Exercise Is Muscle Mitochondrial Medicine

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OLIVEIRA, A.N., B.J. RICHARDS, M. SLAVIN, and D.A. HOOD. Exercise is muscle mitochondrial medicine. *Exerc. Sport Sci. Rev.*, Vol. 49, No. 2, pp. 67–76, 2021. *Exercise stimulates the biogenesis of mitochondria in muscle. Some literature supports the use of pharmaceuticals to enhance mitochondria as a substitute for exercise. We provide evidence that exercise rejuvenates mitochondrial function, thereby augmenting muscle health with age, in disease, and in the absence of cellular regulators. This illustrates the power of exercise to act as mitochondrial medicine in muscle.* **Key Words:** exercise training, mitochondrial function, mitochondrial biogenesis, mitophagy, aging, muscle disuse, lysosomal disease

Key points

- Exercise is a potent stimulus for mitochondrial remodeling, which signals an increased drive for mitochondrial biogenesis and the activation of its key regulator PGC-1α.
- Growing evidence illustrates the multifaceted ways through which exercise can promote an optimization of the quality of the mitochondrial pool. A key pathway involved is mitophagy, the process through which dysfunctional mitochondria are removed from the reticulum.
- Aging and pathological conditions such as disuse and disease have overt mitochondrial phenotypes that contribute to poor muscle oxidative status and metabolic inflexibility.
- Exercise is uniquely capable of improving mitochondrial content and function through a variety of signaling pathways, providing an abundance of therapeutic potential and making it an ideal mitochondrial medicine that likely cannot be replicated pharmaceutically.
- This review describes how mitochondria are involved in disuse-induced muscle atrophy, the aging phenotype, lysosomal and mitochondrial DNA diseases, and how exercise alone or as an adjunct therapy can ameliorate the pathophysiology by repairing and restoring mitochondria.

INTRODUCTION TO MUSCLE CONDITIONS WITH MITOCHONDRIAL DYSFUNCTION

The growth of interest in mitochondrial research over the last decade is related to the knowledge that mitochondrial content and function exhibit plasticity in response to changes in metabolic energy demand. Exercise is a stimulus that is well known to induce increases in mitochondrial content within skeletal muscle. However, decrements in mitochondrial content and function can also take place, forcing muscle to derive a greater fraction of its energy from glycolysis during the stress of energy demands. Such decreases in mitochondria are readily apparent in muscle fibers subject to chronic disuse, providing evidence that muscle activity is critical to maintenance of a normal, healthy mitochondrial pool. Independent of inactivity, evidence exists for decrements in mitochondria with advancing age, as well as in a variety of conditions such as mitochondrial DNA (mtDNA) diseases, lysosomal defects, and cancer cachexia and in the absence of critically important regulatory proteins, such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), p53, and SirT1. Given the ability of exercise to augment the mitochondrial pool in healthy muscle, this review will highlight the role of exercise in rejuvenating muscle organelle content and function under conditions of deteriorating muscle health, thereby acting as mitochondrial "medicine."

ADAPTIVE PLASTICITY OF MITOCHONDRIA IN MUSCLE

Mitochondria are highly dynamic, interconnected organelles that continuously undergo events of renewal, removal, and reorganization. At any given time, mitochondrial content is governed by two opposing processes: mitochondrial biogenesis, the synthesis of new mitochondria, and mitophagy, the selective degradation of dysfunctional mitochondria. Together, these processes are referred to as mitochondrial turnover, and in conjunction, they regulate mitochondrial quantity and quality. Signals for mitochondrial biogenesis, including increases in adenine nucleotide turnover (1), changes in cytosolic Ca^{2+} concentration (2), and elevations in reactive oxygen species (ROS) emission (3), all converge on the protein PGC-1α to promote its transcription, synthesis, and activation. Once in the nucleus, PGC-1α itself serves as a transcriptional coactivator by binding transcription factors such as nuclear respiratory factor-1 and NRF-2, peroxisome proliferator-activated receptor,

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and estogen-related receptor isoforms to up-regulate a wide host of nuclear genes encoding mitochondrial proteins, otherwise known as NuGEMPs (4). As NuGEMPs are transcribed and the mRNAs are translated in the cytosol, they are then shuttled to the mitochondrion and imported through the tightly regulated protein import machinery (5). One of these NuGEMPs encodes Tfam, which, upon entry into mitochondria, helps stabilize, transcribe, and replicate mtDNA. mtDNA encodes a small number of proteins, precisely 13, which are essential for the function and integrity of the organelle (6). This is especially evident in mtDNA-derived diseases, resulting from point mutations or deletions in the mtDNA sequence.

In skeletal muscle, mitochondria are often described as subsarcolemmal located beneath the sarcolemmal membrane, whereas intermyofibrillar (IMF) is dispersed between the myofibrils (7–9). Despite these geographical distinctions, and despite possessing somewhat subtle biochemical differences, these mitochondria exhibit a degree of connectivity. Mitochondria exist in an interconnected network in skeletal muscle, known as the mitochondrial reticulum (10,11). This stands to reason as mitochondria are highly motile organelles, and they continuously undergo events of fusion and fission to fine-tune the organization of the reticulum within the cell (12,13). Fusion refers to the assimilation of relatively new mitochondrial fragments to the network and is carried out by GTPases, Mfn1/2, which tether and fuse the outer mitochondrial (OM) membranes, and Opa1, which is largely responsible for fusion of the inner membrane (IM) (13). Fusion helps expand and elongate the mitochondrial reticulum, and more complex and extensive morphologies are characteristic of healthy, exercise-trained individuals (14). This is associated with greater metabolic flexibility, lipid metabolism, and the broad distribution of mtDNA and metabolites within muscle cells. Conversely, fission refers to the removal of sections from the reticulum and is governed by the dynamin-related GTPase Drp1 and its receptor Fis1 (13). Fission events are critical for the maintenance of a healthy network, as this process can selectively excise portions of the reticulum that have become dysfunctional, serving to limit the spread of toxic by-products such as ROS or mtDNA mutations. However, an overreliance on fission can lead to small fragmented mitochondria that have been shown to generate more ROS and less adenosine triphosphate (ATP). Thus, mitochondrial morphology is inherently tied to mitochondrial function and quality.

As the organization of the mitochondrial network is important, so too is its internal morphology. The IM displays varying degrees of internal folding, referred to as the cristae, providing increased surface area to house the electron transport chain (ETC). Cristae density is often used as an indication of mitochondrial quality, as highly dense organelles are common in healthy individuals, whereas those devoid of folding are characteristic of dysfunction. Another unique feature of the IM is the presence of cardiolipin, a phospholipid found only in mitochondrial membranes and which serves to promote cristae folding due to its irregular shape. It also seems to serve as the "glue" of the ETC, serving to interact with several membrane complexes to maintain their functional integrity (15,16).

The permeability of the IM is tightly regulated. However, a pronounced increase in mitochondrial permeability can be produced upon the opening of the mitochondrial permeability transition pore (mtPTP), a channel comprising a heavily debated number of IM proteins, possibly including cyclophilin, and subunits of the ATP synthase (Complex V). Opening of the mtPTP can be provoked by excessive ROS production accompanied by organelle calcium influx, and the result is a loss of membrane potential, reduced ATP synthesis, mitochondrial swelling, and the release of a number of proapoptotic proteins into the cytosol, including cytochrome *c* and apoptosis-inducing factor (17). Release of these components initiates caspase-dependent and caspase-independent apoptotic pathways, leading to nuclear DNA fragmentation. In a mononucleated cell, the result would be cell death. Larger, multinucleated cells such as skeletal muscle do not die, but rather suffer myonuclear decay and localized atrophy (18,19).

