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Mitochondria, Muscle Health, and Exercise with Advancing Age

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Skeletal muscle health is dependent on the optimal function of its mitochondria. With advancing age, decrements in numerous mitochondrial variables are evident in muscle. Part of this decline is due to reduced physical activity, whereas the remainder appears to be attributed to age-related alterations in mitochondrial synthesis and degradation. Exercise is an important strategy to stimulate mitochondrial adaptations in older individuals to foster improvements in muscle function and quality of life.

The process of aging exploits the malleable nature of skeletal muscle. The age-related loss of muscle mass was termed sarcopenia, based on the Greek (*sarx*, flesh) and (*penia*, poverty) in 1988 (139). Accompanying this loss are profound architectural and molecular changes that alter muscle quality and are manifested in functional limitations. Decreases in muscle fiber number as well as fiber cross-sectional area are both contributing factors to sarcopenia (92) and consequently adversely affect force production (strength) (46, 63) and endurance of the older individual (12). Muscle mass typically peaks in the mid-20s (12, 34, 92), and thereafter several distinct phases of muscle loss have been identified. In the third to fifth decade of life, a slow rate of muscle mass loss is noted, amounting to ~10% in total (34, 92). In later adulthood (>45 yr), the rate of muscle loss increases, with appraisals ranging between 0.5 and 1.4% per year (12, 34, 69). Even more dramatic changes are noted beyond the sixth decade of life. Along with the functional impairments imposed by sarcopenia, are the associated escalations in health care costs, along with coincident rises in metabolic diseases (e.g., Type 2 diabetes, obesity) and a greater risk of falls (67). In the U.S., it is expected that those 65 years of age and over will comprise ~20% of the population, or ~72 million people, by the year 2030 (20). Since the proportion of older adults is increasing, continued research into the mechanisms of muscle loss is warranted, along with the investigation of therapeutic strategies that can mitigate muscle atrophy during aging.

Mitochondria have been implicated as potential mediators of sarcopenia. Recently, it has been suggested that dysfunction of these organelles can be considered a feature of aging (105). However, considerable controversy exists regarding the extent to which muscle mitochondria may be dysfunctional with aging, and thereby contribute to the loss of this tissue. Thus the purpose of this review is to examine the literature with respect to mitochondrial content and function in muscle with advancing age, and provide a perspective on the effectiveness of endurance/aerobic exercise as an intervention for mitochondrial biogenesis and muscle homeostasis in older individuals.

Structural Features of Muscle Relevant to Sarcopenia

In young, healthy individuals, skeletal muscle comprises ~40% of total body mass and is important for locomotion and whole body metabolism. Myosin ATPase histochemistry and electrophoretic analyses of myosin heavy chain isoforms have revealed the presence of three different "fiber types" that, in varying proportions, make up skeletal muscles. Type I fibers are small, generate the least amount of force, but contain the most mitochondria, making them fatigue-resistant. Type II fibers (IIa or IIx), are larger, contain fewer mitochondria, generate more force, and are more fatigueable (144). Although useful for classification purposes, it has been recognized for some time that this represents an oversimplification of the true distribution of myosin isoforms within muscle cells. Muscle fibers are known to co-express more than one myosin heavy chain isoform (50), resulting in "hybrid" fiber types. The incidence of hybrid fibers has been recognized to increase in aging muscle (5, 37), and this adds to the complexity of fiber-type classification in muscle of aging individuals. As such, the long-held concept that age-related muscle atrophy can be attributed mainly to a decrease in the size of only type II fibers (93, 117), whereas type I fibers remain spared from this decline, may need to be revisited (131, 132).

Electron microscopy provides pictorial evidence that mitochondria are largely localized in distinct regions of the muscle, below the sarcolemma, termed subsarcolemmal (SS) mitochondria, and between the myofibrils, called intermyofibrillar (IMF) mitochondria. Although controversy exists

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on the distinction between these organellar fractions (58), they appear to have divergent biochemical (26) and morphological (129) characteristics, and they also adapt differentially to exercise and disease (60). As discussed below, mitochondrial form and function in muscle are both age- and physical activity-dependent.

Characteristics of Mitochondria

An appreciation of how mitochondria contribute to muscle health and sarcopenia obligates an understanding of organelle function, fusion, and synthesis (biogenesis), as well as fission and degradation (mitophagy).

Mitochondrial Function

Mitochondrial respiration and ATP production are driven mainly by cytosolically derived ADP, a product of ATP-consuming reactions. In a resting muscle cell, ATP demand is low. Thus substrate oxidation and electron transport are minimized, restrained by a high proton motive force across the inner membrane (106). In this state, electrons can reduce oxygen and form reactive oxygen species (ROS). When muscle cells contract, myosin ATPase generates free ADP, which enters mitochondria to interact with ATP synthase, thereby dissipating the proton gradient and permitting an increased rate of electron transfer to cytochrome oxidase. As a consequence, both oxygen consumption and ATP synthesis increase, while mitochondrial ROS production per unit of O_2 consumption diminishes, whether expressed per organelle or per unit of muscle mass (99). Thus mitochondria are the main suppliers of cellular energy, and can, along with other reactions, contribute to the formation of ROS (see FIGURE 2) (106). Apart from potential signaling effects or damaging consequences of ROS, these molecules also serve to promote the opening of the permeability transition pore (mtPTP) to cause organelle swelling and allow the release of pro-apoptotic proteins to the cytosol. Once released, these factors initiate apoptotic signaling and ultimately myonuclear decay, which can contribute to muscle atrophy. In addition, mitochondria are also active in the sequestration of calcium, which can modulate cytosolic levels of this cation to affect intracellular signaling. Therefore, mitochondria serve multiple functions in the myocytes, and the diversity of these roles must be considered when mitochondrial dysfunction, and its relation to muscle mass, are analyzed.

Fusion and Biogenesis

Mitochondrial synthesis is dependent on the ability of the cell to transcribe, translate, and import new proteins into preexisting organelles. The vast majority of genes that encode the ~1,000 mitochondrial proteins are found within the nuclear genome; however, 13 critical genes encoding components of the ETC are confined to mtDNA (122). Exquisite coordination of cellular communication is therefore necessary to ensure the proper stoichiometry of nuclear and mitochondrially encoded proteins that make up the organelle. Nuclear genes encoding mitochondrial proteins (NUGEMPs) are transcribed through a process that is thought to be largely controlled by the peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC-1α) family of transcriptional coactivators (FIGURE 2) (51). PGC-1α interacts with and activates numerous transcription factors for gene expression, including nuclear respiratory factors 1 and 2 (NRF-1 and -2), estrogen-related receptor family (ERRs), and the PPAR family, among others. These produce gene products involved in fatty acid oxidation, the ETC, oxidative phosphorylation, Krebs' cycle, as well as antioxidant enzymes (145, 146). Importantly, PGC-1α controls the expression of mitochondrial transcription factor A (Tfam), the regulator of mtDNA expression, making this coactivator responsible for the link between the nuclear and mitochondrial genomes (173).

