TOPICAL REVIEW

Beneficial effects of exercise on age-related mitochondrial dysfunction and oxidative stress in skeletal muscle

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Abstract

Mitochondria are negatively affected by ageing leading to their inability to adapt to higher levels of oxidative stress and this ultimately contributes to the systemic loss of muscle mass and function termed sarcopenia. Since mitochondria are central mediators of muscle health, they have become highly sought-after targets of physiological and pharmacological interventions. Exercise is the only known strategy to combat sarcopenia and this is largely mediated through improvements in mitochondrial plasticity. More recently a critical role for mitochondrial turnover in preserving muscle has been postulated. Specifically, cellular pathways responsible for the regulation of mitochondrial turnover including biogenesis, dynamics and autophagy may become dysregulated during ageing resulting in the reduced clearance and accumulation of damaged organelles within the cell. When mitochondrial quality is compromised and homeostasis is not re-established, myonuclear cell death is activated and muscle atrophy ensues. In contrast, acute and chronic exercise...
attenuates these deficits, restoring mitochondrial turnover and promoting a healthier mitochondrial pool that leads to the preservation of muscle. Additionally, the magnitude of these exercise-induced mitochondrial adaptations is currently debated with several studies reporting a lower adaptability of old muscle relative to young, but the processes responsible for this diminished training response are unclear. Based on these observations, understanding the molecular details of how advancing age and exercise influence mitochondria in older muscle will provide invaluable insight into the development of exercise protocols that will maximize beneficial adaptations in the elderly. This information will also be imperative for future research exploring pharmacological targets of mitochondrial plasticity.

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Abstract figure legend Mitochondria produce reactive oxygen species (ROS) during normal respiration, but when these free radicals accumulate, this leads to progressive damage to mitochondrial constituents including DNA, proteins and lipids. Mitochondrial DNA (mtDNA) mutations impair the synthesis of electron transport chain sub-units and reduce oxidative phosphorylation. These changes cause mitochondrial dysfunction that affects a number of pathways vital for maintaining mitochondrial turnover and integrity such as biogenesis, dynamics (fusion and fission), autophagy–lysosomal degradation, and programmed cell death (apoptosis). Additionally, mitochondrial dysfunction through a vicious cycle causes further increases in ROS and oxidative damage, ultimately leading to a decline in muscle mass and strength, reduced physical function, and ageing.

Abbreviations AMPK, 5′-AMP-activated protein kinase; DAMP, damage-associated molecular pattern; ERRα, oestrogen-related receptor α; ETC, electron transport chain; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; IL-6, interleukin 6; MAPK, mitogen-activated protein kinase; mtPTP, mitochondrial permeability transition pore; OXPHOS, oxidative phosphorylation; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; PRR, pattern recognition receptor; ROS, reactive oxygen species; Tfam, mitochondrial transcription factor A; TLR, Toll-like receptor; TNF-α, tumour necrosis factor α.

Introduction

Ageing leads to the steady accumulation of detrimental cellular and molecular changes within tissues that reduce the body’s ability to respond to stress (Kowald & Kirkwood, 1996). In particular, advancing age causes progressive but significant losses in skeletal muscle mass and strength termed sarcopenia, which is believed to be the precipitating factor contributing to a decline in physical function, increased risk of frailty, disability and death observed in the elderly (Visser & Schaap, 2011). Thus, healthcare professionals and researchers are expeditiously trying to unravel the molecular underpinnings of this debilitating condition, and while slow and steady progress has been made, an efficacious treatment strategy has yet to be developed. Currently, exercise is considered to have numerous health benefits that include maintaining muscle mass and function with age, but the underlying molecular details have yet to be fully elucidated (Konopka & Nair, 2013). The last few years have seen an upsurge of exciting novel data enlightening the highly complex and tightly regulated nature of mitochondrial plasticity involving biogenesis, dynamics/morphology, autophagy–lysosomal degradation and programmed cell death. In this review, we focus on recent findings pertaining to the mitochondrial adaptive responses stimulated by various types of exercise paradigms in human skeletal muscle, although preclinical studies are discussed to support and/or complement limited human data. Additionally, we will highlight novel areas related to oxidative stress and mitochondria, and contemplate their potential influence in the exercise-induced metabolic adaptations observed in older adults.

Mechanisms associated with mitochondrial dysfunction in age-related muscle loss

The overall aetiology of sarcopenia is multifactorial and includes alterations in variables such as nutritional status, physical activity levels and hormone concentrations (Fig. 1). At a cellular level, impaired mitochondrial function and metabolic capacity contributes to reduced muscle health observed in a number of species including humans (Peterson et al. 2012; Calvani et al. 2013). Mitochondria are unique cellular organelles that are dependent upon the coordinated expression of both the nuclear and mitochondrial genome. Although mitochondrial DNA (mtDNA) encodes only 13 of the 1000 or so polypeptides required for organelle biogenesis, these include subunits of the electron transport chain (ETC) that are essential for organelle biogenesis and...
homeostasis. The remainder of proteins required for biogenesis are derived from nuclear DNA and imported into mitochondrial subcompartments via the protein import machinery (Hood, 2001; Lagouge & Larsson, 2013). A variety of muscle wasting conditions have been shown to be the result of dysregulation at various levels in this intricate mitochondrial biogenesis pathway (Dimauro & Davidzon, 2005). The most prominent age-associated mitochondrial profile alterations include reduced overall volume density, ATP production and protein synthesis, and these decrements are strongly linked to impaired skeletal muscle function, aerobic capacity and walking speed (Rooyackers et al. 1996; Conley et al. 2000; Petersen et al. 2003; Short et al. 2005; Safdar et al. 2010; Coen et al. 2013). The underlying mechanisms associated with these age-induced mitochondrial deficiencies have yet to be fully elucidated, but the accumulation of free radical species and elevations in oxidative damage have gained much attention and are discussed in detail below.

