal axis theory of aging (Brunk and Terman 2002).

Members of our group have shown that advanced age is associated with reduced transcript levels of the autophagic factor lysosomal-associated membrane protein 2 (LAMP-2) in the rat *plantaris* muscle, suggestive of decreased autophagy (Wohlgemuth et al. 2010). Life-long mild calorie restriction prevented the age-related decline in LAMP-2 expression, which was associated with an attenuation of oxidative stress and apoptotic DNA fragmentation. These data are in agreement with those by Russ et al. (2012) who showed that the ratio between microtubule-associated protein 1 light chain 3 (LC3)-II and LC3-I, an index of ongoing autophagy (Kadowaki and Karim 2009), was reduced in the *gastrocnemius* muscle of old rats, which was partially restored by voluntary wheel running. In another recent study, a 6-month weight loss program combined with moderate intensity exercise increased the mRNA abundance of LC3B, autophagy-related (Atg) protein 7 and LAMP-2 in the VL muscle of overweight older women (Wohlgemuth et al. 2011). The intervention also upregulated the gene expression of the mitochondrial biogenesis markers PGC-1 α and mitochondrial transcription factor A (TFAM). Finally, Luo et al. (2013) found that 9 weeks of resistance training increased the expression of several autophagy regulatory proteins, including Beclin1, Atg5 and Atg7, and the lysosomal hydrolase cathepsin L in the *gastrocnemius* muscle of old rats. These adaptations were accompanied by downregulation of mitochondrial apoptotic signaling and improvements in muscle mass and strength. Hence, the stimulation of mitochondrial turnover appears to mediate, at least partly, the beneficial effect of exercise and weight loss on the skeletal muscle. In keeping with this idea, the abrogation of stress-induced autophagy (He et al. 2012) or the inhibition of mitochondrial biogenesis (Geng et al. 2010) has been shown to prevent several muscular metabolic adaptations to exercise training.

In contrast to the aforementioned studies, Wenz et al. (2009) found that the LC3-II/LC3-I ratio was increased in the *biceps femoris* muscle of old mice compared with younger controls. Mitochondrial damage and upregulation of autophagic markers were not detected in muscles of old mice with PGC-1 α overexpression. It is possible that PGC-1 α could intervene in the mitochondrial-lysosomal vicious cycle by promoting mitochondrial renewal and improving antioxidant defenses, eventually preventing the generation of lipofuscin. Consistent with these findings, the protein expression of Atg7 was shown to be elevated in the *extensor digitorum longus* muscle of old rats (O'Leary et al. 2013). The mitochondrial localization of Parkin, a mediator recruited to damaged mitochondria assisting in their mitophagic removal (Narendra et al. 2008), was also increased in aged animals (O'Leary et al. 2013). Finally, the protein content of the autophagic regulators Atg7 and Beclin1 was found to be higher in VL muscle samples of healthy older persons compared with younger controls, although the LC3-II/LC3-I ratio was unvarying (Fry et al. 2013).

These contrasting findings reflect the intricate relationships between mitochondrial (dys)function and autophagy during muscle aging. The interpretation of the effects of aging and behavioral interventions on mitochondrial autophagy in muscle is also hampered by the lack of use in most studies of standardized methods for monitoring this cellular pathway (Klionsky et al. 2012). The need for reliable biomarkers of the autophagic flux, combined with measures of mitochondrial network function, is especially stringent when considering that mitochondrial autophagy could follow a biphasic trajectory during muscle aging. A

compensatory upregulation of autophagy may be put forward to cope with the progressive accumulation of dysfunctional mitochondria. Over the long term, this adaptation may impair the reserve capacity of the autophagic system that could therefore become unable to face an eventual additional perturbation (Kriete 2013). This view is supported by the observation that, in spite of an increased basal autophagic activation, old rat muscles cannot further upregulate autophagy during protracted immobilization (O'Leary et al. 2013).

The accumulation of lipofuscin granules into a growing number of lysosomes contributes to impairing the autophagosomal degradative capacity (Terman et al. 2010). Lipofuscin-loaded lysosomes consume a large part of newly produced hydrolases that, however, cannot digest lipofuscin (Fig. 2). At the same time, a smaller amount of lysosomal enzymes remains available for autophagic pathways, including mitophagy. The “futile cycle” of autophagic activation eventually leads to the collapse of the cell's catabolic machinery (Brunk and Terman 2002). Bearing these considerations in mind, a “snapshot” assessment of protein or gene expression levels of autophagic mediators may not provide sufficient information on the efficiency of this pathway. Future studies adopting standardized methods to measure autophagy (Klionsky et al. 2012), in combination with assessments of mitochondrial network health, are required to conclusively establish whether targeting mitophagy to ameliorate MQC provides therapeutic gain against sarcopenia.