The mRNAs produced by transcription are exported from the nucleus and translated into precursor proteins in the cytosol. They are guided to the mitochondria by cytosolic chaperones and imported via the protein import machinery (PIM). Once inside, the proteins can be incorporated into various compartments of the organelle, or they can serve as transcriptional regulators for mtDNA, like Tfam. The coordinated regulation between nuclear gene expression, protein import, and transcription of mtDNA represents mitochondrial biogenesis, and this leads to the expansion of the organelle reticulum into larger membranous networks (FIGURE 2). This mitochondrial network, or reticulum, is particularly evident in the region between the myofibrils (81, 120) and is advantageous for the distribution of mtDNA, proteins, and metabolites within the depths of the myocyte. More discrete, smaller mitochondrial structures exist in the subsarcolemmal region. Mitochondrial morphology is a result of organelle movement, accompanied by the actions of fusion and fission proteins, which are capable of linking or dividing the mitochondrial membranes, respectively (FIGURE 2). Mitofusins 1 and 2 (Mfn1 and 2) and Optic atrophy 1 (Opa1) are proteins that are integral to facilitating mitochondrial fusion, whereas dynamin-related protein 1 (Drp1), fission protein 1 (Fis1), and mitochondrial fission factor (Mff), are factors that promote fission (128). Little is known, thus far,

about the regulation of mitochondrial movements within the dense myofibrillar network that comprises skeletal muscle.

Fission and Mitophagy

When regions of the mitochondrial reticulum lose their membrane potential, and hence their capacity for ATP provision, or when ROS production is high, fission is activated, leading to cleavage of the dysfunctional segment of the organelle network. Removal and degradation of this dysfunctional portion, termed mitophagy, is essential to maintain a healthy population of organelles within the muscle. This catabolic process requires the recognition of the organelle segment that is to be digested and its subsequent enclosure in a double-membrane autophagosome. The autophagosome then fuses with the lysosome, and its contents are degraded by proteolytic enzymes (FIGURE 2). Deficiencies or defects in the autophagy pathway, thus an inability to effectively remove dysfunctional mitochondria, have been linked to pathology (48) and to the maintenance of muscle mass (108).

Aging and Mitochondria

The molecular basis underlying the relationship between mitochondrial function and sarcopenia is multi-factorial and poorly understood. Recent reviews by Nair and colleagues (54, 72) have summarized the factors that contribute to changes in mitochondrial function in aging muscle. A decline in organelle content is supported by many studies that report reduced enzymatic activities [i.e., citrate synthase, cytochrome oxidase (COX) activity] and protein markers (21, 102, 147, 148), mtDNA content (78, 166), along with electron micrograph evidence of diminished IMF mitochondrial size and a reduced thickness of the SS mitochondrial layer (FIGURE 1) (68). Furthermore, mitochondrial functions are impaired with aging (54, 72), including reduced mitochondrial protein synthesis (138), respiration (11, 161), and maximal ATP production rate (MAPR) (89, 147), partly a result of increased uncoupling of oxygen consumption to ATP synthesis (FIGURE 2) (29, 71, 107). Importantly, some of these declines have been noted even when physical activity levels between young and old subjects have been carefully matched (45, 71, 89, 138, 147), suggesting true age-related deficits in mitochondrial function. However, this conclusion remains controversial, since some studies have reported no changes in mitochondrial respiratory function with age or have noted that the changes are muscle type-specific and related to motor unit recruitment patterns and levels of physical activity (72, 80, 89, 127). As discussed below, the level of physical activity of the individual is certainly one of the most important determinants of organelle function in aging muscle.

Transcriptomic and Proteomic Mitochondrial Characteristics of Aged Muscle

One approach toward a better comprehension of aging is to evaluate the molecular signature of old vs. young muscle in an effort to identify biomarkers related to aging. Transcriptomic assessments of muscle have universally revealed the downregulation of many genes involved in mitochondrial metabolism. A number of studies using microarrays, starting in the early 2000s, observed consistent decreases in transcripts encoding fatty acid and mitochondrial metabolism genes in older male and female humans, as well as in monkeys and rodents (FIGURE 3; Table 1). For example, Melov et al. (111) compared relatively active older subjects with inactive younger individuals in an attempt to match them for physical activity patterns. They identified 596 genes (out of >24,000) that were differentially affected by age. Some upregulated genes included

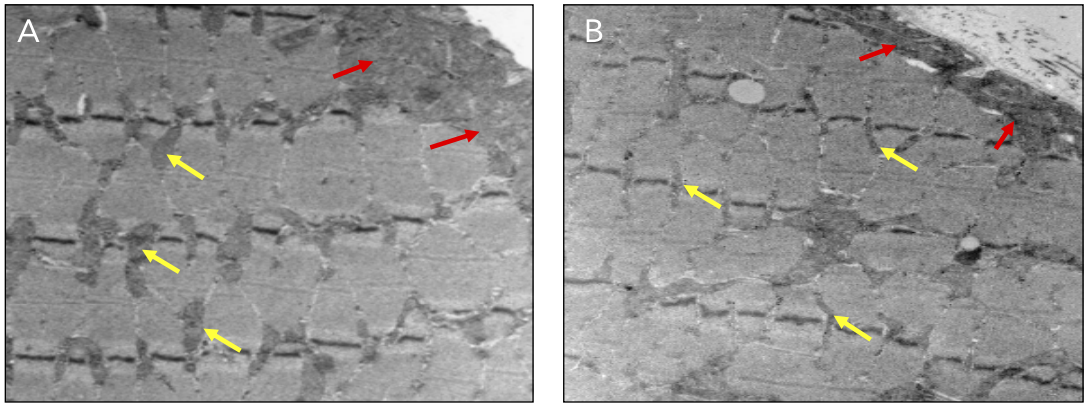

FIGURE 1. **Subsarcolemmal and intermyofibrillar mitochondria in young and aged muscle**
A: in young, healthy muscle, there is a dense layer of SS mitochondria (red arrows), and IMF mitochondria are thick and reticular (yellow arrows). *B*: aged muscle contains a thinner layer of more fragmented SS mitochondria (red arrows), and IMF mitochondria are less reticular (yellow arrows).

those involved in DNA repair, cell death, transcription, and cell cycle control, whereas the 306 genes reduced with age were mainly implicated in mitochondrial function and metabolism. Remarkably, of the 596 genes associated with age, 179 were normalized toward a younger transcriptomic signature with 6 mo of resistance training. Phillips et al. (126) also recently compiled a list of 500 genes that track with age and distinguished these genes from those responsive to various forms of physical activity. However, although these analyses are important for discovery of novel pathways with aging, it should be acknowledged that changes at the mRNA level are not always reflected at the level of proteins, and a greater divergence of results has been observed when proteomic approaches have been employed (Table 2). Theron et al. (159) identified 35 proteins that were differentially expressed during aging, most of which were downregulated and involved in energy metabolism, the myofilament, or the cytoskeleton. In reviewing the literature, Baraibar et al. (8) concluded that most proteomic studies reported decrements in key mitochondrial enzymes in aging muscle, although some investigators have indicated increased levels (Refs. 118, 119; Table 2). This discrepancy may be related to the type of extract employed for analysis, either whole muscle or isolated mitochondria. Indeed, more research on the investigation of the mitochondrial proteome from pure fractions of mitochondria appears to be required to help us understand the role of this organelle in the etiology of