Age-induced mitochondrial oxidative stress

Mitochondria are an important source of reactive oxygen species (ROS) in the cell and are produced via inappropriate electron leakage at ETC complexes I and III during normal respiration (Chance et al. 1979). The association of free radicals and ageing was introduced by Harman in 1956 and later extended to become the mitochondrial theory of ageing, which postulates that the steady accumulation of mitochondrial ROS with age leads to irreversible cell and tissue damage and ultimately impacts lifespan (Harman, 1972; Miquel et al. 1980). Furthermore, ROS-induced oxidative damage to macromolecules, particularly within mitochondria (i.e. mtDNA, ETC components) can lead to additional mitochondrial dysfunction and further elevations in ROS to create a ‘vicious cycle’ scenario that contributes to cell death and sarcopenia (Cadenas & Davies, 2000; Fulle et al. 2004) (Fig. 2). The mitochondrial genome is particularly vulnerable to age-induced damage due to the lack of protected histones and its proximity to the ETC (Yakes & Van Houten, 1997; Bohr et al. 2002). In fact, age-induced mtDNA rearrangements are evident in a number of mammalian tissues after the age of 30 and progressively increase with age, with post-mitotic tissues such as skeletal muscle being highly affected (Katayama et al. 1991; Cortopassi et al. 1992; Zhang et al. 1992). Oxidative DNA and protein damage assessed by measuring 8-hydroxy-2’-deoxyguanosine (8-OHdG) and protein carbonylation, respectively, are elevated in vastus lateralis muscle of older individuals when compared to young adults (Gianni et al. 2004; Short et al. 2005). In addition to dysfunctional mitochondria, evidence suggests reduced intracellular antioxidant defences as also contributing to elevations in ROS with age (Pansarasa et al. 1999; Ji, 2001; Safdar et al. 2010). Furthermore, limited data from mice suggest oxidative DNA damage repair mechanisms are lower in numerous tissues including skeletal muscle with advancing age (Szczesny et al. 2010). Adding to the complexity of sarcopenia is the finding that larger fast-twitch glycolytic fibres (Type II) with inherently lower overall mitochondrial content are

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Figure 1. Factors contributing to age-related muscle mass loss

Age-related skeletal muscle loss is attributed to a wide range of factors including inadequate nutrient intake of proteins and vitamins, a sedentary lifestyle, declines in anabolic hormone levels, loss of motor neuron number and/or activation, and increased levels of inflammatory cytokines (e.g. interleukin 6 (IL-6) and tumour necrosis factor α (TNF-α)). Of the contributory factors involved in sarcopenia, decline in metabolic capacity and mitochondrial function are among the most important molecular changes proposed to have significant consequences for skeletal muscle tissue, due to higher levels of oxidative stress and damage and a reduced oxidative capacity (highlighted in red). The majority of the aetiological factors depicted can be improved and/or prevented with chronic endurance exercise and/or resistance training.
more susceptible to fibre atrophy than small slow-twitch oxidative fibres (Type I) with high mitochondrial content (Lexell et al. 1988). The faster age-induced decline in Type II fibres is partially attributable to greater oxidative damage and apoptosis, as well as enhanced degradation via the ubiquitin–proteasome protein pathway (Cai et al. 2004; Phillips & Leeuwenburgh, 2005). Another intricacy of skeletal muscle is that there are two heterogeneous populations of mitochondria displaying differential responses to ageing. Subsarcolemmal (SS) mitochondria display greater reductions in mitochondrial membrane potential, higher proton leak, enhanced ROS production, increased lipid peroxidation compared to intermyofibrillar mitochondria with age putting forth the idea that subsarcolemmal mitochondria may be particularly relevant to sarcopenia (Lal et al. 2001; Chabi et al. 2008; Ljubicic et al. 2010; Crescenzo et al. 2014). Taken together, these observations emphasize the need for more mitochondrial-based sarcopenia research in humans focusing on different fibre types and mitochondrial subpopulations.

Evidence supporting a causal role for mitochondrial dysfunction in ageing is provided by the mtDNA mutator mouse that harbours a proofreading-deficient version (D257A) of mitochondrial DNA polymerase γ (PolG) and exhibits profound premature ageing. PolG mice accumulate somatic mtDNA mutations consistent with systemic mitochondrial dysfunction including reduced respiratory chain function, increased levels of oxidative damage and apoptosis, as well as compensatory changes in mitochondrial biogenesis and mitochondrial fission proteins (Trifunovic et al. 2004; Kujoth et al. 2005; Hiona et al. 2010; Joseph et al. 2013; Kolesar et al. 2014). These mitochondrial changes precede accelerated ageing characterized by alopecia, kyphosis, hearing loss, osteoporosis and sarcopenia (Trifunovic et al. 2004; Kujoth et al. 2005). Interestingly, 5 months of endurance training prevented premature ageing in most tissues and was associated with reduced mtDNA depletion/mutations, and apoptosis, as well as higher mitochondrial biogenesis and oxidative capacity suggesting chronic exercise can attenuate the ageing phenotype in this progeroid mouse model (Safdar et al. 2011). Furthermore, segmental fibre analysis performed originally in muscle from aged rodents (Wanagat et al. 2001) and muscle of older humans (Bua et al. 2006) elegantly demonstrated that ETC abnormalities colocalized with mtDNA deletion mutations and fibre atrophy making a direct link between mtDNA mutations and muscle loss. Interestingly, despite these reports, several studies have shown contrasting results, with some failing to detect changes in mitochondrial function and enzyme activities in aged human muscle (Barrientos et al. 1996; Rasmussen et al. 2003). These equivocal results may stem from differing experimental factors including mitochondrial purity, assays and techniques measuring mitochondrial function, data analyses, and ages and physical activity levels of participants.