3.2.3. Mitochondrion-mediated apoptosis: a final common pathway in

sarcopenia of aging?—According to the myonuclear domain paradigm, the elimination of myonuclei via an apoptosis-like process mediates the removal of DNA units during muscle atrophy (Allen et al. 1999) (Fig. 2). The hypothesis is supported by numerous studies showing that the extent of apoptotic DNA fragmentation increases in skeletal muscle over the course of aging, paralleling the development of sarcopenia and physical frailty (reviewed by Marzetti et al. 2010b). Given the central role of mitochondria in the integration of apoptotic signaling and the decline in muscle mitochondrial function with age, a great deal of research has been devoted to exploring the contribution of mitochondrion-mediated apoptosis to the pathogenesis of sarcopenia. Detailed descriptions of the mitochondrial apoptotic machinery are available in specialized reviews (for instance, Wang and Youle 2009).

Studies in rodent muscles have shown that advanced age is associated with changes in the expression pattern of B-cell leukemia-2 (Bcl-2) family proteins that favor outer mitochondrial membrane (OMM) permeabilization (Alway et al. 2002; Pistilli et al. 2006; Rice and Blough 2006; Song et al. 2006; Braga et al. 2008; Carter et al. 2011). An enhanced susceptibility towards opening of the permeability transition pore has also been demonstrated in muscles from old rats (Chabi et al. 2008; Seo et al. 2008; Picard et al. 2011). Noticeably, markers pertaining to both mitochondrial caspase-dependent (e.g., cytosolic cytochrome *c* and active caspase-9) and independent pathways [i.e., cytosolic and/or nuclear levels of apoptosis-inducing factor (AIF) and endonuclease G (EndoG)] are elevated in aged muscles (reviewed by Marzetti et al. 2010a).

Initial evidence on the possible involvement of mitochondrion-mediated apoptosis in human muscle aging has recently been reported. Park et al. (2010) found increased gene expression of AIF in the *semitendinosus* muscle of middle-aged men relative to younger controls, with no changes in caspase-dependent apoptotic signaling. However, since only mRNA levels of apoptotic regulators were assessed, these findings do not allow drawing conclusions about the actual activation of apoptosis. The involvement of caspase-independent apoptosis in human muscle aging is not supported by a recent study from our group, showing that only mediators pertaining to caspase-dependent apoptotic signaling are correlated with sarcopenia indices (i.e., muscle volume and gait speed) in VL muscle samples from community-

dwelling elders (Marzetti et al. 2012d). The divergence between the two studies may be ascribed to methodological differences as well as to the different age of participants. The discrepancy also reflects the complexity of mitochondrion-mediated apoptosis regulation in the setting of human muscle aging.

Although substantial evidence supports the involvement of mitochondrial apoptotic signaling in sarcopenia, recent studies reported no apoptotic loss of myonuclei in murine muscles atrophied by denervation, nerve impulse block, or mechanical unloading (Bruusgaard and Gundersen 2008; Bruusgaard et al. 2012). Imaging of single fibers by *in vivo* time-lapse microscopy revealed no myonuclear depletion in either slow- or fast-twitch muscles, despite a 50% reduction in muscle CSA. The different timeframes during which acute atrophy and sarcopenia develop could explain these controversial findings. While apoptosis of myonuclei may be negligible in the short term, chronic atrophying stimuli have shown to induce a significant decrease in myonuclear count (Viguie et al. 1997). In addition, the young age of the mice used by Bruusgaard and colleagues could have protected skeletal myofibers against myonuclear apoptosis. In this regard, Salucci et al. (2013) found that well-functioning autophagy preserved the viability of C2C12 myotubes by conferring resistance towards pro-apoptotic stimuli. Accordingly, the induction of autophagy by resistance exercise training has been associated with downregulation of mitochondrial apoptotic signaling in the *gastrocnemius* muscle of old rats (Luo et al. 2013). It may therefore be hypothesized that the age-related decline in autophagic efficiency could render skeletal myocytes more prone to apoptosis, therefore providing an additional explanation for the increased severity of apoptosis experienced by old muscles.

The contribution of apoptosis to sarcopenia is strongly supported by the observation that both the activation of apoptosis and the severity of atrophy are generally greater in muscles with fast-twitch fiber dominance relative to those mainly populated by slow-twitch fibers (Marzetti and Leeuwenburgh 2006). Moreover, behavioral, pharmacological or genetic interventions proven to be effective at rescuing muscle mass and function in old age have also been shown to mitigate the severity of apoptosis (Marzetti et al. 2012b). Should the myonuclear domain paradigm be confuted, apoptotic signaling could still be involved in the pathogenesis of sarcopenia. Indeed, the activation of caspase-3, the final effector of intrinsic and extrinsic apoptotic pathways, is required for the initial breakdown of actomyosin complexes, yielding protein fragments of manageable size for degradation by the UPS (Du et al. 2004) (Fig. 2).