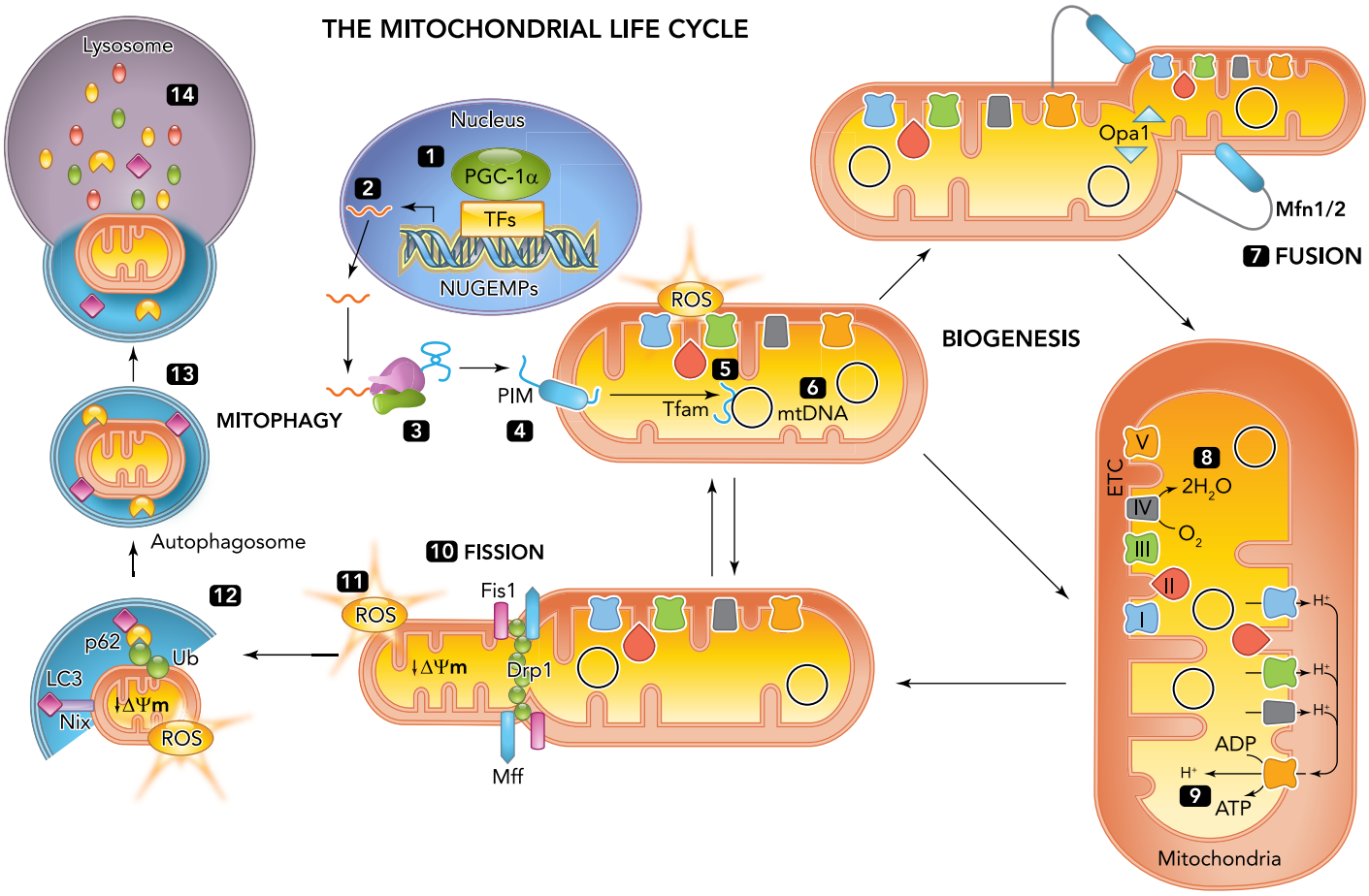

FIGURE 2. **The mitochondrial life cycle**
Under steady-state conditions, mitochondrial turnover occurs through a combination of organelle synthesis and degradation. Binding of transcriptional coactivators, such as PGC-1α, to transcription factors (TFs) results in the expression of nuclear genes encoding mitochondrial proteins (NUGEMPs; *1*) and production of multiple mRNAs (*2*). These mRNAs are exported from the nucleus and translated into protein in the cytosol (*3*). With the assistance of chaperones, proteins destined for the mitochondria will be directed to the protein import machinery (PIM) for import into the organelle (*4*). Once inside, the proteins are directed to the appropriate mitochondrial compartment, and the organelle can expand to contain more ETC machinery. Tfam is a matrix-destined protein that binds mtDNA (*5*) to regulate transcription and mtDNA replication (*6*). Two adjacent organelles can be tethered and fused together through the fusion proteins (Mfn1/2 and Opa1) as an additional mechanism for organelle expansion (*7*). Healthy mitochondria consume oxygen (*8*) and produce ATP (*9*) in the electron transport chain (ETC), in accordance with the cellular demand. When a portion of the mitochondrial network becomes damaged, fission proteins (Fis1, Mff, and Drp1) can be recruited to the dysfunctional site to cleave off the damaged portion (*10*). Typically, dysfunctional mitochondria are recognized through an increase in ROS emission and lower membrane potential (*11*). Once the damaged mitochondrion is separated from the network, it can be flagged for mitophagic degradation by ubiquitination of outer membrane proteins and binding of p62, LC3II, and NIX (*12*). Lipidated LC3II will initiate autophagosome formation to surround the organelle (*12*). Once fully encapsulated, the autophagosome (*13*) is directed to the lysosome. Fusion of the autophagosome with the lysosome results in the degradation of the organelle by proteolytic enzymes to its basic constituents (*14*).

sarcopenia. Additionally, a limited number of the studies that have performed transcriptomic and proteomic assessments in young and aged muscle (Tables 1 and 2) have considered physical activity levels when making comparisons between age groups (44, 45, 97, 111, 167-169). Thus future work should attempt to match subjects for physical activity to tease apart the effect of aging per se from the consequences of physical inactivity on the transcriptome and the proteome.

Morphology and Aging

In aging skeletal muscle, electron micrographs have portrayed images of two very distinct mitochondrial morphologies (FIGURE 1). This includes evidence of the presence of small, fragmented mitochondria compared with that found in young muscle (68, 102), as well as occasional instances of giant mitochondria (10, 116). This puzzling combination forces us to consider the ratio of the expression of fusion and fission regulatory proteins that govern mitochondrial morphology. Based on this, it appears that the rate of mitochondrial dynamics (i.e., fusion and fission) is suppressed in sarcopenic rat muscle, since transcript and protein expression of Drp1, Fis1, Mfn1, Mfn2, and Opa1 were reduced (66). Although some variability exists between studies, other reports from muscle of aged rodents and humans have observed that the balance of these regulatory factors is skewed toward favoring greater rates of fission, compared with fusion, within aged muscle (68, 73, 177). This appears to coincide with the preponderance of data that document a more fragmented organelle phenotype in muscle with age (FIGURE 3).