Molecular mechanisms mediating exercise-induced mitochondrial plasticity in ageing muscle

Mitochondrial regulation and oxidative stress. Pioneering work by Holloszy (1967) demonstrated that exercise can improve mitochondrial function and content in muscle to meet the increasing energy demands of active cells. Since then, there is definitive evidence that endurance exercise improves muscle health by increasing oxidative phosphorylation (OXPHOS), oxidative enzyme activities, and mitochondrial content in both young and elderly individuals (Coggan et al. 1992; Short et al. 2003). Over the years, the intricate molecular details of coordinating the nuclear and mitochondrial genomes for correct stoichiometric assembly of nascent mitochondria have been extensively reported and are therefore only briefly discussed here (Hood, 2001).
Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is a central regulator of exercise-induced mitochondrial adaptations and its expression is activated by upstream kinases including mitogen-activated protein kinase (MAPK) and SIRT1 (NRF-1/2) and oestrogen-related receptor α (ERRα), PGC-1α trans-activates a number of genes involved in OXPHOS, fatty acid oxidation and antioxidant capacity (Puigserver & Spiegelman, 2003). Importantly, PGC-1α upregulates mitochondrial transcription factor A (Tfam), which is imported into mitochondria and responsible for driving the expression of mtDNA-encoded ETC subunit proteins (Hood, 2001). Several lines of evidence have linked PGC-1α with muscle maintenance. First, PGC-1α and its downstream targets are reduced in skeletal muscle from older individuals when compared to young adults (Conley et al. 2000; Short et al. 2005; Safdar et al. 2010; Joseph et al. 2012). Second, PGC-1α content is positively correlated with oxidative capacity and training status in healthy young individuals (Garnier et al. 2005) and similar correlations have been made with gait speed in older adults (Joseph et al. 2012). Lastly, mouse overexpression studies demonstrate that mildly increasing PGC-1α levels in skeletal muscle prevents age-related muscle atrophy by augmenting processes related to mitochondrial turnover and quality control (Wenz et al. 2009).

The general consensus is that deficits in mitochondrial function and biogenesis programmes compromise muscle integrity whereas exercise attenuates these declines and improves muscle function (Carter et al. 2015) (Fig. 3). For example, 16 weeks of chronic aerobic exercise training increased peak oxygen uptake in muscle from older individuals and this occurred coincident with higher citrate synthase activity and mRNA levels of ETC subunits, PGC-1α, and its downstream effector proteins NRF-1 and Tfam (Short et al. 2003). In addition to the beneficial adaptations evoked by endurance-type training on muscle mitochondria, data also support the use of resistance training in older adults (Ades et al. 1996; Jubrias et al. 2001). A recent study investigating the effects of 8 weeks of combined endurance and resistance training in young and older adults reported greater changes in body composition, skeletal muscle mass and strength, and mitochondrial OXPHOS and biogenesis markers when compared to each of the interventions alone (Irving et al. 2015). These data support the assertion that employing a combined resistance and endurance exercise paradigm may be superior to traditional endurance-based exercise prescriptions with respect to beneficial mitochondrial adaptations and functional outcomes but more research in humans is warranted to substantiate these findings.

The beneficial effects of PGC-1α on mitochondrial biogenesis induced by both diet and exercise are largely mediated by the NAD⁺-dependent deacetylase Sir2 (SIRT1), the product of one of the seven mammalian homologues of the yeast Sir2 gene (Haigis & Sinclair, 2010). Another member of the mammalian sirtuin family implicated in sarcopenia includes the mitochondrial-localized SIRT3 (Onyango et al. 2002). The abundance of SIRT3 is reduced in ageing muscle and induced with oxidative stress caused by endurance training in young and older adults (Lanza et al. 2008). While the physiological details of SIRT3 in muscle are still under debate, data from animals showed that it is a downstream target of PGC-1α and an important regulator of PGC-1α’s effects on mitochondrial metabolism and ROS production (Kong et al. 2010). Several pathways, however, have been identified that function independently of PGC-1α to modulate mitochondrial OXPHOS. One such pathway involves SIRT1 and hypoxia-inducible factor 1α (HIF-1α). In particular, these experiments found that skeletal muscle of aged mice display lower levels of nuclear NAD⁺, reduced SIRT1 activity, and higher levels of HIF-1α. The consequences of these changes were reduced transcriptional activation of the critical mitochondrial regulatory gene Tfam by c-myc and a downregulation in the expression of mitochondrial-encoded OXPHOS subunits. Increasing NAD⁺ levels pharmacologically in vivo with nicotinamide riboside or using calorie restriction (30–40%) reduces HIF-1α levels and improves mitochondrial and muscle health in elderly mice (Gomes et al. 2013). Additionally, boosting NAD⁺ levels with poly (ADP-ribose) polymerase (PARP) inhibitors has also been shown to protect against muscle dysfunction caused by aberrant mitochondria (Pirinen et al. 2014). Another potential signalling mechanism regulating mitochondrial metabolism during ageing may involve insulin-like growth factor 1 (IGF-1) and its ability to phosphorylate ATP citrate lyase (ACL). Higher activity of ACL, an enzyme that catalyses mitochondria-derived citrate into oxaloacetate and acetyl CoA, was found to upregulate ETC complexes leading to improvements in oxygen consumption. ACL activity is reduced in skeletal muscle from aged mice, suggesting that age-induced reductions in IGF-1 levels (Harris et al. 1997) may suppress mitochondrial ETC activity via ACL and that increasing levels of this enzyme may help to attenuate mitochondrial dysfunction and sarcopenia (Das et al. 2015). These studies underscore the complexity involved in mitochondrial regulation and demonstrate the need for more research to identify additional mitochondrial upstream effector molecules.