In conclusion, further research is required to clearly establish a mechanistic link between apoptosis and muscle atrophy, identify the most relevant apoptotic pathway(s) to target, and determine the optimal timing for intervention. Studies are also needed to investigate whether the network arrangement of mitochondria facilitates the propagation of the apoptotic signaling under severe muscle-atrophying conditions. The occurrence of such an amplification could provide a mechanistic explanation for the higher degree of atrophy experienced by older adults during periods of protracted disuse (e.g., prolonged bed rest) (Kortebein et al. 2007), therefore indicating new targets for treatment.

4. Impact of age-related conditions on muscle mitochondrial function: targeting extramuscular processes to treat sarcopenia

Muscle trophism and mitochondrial homeostasis are governed by complex interactions between intrinsic and extramuscular processes. Discerning the relative contribution of these factors to the pathogenesis of sarcopenia is a crucial issue researchers in the field need to address. The existence of an extramuscular component in the sarcopenia syndrome is supported by studies on muscle regeneration showing that the reduced regenerative potential

of cross-transplanted muscle grafts is largely dictated by the age of the host rather than by the donor's age (Carlson and Faulkner 1989 and 1996). The involvement of extrinsic factors in muscle aging is reinforced by findings in a model of heterochronic parabiosis where young and old mice shared the circulatory system (Conboy et al. 2005). The exposure of old muscles to blood supply from a young mouse restored the proliferative and regenerative capacity of aged satellite cells without recruitment of young cells from the shared circulation. These observations indicate that extramuscular factors have a great impact on muscle trophism and may therefore provide valuable targets for the prevention and treatment of sarcopenia. As discussed in the following subsections, several extrinsic processes may impact muscle trophism by acting directly or indirectly on mitochondria.

4.1. Chronic, low-grade inflammation

Systemic subacute inflammation is a salient characteristic of the aging process that has been implicated in the development of a number of chronic degenerative diseases, including atherosclerosis, osteoarthritis, cancer, diabetes, osteoporosis, cardiovascular diseases, and dementia (reviewed by Chung et al. 2009). Chronically elevated circulating levels of several pro-inflammatory mediators, most notably interleukin 6, C-reactive protein and tumor-necrosis factor α (TNF- α), have also been associated with the development of sarcopenia and physical frailty (Ferrucci et al. 2002; Visser et al. 2002; Cesari et al. 2005).

The induction of muscle protein breakdown has long been considered to be the major pathway underlying the relationship between inflammation and sarcopenia (Combarret et al. 2009). More recently, a link among inflammation, mitochondrial dysfunction and oxidative stress has been proposed (Salvioli et al. 2006; De la Fuente and Miquel 2009), possibly providing a further mechanistic explanation for the association between inflammation and sarcopenia.

One pathway through which inflammation may impact muscle mitochondrial function is the nitric oxide (NO $\cdot$) signaling. NO $\cdot$ exerts important actions on mitochondria by modulating biogenesis, O $_2$ consumption and redox homeostasis (Dai et al. 2013). Within the ETC, NO $\cdot$ competes with O $_2$ for the substrate-binding site of complex IV (Carreras and Poderoso 2007). The outcome of such competition is dependent on intramitochondrial NO $\cdot$ concentrations, which in turn are determined by the NO $\cdot$ synthase (NOS) isoform expressed. Low levels of NO $\cdot$ -mediated ETC inhibition induced by endothelial and neuronal NOS (eNOS and nNOS) may be beneficial through dispensing O $_2$ to cells at varying distances from blood vessels and by reducing oxidant generation (Gladwin and Shiva 2009). In skeletal myofibers, this mechanism could optimize O $_2$ repartition between SSM and IFM (Marzetti et al. 2012c). On the other hand, excessive NO $\cdot$ production by inducible NOS (iNOS) leads to extensive ETC inhibition, amplification of oxidant production, and induction of apoptosis via OMM permeabilization (Boczkowski et al. 1999; Boyd and Cadenas 2002). Noteworthy, TNF- α is a potent inducer of iNOS (Nussler and Billiar 1993), which establishes a mechanistic link between inflammation and mitochondrial dysfunction.

To this end, we recently showed that downregulation of TNF- α expression was associated with upregulation of eNOS and repression of iNOS in the TA muscle of old rats treated with the angiotensin-converting enzyme inhibitor enalapril (Marzetti et al. 2012c). These adaptations were accompanied by decreased SSM emission of H $_2$ O $_2$. In an earlier study from our group, enalapril administration downregulated mitochondrial caspase-dependent apoptotic signaling in the *gastrocnemius* of old rats, while attenuating the loss of muscle mass and strength (Carter et al. 2011).