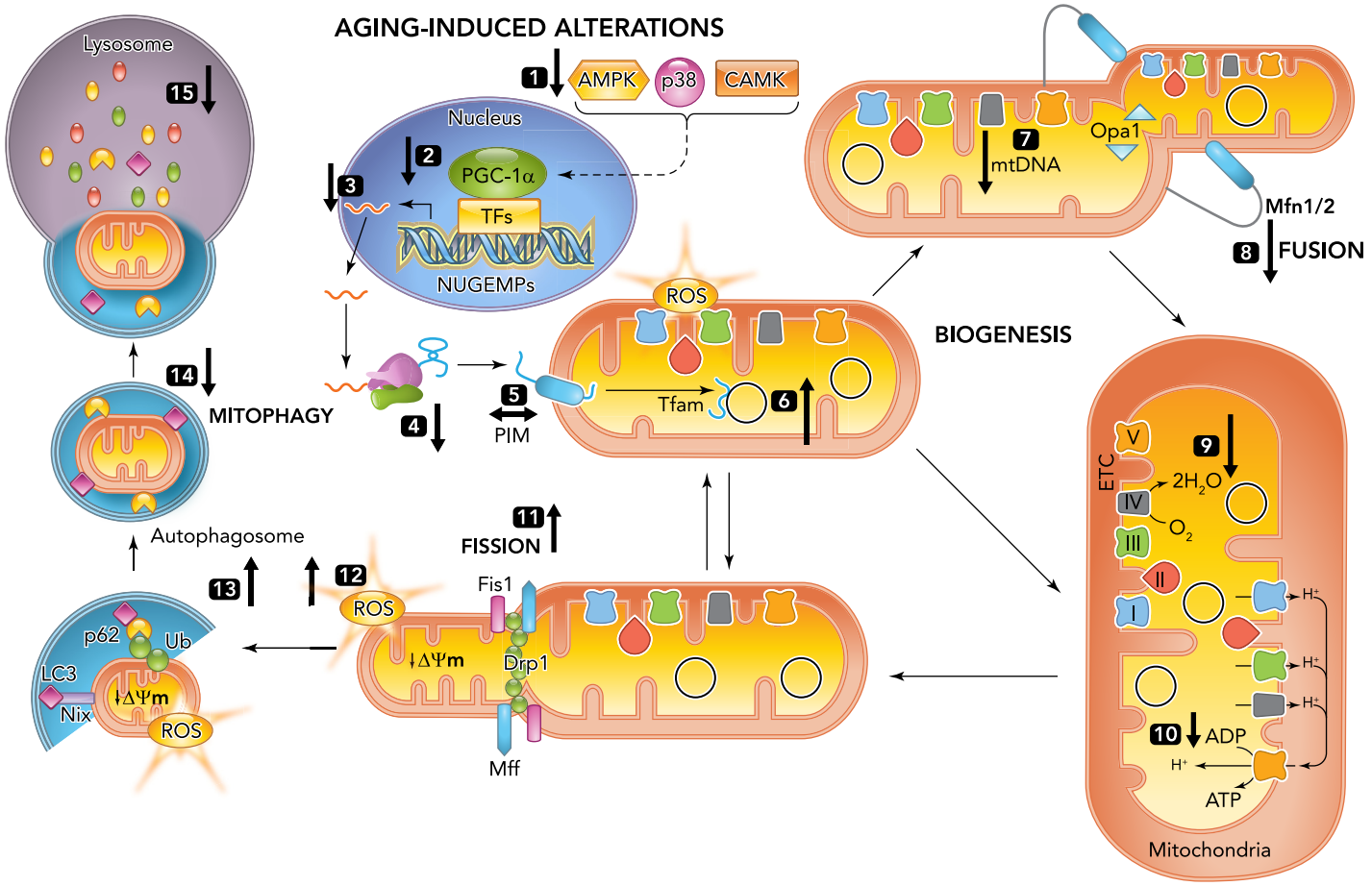

FIGURE 3. **Aging-induced effects on the mitochondrial life cycle**
Signaling kinase activation in response to exercise is reduced in aging muscle (*1*). PGC-1α expression is decreased (*2*), coinciding with the reduced expression of NUGEMPs (*3*), leading to decreased protein levels (*4*). Despite this reduction, the import of synthesized proteins remains intact in aged skeletal muscle (*5*). Interestingly, Tfam protein expression and binding to mtDNA have been observed to be increased in aged muscle (*6*). However, aged muscle contains reduced copies of mtDNA, of which the mutation/deletion load is increased (*7*). Mitochondria in aged muscle exhibit fragmentation, suggestive of an imbalance in mitochondrial dynamics [fusion (*8*) and fission (*11*)]. Although it remains controversial, oxygen consumption (*9*) and ATP production (*10*) have been observed to be compromised in many studies comparing aged and younger counterparts. These defects may be mediated through the accumulation of mtDNA defects, increased ROS production (*12*), and defective ETC machinery. Ideally, these defective mitochondria would be cleared from the cell, and aged muscle retains a high expression of autophagic and mitophagic proteins (*13*). Whether the encapsulation of mitochondria into autophagosomes is impaired in aging muscle remains unknown (*14*). At the lysosome, lipofuscin accumulation is evident with aging, suggestive of an impaired ability of the lysosome to degrade defective organelles, potentially leading to their accumulation (*15*).

Table 1. *Transcriptomic comparison between young and aged muscle*

Species and Sex	Age Groups Compared	Muscle Analyzed	Age Effect on Mitochondrial Genes	Reference
Human, M, F	20–22 vs. 65–70 yr	V. lateralis	↓	111
Human, M	21–24 vs. 66–77 yr	V. lateralis	↓	167
Human, F	20–29 vs. 65–71 yr	V. lateralis	↓	168
Human, M, F	22–24 vs. 70–73 yr	Biceps brachii	↓	97
Human, M	21–27 vs. 67–75 yr	V. lateralis	↓	169
Rhesus Monkey, M	8 vs. 26 yr	V. lateralis	↓	79
Rat, M	3–4 vs. 30–31 mo	Soleus	↓	123
Rat, M	6, 12, 18, 21, 24, and 27 mo	Gastrocnemius	↓	66
Rat, M	3 vs. 24 mo	Gastrocnemius	↓	103
Rat, M	4 vs. 30 mo	Gastrocnemius	↓	3

M, male; F, female.

PGC-1α and Aging

PGC-1α mRNA and protein content are reduced in both slow- and fast-twitch muscles with age (21, 30, 45, 82, 147), suggesting that reductions in mitochondrial function or content could be attributable to the loss of this coactivator. Lessons from PGC-1α transgenic and knockout animals have been instrumental in the understanding of the role that this coactivator plays in mitochondrial biogenesis and aging. For example, Wenz et al. (170) overexpressed PGC-1α in muscle in both young (6 mo) and aged (22 mo) mice. The elevated levels of PGC-1α prevented atrophy and preserved muscle fiber integrity, retained mitochondrial content and function, increased antioxidant capabilities, and subdued markers of apoptosis. The importance of PGC-1α with age is further supported by the study of Leick et al. (91), which examined whether the coactivator was necessary to extend the benefits of exercise training into older age. Markers of mitochondrial content were decreased with age as expected, and this was prevented with endurance exercise training. However, training was incapable of rescuing the decline of mitochondrial markers in animals that lacked PGC-1α, indicating that PGC-1α is necessary to stimulate the beneficial effects of exercise on mitochondrial content during the aging process. Thus elucidation of the mechanisms that cause reductions in PGC-1α with age are needed.

Decreases in PGC-1α expression with age could be attributable to reduced transcriptional drive (FIGURE 3), a result of impaired signaling, or to epigenetic modifications. Gene expression is a tightly regulated process, influenced by the availability of transcription factors and coactivators, and by the interaction of DNA with proteins within chromatin. DNA is subject to methylation on cytosine nucleotides, which, if in the promoter region, suppresses gene transcription, thereby reducing the amount of mRNA available for translation (6). A recent study examining genome-wide methylation in young and aged human muscle demonstrated an increased frequency of hypermethylated sites in aged muscle compared with the younger subjects (178). Since 99% of the genes required for mitochondrial function are derived from the nuclear genome, DNA methylation-induced gene-silencing could potentially impair the expression of genes critical for mitochondrial maintenance. Indeed, in a study examining the COX7A1 gene, a subunit of cytochrome oxidase, aging subjects displayed increased methylation and reduced gene expression (137). However, acute exercise appears to produce a counteractive effect, inducing the demethylation of promoters critical for exercise adaptation, including PGC-1α (9). Demethylation preceded the rise in gene expression, suggesting that this change in DNA conformation is an early response to exercise. Whether this mechanism can operate within aged skeletal muscle to enhance PGC-1α expression has not been studied.