Despite the ability of older muscle to adapt to exercise there have been reports that this response may be blunted when compared to young muscle. A study conducted by Lanza and colleagues of young and older sedentary and chronically endurance trained (> 4 years) participants found training increased mitochondrial oxidative capacity to a similar extent in old and young muscle but that changes in mtDNA content and mitochondrial
transcription factors were still markedly reduced in older individuals (Lanza et al. 2008). A follow-up study revealed that chronic endurance exercise led to nearly twice as many genes being affected in young participants compared with their older trained counterparts. Interestingly, the genes differentially regulated were those involved in protein degradation and cellular stress pathways (Johnson et al. 2014b). Additionally, a separate investigation of proteomic changes using a mass spectrometry approach in young and older adults following 8 weeks of endurance exercise found training reduced muscle and mitochondrial damage and increased protein degradation in young but not older participants. This study further corroborates the gene microarray data showing that older muscle has an

![Figure 3. Molecular adaptations in ageing skeletal muscle mitochondria with exercise](image)

1) Biogenesis. Following an acute exercise stimulus, signalling through AMP and MAPK leads to the upregulation of PPAR γ co-activator 1α (PGC-1α) and together with transcription factors (TFs) activate nuclear genes encoding mitochondrial proteins such as the mitochondrial transcription factor A (Tfam). Tfam is then targeted and imported via the protein import machinery (PIMs) to its final destination on mitochondrial DNA (mtDNA) where it upregulates genes encoding electron transport chain (ETC) subunits, which results in higher oxygen consumption, ATP synthesis and mitochondrial content. (2) Dynamics. Exercise also evokes changes in mitochondrial morphology by increasing the abundance of fusion (Mfn1 and Opa1) and fission (Drp1 and Fis1) proteins. These changes increase mitochondrial turnover to facilitate the dilution and clearance of damaged mitochondria, and also help dissipate energy to all parts of the muscle cell. (3) Autophagy. Acute and chronic endurance training alters the levels of key autophagy markers such as LC3-II and the ubiquitin (Ub) binding protein p62, leading to greater autophagosome formation and degradation of damaged mitochondria via mitophagy. (4) Apoptosis. Exercise-induced improvements in mitochondrial function lead to reduced levels of pro-apoptotic release (cytochrome c, cyto c; apoptosis inducing factor, AIF; and endonuclease G, Endo G) and attenuated activation of caspase-dependent and -independent signalling cascades ultimately decreasing DNA fragmentation to maintain myofibre number and size with age. For simplicity, the signalling pathways are depicted as distinct processes, but the dashed lines indicate the interconnectivity of these four processes as discussed in the text.
events. Although there is no consensus regarding the in the absence of cytosolic signalling and/or cleavage et al. (AIF) (Susin et al. 2006; Frezza et al. 2010). The lack of responsiveness of old muscle to exercise may be due to a number of factors including (1) old muscle being inherently less adaptable than young muscle, and (2) training volume and duration of the exercise programme not being optimal to induce maximal muscle and mitochondrial adaptations in both young and old muscle. More exercise studies will be crucial to address these shortcomings as it is evident from the current literature that a 'one size fits all' approach to exercise training in older individuals will not be sufficient to elicit maximal adaptations in muscle.

**Mitochondria-mediated apoptosis.** Mitochondria are vital components of the intrinsic apoptotic pathway since they (1) contain a variety of pro-apoptotic proteins that upon release can lead to myonuclear death and (2) produce ROS that can activate apoptotic signalling mechanisms (Calvani et al. 2013). Induction of apoptotic signalling pathways in muscle can lead to reductions in overall fibre number and/or decreases in the nuclear-to-cytoplasm ratio by targeted myonuclei removal, and both are likely to contribute to decreased muscle size and/or function observed with sarcopenia (Dupont-Versteegden, 2005). Mitochondria can execute myonuclear apoptosis via two independent intracellular death-signalling pathways: caspase dependent and caspase independent. Release of pro-apoptotic factors from mitochondria occurs via the formation of the mitochondrial apoptosis-inducing channel (MAC) and/or the mitochondrial permeability transition pore (mtPTP) and both of these complexes are regulated by the ratio of pro- and anti-apoptotic Bcl-2 family members (Adhihetty et al. 2008; Calvani et al. 2013). The caspase-dependent pathway is mediated by the release of cytochrome c from the mtPTP or MAC and its association with other cytosolic factors forms the apoptosome complex, which leads to multiple proteolytic caspase cleavage events and eventually DNA fragmentation, a hallmark feature of apoptosis (Marzetti et al. 2010). Important to this process is optic atrophy protein 1 (Opa1), which is required for the remodelling of mitochondrial cristae junctions and the subsequent escape of proapoptotic factors into the cytosol (Cipolat et al. 2006; Frezza et al. 2006). The caspase-independent pathway involves the release of apoptosis inducing factor (AIF) (Susin et al. 1999) and endonuclease G (Endo G) (Li et al. 2001), which both directly evoke DNA fragmentation in the absence of cytosolic signalling and/or cleavage events. Although there is no consensus regarding the preferential importance of one pathway over the other, data from our group suggest that caspase-dependent and not caspase-independent apoptotic signalling is correlated with sarcopenic indices (i.e. muscle volume and gait speed) in vastus lateralis muscle samples obtained from community-dwelling older adults (Marzetti et al. 2012).