Apart from its actions on NO $\cdot$ signaling, TNF- α can also trigger apoptosis through the death-receptor signaling pathway (Lavrik et al. 2005). Downstream of TNF- α , caspase-8

initiates the caspase cascade and also mediates crosstalk between the extrinsic and intrinsic apoptotic pathways via Bid truncation (Li et al. 1998). Once cleaved, Bid induces OMM permeabilization and release of apoptogenic factors from the intermembrane space (Scorrano et al. 2002). Interestingly, Bid localization to mitochondria was found to be increased in the *gastrocnemius* muscle of old rats, accompanied by elevation of markers pertaining to mitochondrial caspase-dependent and independent apoptotic signaling (Marzetti et al. 2008b).

Impairments in mitochondrial turnover have recently emerged as an additional mechanism linking inflammation to mitochondrial dysfunction. Elevated levels of TNF- α decrease the mRNA abundance of PGC-1 α , TFAM and nuclear respiratory factor 1 in cultured C2C12 myoblasts, indicative of suppressed mitochondriogenesis (Remels et al. 2010). The effect, mediated by NF- κ B, has been implicated in muscle wasting in patients with chronic obstructive pulmonary disease (COPD) (Remels et al. 2010). Inflammation may also inhibit autophagy, which in turn could amplify mitochondrial dysfunction by impairing the disposal of damaged organelles (Salminen et al. 2012).

In conclusion, the negative effect of chronically elevated levels of inflammatory cytokines on muscle mitochondrial function may underlie the well-known association between low-grade inflammation and sarcopenia. This implies that interventions targeting inflammation might help preserve skeletal muscle mass and function in advanced age, a concept starting to be supported by preclinical and epidemiological studies (Rieu et al. 2009; Landi et al. 2013). Further research is necessary to decipher the complex interaction between mitochondrial dysfunction and inflammation, select the most relevant inflammatory pathways to target, and identify circulating biomarkers that reflect the relationship among inflammation, muscle mitochondrial damage and sarcopenia.

4.2. Endocrine aging

The nature and magnitude of age-related alterations in circulating hormones and target tissue responsiveness have taken center stage in sarcopenia research (Sakuma and Yamaguchi 2012). Interestingly, recent studies have shown that the vast majority of endocrine factors associated with muscle aging may act on mitochondria. For instance, glucocorticoids, sex steroids and thyroid hormones regulate a wide range of muscle mitochondrial processes, including MQC pathways, OXPHOS activity, redox balance, and apoptotic signaling (Weber et al. 2002; Pitteloud et al. 2005; Casas et al. 2009; Musa et al. 2011; Weitzel and Iwen 2011; Guo et al. 2012; La Colla et al. 2013). The detection of steroid and thyroid hormone receptors in mitochondria and the presence of sequences similar to nuclear hormone responsive elements in the mitochondrial genome suggest these hormones may coordinate nuclear and mitochondrial gene transcription for adapting the cell to variable energy demands (Psarra and Sekeris 2008).

Studies have begun to unveil the role of sex steroid and thyroid hormones on mitochondrial function in the context of muscle aging. Casas et al. (2009) found that overexpression of p43, the mitochondrial triiodothyronine receptor, aggravated muscle loss in old mice. After an initial increase in mitochondrial mass, the mtDNA abundance declined significantly in the *quadriceps* muscle of aged transgenic mice. In addition, p43 overexpression was associated with enhanced oxidative stress in spite of increased antioxidant enzyme activities. As for sex steroids, Guo et al. (2012) showed that testosterone administration combined with low-intensity treadmill training ameliorated muscle mass and strength in old male mice to a greater extent than exercise alone. Such an effect was associated with upregulation of mitochondrial biogenesis, increased expression of markers of mitochondrial fission/fusion and mitophagy, and reduced oxidative stress.

Concurrent impairments in muscle mitochondrial function and insulin sensitivity are typically observed in advanced age (reviewed by Abbatecola et al. 2011). However, the association between insulin resistance and mitochondrial dysfunction and the actual direction of the relationship remain controversial (Dela and Helge 2013). Although mitochondrial decay has traditionally been attributed a causative role in insulin resistance, some intriguing findings point to impaired insulin sensitivity as a determinant of muscle mitochondrial dysfunction rather than the opposite (Stump et al. 2003; Asmann et al. 2006). Moreover, insulin sensitivity is similar in young and older men engaged in regular endurance exercise, in spite of a significant age-related reduction in mitochondrial oxidative capacity (Lanza et al. 2008). Declining insulin sensitivity is therefore likely related to changes in adiposity and to physical inactivity, rather than to alterations of mitochondrial function or mass.