Mitochondrial Protein Import and Aging

A defect in the protein import machinery, comprising the cytosolic molecular chaperones, the translocases of the outer membrane (TOM), and the translocases of the inner membrane (TIM) could seriously compromise the process of organelle biogenesis. Indeed, import of nuclear-encoded mitochondrial proteins is strongly correlated with indexes of biogenesis (149), and defects in the pathway are a cause of disease (59, 151). Thus it is possible that alterations in aged muscle arising in the protein-import process could underlie mitochondrial dysfunction. Interestingly, this does not appear to be the case (FIGURE 3). In fact, Craig and Hood (33) demonstrated greater rates of protein import into the matrix of mitochondria isolated from cardiac muscle of aged animals compared with younger ones, whereas Huang et al. (62) showed no difference in the import of matrix-destined proteins in skeletal muscle mitochondria between young and aged animals. With regard to import into the outer mitochondrial membrane, Joseph et al. (75) found that the import of Tom40

Table 2. *Proteomic assessment between young and aged muscle*

Species and Sex	Age Groups Compared	Muscle Analyzed	Extract Type	Age Effect on Mitochondrial Proteins	Reference
Human	47–62 vs. 76–82 yr	V. lateralis	Muscle	↑	154
Human, F	53 vs. 77 yr	V. lateralis	Muscle	↓	159
Human	18–30 vs. 65+ yr	V. lateralis	Mitochondria	↓	45
Human	20–25 vs. 70–76 yr	V. lateralis	Muscle	↑	44
Rat, M	3 vs. 24 mo	Gastrocnemius	Muscle and Mitochondria	↓	103
Rat, M	7 vs. 18 vs. 30 mo	Gastrocnemius	Muscle	↓	130
Rat, M	6 vs. 18 vs. 27 mo	Gastrocnemius	Muscle	↓	66
Rat, M	3 vs. 24 mo	Soleus, EDL	Muscle	Soleus ↓, EDL ↑	23
Rat, F	6–7 vs. 24–25 mo	Gastrocnemius	Mitochondria	↑	22
Rat	3 vs. 26 mo	Gastrocnemius	Mitochondria	↑	118
Rat, M	4 vs. 30 mo	Gastrocnemius	Muscle	↓	3
Rat, M	8 vs. 22 mo	Gastrocnemius	Muscle	↑	17
Mouse	6 vs. 27 mo	Gastrocnemius	Muscle	↑ ↓	65

Some studies did not indicate the sex investigated.

did not differ between young and aged animals, and that Tom40 assembly into the TOM complex was higher in mitochondria from aged compared with young animals. Thus a possible defect in this important pathway leading to expansion of the organelle network is not the reason for mitochondrial dysfunction or reduced content in aging muscle.

mtDNA, Tfam, and Aging

Mammalian mtDNA encodes 13 polypeptides that are critical components of the ETC. With mutations or deletions in mtDNA, the ETC defects that ensue can affect mitochondrial ATP production, leading to enhanced reliance on glycolysis, significant acidosis, and poor exercise tolerance. Mitochondria are unique since multiple copies of DNA can exist within an organelle, some of which may be mutated, whereas others may be normal. This distribution is referred to as heteroplasmy. In addition to mutations, mtDNA levels can be depleted, and evidence exists for this in aged muscle (15, 90, 104, 110), contributing to a reduced ETC capacity. A variety of mtDNA mutations accumulate with age, with exponential growth occurring with the seventh decade of life (15, 77, 162). One of the most common of these is the 4,977-bp deletion, detected at higher frequency in postmitotic tissues that have high ATP requirements, such as brain, heart, and skeletal muscle (31, 32, 95, 110). Common mutations in mtDNA also occur at the displacement loop (D-loop) structure and contain promoter regions for transcriptional initiation (see review in Ref. 39). For example, Wang et al. (165) found two point mutations in the D-loop region that negatively affected the transcription and replication of mtDNA in human skeletal muscle biopsies from older individuals. These mtDNA mutations tend to persist as an organism ages because of the preferential replication of mutated or deleted mtDNA (84).

Faulty proofreading mechanisms can also result in the accumulation of mtDNA mutants. Using mice that were deficient in DNA polymerase γ, an enzyme responsible for proofreading, both Trifunovic et al. (160) and Kujoth et al. (85) demonstrated an accretion of mtDNA mutations and phenotypic features of aging and sarcopenia. Further support for this comes from the work of Aiken and colleagues, who used longitudinal single muscle fibers from both aged animals (56, 164) and human subjects (15), and demonstrated that certain sites in aged fibers were highly susceptible to breakage, atrophy, and dysfunction when mutations exceeded 80% of total mtDNA. Thus the proper maintenance and function of mtDNA, along with the mtDNA mutation load, are important in determining regional changes in fiber size and the preservation of muscle mass with age. An argument has been made that mtDNA mutations likely do not account for the majority of organelle dysfunction characteristics that are evident with age, because the onset of organelle dysfunction, measured using whole muscle imaging techniques, occurs at earlier stages in life than the detectable accumulation of mtDNA defects in muscle homogenates (27). Indeed, Brierley et al. (13) questioned the importance of mtDNA mutations for the age-related decline of muscle function, given the relatively low abundance of mtDNA abnormalities leading to COX^- ragged red fibers with age and the closer association of mitochondrial defects with self-reported physical activity levels rather than aging per se. However, other studies indicate that ETC abnormalities detected at the single-fiber level can be found in ~6% of muscle fibers at age 49, a fraction of which increases to 31% by age 92 (15). This represents an increase of ~0.5% per year in the number of ETC abnormal fibers, a fraction that

could contribute to the 1–2% decline in muscle mass per year (12) between 50 and 90 years of age.

Could an enhancement of mitochondrial biogenesis improve, or deteriorate, the level of mtDNA mutations and ETC abnormalities that are evident with age? Because mutated or deleted mtDNA appears to be preferentially replicated in cells (84), the accumulation of such deletion mutants could be enhanced when replication factors such as Tfam (below) are increased with mitochondrial biogenesis stimuli. Recently, Herbst et al. (55) used β-GPA to increase mitochondrial biogenesis in aged animals, and this resulted in a greater incidence of ETC abnormal fibers with mtDNA deletion mutations. With respect to the use of exercise, Taivassalo et al. (157) originally found that endurance training improved performance and mitochondrial function in mtDNA patients, but also increased the fraction of mutated mtDNA. However, subsequent studies have found that the mutation load remained unchanged with endurance training (70, 156) or was actually reduced in the mutator mouse model of aging (141). Clearly, further research is required to clarify the role of endurance exercise-induced biogenesis on mtDNA mutation load in muscle. Interestingly, resistance training may offer a more optimal solution for patients or elderly individuals with mtDNA mutations. The idea comes from the identification that satellite muscle cells harbor no mtDNA mutation load, thus their activation by resistance exercise, which promotes fusion of these cells and their organelles with exercised myofibers, will thereby shift mtDNA toward a healthier genotype, thus improving mitochondrial quality and function (155). One recent study indicated that the mutation load was not significantly altered in mtDNA mutation patients with resistance training (115), but further research is needed in this area, particularly with aging muscle. Clearly, resistance training is an effective modality for improving muscle strength and function, but whether it has an impact on mtDNA mutation load in aging muscle remains to be determined.