Mitochondrial dysfunction does not always culminate in nuclear decay, because mitophagy serves as a cytoprotective mechanism through which dysfunctional organelles can be removed before the onset of irreversible damage (20,21). Mitophagy is a mitochondria-specific form of autophagy that starts with the tagging of dysfunctional organelles to be engulfed in a membranous structure known as the autophagosome. Autophagosomes are then transported to lysosomes, which degrade the cargo into its basic lipids and amino acids to support future macromolecular synthesis. Several mechanisms have been described that initiate this process; however, the PINK1/Parkin pathway is the most well studied and is initiated by elevations in ROS emission and losses in membrane potential, which are common characteristics of mitochondrial dysfunction. These signals perturb the import capacity of the organelle, causing PINK1, a kinase that is regularly imported into the mitochondrial matrix and degraded, to accumulate on the OM and recruit Parkin (22). As an E3 ubiquitin ligase, Parkin ubiquitinates various OM proteins to flag the organelle for degradation. These ubiquitin chains then signal autophagosome formation to surround the dysfunctional material. Once mitochondria are fully engulfed within the autophagosome, this structure is transported to the lysosome, where it fuses with the aid of lysosome associated membrane proteins (LAMPs) to sequester and degrade the dysfunctional cargo (23). Therefore, the function of the lysosome is essential for the maintenance of mitochondrial quality within muscle cells.

AGING MUSCLE

It is estimated that after the age of 30, aerobic capacities decline ~10% each decade (24). In contrast, strength begins to decline ~1% annually after the age of 40 and accelerates in later years (25). The age-related declines in muscle strength, which coincide with losses in muscle mass, are known as sarcopenia. Sarcopenia is inherently tied to frailty in older adults and results in increased falls and diminished quality of life. This is concerning, as projections suggest that 22% of the world population will be over the age of 60 by 2050 (26). Like the rate of sarcopenia, sedentary behavior also is on the rise, and with advancing age, this may exacerbate the sarcopenic phenotype in the elderly population. Thus, to relieve the aging-associated burden on health care systems and to improve the quality of life of older individuals, the promotion of lifelong exercise behavior is imperative.

Mitochondria have long been central in the discussions of sarcopenia, in part because they are major contributors to oxidative stress with age (27), leading to ETC dysfunction (28), reduced membrane potential (29), and the activation of apoptotic

pathways (29). Electron microscopy images have identified both small, fragmented mitochondria (30,31) and giant mitochondria (32) in aging muscle. These morphological alterations may be a result of imbalanced mitochondrial dynamics, with rates of fusion and fission being out of balance (30,33). Interestingly, controversy exists surrounding mitochondrial content and advancing age. Some studies indicate that mitochondrial content declines with advancing age, whereas others suggest that it increases, although the latter includes a larger proportion of dysfunctional mitochondria (surveyed in 34). Despite the inconsistencies in overall content, several studies have demonstrated that with advancing age, mitochondrial dysfunction becomes more apparent (35). These age-related mitochondrial impairments can include reduced respiration (28), protein synthesis (36), and maximal rates of ATP production (37,38). Interestingly, the alterations in mitochondrial content and function occur independently of changes in mitochondrial protein import (39).

An elevation in the proportion of dysfunctional mitochondria within muscle with age could be a consequence of reduced biogenesis or impaired removal via mitophagy. Although aged muscle mitochondria have no obvious protein import deficiencies, the stability of precursor proteins in the cytosol is reduced in aged muscle, providing less substrate for the import pathway (39). In addition, the transcription of the regulator PGC-1α is decreased, and PGC-1α mRNA decay rates are accelerated in aged muscle (40). These changes can contribute to a reduction in organelle biogenesis. From a mitophagy perspective, our laboratory has shown that mitophagy flux increases with advancing age, at least up to the point of autophagosome engulfment (20). These increases in flux are accompanied by evidence of the accumulation of indigestible material, also known as lipofuscin, indicative of lysosomal defects (20,41). Thus, lysosomal dysfunction may limit the capacity to ultimately remove damaged organelles. Exercise has been shown to reverse many of these defects and to promote autophagy in general (42). For example, chronic contractile activity (CCA) reverses the accelerated ROS production evident in mitochondria from aged muscle and increases the transcription of PGC-1α toward levels found in muscle from younger individuals (31,40,42). In addition, exercise has been shown to increase lysosomal content irrespective of age, and this may help circumvent the declines in lysosomal function that are apparent with age (20). Interestingly, mitophagy flux, although elevated with age, was reduced after training. This is thought to reflect the improvement in mitochondrial quality elicited by the training paradigm, resulting in an attenuation of mitophagy signaling (20,43).

These mechanistic studies fortify the concept that regular exercise over the long term can act as a protective mechanism against the risk of disease and is associated with greater maximal oxygen uptake, longer time to exhaustion, and higher mitochondrial enzyme activities compared with sedentary counterparts (44,45). Lifelong exercise has also been shown to act as a protective mechanism against oxidative damage (46). Several studies of older adults aged 60–75 yr have demonstrated acute, exercise-induced increases in PGC-1α mRNA, Tfam, and phosphorylated AMPK (p-AMPK) content (44), as well as increases in mitochondrial markers at both the mRNA and protein levels after typical endurance training paradigms (47). This latter study also confirmed a reduction in oxidative damage and inflammatory markers with training (47). Furthermore, an exercise intervention in older obese women has also been shown to promote gene expression of autophagy-related genes (48), although definitive measurements of flux are challenging in humans. Collectively, these studies of animal models and humans suggest that introducing exercise at any age can be advantageous and that aged muscle retains the ability to adapt to exercise and restore possible defects in mitochondrial content/function (Fig. 1), although the degree of this adaptation seems to be attenuated at an older age (31,44).

MUSCLE DISUSE

In the absence of contractile activity, mitochondria in muscle exhibit functional and morphological impairments. They also become fragmented as a result of fission-fusion regulatory imbalances (30). Declines in mitochondrial volume have been shown after inactivity as evidenced by reductions in expression of mitochondrial proteins (19,49–51), whereas impairments in oxidative phosphorylation and increased ROS production demonstrate how disuse also significantly affects the organelle's bioenergetic function (49,52,53). Importantly, mitochondrial biogenesis signaling also is disrupted early on in disuse, characterized by reductions in PGC-1α gene expression (49,54,55). In rodent models of disuse, the increase in ROS and a loss of mitochondrial function create an energy deficit, thus, enhancing AMPK-mediated catabolic signaling and proteolysis (19,49,52,56). This promotes the rapid up-regulation of a host of atrophy genes mediated by the Foxo3 family of transcription factors, including Atrogin-1 and MuRF1 at both the mRNA (55) and protein levels (49,56). The ensuing energy deficiency and enhanced mitochondrial dysfunction are partly responsible for the activation of proteolytic pathways, greater apoptotic susceptibility, and the possible loss of myonuclei (19). Phenotypically, this translates into muscle atrophy and a reduction in contractile function (55,57).

Exercise Preconditioning Serves as Mitochondrial Medicine for Disused Muscle

"Exercise preconditioning" is used to describe exercise-induced adaptations obtained before a period of inactivity that serve to protect skeletal muscle against atrophy and dysfunction during disuse (58–60). This protection is achieved through 1) increasing cytosolic and mitochondrial antioxidants and chaperones (60), 2) increasing mitochondrial gene expression and protein levels (61), and 3) reducing signaling toward apoptotic and atrophy pathways (Fig. 1) (62).