Echoing these findings, a recently published cross-sectional study found a combination of muscle mitochondrial dysfunction, reduced muscle mass, ectopic lipid deposition and impaired glucose tolerance in sedentary non-obese elderly subjects (Johannsen et al. 2012). In this regard, crosstalk between muscle and adipose tissue, mediated by cytokines and peptides collectively named myokines and adipokines, may represent another aspect to take into account in the study of age-related mitochondria derangements. Indeed, leptin and adiponectin have shown to affect mitochondrial biogenesis and function in muscle (Iwabu et al. 2010; Li et al. 2011).

Although declining levels of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) are often considered “the usual suspects” responsible for the deleterious changes in body composition and physical function with aging (Giovannini et al. 2008), controversies exist about the role of GH/IGF-1 axis modulation as a strategy to improve muscle strength and function (Taaffe et al. 1994; Yarasheski et al. 1995; Lange et al. 2002; Marzetti et al. 2008a). Notwithstanding, GH infusion was found to enhance mitochondrial bioenergetics in skeletal muscle of healthy young persons (Short et al. 2008). Whether GH supplementation produces a similar adaptation in older, sarcopenic individuals has not been explored. In any case, given the number of severe side effects associated with long-term GH supplementation, the Growth Hormone Research Society has warned against the use of GH or GH secretagogues as an anti-aging strategy (Thorner 2009).

Alterations in the renin-angiotensin system are indicated as potential contributors to sarcopenia through the promotion of muscular inflammation, mitochondrial dysfunction, and apoptosis (Carter et al. 2005). Angiotensin II (AngII) reduces mitochondrial mass in C2C12 cells in a dose-dependent manner by directly inhibiting the expression of genes involved in mitochondrial biogenesis (Mitsuishi et al. 2009). Similar effects were observed in skeletal muscle of mice after AngII infusion. Moreover, recent reports by our group show that the administration of enalapril to old rats protects against sarcopenia likely by attenuating mitochondrial oxidant generation and apoptosis (Carter et al. 2011; Marzetti et al. 2012c).

An extensive literature exists on the association between vitamin D deficiency and sarcopenia (reviewed by Cesari et al. 2011), but proof of causality and pathogenetic mechanisms are still controversial (Stockton et al. 2011). Nevertheless, a recent study reported that vitamin D supplementation to severely deficient persons produced marked improvements in fatigue that were accompanied, and perhaps mediated by augmented mitochondrial function, as measured *in vivo* by ³¹P-NMR spectroscopy (Sinha et al. 2013). Similar to steroid and thyroid hormones, the constitutive presence within mitochondria of vitamin D receptor (VDR) and its heterodimer partner retinoid X receptor has been detected in several cell types (Silvagno et al. 2010 and 2013). However, the function of mitochondrial VDR and its actual existence in skeletal myocytes have not yet been established.

In conclusion, although hormonal changes are likely involved in the pathogenesis of sarcopenia (Morley and Malmstrom 2013), several unresolved queries remain about the relationship between endocrine function and muscle mitochondrial physiology. Studies are also needed to identify possible differential effects of various hormones on SSM and IFM. Lastly, further research is warranted to determine if functional differences exist between systemic and local hormonal systems in advanced age, which could explain some of the controversial and disappointing results of hormonal supplementation in sarcopenic individuals.

4.3. Vascular aging

Vascular aging encompasses alterations in large conduit arteries as well as in smaller resistance arteries across multiple tissues. In skeletal muscle, aging is accompanied by reduced blood flow capacity (Lawrenson et al. 2003; Donato et al. 2006; DeVan et al. 2013) and impairment in endothelium-dependent vasodilation, due, in part, to altered ROS signaling and diminished NO^{*} bioavailability (Muller-Delp et al. 2002).

Although the precise mechanisms underlying the alterations of ROS signaling within the aged microcirculation are not fully understood, emerging evidence suggests a fundamental role for mitochondrial dysfunction (Ungvari et al. 2008). Defective autophagy in endothelial cells (LaRocca et al. 2012) as well as increased ROS production (van der Loo et al. 2000; Ungvari et al. 2007) and impaired mitochondrial biogenesis (Ungvari et al. 2008) have been detected in vascular endothelium and smooth muscle cells. Enhanced mitochondrial ROS generation contributes to the adoption of a senescent phenotype in endothelial cells and to the activation of the redox-sensitive transcription factor NF- κ B (Erusalimsky 2009). The latter leads to diminished endothelium-dependent vasodilation and upregulation of inflammatory gene expression in the aged vasculature (Ungvari et al. 2007; Pierce et al. 2009).

Given the role of mitochondrion-derived H₂O₂ from the myocyte as a potential feed-forward regulator of blood flow (Saitoh et al. 2006), an altered ROS signaling linked to age-related muscle mitochondrial dysfunction is more likely to affect the structural and dynamic behavior of the surrounding vasculature rather than the opposite. Regardless of the mechanisms responsible for vascular dysfunction in advanced age, the subsequent alteration in tissue perfusion may potentiate mitochondrial decay within skeletal muscle. In particular, age-dependent vascular alterations may disrupt the matching of substrate delivery (i.e., O₂) with requirements in skeletal muscle both at rest and during exercise (DeLorey et al. 2004; Behnke et al. 2005; Herspring et al. 2008), resulting in a significantly lower microvascular oxygenation (and, by extension, intracellular oxygenation to maintain a given O₂ uptake according to Fick's law).