Independent of mtDNA mutations, the sufficient expression of mtDNA gene products is required for optimal ETC function. Tfam is the most important transcription factor involved in the replication and transcription of mtDNA. With aging, some studies have reported enhanced protein content of Tfam in skeletal muscle (21, 82, 94, 124), whereas others have described decreases (74, 109). Investigators have speculated that increases represent a cellular compensatory response in the face of mitochondrial enzyme deficiencies in an attempt to preserve organelle integrity (35). However, studies reporting declines in Tfam have also documented increased binding of Tfam to distinct regions of mtDNA (74). Indeed, binding may be a more informative indicator of Tfam activity, but the consequences of this increased binding, in the presence of declining mtDNA levels in aging muscle, are not clear.

Apoptosis and Aging

Apoptosis, in the form of myonuclear decay, is a likely mechanism that contributes to sarcopenia (4, 21, 36). Mitochondria are regulators of apoptosis, since ROS emission from the ETC can trigger the opening of the mtPTP, resulting in a loss of membrane potential, a decrease in ATP synthesis, and swelling of organelles. Pro-apoptotic proteins such as cytochrome c, apoptosis-inducing factor (AIF), and endonuclease G (Endo G) are then released to the cytosol, leading to DNA fragmentation in a caspase-dependent (cytochrome c) or -independent manner (AIF, Endo G). In aging muscle, mitochondrial ROS production is elevated (FIGURE 3) and calcium retention is reduced, leading to an increased release of cytochrome c and Endo G from the organelle, compared with young muscle (21, 47). The result is an approximately threefold greater rate of myonuclear DNA fragmentation in aged muscle. If localized within a specific region of a fiber, this nuclear decay could lead to regional atrophy and possible disappearance of this fiber segment, as documented by Bua et al. (16). Interestingly, this myonuclear decay in aging muscle can be completely reversed with chronic contractile activity (102, 133). Indeed, chronic exercise can increase anti-apoptotic Bcl-2 levels, reduce apoptotic protein release from mitochondria, and decrease DNA fragmentation (1, 152), illustrating at least one mechanism through which exercise can potentially preserve muscle mass through improved mitochondrial function.

Mitophagy and Aging

The specific removal of dysfunctional mitochondrial segments via mitophagy is essential to maintain a healthy population of organelles within the muscle. Aberrations in the ability of muscle cells to effectively remove dysfunctional mitochondria can contribute to enhanced ROS production, and impairments in this pathway have been linked to sarcopenia. Indeed, inhibition of the autophagy cascade through deletion of the crucial regulator Atg7 in skeletal muscle promotes muscle wasting (108), along with the accumulation of dysfunctional mitochondria that display impaired respiration and increased ROS production (172).

Considerable information on the regulation of autophagy/mitophagy in muscle has been gleaned from rodent models. However, divergent results exist, reporting either increases (119), decreases (43), or mixed changes (74, 171) in autophagic/mitophagic regulators with aging. A recent study

from our laboratory documented an increase in the localization of Parkin and p62 to the mitochondria, suggestive of an increase in mitophagy with age (119). However, also evident was a marked accumulation of lipofuscin granules, suggestive of lysosomal dysfunction (119). This could directly impact the removal of mitochondria in aged muscle by preventing the fusion of the autophagosome with the lysosome (FIGURE 3). Lipofuscin has previously been suggested to be a robust marker of aging human muscle (64). This is consistent with the mitochondrial-lysosomal theory of aging, which suggests that lysosomal dysfunction leads to an accumulation of aberrant mitochondria (158). In addition, defects in the process of mitochondrial removal may be a cause of the presence of large, mega-mitochondria, which are occasionally observed in aged muscle.

Research examining autophagy/mitophagy with aging, particularly in human skeletal muscle, is not well described, and this represents an area for intensified focus. Cross-sectional comparisons of autophagy/mitophagy markers from young and aged men have shown that older men exhibited increases in the protein markers Beclin-1, Bnip3, and p62 compared with young adults (174), whereas aged-match seniors who engaged in life-long exercise had decreased levels of these same factors. In contrast, it has also been reported that inactive older women had reduced expression of autophagy genes compared with a more active older cohort (38). Thus sex-specific differences in the regulation of mitochondrial breakdown with aging may be evident.

Exercise is a potential candidate treatment to improve mitophagy and thus muscle health. Recent evidence in young animals has demonstrated that a single acute bout of endurance exercise increases autophagy/mitophagy markers (49, 53, 143) and may assist in the removal of defective mitochondria postexercise (FIGURE 4). Whether successive bouts of exercise continue to stimulate autophagy and mitochondrial turnover is not well described. One month of treadmill training resulted in an increase in the ratio of LC3-II to LC3-I expression and decreased p62, suggestive of activated autophagy (96). However, longer term training led to a reduction in total LC3, and no change in the ratio of the activated to inactive form (49). These data suggest that large changes in autophagy occur early on with an exercise training stimulus. When sufficient adaptation has occurred, there may be a reduced requirement for autophagy, likely due to the presence of a healthier population of organelles. Thus engagement in exercise for older individuals can likely promote mitochondrial benefits by reversing the attenuated rates of mitophagy evident with age and by stimulating the removal of damaged organelles. However, this is an area that requires considerable clarification, especially with respect to the effects of exercise and possible sex-specific differences.

Mitochondrial Adaptations With Exercise in Aging Muscle

It is well established that exercise is a potent inducer of mitochondrial biogenesis in younger individuals. In the 1980s, the paucity of data regarding the mechanisms by which exercise may improve mitochondrial content and muscle health in the aging individual led to an urgent call to identify and further explore how exercise may benefit the aging population (57). This is now a very active field of exercise physiology research.

Is aging muscle equally adaptive to an exercise regimen as muscle from younger individuals? An important issue involved in comparing the adaptive response of young and old subjects to exercise is ensuring that the training workloads are comparable. An exercise training program that employs the same progressive, relative workloads (i.e., $\dot{V}O_{2max}$) over time would represent an ideal experimental design. This would control for age-related differences in $\dot{V}O_{2max}$. A compilation of exercise training studies in both humans and rodents in which the same absolute or relative workloads can be readily compared between young and older subjects is provided in Table 3. Although basal differences in organelle content were not always observed, these studies suggest that the mitochondrial adaptations that occur are comparable in magnitude when training intensity is matched between young and old groups. Similarly, in scenarios where the absolute training intensity is identical between young and aged groups, therefore providing the older group with a greater relative exercise intensity, similar adaptations are observed at the completion of a long-term training program.

Many studies have investigated mitochondrial adaptations in older humans; however, they have not consistently employed the use of a younger group for comparison. This makes it difficult to conclude on the degree of mitochondrial adaptation in young compared with older individuals. Nonetheless, many studies using both cross-sectional and longitudinal designs indicate that mitochondrial concentration can increase in both older men and women with exposure to exercise (Table 3). For example, this is evident from the high mitochondrial content evident in male master athletes (25), as well as from the few training studies that have used older female subjects (14, 83). Further support comes from studies that compare active older adults to sedentary counterparts. The active older groups have preserved mitochondrial

content and function, higher PGC-1α expression, and greater capacity to defend against oxidative stress (FIGURE 4) (73, 142).