Exercise training in mice performed before hindlimb suspension or mechanical ventilation (MV) rescued ROS levels and up-regulated antioxidants such as SOD1 and GPx (59,60). Mitochondrially sourced ROS seems to be crucial in causing muscle atrophy during disuse, since the knockdown of SOD2 prevented the protective adaptation of exercise before MV (63). Protection also is conferred by a training-induced attenuation of the expression of catabolic markers, whereas anabolic signaling and antiapoptotic proteins are enhanced in rodent skeletal muscle (52,62,64). These exercise-evoked mechanisms likely have practical consequences. The prescription of a moderate exercise protocol before surgery could contribute to the preservation of muscle mass and mitochondrial function, attenuating

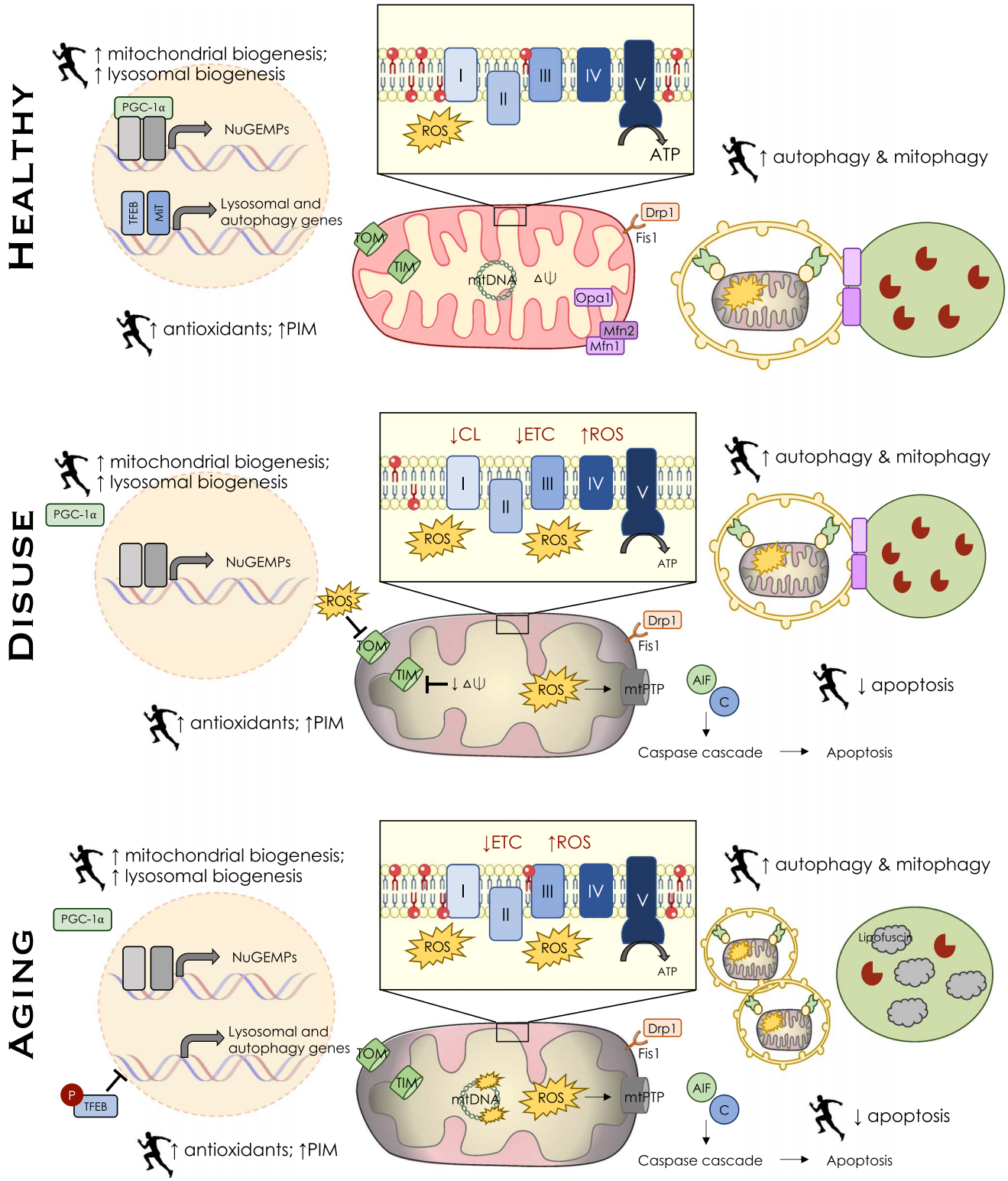

Figure 1. Mitochondrial phenotype with disuse and age. With age and muscle disuse, mitochondria exhibit diminished electron transport chain (ETC) function and elevations in reactive oxygen species (ROS) emission. Reductions in PGC-1α and the drive for mitochondrial biogenesis have been described with both age and disuse. With muscle disuse, protein import is impaired, and this is a fundamental component of the biogenesis pathway. This is likely due to the elevations in ROS. With age, elevations in mitophagy contribute to the reduction in mitochondrial content, but the accumulation of lipofuscin in the lysosomes may be inhibiting lysosomal function, leading to the accumulation of autophagosomes. Together, this can lead to the appearance of mitochondrial dysfunction in these conditions as biogenesis through PGC-1α is impaired, while dysfunctional mitochondria are allowed to accumulate, at least in the context of aging. Mitochondrial fragmentation also is evident with age and disuse. Both aging and disuse have been associated with increases in mitochondrial-mediated apoptosis. This initiates DNA fragmentation and can promote muscle atrophy. Exercise (symbolized by the runner) promotes PGC-1α expression, protein import, mitochondrial biogenesis, lysosomal degradation capacity, and mitophagy to promote mitochondrial turnover, resulting in a more optimal pool of healthy mitochondria. Exercise has also been shown to reduce apoptosis and promote a prosurvival phenotype, in part via the increased expression of antioxidant enzymes, thereby alleviating oxidative stress, preserving muscle mass and promoting muscle health.

the disuse-induced decline in muscle function upon bed rest–induced inactivity during the healing process (65).

MTDNA DISEASE AND COMPLEX ASSEMBLY DEFECTS

The mitochondrial genome encodes 13 of the 87 proteins within the ETC of mitochondria (66). Being in close proximity to the ETC, mtDNA is susceptible to ROS-induced damage and mutations. Mitochondria are heteroplasmic in nature, often containing a mixture of both mutated and wild-type (WT) mtDNA copies. Tissue-dependent heteroplasmy thresholds exist that determine the extent of dysfunction present (67). For example, skeletal muscle harboring a high level of mtDNA mutations or large-scale deletions experiences impaired respiration and elevated ROS levels, thereby increasing the vulnerability of muscle cells to apoptotic nuclear decay. Functionally, this translates into a damaged muscle phenotype with reduced muscle performance and endurance capacity (68,69). When accompanied by chronic physical inactivity, the result is a vicious cycle of deteriorating mitochondrial and whole-muscle function, as evident in patients with mitochondrial myopathy (MM) (67).

Human studies of patients with MM and rodent models with inducible mtDNA mutations are of great use in researching the potential benefits of exercise on muscle function and endurance. It is evident that endurance exercise training can lead to increases in mitochondrial volume, improving peak oxygen utilization of skeletal muscle in human patients with MM and rodent models (67,68,70–74). Thus, regular exercise can improve deficient muscle oxidative metabolism and can serve as a potential therapeutic to alleviate exercise intolerance Fig. 2 (40,41,75–77). Additional studies examining the exercised muscle of patients with MM show improved mitochondrial content and quality occurring independent of changes in mutation load (67,70). Interestingly, resistance exercise has also been found to improve the mtDNA genotype through the transfer of normal mtDNA from the fusion of new satellite cells into the myofiber (78). This "gene shifting" approach suggests an alternative therapeutic route for those with MM to improve the health of the skeletal muscle and improve the associated exercise intolerance.