In turn, the decreased skeletal muscle oxygenation occurring with age could lead to the buildup of reducing equivalents (i.e., NADH and FADH₂) within mitochondria ("reductive stress"), resulting in increased electron availability and enhanced generation of superoxide anions (Clanton 2007). Outside of skeletal muscle, it is possible that an altered vascular function in the adipose tissue (Davis et al. 2013; Padilla et al. 2013) may alter the synthesis and secretion of mitochondrial protective hormones such as IGF-1 (Kern et al. 1989), while enhancing the production of inflammatory cytokines (O'Rourke et al. 2011).

In conclusion, although there is no definite cause-effect evidence that the age-related vascular dysfunction drives muscle mitochondrial dysfunction or *vice versa*, it is likely that the reduced microvascular oxygenation (Behnke et al. 2005) and diminished lineal density of perfused capillaries (Russell et al. 2003) may contribute to impairing muscular mitochondrial function late in life. Furthermore, since H₂O₂ is involved in the regulation of

vascular tone and muscle perfusion (Suvorava et al. 2005; Saitoh et al. 2007; Sindler et al. 2009), it is plausible that this oxidant may act as a paracrine signal regulating the local matching between O₂ delivery and demand (Marvar et al. 2007; Golub and Pittman 2013). Hence, alterations in mitochondrial redox homeostasis may impair the local regulation of O₂ delivery and exacerbate mitochondrial dysfunction with age.

More integrative research is needed to determine whether dysfunction in one compartment compromises the other and to identify targets for interventions that can protect both systems.

5. Towards clinical trials on mitochondrial dysfunction and sarcopenia: opportunities and challenges

The need of developing interventions to prevent or treat sarcopenia has been repeatedly evoked in literature (Brass and Sietsema 2011; Chumlea et al. 2011; Matthews et al. 2011). The design of a clinical trial targeting mitochondrial dysfunction to treat sarcopenia requires to preventively address several important issues. First of all, the effects of a hypothetical intervention on muscle mitochondrial function need to be carefully monitored in order to avoid potentially serious adverse events (Fig. 1). Just like most biological processes, the previously described mitochondrial pathways are not on/off phenomena, but proceed along a continuum with an optimal window of functioning, flanked by (detrimental) defective and excessive levels of operation.

Antioxidant supplementation is a paradigmatic example of how a simplistic way of tackling a complex issue produces unpredictable outcomes. Abnormal ROS emission likely contributes to muscle aging; hence, as predicated by the “Occam’s razor” principle, antioxidant supplementation would be the obvious remedy. Yet, oxidant generation within a hormetic range is needed for intracellular signaling (Handy and Loscalzo 2012) and optimal force production (Reid et al. 1993). Thus, antioxidants may paradoxically be harmful unless their effects on redox balance are closely monitored (Cerullo et al. 2012).

Likewise, while an excessive apoptotic activation may promote muscle atrophy, the indiscriminate abolition of apoptosis could result in the persistence of dysfunctional nuclei or damaged contractile elements, which may further aggravate muscle tissue dyshomeostasis. The consequences of varying levels of activation of mitochondrial fission and fusion on muscle trophism have already been discussed. As for autophagy, its unchecked stimulation could lead to autophagic cell death, while its abolition would abrogate the housekeeping function of this pathway, resulting in the accumulation of dysfunctional mitochondrial and other cellular waste (Gottlieb and Carreira 2010; Sandri 2010). Finally, excessive mitochondrial biogenesis is also maladaptive unless it is compensated by adequate upregulation of mitophagy (Gottlieb and Carreira 2010).

Another important aspect to take into account is the pleiotropic function of nearly all mediators of the mitochondrial pathways described (Gottlieb and Carreira 2010; Sack 2011; Galluzzi et al. 2012; Subramani and Malhotra 2013). Targeting a single molecule might therefore impact multiple processes. Moreover, manipulation of one pathway could elicit compensatory effects, impeding a clear distinction between primary and secondary outcomes and cause-effect relationships. These considerations highlight the need for reliable biomarkers of mitochondrial function to monitor the effects of the intervention under study.