There is considerable evidence from both human and rodent studies to support the idea that aged muscle ultimately retains the capacity to elicit mitochondrial biogenesis in a manner similar to that of young muscle (Table 3). Research conducted using rodent models has frequently employed a variety of exercise modalities, including treadmill running or electrical stimulation-invoked chronic contractile activity (CCA) to elicit mitochondrial biogenesis (Table 3). A common feature of many of these prior studies is that the old animals were ~2 yr old, which is considered to approximate 60 yr in a human being (133a). In this age range, an initial defect in mitochondrial content is not always observed. Thus a potential limitation of some of these studies is that the animals' ages were not most fully representative of "aged" or "senescent" muscle. In addition, although a number of rat strains have been used for the study of aging muscle in the past, a recent review of the rodent models of aging has pointed out that the Fischer 344 Brown Norway F1 Hybrid rat most closely resembles human aging muscle since this species lives to ~3 yr of age, equating to ~90 human years (7).

Rodent models of exercise have provided considerable insight into the molecular regulation of mitochondrial biogenesis in aging muscle and afford the possibility of strict control over the absolute training workload. Several studies using the CCA model (98) have been used to advantage to study the effects of "exercise" on mitochondrial adaptation in aging muscle. Walters et al. (163) found that electrical stimulation-invoked exercise of the flexor digitorum longus in young and aged rodents increased citrate synthase activity; however, the rate of increase in the aged animals was attenuated in the early time course of the experiment.

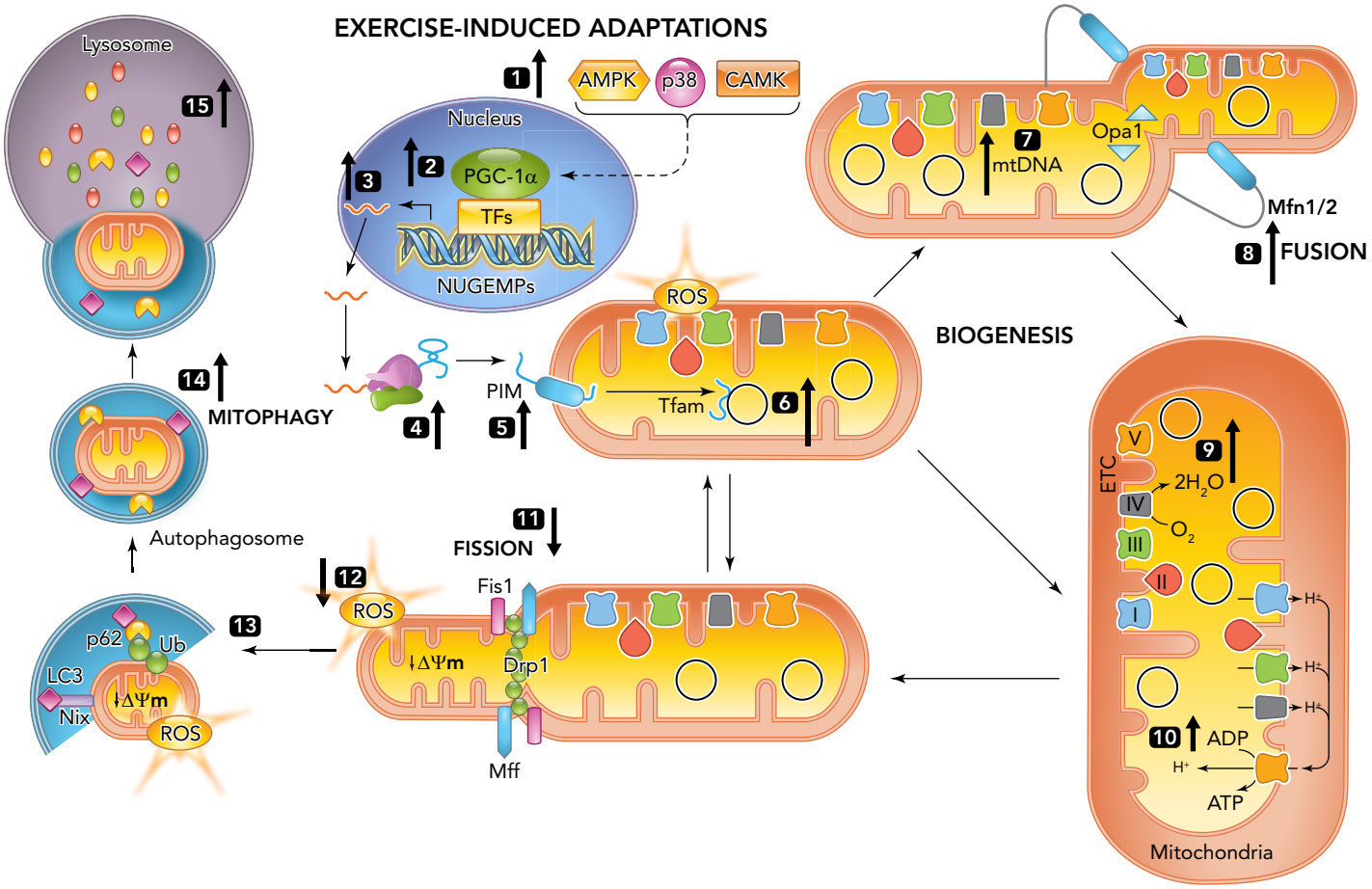

FIGURE 4. **Exercise-induced effects on the mitochondrial life cycle**
With an acute bout of exercise, there is an immediate increase in signaling activation (*1*), which promotes posttranslational and transcriptional increases in PGC-1α (*2*). This leads to an increased expression of NUGEMPs (*3*), and with chronic exercise exposure, these transcriptional increases result in an accruement of mitochondrial proteins (*4*). Protein import machinery (PIM) components (*5*), as well as Tfam (*6*), are also upregulated with chronic exercise facilitating greater rates of organellar import and mtDNA gene expression (*7*), respectively. Electron micrographs portray images of larger, reticular mitochondria, indicating a shift in mitochondrial dynamics to favor fusion (*8*, *11*). These exercise-induced increases in mitochondrial content result in a greater capacity for oxygen consumption (*9*) and ATP synthesis (*10*) per gram of muscle. Improvements in ETC functionality also favor a reduction in ROS production (*12*). Acute exercise increases mitophagy (*13*) as well as autophagosome formation (14), mediating an increased degradation of the organelles (*15*), and when combined with exercise-induced biogenesis, this maintains high-quality mitochondria within the muscle.