Increased apoptosis in skeletal muscle from aged humans typically occurs coincident with elevations in mitochondrial ROS production and/or ROS-induced molecular damage, and this is consistent with the mitochondrial theory of ageing (Capel et al. 2005; Hutter et al. 2007). In fact, a direct causative role for increased mitochondrial ROS production in mitochondrial and muscle dysfunction with age has been shown using a mitochondria-targeted anti-oxidant peptide (SS-31). Old mice treated with a single dose of SS-31 peptide displayed normal levels of mitochondrial energetics including mitochondrial ATP production and oxidative phosphorylation, and these changes were accompanied by improved fatigue resistance and endurance capacity. The efficacious effects of SS-31 were attributed to lower mitochondrial hydrogen peroxide emission and reduced glutathione redox status (Siegel et al. 2013). What is more, SS-31 has been reported to provide significant protection against muscle atrophy and mitochondrial dysfunction in other muscle wasting conditions including limb immobilization and mechanical ventilation (Powers et al. 2011; Talbert et al. 2013). Even though a significant amount of proof exists validating the involvement of apoptosis as a contributing pathway in age-related skeletal muscle wasting in animals (Dirks & Leeuwenburgh, 2002; Leeuwenburgh et al. 2005; Song et al. 2006; Ljubicic et al. 2009), human studies are still scarce. Whitman et al. (2005) showed that older muscle from healthy humans display marked increases in DNA fragmentation as indicated by a greater number of TUNEL-positive cells, although no significant changes were observed in any of the caspases measured or the cross-sectional area of muscle fibres. Additionally, an earlier study of rhabdosphincter muscle reported age-dependent increases in apoptotic nuclei that were associated with a significant loss of muscle cells in older individuals (Strasser et al. 1999). Furthermore, recent in situ analysis of permeabilized fibres from healthy physically active older men found greater sensitization for mtPTP opening and a greater fraction of EndoG-positive myonuclei compared with muscle from young adults, suggesting that muscle from ageing humans may have higher mitochondrially mediated apoptotic susceptibility (Gouspillou et al. 2014). Chronic exercise protects muscle from age-related declines by promoting an anti-apoptotic intracellular environment. Exercise leads to a multitude of changes in apoptotic signalling in animal muscle including reduced levels of pro-apoptotic Bax and higher levels of the anti-apoptotic Bcl-2 protein, and lower caspase

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cleavage and DNA fragmentation, and this has been shown to occur in both fast-twitch and slow-twitch muscle of young and older rats (Payne et al. 2003; Siu et al. 2005; Song et al. 2006; Ljubicic et al. 2009) (Fig. 3). A detailed investigation of the effect of moderate-intensity endurance cycling (2 h) in young healthy adults found no significant changes in any muscle apoptotic signalling molecules measured, suggesting perhaps that the exercise intensity was not high enough to induce detectable differences in apoptosis (Quadrilatero et al. 2010). In a separate study of young untrained individuals, high-intensity cycling exercise (75 min) resulted in alterations in a number of apoptotic-related genes including Bcl-2-like 10, c-myc and BH3 interacting domain death antagonist (Mahoney et al. 2005). Exercise can also evoke beneficial changes in mitochondria by promoting DNA repair. Higher 8-oxoguanine DNA glycosylase (OGG1) activity was observed in human muscle following exercise (Radak et al. 2003) whereas greater OGG1 acetylation was reported in muscle from older active compared with sedentary individuals (Radak et al. 2011). OGG1 is the main basic excision repair (BER) enzyme within mitochondria and mediates the excision of 8-OHdG, the most commonly formed oxidative lesion in the cell (Bohr et al. 2002). Upregulation of OGG1 activity during exercise may be linked to SIRT3, which has been shown to deacetylate OGG1 and prevent its degradation (Cheng et al. 2013). Taken together, exercise represents a physiological perturbation that prevents age-induced muscle loss, in part through enhancement of DNA damage repair pathways which in turn may attenuate mitochondrial dysfunction and apoptosis.

Mitochondrial turnover. In recent years, considerable interest has been dedicated to elucidating the cellular mechanisms regulating mitochondrial quality control in muscle, which primarily involve biogenesis, dynamics and lysosomal degradation. With regard to ageing muscle, organelle turnover has been shown to be significantly impaired leading to altered redox status, reduced mtDNA integrity and organelle function, as well as increased cell death (Calvani et al. 2013). Below we discuss some of the primary cellular pathways involved in maintaining mitochondrial turnover and homeostasis and address how they adapt to accommodate the higher oxidative demands imposed by exercise on active muscle.

Mitochondrial dynamics – fusion and fission. Ongoing mitochondrial fusion and fission events are important for organelle function, genetic complementation, and the proper distribution of newly synthesized mitochondria during cell division (Seo et al. 2010). Support for the dysregulation of mitochondrial dynamic remodelling has been provided by electron microscopy of animal and human muscle fibres showing enlarged and swollen mitochondria (Tandler & Hoppel, 1986; Beregi et al. 1988; Navratil et al. 2008), as well as mitochondria that appear punctate and fragmented (Ljubicic et al. 2009; Iqbal et al. 2013). The modulation of mitochondrial ultrastructure within ageing muscle is thought to be due to alterations in the levels of key fusion and fission proteins including dynamin-related GTPases, mitofusins 1 and 2 (Mfn1/2) and optic atrophy protein 1 (Opa1), and dynamin-related protein 1 (Drp1) and fission protein 1 (Fis1), respectively (Crane et al. 2010; Joseph et al. 2012; Ibebujo et al. 2013; Zhao et al. 2014). Mitochondrial dynamics are also an important contributor to muscle adaptations following exercise (Fig. 3), but the impact of both exercise and age on this cellular pathway are currently unknown. One of the first studies investigating the effect of physical activity on mitochondrial morphology gene expression reported higher Mfn1 and Mfn2 mRNA levels following an acute bout of exercise in muscle of healthy young trained cyclists (Cartoni et al. 2005). High intensity interval training has also been shown to increase the abundance of Mfn1 and Fis1 proteins in muscle from healthy young individuals (Perry et al. 2010), whereas ultra-endurance running exercise (24 h) resulted in higher Drp1 activity in male athletes (Jamart et al. 2012b). In one of the few papers examining exercise-induced mitochondrial morphology adaptations in aged muscle, Bori and colleagues (2012) found that Fis1 expression was higher in muscle from both young and older regularly active individuals when compared with their sedentary age-matched counterparts, but that the response was blunted in the older group. This study also observed differential responses in Mfn1 and Fis1 expression levels between young and aged physically active participants following an acute bout of endurance exercise. Additionally, 12 weeks of aerobic exercise training in older men significantly upregulated PGC-1α, Mfn1, Mfn2 and Fis1 proteins, and this was independent of age (Konopka et al. 2014). The upstream signalling factors mediating these changes are currently unclear, but several studies have provided strong evidence for PGC-1α in coordinating mitochondrial biogenesis and dynamics following an exercise stimulus. PGC-1α and ERRα drive the expression of Mfn2 in trained athletes (Cartoni et al. 2005), and PGC-1α transcript levels were positively correlated with Mfn2 and Drp1 expression levels in the vastus lateralis of healthy participants with a wide range of exercise capacities (Garnier et al. 2005). Whether these speculations hold true in skeletal muscle during disease states or with ageing has yet to be confirmed.