The determination of muscle mitochondrial function in clinical research settings poses a relevant challenge (Marzetti et al. 2012a). Invasive approaches, such as muscle biopsy, may render more difficult the participation of older adults in a clinical trial, especially when considering that muscle specimens should be collected at least at two time-points to

determine the effects of an intervention. In addition, sophisticated analyses of muscle samples are generally expensive, besides requiring specific laboratory equipment and solid personnel experience. Some mitochondrial measurements, such as O₂ consumption or oxidant emission, need to be performed on fresh tissue and are highly laborious. This entails that, in the case of a multicenter trial, each of the participating sites must have its own laboratory, possibly introducing significant sources of variability, in addition to increasing the cost of research. Some of these limitations may be overcome by employing noninvasive approaches, like ³¹P-NMR spectroscopy (Wray et al. 2009). However, NMR is not widely available, besides being time-consuming and expensive.

Recent studies have shown that surrogate markers of mitochondrial function can be retrieved in biofluids, including “ATP profile” of peripheral white blood cells (Myhill et al. 2009), plasma creatine (Shaham et al. 2010), serum levels of fibroblast growth factor 21 (Suomalainen et al. 2011), and serum concentrations of acylcarnitines and non-esterified fatty acids (Sampey et al. 2012). If these analytes qualified as biomarkers for mitochondrial dysfunction in the setting of sarcopenia, their determination could serve for screening and follow-up purposes (Marzetti 2012). In this scenario, sophisticated analyses on muscle specimens would represent a second-level assessment for the identification of the pathway(s) compromised.

The design of an informative randomized controlled trial (RCT) necessarily goes through the correct selection of the study sample. The optimal target population of a RCT might be defined as a group of individuals who, for their sociodemographic, biological, and clinical characteristics, may best benefit from the intervention under study, with the lowest risk of experiencing treatment-related adverse events. In the case of sarcopenia, several criteria have been indicated to identify such a target population (Cesari and Pahor 2008). However, it should be well kept in mind that controversies still exist about the clinical identification of sarcopenia in older persons (Cruz-Jentoft et al. 2010; Muscaritoli et al. 2010; Fielding et al. 2011; Morley et al. 2011).

An optimal target individual for interventions against mitochondrial dysfunction and sarcopenia should present the condition of interest at the baseline. This implies that, in addition to mitochondrial parameters, muscle mass and strength must be objectively measured through imaging and physical performance tests (Marzetti 2012). Apart from quantitative parameters, the individual should also be assessed for the qualitative aspect of skeletal muscle. In this regard, the presence of sarcopenia can be judged by measuring the functional capacity expressed by unit of muscle (Goodpaster et al. 2001; Barbat-Artigas et al. 2012). Such approach might sufficiently confine the identification of sarcopenia to the skeletal muscle by reducing more systemic influences.

Since the primary reason for treating sarcopenia is to prevent disability, disabled subjects must be excluded from the trial. Therefore, selection criteria should not only include individuals with some physical impairment (e.g., reduced strength generated per unit of muscle), but also exclude those with overt disability (e.g., incapacity to walk 400 meters) or muscle-wasting disorders independent of aging (e.g., cachexia). It will also be necessary to exclude subjects in whom muscle mitochondrial function may be impaired by comorbid conditions (e.g., mitochondrial myopathies, peripheral vascular disease, type II diabetes, COPD, congestive heart failure, etc.). Such an approach, however, would significantly limit the representativeness of the sample for future generalization of the study results. Alternatively, individuals with comorbidities could still be considered eligible for participation, as long as investigators are aware of potential clinical confounders and take adequate countermeasures for balancing the randomization (Studenski 2009; Pahor and Cesari 2011). Same reasoning applies to medications. In other words, when designing their

study, investigators should evaluate the ratio between “purity” of the future findings and the applicability of the results to a larger population (and real life).

Disquisitions about the optimal timing and duration of the intervention have been published elsewhere (Calvani et al. 2013b). It is however important to bear in mind that six months are typically indicated as the minimum timeframe to expect sizable changes in muscle mass (Cesari et al. 2012).

Taken together, these considerations highlight the number of safety and methodological issues researchers are called to address before embarking in the design of RCTs on mitochondrial dysfunction and sarcopenia. However, the possibility of relieving the individual and societal burden associated with muscle loss and disability in late life makes this challenging task worth pursuing.

6. Conclusion and future perspectives

Sarcopenia is one of the most burdensome geriatric syndromes. As such, the development of effective preventive and therapeutic measures is urgently needed. This task is hampered by the heterogeneity of clinical correlates of sarcopenia, reflecting the complexity of its pathogenesis. Derangements in mitochondrial function are currently invoked as major factors in the sarcopenia syndrome. Yet, exploiting mitochondrial pathways for therapeutic purposes is an ambitious task. First, most processes governing mitochondrial physiology exhibit a “yin-yang behavior”, but their optimal range of functioning is currently unclear. Moreover, no widely-accepted biomarkers of muscle mitochondrial dysfunction are presently available.