LYSOSOMAL DISEASE AND BIOGENESIS

In healthy cells, mitophagy is a tightly regulated process whereby dysfunctional mitochondria are recycled by lysosomes to maintain mitochondrial quality. Lysosomal-related pathological conditions such as Pompe disease and Danon disease have an obstructed degradation pathway, resulting in an accumulation of dysfunctional organelles. Thus, stimuli that increase lysosomal biogenesis to alleviate autophagic blockade would represent effective treatments for these conditions. Common symptoms in patients with Pompe disease include skeletal muscle deterioration and functional impairments, which stem from a deficiency in lysosomal enzyme acid α-glucosidase (GAA) (79). In contrast, Danon disease is caused by a deficiency in lysosome-associated membrane protein 2 (LAMP2), and symptoms may include hypertrophic cardiomyopathy, muscle weakness, and cognitive deficiencies (80). Although little is known regarding the effect of exercise in patients with Danon disease, exercise may be effective to promote the clearance of dysfunctional organelles in patients with Pompe disease, which is characterized by the accumulation of glycogen in autophagic vacuoles and lysosomes. Lysosomal enlargement and rupture along with impaired autophagic flux are evident, leading to abnormal mitochondrial structure (81,82). A common treatment is the use of enzyme replacement therapy (ERT), and when combined with exercise training, it has been shown to result in greater mitochondrial enzyme activity in quadriceps muscles than if ERT therapy was used alone in GAA knockout (KO) mice (81). Autophagy markers were also restored to normal levels in cardiac tissue of GAA KO mice after the combined therapy approach. Another study demonstrated that exercise and ERT, rather than each treatment alone, led to a more effective clearance of autophagic debris and lipofuscin (83). Other benefits of this combined polytherapy approach included improved antioxidant system capacity and reduced oxidative damage, whereas the mitochondrial size distribution was restored. These studies suggest that a combined therapy approach leads to better outcomes than each therapy used individually.

Exercise may effectively alleviate a blockade in autophagy, such as that evident in the diseases above. To study this experimentally, Parousis *et al.* (84) used a cell culture model of chronic exercise in myotubes, known as CCA, while treating them with either vehicle or with bafilomycin A (BafA), a drug that inhibits autophagosome-lysosome fusion. BafA treatment led to impaired mitochondrial respiration, elevated ROS emission, and autophagy/mitophagy blockage. After CCA, ROS emission was reduced, respiration rates were restored, lysosomal proteins increased, and mitophagy flux was ameliorated. Thus, this suggests that "exercise" can serve to restore mitochondrial function and turnover, leading to a healthier mitochondrial pool in the presence of lysosomal dysfunction (Fig. 2).

The microphthalmia (MiT) family of transcription factors, namely, transcription factor EB (TFEB) and its family member TFE3, are widely regarded as master regulators of lysosomal biogenesis as they are responsible for regulating promoter regions on the genome to up-regulate various lysosomal genes (85). It is interesting to note that the absence of TFEB or TFE3 also is associated with a dysfunctional mitochondrial phenotype (86,87). This is attributed to declines in lysosomal biogenesis, leading to impaired organelle clearance, favoring the accumulation of dysfunctional mitochondria. However, independent of their roles as regulators of lysosomal genes, TFEB and TFE3 have been shown to regulate PGC-1α (88,89). Mansueto *et al.* (86) showed that the overexpression of TFEB improved mitochondrial content in a manner that was independent of PGC-1α and PGC-1β. These data suggest that a coordinated relation should exist between lysosomal and mitochondrial biogenesis (75,90), as driven by MiT family transcription factors, an area of research that is ripe for further investigation. Evidence for this coordination can be found in response to chronic exercise. Widely recognized as a potent stimulus for mitochondrial biogenesis, chronic exercise also promotes the synthesis of lysosomes. A number of lysosomal markers including LAMP1/2, MCOLN1, and v-ATPase, were up-regulated early on in the training paradigm (43). Interestingly, these adaptations preceded changes in mitochondrial content, highlighting the importance of the lysosomes in facilitating the onset of an exercise-induced oxidative phenotype. This increase in lysosomal capacity likely aids in the removal of damaged mitochondria to promote an optimally functioning mitochondrial pool.