Studies using a mouse model of controlled Opa1 overexpression provide exciting new data supporting the potential therapeutic use of physiologically or pharmacologically altering the levels of these morphology genes. Specifically, mild overexpression of Opa1 protected tissues from a myriad of conditions that cause tissue
damage including muscle atrophy induced by denervation and genetic mitochondrial defects (Civiletto et al. 2015; Varanita et al. 2015). The anti-atrophic effect caused by Opa1 stabilization of cristae junctions is mediated by increased respiratory chain efficiency, reduced ROS and lower cytochrome c release (Frezza et al. 2006; Varanita et al. 2015). In contrast to humans however, exercise studies in animals have been more controversial with some reporting higher, lower, or no change in the levels of fusion proteins, and these divergent results appear to be largely dependent on the intensity and duration of the training protocol (Ding et al. 2010; Pagano et al. 2014). Nevertheless, collectively these findings suggest that both mitochondrial fusion and mitochondrial fission events may be upregulated during exercise and that the type of exercise programme employed may be an important factor in the response of mitochondrial dynamics in muscle (Fig. 3). More pronounced and interconnected mitochondrial networks may facilitate the mixing of intra-mitochondrial components (i.e. metabolites, proteins and mtDNA), while smaller mitochondria following division may help deliver ATP to other cellular compartments that are in need of energy (Calvani et al. 2013). Also, smaller mitochondria may support the clearance of damaged organelles via the autophagosome–lysosomal pathway as this has been shown to occur in tissues from animals (Twig et al. 2008). Together, these adaptations maintain efficient OXPHOS during exercise and prevent the accumulation of damaged organelles that could negatively impact mitochondrial turnover and muscle function, particularly in ageing muscle (Yan et al. 2012; Calvani et al. 2013).

**Autophagy.** Macroautophagy (hereafter referred to as autophagy) involves the degradation and renewal of cellular components through the fusion of double membrane structures known as autophagosomes with lysosomes. This proteolytic process maintains cellular homeostasis by preventing the accumulation of damaged proteins and organelles, and also provides an alternative source of nutrients during increased states of energy demand through the recycling of degraded products (Yorimitsu & Klionsky, 2005). The aforementioned events are particularly relevant for skeletal muscle given its high metabolic demand and limited regenerative capacity (Ravikumar et al. 2010). What is more, autophagy can be selective, targeting and degrading defective mitochondria, a process termed mitophagy (Sandri, 2010; Mizushima, 2011). Mitochondrial fusion and fission processes in conjunction with mitophagy work together to maintain and control mitochondrial quality. For instance, during conditions of increased oxidative stress, damaged mitochondria with a lower mitochondrial membrane potential will be selectively removed by fission through mitophagy whereas normal organelles will continue to fuse and divide. Aberrant mitochondria also display lower levels of Opa1, facilitating their exit and preventing their re-entry into the mitochondrial turnover cycle (Gomes & Scorrano, 2008; Twig et al. 2008).

It has recently been postulated that autophagy is compromised in ageing muscle and that this may lead to the progression of sarcopenia, and several studies support this hypothesis. First, electron microscopy experiments demonstrate the presence of enlarged mitochondria with abnormal cristae in senescent cells (Murakoshi et al. 1985; Tandler & Hoppel, 1986). Second, genetic inhibition of the key autophagy gene Atg7 in mice increases inflammation, decreases myofibre size and number (preferentially in fast-twitch fibres), impairs muscle function and reduces survival, while Atg7 overexpression rescues the sarcopenic phenotype in aged animals (Masiero et al. 2009). Third, key markers of autophagy are reduced in muscle from both aged rodents and humans when compared to young controls (Wohlgemuth et al. 2010, 2011; Joseph et al. 2013; Carnio et al. 2014). Lastly, autophagy inhibition negates the beneficial lifespan extension properties of exercise and calorie restriction (Morselli et al. 2010; Lira et al. 2013). We would be remiss not to mention that some studies have also reported higher levels of autophagy markers (e.g. LC3-II, Atg7 and Parkin) in ageing muscle (Wenz et al. 2009; O’Leary et al. 2013) and while the discrepancy in these animal findings is unclear, differences in the type of rodent strain and the age of the animals being studied may be a factor.