A deeper understanding of mitochondrial pathophysiology in sarcopenia also requires discerning the relative impact of intrinsic muscular aging from that of lifestyle habits and common age-associated conditions. At the patient level, it is possible that the phenotypic heterogeneity of sarcopenia could be the resultant of the diverse combination of factors acting at multiple levels, ranging from the extramuscular environment to specific mitochondrial processes in skeletal myocytes. Hence, future developments in the field will require the implementation of new analytical tools to achieve a global characterization of the sarcopenic patient. This multidimensional approach will assist in the design of personalized therapeutic programs and monitoring of the intervention effects.

The present review has not the pretension to being exhaustive. Our aim is to provide a *coup d’oeil* on the current evidence about the contribution of specific mitochondrial pathways to sarcopenia and emphasize the complexity of muscle aging. After all, understanding the inner foundations of sarcopenia means discovering why we age and how we can reduce the weathering and wearing effects of time.

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List of abbreviations

³¹P-NMR	³¹ P nuclear magnetic resonance
AIF	apoptosis-inducing factor
AngII	angiotensin II
Atg protein	autophagy-related protein
Bcl-2	B-cell leukemia-2
COPD	chronic obstructive pulmonary disease
COX	cytochrome <i>c</i> oxidase
CSA	cross-sectional area
Drp1	dynamin-related protein 1
EndoG	endonuclease G
eNOS	endothelial nitric oxide synthase
ETC	electron transport chain
Fis1	fission protein 1
FoxO3	Forkhead box O3
GH	growth hormone
IFM	interfibrillar mitochondria
IGF-1	insulin-like growth factor-1
iNOS	inducible nitric oxide synthase
LAMP-2	lysosomal-associated membrane protein 2
LC3	microtubule-associated protein 1 light chain 3
MFRTA	mitochondrial free radical theory of aging
Mfn	mitofusin
MQC	mitochondrial quality control
mtDNA	mitochondrial DNA
NF-κB	nuclear factor κB
nNOS	neuronal nitric oxide synthase
NOS	nitric oxide synthase
OMM	outer mitochondrial membrane
OXPHOS	oxidative phosphorylation
PGC-1α	peroxisome proliferator-activated receptor-γ coactivator-1α
RCT	randomized controlled trial
ROS	reactive oxygen species
SDH	succinate dehydrogenase
SSM	subsarcolemmal mitochondria
TA	<i>tibialis anterior</i>

TFAM	mitochondrial transcription factor A
TNF-α	tumor-necrosis factor α
UPS	ubiquitin-proteasome system
VDR	vitamin D receptor
VL	<i>vastus lateralis</i>

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Fig. 1.

Hypothetical progression of mitochondrial dysfunction during the development sarcopenia and possible windows for interventions. Multiple, interrelated factors can impact muscle mitochondria function over the life course, including intrinsic muscular aging, lifestyle habits, chronic inflammation, vascular dysfunction, hormonal changes, etc. Young individuals should be advised to adopt a healthy lifestyle, avoiding all of the known risk factors for chronic degenerative diseases. At middle age, subjects at risk for sarcopenia should be promptly identified, for instance through the computation of a “sarcopenia risk chart” (Calvani et al. 2013b). Eventual chronic degenerative diseases associated with accelerated development and/or progression of sarcopenia need to be managed appropriately. Periodical assessments of muscle mitochondrial function may allow detecting early signs of dysfunction. Once the presence of sarcopenia has been established, interventions must be implemented. A hypothetical treatment targeting mitochondria requires a close monitoring of mitochondrial response to the intervention. *Artwork by Francesco Antognarelli.*

Fig. 2.

Possible scenarios resulting from mitochondrial quality control failure during the progression of sarcopenia. An imbalance in mitochondrial dynamics towards fusion is associated with the appearance of giant mitochondria, characterized by highly interconnected networks, aberrant morphology, reduced bioenergetic efficiency, and increased ROS production. Enlarged mitochondria cannot be efficiently removed due to their larger size. The accumulation of lipofuscin within lysosomes further contributes to impairing the autophagosomal-lysosomal axis. Oxidants generated by dysfunctional mitochondria compromise the surrounding tissue and amplify mitochondrial damage, eventually triggering apoptosis and proteolysis via ROS-mediated activation of nuclear factor κ B (NF- κ B) and Forkhead box O (FoxO) (Dodd et al. 2010). These transcription factors stimulate the expression of the muscle-specific ubiquitin ligases atrogin-1 and muscle-specific RING finger 1 (MuRF-1). Protein fragments derived from the action of caspase-3 on actomyosin complexes are eventually degraded by the ubiquitin-proteasome system. A shift of dynamics towards fission leads to mitochondrial network disintegration and overactivation of mitophagy. ROS generation by fragmented mitochondria is increased, which together with the upregulation of fission, stimulates muscle protein breakdown and myonuclear apoptosis through mechanisms similar to those described above. *Artwork by Francesco Antognarelli.*