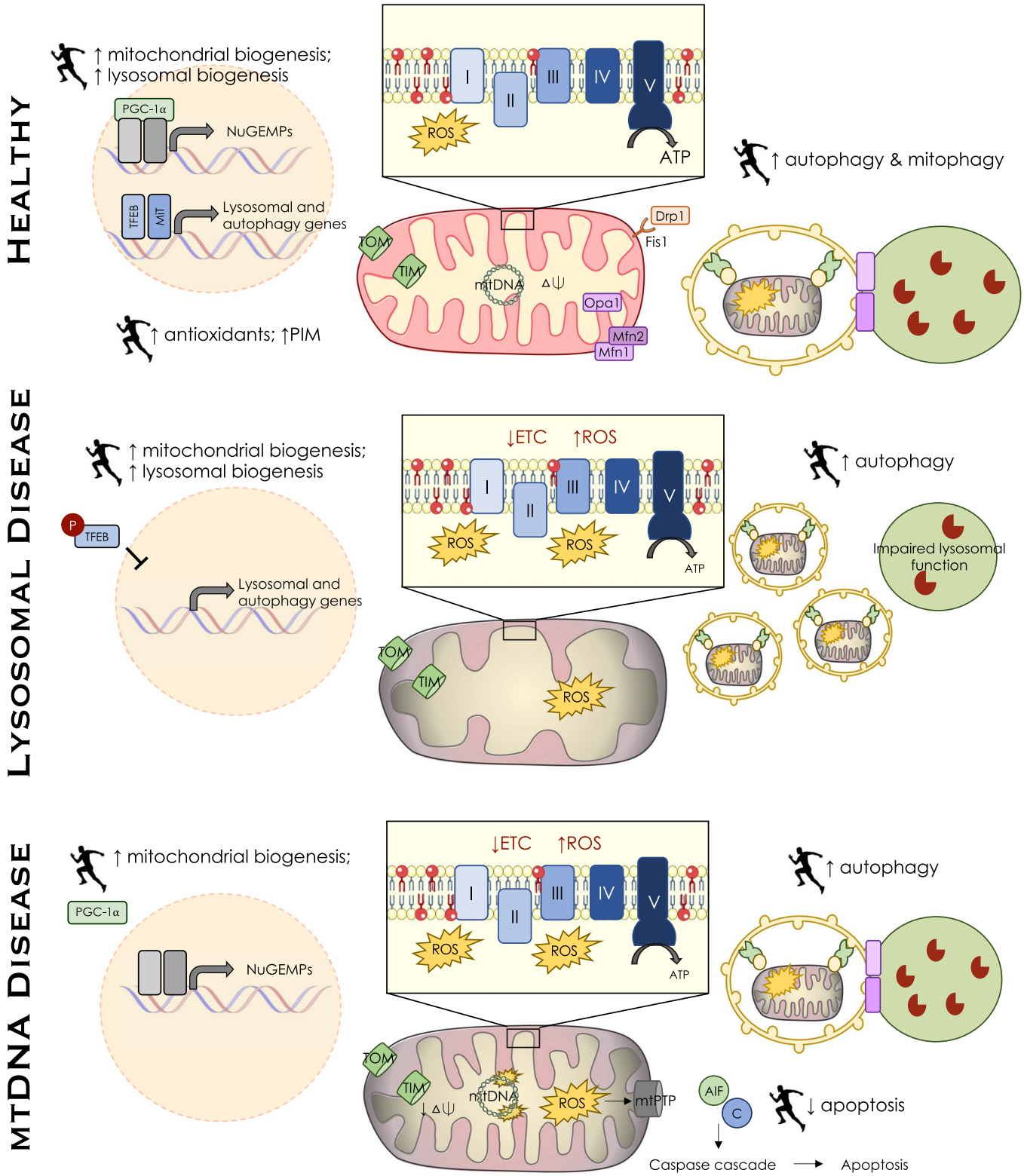

Figure 2. Mitochondrial dysfunction in mtDNA disease and lysosomal disease. The presence of lysosomal disease has repercussions for mitochondria. Mitochondrial dysfunction is present and characterized by declines in oxidative phosphorylation and elevations in reactive oxygen species (ROS) emission. This is likely due to the accumulation of mitochondria that cannot be cleared via the lysosomes. Cristae density is reduced, and mitochondrial swelling has been observed. Interestingly, exercise (symbolized by the runner) has been shown to stimulate lysosomal biogenesis and may offer some promising outcomes, especially in combination therapies. mtDNA disease arises out of genetic mutations in the mitochondrial genome. Because of the heteroplasmic nature of mitochondria, the phenotypes observed in mtDNA disease can vary significantly and correlates positively with mutation load. In general, mitochondrial dysfunction is observed and characterized by decrements in oxidative phosphorylation and elevations in ROS emission. Declines in membrane potential have also been described and likely contribute to the impaired electron transport chain (ETC) function. These organelles also show a greater tendency toward mtPTP opening and, subsequently, apoptosis. Exercise has shown to be a promising therapy in patients with mtDNA disease, promoting an increase in work capacity, $\dot{V}O_{2\ peak}$, and mitochondrial enzyme activities, with no apparent change in mutation load. Despite optimism, more research is needed to evaluate mitophagy in these two disease conditions, as well as the full spectrum of benefits brought about by exercise.

REGULATORY PROTEIN DEFECTS

p53

The tumor suppressor protein p53 and its role in the development of cancer have been well documented. p53 drives cellular apoptosis and DNA damage/repair, whereas its functional absence is associated with several cancers (91). Interestingly, p53 can also regulate and maintain mtDNA by binding directly to the D-Loop region or with Tfam, the main transcription factor for mtDNA (92). p53 also can bind to the promoter region of PGC-1α and several NuGEMPs. Our laboratory has shown that whole-body p53 null mice exhibit a pronounced mitochondrial phenotype characterized by reduced PGC-1α expression, cytochrome c oxidase activity, IMF mitochondrial respiration, and elevated IMF ROS production, suggesting that p53 is important for maintaining mitochondrial content and function (93). Interestingly, the absence of p53 did not hinder the ability of these mice to adapt to exercise (76). p53 muscle-specific knockout (mKO) mice have been shown to display a less severe phenotype (77). Indeed, our studies have shown that p53 mKO mice subjected to exercise improved their endurance capacity to the same level as WT animals, despite having lower basal COX activity, PGC-1α protein, and Tfam protein before training (76). Furthermore, exercise training was enough to reverse excessive ROS production. Autophagy clearance also seemed to be disturbed in mKO mice, demonstrated by elevated basal levels of p62, Parkin, and Beclin-1. However, with exercise, autophagy markers were restored toward normal levels. These studies demonstrate that p53 may have important ramifications for the maintenance of basal mitochondrial content and function but that this can be reversed by exercise training, again illustrating the breadth of signaling that exercise evokes to produce a healthy muscle phenotype.

Sirt1

Sirt1 belongs to a family of deacetylases that gained notoriety for its role in the regulation of mitochondria, as well as its involvement in the NAD+ synthesis pathway. Sirt1 is responsive to the NAD+/NADH status of the cell, as NAD+ acts as a coenzyme or substrate for Sirt1 action. Sirt1 deacetylates PGC-1α, thereby increasing its activity and indirectly promoting its transcription (94). This uniquely positions Sirt1 as a homeostatic sensor, as it, therefore, is sensitive to the energy and redox status of the cell. As such, Sirt1 is appropriately situated to aid in the regulation of mitochondria, both by stimulating mitochondrial biogenesis and mitophagy.

Manipulation of Sirt1 both pharmacologically and genetically results in a dramatic mitochondrial phenotype. Sirt1 null animals have poor endurance capacity that is attributed to the decline in mitochondrial content within skeletal muscle, and poor mitochondrial function characterized by elevations in ROS emission along with reductions in basal and maximal respiration (95). Despite the mitochondrial decrement caused by the absence of SirT1, endurance training in the form of treadmill running was able to improve this mitochondrial defect. These data demonstrate the importance of SirT1 in the maintenance of mitochondria but highlight its redundancy in exercise-induced adaptations while demonstrating the value of exercise in rescuing mitochondrial dysfunction. Interestingly, activation of Sirt1 through resveratrol partially restores the mitochondrial phenotype, and this effect is synergistic when combined with endurance training (95). This is an important finding as resveratrol treatment in older men and women enhances exercise-induced increases in mitochondrial density and further elevates the improvements seen in functional measures such as fatigue resistance and power (96). This illustrates the value of seeking pharmaceutical interventions that can restore mitochondrial health in skeletal muscle when combined with exercise training.

Parkin

Mutations in the Parkin gene are the predominant cause of familial Parkinson disease. Parkin is a key player in the widely studied PINK1/Parkin pathway of mitophagy, discussed above. As an E3 ubiquitin ligase, it is responsible for tagging dysfunctional mitochondria for their degradation. Although Parkin is one of many E3 ubiquitin ligases within the cell, in *Drosophila*, the absence of Parkin is associated with a dramatic mitochondrial phenotype and results in the degeneration of flight muscles (97). In rodent skeletal muscle, the absence of Parkin confers a more nuanced mitochondrial phenotype, whereby mitochondrial content is maintained but the organelles are functionally impaired (98–100). This deficit is characterized by a decline in maximal respiration and occurs independent of a disruption in basal mitophagy (98). In contrast, Parkin overexpression has been associated with the extension of life span and improved mitochondrial function in *Drosophila* (101). In mammalian tissue, overexpression of Parkin attenuated age-related declines in muscle mass and strength and, surprisingly, promoted hypertrophy in adult skeletal muscle (102). Parkin overexpression also improved mitochondrial content and function, thus, protecting against aging-associated elevations in oxidative stress and apoptosis (102). However, Parkin is required to facilitate the normal stress-induced increase in mitophagy promoted by acute exercise (98,103). This does not seem to be dependent on Pink1 accumulation on the OM membrane (104), suggesting that Parkin may be recruited to the mitochondria to facilitate clearance independent of PINK1 after exercise. Therefore, the acute exercise effect on mitophagy serves as a first step in the maintenance of a healthy mitochondrial pool. With repeated bouts, this effect tapers off, likely reflecting early remodeling and subsequent adaptation, attenuating the signaling that stimulates further mitophagy. However, because the acute response is lost in the absence of Parkin, mitophagy remains low after training, and functional improvements are not seen in the mitochondria of Parkin KO animals (98). Endurance training is still capable of increasing mitochondrial content but is not able to rescue the functional impairment (94). This highlights a role for Parkin in mediating exercise-induced mitochondrial adaptations through mitophagy, as well as via noncanonical routes of mitochondrial biogenesis.

FUTURE PERSPECTIVES

Mitochondria are highly plastic organelles that are inherently tied to the health of skeletal muscle. As such, mitochondria are often central in discussions of the muscle pathology observed with atrophy, aging, and disease. Exercise provides a compelling avenue for therapy, as it evokes the activation of a broad array of redundant signaling pathways that stimulate mitochondrial biogenesis to maintain organelle content and

function. Recent studies also indicate that exercise has the unique ability to promote the removal of dysfunctional mitochondria that are often central in the etiology of disease, via a simultaneous increase in mitophagy and in lysosomal degradation capacity. The complexity and multifaceted nature of the adaptations elicited through exercise training illustrate the therapeutic potential of "exercise as mitochondrial medicine" that may be impossible to replicate pharmaceutically. Future work in this area could focus on 1) a better understanding of novel, yet independent, biogenesis pathways that respond to both exercise and pharma/nutriceuticals, with the hope of achieving synergistic responses when both are used in combination; 2) the activation of mitophagy as an equal partner with biogenesis in maintaining a healthy organelle pool; 3) the role of exercise in stimulating mitochondrial motility and dynamics via fission and fusion, as mitochondrial morphology often determines organelle function; and finally, 4) lysosomal degradation capacity as a determinant of mitochondrial health, currently a poorly studied area of muscle biology. Knowledge of these pathways would allow greater possibilities for intervention, especially when exercise is impossible or challenging.