Exercise places a tremendous amount of metabolic stress on the cell leading to greater ROS production and damage to tissues and cells (Powers & Jackson, 2008). Therefore, autophagy promotes cell renewal by clearing aberrant proteins and organelles and recycling macromolecules as a potential source of nutrients and energy for the working muscle. The signal transduction pathways regulating autophagy during exercise have been extensively reviewed (Sanchez et al. 2014) and therefore only some events will be highlighted here. Mammalian target of rapamycin (mTOR) is a central mediator of autophagy responses in muscle and is regulated by AKT and AMPK. Increased AKT activity following an exercise stimulus phosphorylates mTOR and blocks autophagy induction by inhibiting the serine/threonine-protein kinase ULK1/2, while activation of AMPK suppresses mTOR, removing its inhibitory effect on ULK1/2 (Sanchez et al. 2014). Forkhead box 03 (FOXO3) transcription factor is another crucial regulator of autophagy in vivo. Phosphorylation of FOXO3 by AMPK mediates the upregulation of several autophagy genes including those for LC3, Bcl2/adenovirus E1B (BNIP3) and Atg12 (Sanchez et al. 2012), as well as the muscle-specific E3 ubiquitin ligases, muscle RING finger 1 (MuRF1) and muscle atrophy F-box (MAFbx)/atrogin-1 (Zhao et al. 2008).
Currently the majority of research elucidating the molecular details of exercise-induced changes in autophagy pathways has been conducted in animal models. Very early evidence in young mice following a single bout of strenuous exercise (9 h running) demonstrated the presence of higher mitochondria-containing autophagic vacuoles in regenerating muscle fibres suggesting that autophagy may be a vital component of muscle damage repair (Salminen & Vihko, 1984). Data characterizing the impact of more common exercise protocols have yielded mixed results. For example, acute endurance exercise in young mice results in rapid changes in upstream signalling molecules such as AMPK, AKT and mTOR, as well as in early and late autophagy markers including ULK1, LC3-II, and p62/SQSTMQ (a ubiquitin and LC3 binding protein) (He et al. 2012; Pagano et al. 2014), whereas mice subjected to shorter moderate to high intensity treadmill exercise showed a general decline or no change in key autophagy markers including LC3-II, Beclin-1, Atg7 and LAMP2a (Kim et al. 2012; Saleem et al. 2014). Interestingly, transgenic mice deficient in stimulus-induced autophagy displayed attenuated autophagy responses and also lacked the beneficial metabolic effects observed with exercise (He et al. 2012). With regard to chronic endurance training, long-term voluntary wheel running in young mice (4 weeks) increased basal autophagy flux (higher LC3-II, Beclin1 and BNIP3, and reduced p62/SQSTM1 immediately following only high-intensity exercise and this was accompanied by greater AMPK and ULK-1 activity, and reduced p62/SQSTM1 content 1 h post-exercise. Moreover, life-long exercise (regular exercise in past 30 years) was found to attenuate the deficiencies in LC3-II and Atg7 proteins in elderly sportsmen when compared with age-matched healthy sedentary and active young adults (Carnio et al. 2014) and these changes were associated with improved muscle ultrastructure, as well as greater muscle mass and strength (Zampieri et al. 2015).

Collectively, these findings point to the central role of mitochondrial quality control in maintaining cellular homeostasis in muscle and also strongly suggest that exercise intensity is an important determinant of the autophagic response of muscle to exercise. However, interpreting the outcome of protein changes in mitochondrial dynamics and autophagy in response to ageing and exercise has been a challenge. Despite advancements in live cell imaging techniques assessing these processes in vitro, we are still limited in the tools available to monitor these events in vivo, particularly in human tissue. Therefore, we need to continue developing strategic instruments and experimental assays that will allow the accurate visualization of mitochondria in living tissue in real time, which together with biochemical data will provide a better understanding of mitochondrial turnover.

Mitochondria as mediators of immune responses and implications for sarcopenia. Chronic inflammation termed ‘inflamm-aging’ has long been proposed to be an important contributor to sarcopenia (Chung et al. 2006). Indeed, levels of circulating inflammatory cytokines including tumour necrosis factor α (TNF-α) and interleukin 6 (IL-6) are increased in older individuals compared with young and these changes are highly correlated with decreased muscle mass and strength, and increased frailty (Conley et al. 2000; Ferrucci et al. 2002; Visser et al. 2002; Pedersen & Bruunsgaard, 2003; Reuben et al. 2003; Beyer et al. 2012). Similar elevations in the expression of pro-inflammatory cytokines have also been documented within skeletal muscle of older individuals (Przybyla et al. 2006; Leger et al. 2008; Thalacker-Mercer et al. 2010),
but to date the influence of mitochondria on muscle inflam-aging has remained relatively unexplored.

Mitochondria have recently been coined ‘master regulators of danger signalling’ due to their ability to initiate and promote innate and adaptive immune responses to protect the cell against harmful stimuli (Galluzzi et al. 2012). Mitochondria can themselves serve as a major source of endogenous danger signals, termed damage-associated molecular patterns (DAMPs, or ‘alarmins’), which are released during times of cellular stress and damage to promote a local and/or systemic immune response. Mitochondrial DAMPs such as mtDNA, N-formyl peptides and ROS bind to a number of pattern recognition receptors (PRRs) including Toll-like receptors (TLRs), formyl peptide receptors (FPRs), and the LRR and pyrin domain-containing 3 (NLRP3) inflammasome, which upon ligation lead to activation of immune responses (Kryska et al. 2011). Numerous mammalian TLRs have been identified (TLR 1–13), and these display ligand and tissue specificity (Nishimura & Naito, 2005). TLR9, for example, is located almost exclusively intracellularly in endosomes and lysosomes and has been given much attention due to its ability to recognize and be stimulated by non-methylated CpG motifs contained in mtDNA (Hemmi et al. 2003). Therefore, given their prokaryotic ancestry and the similarities between mitochondrial and bacterial constituents (Sagan, 1967), mtDNA can be inflammatogenic, stimulating TLR9-mediated immune responses that can have negative consequences on cells and tissues. Below we primarily focus on danger signals related to the release of mtDNA and discuss what is known about their involvement in the inflammation response observed with ageing and exercise in muscle.

As mentioned above, while mitochondria exert a protective function in host defence, in some cases they can also negatively impact cellular homeostasis and immunity. Of note, recent data have shown that release of mitochondrial DAMPs into the systemic circulation following significant injury and cell death is linked to a number of chronic conditions including systemic inflammatory response syndrome (SIRS), rheumatoid arthritis, HIV and cancer (Collins et al. 2004; Kohler et al. 2009; Zhang et al. 2010; Cossarizza et al. 2011). A seminal paper by Zhang et al. (2010) eloquently demonstrated the immunostimulatory and pathogenic effects of mitochondrial DAMPs in vitro and in vivo. More specifically, analysis of mtDNA levels in plasma from trauma patients was found to be several thousand-fold higher than that of controls while intravenous injection of mitochondrial DAMPs in rats increased neutrophil infiltration, inflammation and oxidative damage in lung tissue. In one of the only papers examining mitochondrial DAMPs in the context of ageing, Pinti et al. (2014) reported higher mtDNA plasma levels in humans after the fifth decade of life, and these changes were correlated with higher levels of circulating pro-inflammatory cytokines suggesting the involvement of circulating mtDNA in the chronic low-grade inflammation observed in the elderly. However, this study did not elucidate the source(s) of the mitochondrial DAMP release, the downstream signalling pathways promoting the expression and production of pro-inflammatory molecules, or the influence of these circulating factors on peripheral tissues.