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References

1. Winder WW, Holmes BF, Rubink DS, Jensen EB, Chen M, Holloszy JO. Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. *J. Appl. Physiol.* 2000; 88(6):2219–26.
2. Wu H, Olson EN. Activation of the MEF2 transcription factor in skeletal muscles from myotonic mice. *J. Clin. Invest.* 2002; 109(10):1327–33.
3. Pogozelski AR, Geng T, Li P, et al. p38gamma mitogen-activated protein kinase is a key regulator in skeletal muscle metabolic adaptation in mice. *PLoS One.* 2009; 4(11):e7934.
4. Puigserver P, Adelmant G, Wu Z, et al. Activation of PPARgamma coactivator-1 through transcription factor docking. *Science.* 1999; 286(5443):1368–71.
5. Wiedemann N, Pfanner N. Mitochondrial machineries for protein import and assembly. *Annu. Rev. Biochem.* 2017; 86(1):685–714.
6. Calvo SE, Clauser KR, Mootha VK. MitoCarta2.0: an updated inventory of mammalian mitochondrial proteins. *Nucleic Acids Res.* 2016; 44(D1): D1251–7.
7. Hoppeler H. Exercise-induced ultrastructural changes in skeletal muscle. *Int. J. Sports Med.* 1986; 7(4):187–204.
8. Kirkwood SP, Munn EA, Brooks GA. Mitochondrial reticulum in limb skeletal muscle. *Am. J. Phys.* 1986; 251(3 Pt 1):C395–402.
9. Picard M, White K, Turnbull DM. Mitochondrial morphology, topology, and membrane interactions in skeletal muscle: a quantitative three-dimensional electron microscopy study. *J. Appl. Physiol.* 2013; 114(2):161–71.
10. Glancy B, Hartnell LM, Malide D, et al. Mitochondrial reticulum for cellular energy distribution in muscle. *Nature.* 2015; 523(7562):617–20.
11. Cogswell AM, Stevens RJ, Hood DA. Properties of skeletal muscle mitochondria isolated from subsarcolemmal and intermyofibrillar regions. *Am. J. Phys.* 1993; 264(2 Pt 1):C383–9.
12. Iqbal S, Hood DA. Cytoskeletal regulation of mitochondrial movements in myoblasts. *Cytoskeleton.* 2014; 71(10):564–72.
13. Chan DC. Fusion and fission: interlinked processes critical for mitochondrial health. *Annu. Rev. Genet.* 2012; 46(1):265–87.
14. Kirkwood SP, Packer L, Brooks GA. Effects of endurance training on a mitochondrial reticulum in limb skeletal muscle. *Arch. Biochem. Biophys.* 1987; 255(1):80–8.
15. Dudek J. Role of cardiolipin in mitochondrial signaling pathways. *Front. Cell Dev. Biol.* 2017; 5:90.
16. Ostojic O, O'Leary MF, Singh K, Menzies KJ, Vainshtein A, Hood DA. The effects of chronic muscle use and disuse on cardiolipin metabolism. *J. Appl. Physiol. (1985).* 2013; 114(4):444–52.
17. Baines CP. The molecular composition of the mitochondrial permeability transition pore. *J. Mol. Cell. Cardiol.* 2009; 46(6):850–7.
18. Cheema N, Herbst A, Mckenzie D, Aiken JM. Apoptosis and necrosis mediate skeletal muscle fiber loss in age-induced mitochondrial enzymatic abnormalities. *Aging Cell.* 2015; 14(6):1085–93.
19. Adhihetty PJ, O'Leary MF, Chabi B, Wicks KL, Hood DA. Effect of denervation on mitochondrially mediated apoptosis in skeletal muscle. *J. Appl. Physiol. (1985).* 2007; 102(3):1143–51.
20. Carter HN, Kim Y, Erlich AT, Zarrin-khat D, Hood DA. Autophagy and mitophagy flux in young and aged skeletal muscle following chronic contractile activity. *J. Physiol.* 2018; 596(16):3567–84.
21. Frank M, Duvezin-Caubet S, Koob S, et al. Mitophagy is triggered by mild oxidative stress in a mitochondrial fission dependent manner. *Biochim. Biophys. Acta.* 2012; 1823(12):2297–310.
22. Matsuda N, Sato S, Shiba K, et al. PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. *J. Cell Biol.* 2010; 189(2):211–21.
23. Huynh KK, Eskelinen EL, Scott CC, Malevanets A, Saftig P, Grinstein S. LAMP proteins are required for fusion of lysosomes with phagosomes. *EMBO J.* 2007; 26(2):313–24.
24. Pimentel AE, Gentile CL, Tanaka H, Seals DR, Gates PE. Greater rate of decline in maximal aerobic capacity with age in endurance-trained than in sedentary men. *J. Appl. Physiol.* 2003; 94(6):2406–13.
25. Doherty TJ. Invited review: aging and sarcopenia. *J. Appl. Physiol.* 2003; 95(4):1717–27.
26. Bloom DE, Chatterji S, Kowal P, et al. Macroeconomic implications of population ageing and selected policy responses. *Lancet.* 2015; 385(9968):649–57.
27. Harman D. The biologic clock: the mitochondria? *J. Am. Geriatr. Soc.* 1972; 20(4):145–7.
28. Figueiredo PA, Powers SK, Ferreira RM, Appell HJ, Duarte JA. Aging impairs skeletal muscle mitochondrial bioenergetic function. *J. Gerontol. A Biol. Sci. Med. Sci.* 2009; 64(1):21–33.
29. Chabi B, Ljubicic V, Menzies KJ, Huang JH, Saleem A, Hood DA. Mitochondrial function and apoptotic susceptibility in aging skeletal muscle. *Aging Cell.* 2008; 7(1):2–12.
30. Iqbal S, Ostojic O, Singh K, Joseph A-M, Hood DA. Expression of mitochondrial fission and fusion regulatory proteins in skeletal muscle during chronic use and disuse. *Muscle Nerve.* 2013; 48(6):963–70.
31. Ljubicic V, Joseph A-M, Adhihetty PJ, et al. Molecular basis for an attenuated mitochondrial adaptive plasticity in aged skeletal muscle. *Aging (Albany NY).* 2009; 1(9):818–30.
32. Beregi E, Regius O, Huttl T, Gobl Z. Age-related changes in the skeletal muscle cells. *Z. Gerontol.* 1988; 21(2):83–6.
33. Joseph A-M, Adhihetty PJ, Buford TW, et al. The impact of aging on mitochondrial function and biogenesis pathways in skeletal muscle of sedentary high- and low-functioning elderly individuals. *Aging Cell.* 2012; 11(5):801–9.
34. Carter HN, Chen CC, Hood DA. Mitochondria, muscle health, and exercise with advancing age. *Physiology (Bethesda).* 2015; 30(3):208–23.
35. Johnson ML, Robinson MM, Nair KS. Skeletal muscle aging and the mitochondrion. *Trends Endocrinol. Metab.* 2013; 24(5):247–56.
36. Rooyackers OE, Adey DB, Ades PA, Nair KS. Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle. *Proc. Natl. Acad. Sci. U. S. A.* 1996; 93(26):15364–9.
37. Larsen RG, Callahan DM, Foulis SA, Kent-Braun JA. Age-related changes in oxidative capacity differ between locomotory muscles and are associated with physical activity behavior. *Appl. Physiol. Nutr. Metab.* 2012; 37(1):88–99.
38. Short KR, Bigelow ML, Kahl J, et al. Decline in skeletal muscle mitochondrial function with aging in humans. *Proc. Natl. Acad. Sci. U. S. A.* 2005; 102(15):5618–23.
39. Huang JH, Joseph AM, Ljubicic V, Iqbal S, Hood DA. Effect of age on the processing and import of matrix-destined mitochondrial proteins in skeletal muscle. *J. Gerontol. A Biol. Sci. Med. Sci.* 2010; 65(2):138–46.
40. Carter HN, Pauly M, Tryon LD, Hood DA. Effect of contractile activity on PGC-1α transcription in young and aged skeletal muscle. *J. Appl. Physiol. (1985).* 2018; 124(6):1605–15.

41. O'Leary MF, Vainshtein A, Iqbal S, Ostojic O, Hood DA. Adaptive plasticity of autophagic proteins to denervation in aging skeletal muscle. *Am. J. Physiol. Cell Physiol.* 2013; 304(5):C422–30.
42. Wohlgemuth SE, Seo AY, Marzetti E, Lees HA, Leeuwenburgh C. Skeletal muscle autophagy and apoptosis during aging: effects of calorie restriction and life-long exercise. *Exp. Gerontol.* 2010; 45(2):138–48.
43. Kim Y, Triolo M, Erlich AT, Hood DA. Regulation of autophagic and mitophagic flux during chronic contractile activity-induced muscle adaptations. *Pflugers Arch.* 2019; 471(3):431–40.
44. Iversen N, Krustrup P, Rasmussen HN, Rasmussen UF, Saltin B, Pilegaard H. Mitochondrial biogenesis and angiogenesis in skeletal muscle of the elderly. *Exp. Gerontol.* 2011; 46(8):670–8.
45. Nilsson MI, Bourgeois JM, Nederveen JP, et al. Lifelong aerobic exercise protects against inflammaging and cancer. *PLoS One.* 2019; 14(1):e0210863.
46. Figueiredo PA, Powers SK, Ferreira RM, Amado F, Appell HJ, Duarte JA. Impact of lifelong sedentary behavior on mitochondrial function of mice skeletal muscle. *J. Gerontol. A Biol. Sci. Med. Sci.* 2009; 64(9):927–39.
47. Olesen J, Gliemann L, Biensø R, Schmidt J, Hellsten Y, Pilegaard H. Exercise training, but not resveratrol, improves metabolic and inflammatory status in skeletal muscle of aged men. *J. Physiol.* 2014; 592(8):1873–86.
48. Wohlgemuth SE, Lees HA, Marzetti E, et al. An exploratory analysis of the effects of a weight loss plus exercise program on cellular quality control mechanisms in older overweight women. *Rejuvenation Res.* 2011; 14(3):315–24.
49. Trevino MB, Zhang X, Standley RA, et al. Loss of mitochondrial energetics is associated with poor recovery of muscle function but not mass following disuse atrophy. *Am. J. Physiol. Endocrinol. Metab.* 2019; 317(5):E899–910.
50. Tryon LD, Crilly MJ, Hood DA. Effect of denervation on the regulation of mitochondrial transcription factor A expression in skeletal muscle. *Am. J. Physiol. Cell Physiol.* 2015; 309(4):C228–38.
51. Vainshtein A, Desjardins EM, Armani A, Sandri M, Hood DA. PGC-1α modulates denervation-induced mitophagy in skeletal muscle. *Skelet. Muscle.* 2015; 5:9.
52. O'Leary MF, Hood DA. Effect of prior chronic contractile activity on mitochondrial function and apoptotic protein expression in denervated muscle. *J. Appl. Physiol. (1985).* 2008; 105(1):114–20.
53. Singh K, Hood DA. Effect of denervation-induced muscle disuse on mitochondrial protein import. *Am. J. Physiol. Cell Physiol.* 2011; 300(1): C138–45.
54. Liu J, Peng Y, Cui Z, et al. Depressed mitochondrial biogenesis and dynamic remodeling in mouse tibialis anterior and gastrocnemius induced by 4-week hindlimb unloading. *IUBMB Life.* 2012; 64(11):901–10.
55. Talbert EE, Smuder AJ, Min K, Kwon OS, Szeto HH, Powers SK. Immobilization-induced activation of key proteolytic systems in skeletal muscles is prevented by a mitochondria-targeted antioxidant. *J. Appl. Physiol.* 2013; 115(4):529–38.
56. Smuder AJ, Sollanek KJ, Nelson WB, et al. Crosstalk between autophagy and oxidative stress regulates proteolysis in the diaphragm during mechanical ventilation. *Free Radic. Biol. Med.* 2018; 115:179–90.
57. Hyatt H, Deminice R, Yoshihara T, Powers SK. Mitochondrial dysfunction induces muscle atrophy during prolonged inactivity: a review of the causes and effects. *Arch. Biochem. Biophys.* 2019; 662:49–60.
58. Powers SK, Bomkamp M, Ozdemir M, Hyatt H. Mechanisms of exercise-induced preconditioning in skeletal muscles. *Redox Biol.* 2020; 35:101462.
59. Smuder AJ, Min K, Hudson MB, et al. Endurance exercise attenuates ventilator-induced diaphragm dysfunction. *J. Appl. Physiol.* 2012; 112(3):501–10.
60. Theilen NT, Jeremic N, Weber GJ, Tyagi SC. Exercise preconditioning diminishes skeletal muscle atrophy after hindlimb suspension in mice. *J. Appl. Physiol.* 2018; 125(4):999–1010.
61. Leick L, Lyngby SS, Wojtaszewski JF, Pilegaard H. PGC-1alpha is required for training-induced prevention of age-associated decline in mitochondrial enzymes in mouse skeletal muscle. *Exp. Gerontol.* 2010; 45(5):336–42.
62. Liu J, Peng Y, Feng Z, et al. Reloading functionally ameliorates disuse-induced muscle atrophy by reversing mitochondrial dysfunction, and similar benefits are gained by administering a combination of mitochondrial nutrients. *Free Radic. Biol. Med.* 2014; 69:116–28.
63. Morton AB, Smuder AJ, Wiggs MP, et al. Increased SOD2 in the diaphragm contributes to exercise-induced protection against ventilator-induced diaphragm dysfunction. *Redox Biol.* 2019; 20:402–13.
64. Fujino H, Ishihara A, Murakami S, et al. Protective effects of exercise preconditioning on hindlimb unloading-induced atrophy of rat soleus muscle. *Acta Physiol (Oxf.).* 2009; 197(1):65–74.
65. Kneppers A, Leermakers P, Pansters N, et al. Coordinated regulation of skeletal muscle mass and metabolic plasticity during recovery from disuse. *FASEB J.* 2019; 33(1):1288–98.
66. Kujoth CC, Hiona A, Pugh TD, et al. Medicine: mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science.* 2005; 309(5733):481–4.
67. Jeppesen TD, Schwartz M, Olsen DB, et al. Aerobic training is safe and improves exercise capacity in patients with mitochondrial myopathy. *Brain.* 2006; 129(12):3402–12.
68. Safdar A, Bourgeois JM, Ogborn DI, et al. Endurance exercise rescues progeroid aging and induces systemic mitochondrial rejuvenation in mtDNA mutator mice. *Proc. Natl. Acad. Sci. U. S. A.* 2011; 108(10):4135–40.
69. Dillon LM, Williams SL, Hida A, et al. Increased mitochondrial biogenesis in muscle improves aging phenotypes in the mtDNA mutator mouse. *Hum. Mol. Genet.* 2012; 21(10):2288–97.
70. Taivassalo T, Gardner JL, Taylor RW, et al. Endurance training and detraining in mitochondrial myopathies due to single large-scale mtDNA deletions. *Brain.* 2006; 129(12):3391–401.
71. Taivassalo T, Shoubridge EA, Chen J, et al. Aerobic conditioning in patients with mitochondrial myopathies: physiological, biochemical, and genetic effects. *Ann. Neurol.* 2001; 50(2):133–41.
72. Adhihetty PJ, Taivassalo T, Haller RG, Walkinshaw DR, Hood DA. The effect of training on the expression of mitochondrial biogenesis- and apoptosis-related proteins in skeletal muscle of patients with mtDNA defects. *Am. J. Physiol. Endocrinol. Metab.* 2007; 293(3):E672–80.
73. Jeppesen TD. Aerobic exercise training in patients with mtDNA-related mitochondrial myopathy. *Front. Physiol.* 2020; 11:349.
74. Siciliano G, Simoncini C, Lo Gerfo A, Orsucci D, Ricci G, Mancuso M. Effects of aerobic training on exercise-related oxidative stress in mitochondrial myopathies. *Neuromuscul. Disord.* 2012; 22 Suppl 3(3–3):S172–7.
75. Erlich AT, Brownlee DM, Beyfuss K, Hood DA. Exercise induces TFEB expression and activity in skeletal muscle in a PGC-1α–dependent manner. *Am. J. Physiol. Cell Physiol.* 2017; 314(1):C62–72.
76. Beyfuss K, Erlich AT, Triolo M, Hood DA. The role of p53 in determining mitochondrial adaptations to endurance training in skeletal muscle. *Sci. Rep.* 2018; 8(1):14710.
77. Stocks B, Dent JR, Joanisse S, McCurdy CE, Philp A. Skeletal muscle fibre-specific knockout of p53 does not reduce mitochondrial content or enzyme activity. *Front. Physiol.* 2017; 8:941.
78. Taivassalo T, Fu K, Johns T, Arnold D, Karpati G, Shoubridge EA. Gene shifting: a novel therapy for mitochondrial myopathy. *Hum. Mol. Genet.* 1999; 8(6):1047–52.
79. Hagemans ML, Winkel LP, Van Doorn PA, et al. Clinical manifestation and natural course of late-onset Pompe's disease in 54 Dutch patients. *Brain.* 2005; 128(Pt 3):671–7.
80. Danon MJ, Oh SJ, DiMauro S, et al. Lysosomal glycogen storage disease with normal acid maltase. *Neurology.* 1981; 31(1):51–7.
81. Nilsson MI, Samjoo IA, Hettinga BP, et al. Aerobic training as an adjunctive therapy to enzyme replacement in Pompe disease. *Mol. Genet. Metab.* 2012; 107(3):469–79.
82. Thurberg BL, Lynch Maloney C, Vaccaro C, et al. Characterization of pre- and post-treatment pathology after enzyme replacement therapy for Pompe disease. *Lab. Investig.* 2006; 86(12):1208–20.
83. Nilsson MI, MacNeil LG, Kitaoka Y, et al. Combined aerobic exercise and enzyme replacement therapy rejuvenates the mitochondrial-lysosomal axis and alleviates autophagic blockage in Pompe disease. *Free Radic. Biol. Med.* 2015; 87:98–112.
84. Parousis A, Carter HN, Tran C, et al. Contractile activity attenuates autophagy suppression and reverses mitochondrial defects in skeletal muscle cells. *Autophagy.* 2018; 14(11):1886–97.
85. Palmieri M, Impey S, Kang H, di Ronza A, Pelz C, Sardiello M. Characterization of the CLEAR network reveals an integrated control of cellular clearance pathways. *Hum. Mol. Genet.* 2011; 20(19):3852–66.
86. Mansueto G, Armani A, Viscomi C, et al. Transcription factor EB controls metabolic flexibility during exercise. *Cell Metab.* 2017; 25(1):182–96.
87. Pastore N, Vainshtein A, Klisch TJ, et al. TFE3 regulates whole-body energy metabolism in cooperation with TFEB. *EMBO Mol. Med.* 2017; 9(5):605–21.