Recent data have revealed the deleterious consequences of mitochondria-derived DAMPs in heart and skeletal muscle. First, in vitro studies in cardiomyocytes treated with bacterial DNA (CpG ODNs) for 30 min resulted in inflammation and reduced contractility, and this response was abolished in TLR9-deficient mice (Knieuermann et al. 2008). Second, inhibition of TLR7/9 markedly reduced skeletal muscle inflammation and improved muscle force in a mouse model of Duchenne muscular dystrophy (Henriques-Pons et al. 2014). Lastly, in a series of experiments conducted by Otsu’s group investigating the mechanism of DAMP release, impaired mitophagy was directly linked to the accumulation of cytosolic mtDNA, the activation and induction of TLR9-stimulated inflammatory cytokines, and increased mortality in a mouse model of cardiac failure. Administration of a TLR9 antagonist attenuated inflammation and cardiac dysfunction in these mice (Oka et al. 2012). This was the first study demonstrating that inefficient clearance of mitochondria by autophagy mediates the release of mitochondrial DAMPs and the induction of TLR9-mediated inflammatory responses that cause muscle damage within the same cell. Moreover, there is evidence suggesting that the response of muscle to mitochondrial DAMPs may be linked to muscle fibre type. For example, addition of slow-twitch muscle homogenates to isolated rat hearts leads to reduced cardiac function whereas no changes were detected with fast-twitch muscle samples (Di Battista & Locke, 2013). The fibre type-dependent effect was postulated to be due to the inherently higher levels of mitochondria and mitochondria-associated DAMPs (e.g. heat shock proteins) present within slow-twitch muscle (Staron et al. 1984; Locke et al. 1991) that can be released into the extracellular space to activate TLR-mediated inflammation signalling pathways and negatively impact tissue function (Vabulas et al. 2002; Kim et al. 2009; Mathur et al. 2011).

It is well known that exercise exerts anti-inflammatory effects leading to the local and systemic adaptations that are observed in older individuals with training, and one of the proposed mechanisms mediating this protection is through attenuated TLR signalling (Gleeson et al. 2011). Support for this hypothesis comes from evidence that expression of TLR4, the primary receptor for lipopolysaccharide, and IL-6 and TNF-1α mRNA levels
are significantly reduced in skeletal muscle from obese elderly physically frail individuals following 12 weeks of chronic exercise (Lambert et al. 2008). Similar findings of attenuated TLR4 signalling have also been noted in whole blood cultures obtained from active (young and old) compared with inactive individuals following LPS stimulation (McFarlin et al. 2006). Higher levels of TLR4 signalling in muscle are associated with reduced muscle volume and strength in older adults; however, these markers were not improved following 16 weeks of aerobic exercise training (Ghosh et al. 2015). While to our knowledge the effect of exercise on mtDNA–TLR9 signalling has not been explored in skeletal muscle, a very recent study of professional male athletes (volleyball players) reported lower levels of circulating mtDNA when compared to non-athlete volunteers (Nasi et al. 2016). These preliminary findings indicate that increased TLR signalling may be correlated with physical inactivity and a sedentary lifestyle and supports the potentially important influence of mitochondria-driven inflammation pathways in the beneficial adaptations observed with training (Gleeson et al. 2011). Thus, in light of this newly discovered function of mitochondria as rheostats for danger signalling and proof that mitochondrial DAMPs can initiate and drive pathogenic states, it is tempting to speculate that similar events may underscore the chronic inflammation implicated in sarcopenia. For instance, it is plausible that mtDNA that escape autophagic degradation can leak into the intracellular and extracellular space and activate PRR immune response pathways. The production of pro-inflammatory molecules can, in turn, impair protein synthesis and degradation and promote a state of chronic inflammation that eventually leads to myocyte death and sarcopenia. Although still in its infancy, this new line of mitochondrial research may provide the missing link between mitochondrial dysfunction, chronic inflammation and muscle loss during ageing.

Conclusions and perspectives

The plasticity of mitochondria constitutes a fundamental cellular mechanism in understanding the pathogenesis of sarcopenia and the development of strategies to treat this debilitating condition. While a plethora of research and resources have been allocated to developing pharmacological agents to reduce the progression of muscle wasting, exercise strategies combined with nutritional support currently remain the most effective intervention (Brook et al. 2016). Although muscle is highly adaptable to exercise training, the anabolic responsiveness to this stimulus appears to be highly variable and sub-optimal in older adults. It is becoming increasing clear that more studies are required to (1) investigate the combination of exercise training regimens (e.g. aerobic and resistance), (2) distinguish the effects of different muscle-loading paradigms (e.g. volume, workload, intensity, duration), and (3) better characterize older subjects (e.g. lifestyle factors, co-morbidities). Over the past few years our understanding of the mechanisms regulating mitochondria have also tremendously expanded with findings of a highly regulated and complex induction of mitochondrial pathways that coordinate the upregulation of mitochondrial energy production and the clearance of dysfunctional mitochondria during times of altered homeostasis. Additionally, new and exciting data on the involvement of mitochondria as potential mediators of inflammatory signalling pathways in muscle shed light on previously unexplored areas of mitochondrial biology (Oka et al. 2012; Henriques-Pons et al. 2014) and may be another clue to understanding the impact of ageing on exercise-induced mitochondrial adaptations. However, more work in this area is required to confirm these hypotheses. In conclusion, investigating mitochondrial alterations evoked by exercise in aged muscle will be instrumental for cultivating novel and innovative pharmacological and/or physiological interventions that will delay sarcopenia and prolong healthy life in older adults.

References


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Effect of exercise-induced adaptations in muscle mitochondria with ageing

Additional information

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There is no conflict of interest.

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