88. Settembre C, De Cegli R, Mansueto G, et al. TFEB controls cellular lipid metabolism through a starvation-induced autoregulatory loop. 2015; 230(10):2330–6.
89. Salma N, Song JS, Arany Z, Fisher DE. Transcription factor Tfe3 directly regulates Pgc-1alpha in muscle. *J. Cell. Physiol.* 2015; 230(10):2330–6.
90. Lynch MR, Tran MT, Ralto KM, et al. TFEB-driven lysosomal biogenesis is pivotal for PGC1alpha-dependent renal stress resistance. *JCI Insight*. 2019; 5(8):e126749.
91. Lane DP. p53, guardian of the genome. *Nature*. 1992; 358(6381):15–6.
92. Saleem A, Hood DA. Acute exercise induces tumour suppressor protein p53 translocation to the mitochondria and promotes a p53-Tfam-mitochondrial DNA complex in skeletal muscle. *J. Physiol.* 2013; 591(14):3625–36.
93. Saleem A, Adhihetty PJ, Hood DA. Role of p53 in mitochondrial biogenesis and apoptosis in skeletal muscle. *Physiol. Genomics*. 2009; 37(1):58–66.
94. Mouchiroud L, Houtkooper RH, Moullan N, et al. The NAD(+)/Sirtuin pathway modulates longevity through activation of mitochondrial UPR and FOXO signaling. *Cell*. 2013; 154(2):430–41.
95. Menzies KJ, Singh K, Saleem A, Hood DA. Sirtuin 1–mediated effects of exercise and resveratrol on mitochondrial biogenesis. *J. Biol. Chem*. 2013; 288(10):6968–79.
96. Alway SE, McCrory JL, Kearcher K, et al. Resveratrol enhances exercise-induced cellular and functional adaptations of skeletal muscle in older men and women. *J. Gerontol. A Biol. Sci. Med. Sci.* 2017; 72(12):1595–606.
97. Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ. Mitochondrial pathology and apoptotic muscle degeneration in *Drosophila* Parkin mutants. *Proc. Natl. Acad. Sci*. 2003; 100(7):4078–83.
98. Chen CCW, Erlich AT, Hood DA. Role of Parkin and endurance training on mitochondrial turnover in skeletal muscle. *Skelet. Muscle*. 2018; 8(10):10.
99. Rosen KM, Veereshwarayya V, Moussa CE, et al. Parkin protects against mitochondrial toxins and beta-amyloid accumulation in skeletal muscle cells. *J. Biol. Chem*. 2006; 281(18):12809–16.
100. Gouspillou G, Godin R, Piquereau J, et al. Protective role of Parkin in skeletal muscle contractile and mitochondrial function. *J. Physiol.* 2018; 596(13):2565–79.
101. Rana A, Rera M, Walker DW. Parkin overexpression during aging reduces proteotoxicity, alters mitochondrial dynamics, and extends lifespan. *Proc. Natl. Acad. Sci. U. S. A.* 2013; 110(21):8638–43.
102. Leduc-Gaudet JP, Reynaud O, Hussain SN, Gouspillou G. Parkin overexpression protects from ageing-related loss of muscle mass and strength. *J. Physiol.* 2019; 597(7):1975–91.
103. Chen CCW, Erlich AT, Crilly MJ, Hood DA. Parkin is required for exercise-induced mitophagy in muscle: impact of aging. *Am. J. Physiol. Endocrinol. Metab.* 2018; 315(3):E404–15.
104. Drake JC, Laker RC, Wilson RJ, Zhang M, Yan Z. Exercise-induced mitophagy in skeletal muscle occurs in the absence of stabilization of Pink1 on mitochondria. *Cell Cycle*. 2019; 18(1):1–6.