Table 3. *Mitochondrial adaptation to training in young and aged subjects*

Species and Sex[1]	Age Groups Compared	Matched Training Intensity[2]	Muscle Used	Training Protocol[3]	Mitochondria Decreased With Age[4]	Mitochondrial Adaptation in Young[4]	Mitochondrial Adaptation in Aged[4]	Reference
Human, M	22 vs. 69 yr	Relative	V. lateralis	Cycling, 12 wk	No	Yes	Yes, equal	114
Human, M, F	21 vs. 87 yr	Relative	V. lateralis	Cycling, 16 wk	Yes	Yes	Yes, equal	148
Human	18–30 vs. 65+ yr	Relative	V. lateralis	Cycling, 16 wk	Yes	Yes	Yes, equal	45
Rat, M	10 vs. 24 mo	Absolute	Gastrocnemius	Treadmill running, 6 mo	No	Yes	Yes, equal	40
Rat, M	4 vs. 18 mo	Absolute	Gastrocnemius	Treadmill running, 6 mo	Yes	Yes	Yes, equal	18
Rat, M	5 vs. 24 mo	Absolute	EDL	CCA, 50 days	No	Yes	Yes, equal	150
Rat, M	6–8 vs. 26–28 mo	Absolute	FDL	CCA, 90 days	No	Yes	Yes, less than young	163
Rat, M	6 vs. 36 mo	Absolute	TA	CCA, 7 days	Yes	Yes	Yes, less than young	102
Rat, F	6 vs. 24 mo	Absolute, relative	Gastrocnemius	Treadmill running, 8 wk	Yes	Yes	Yes, equal or better	140
Rat, M	2 vs. 21 mo	Relative	Soleus	Treadmill running, 12–13 wk	No	Yes	Yes, equal	88
Rat, M	18 vs. 24 mo	Relative	Plantaris	Treadmill running, 8 wk	No	Yes	Yes, equal	121

[1]Some studies did not indicate sex investigated. [2]Training intensity compared between studies and indicated whether relative (i.e., % $\dot{V}O_{2max}$) or absolute (identical exercise protocols) were used for young and aged subjects. [3]CCA, electrical stimulation-invoked chronic contractile activity. [4]Measures examined included mitochondrial content (COX activity, citrate synthase) and/or function (i.e., respiration, ATP synthesis).

Yet, by 90 days, young and aged animals had equivalent levels of this mitochondrial enzyme marker. In another study, CCA over 50 days effectively increased mitochondrial content and reduced the number of COX-deficient fibers in aged muscle (125, 150). These data illustrate the potential corrective nature of chronic exercise in ameliorating ETC dysfunction but also suggest that the kinetics of mitochondrial adaptations in old muscle are delayed in response to an exercise regimen.

Support for the idea of reduced adaptive kinetics of aged muscle has been found in other studies (24), particularly at the onset of exercise-induced adaptations (100). Work from our laboratory using a short-term CCA protocol (7 days) of the rat tibialis anterior muscle indicated reduced mitochondrial biogenesis in old muscle compared with young muscle (102). This blunted response in mitochondrial proliferation was attributable to reduced elevations of PGC-1α and Tfam, in addition to a lack of alterations in protein import machinery components in aged muscle. Despite evidence of an attenuated adaptation of important transcriptional regulators to CCA, we and others have nonetheless found that the decrements in PGC-1α expression can be at least partially recovered with exercise (78, 82, 102, 148). We subsequently questioned whether this reduced early adaptive response could be a result of disrupted exercise signals to the coactivator in aging muscle. Acute exercise is known to elicit the activation of p38 mitogen-activated protein kinase (MAPK), AMP-activated protein kinase (AMPK), and Ca^{2+}/calmodulin-dependent protein kinase IV (CAMKIV), which impinge on PGC-1α transcription and activity (176). Our studies, and those of others, have revealed that, after acute exercise, aged muscle is less capable of activating these upstream kinases (FIGURE 3) (52, 100, 135). Interestingly, this attenuated signaling, leading to a reduced mRNA response, has also been repeatedly demonstrated in response to resistance exercise protocols in old compared with young subjects (41, 42, 86, 134, 136). Thus the reduced activation of important kinases regulating mitochondrial biogenesis may be partly responsible for the delayed and diminished adaptation of mitochondria to exercise in senescent muscle.

In addition to increasing organelle content, can exercise reverse potential mitochondrial dysfunction at the organelle level? Many studies examining the effect of exercise training on mitochondrial respiratory function have found no changes in well coupled organelles. However, plentiful data now exist supporting the idea that, when mitochondrial respiration is impaired below normal, chronic exercise can serve to reverse this back toward a healthy functional status. This has been documented in aging humans (28, 76), patients with mtDNA defects (157), mice lacking PGC-1α (2) or SirT1 (112), and myotubes deficient in mTOR activity (19). Chronic exercise can also repair mitochondrial protein import defects via the increased expression of import machinery components (175).

Taken together, these data suggest that aged muscle is capable of increasing mitochondrial content, although the rate of onset at which this increase takes place may be reduced. The extent of this change is clearly dependent on the age of the subjects, their health, the presence or absence of various co-morbidities, as well as the intensity, duration, and frequency of the exercise dose, as expected. Exercise also can be used as an intervention to repair dysfunctional mitochondria and restore coupling efficiency, ultimately improving aerobic energy provision in aging muscle.

Concluding Perspectives

Mitochondrial functional decline with age and restoration with exercise. In sedentary individuals, a decline in mitochondrial content and

Table 4. *Calculated rates of change for human muscle and mitochondrial parameters between 50 and 80 yr*

Location	Parameter	Approx. Age of Onset, yr	Estimated Rate of Change Between 50 and 80 yr	Reference	Can Exercise Partially Reverse the Change?	Reference
Whole body	$\dot{V}O_{2max}$	25	↓ 1%/yr	12	Yes	61, 113
Muscle	Fiber no.	50	↓ 1.4%/yr	12, 92	Unlikely	
	Motor unit no.	50	↓ 1–2%/yr	12	Unlikely	
	Mass	45	↓ 0.5–1.4%/yr	12, 34, 69	Yes	87
	Strength	50	↓ 1.5–4%/yr; ↓ 3%/yr after age 70	12, 46, 63	Yes	87
Mitochondria	Content	25	↓ 0.6%/yr	147	Yes	See Table 3
	Respiration	20–25	↓ 0.3–1.4%/yr	11,147,161	Yes	See text
	Fibers with ETC abnormalities	25	↑ 0.5%/yr	15	Controversial	See text
	Mutant mtDNA (% WT)	25	↑ 0.05%/yr	77	Controversial	See text

function with advanced age is a common finding, revealed at the mRNA, protein, morphological, and functional (i.e., respiration, apoptosis) levels. Part of this decline is reversible with a program of regular exercise, observed in humans and rodent models of aging, suggesting that at least a portion of the deterioration observed is caused by decreases in physical activity levels. Indeed, mitochondria within muscle of previously sedentary aged individuals are responsive, over the long term, to a diversity of contractile activity stimuli, including resistance training, which would otherwise have considerably less effect on mitochondria in younger subjects.

Why only partial restoration of function with exercise? A lack of complete restoration of mitochondrial content and/or function with exercise could be a result of an insufficiently applied exercise stimulus (i.e., duration, frequency, or intensity of the program). Evidence from master athletes or chronic training experiments in rodents suggests that, if pursued long enough, the extent of the mitochondrial adaptations can potentially be similar between younger and older subjects (Table 3). The fact that chronic exercise can, at least in part, restore mitochondrial function and the upregulation of NUGEMPs leading to improved functional consequences for mitochondria suggests that these are physical activity-responsive genes and that only a portion of the dysfunction is related to aging per se. This partial restoration of function underscores the value of exercise in attenuating mitochondrial dysfunction observed with aging. However, studies reporting either *1*) age-associated decrements in mitochondrial function or *2*) no difference in function between aged and younger individuals will continue to be found in the literature, with the distinction being dependent on how well the young and older groups were matched based on prior physical activity (Table 3).

One notable difference between young and aged muscle appears to be in the kinetics of the adaptive response to the same, or even higher, relative workload. This divergent response is initiated by the blunted signaling observed as a result of acute endurance or resistance exercise. Typically, muscle with a lower oxidative capacity generates a more robust increase in the kinase signaling response to acute exercise (101). This is not the case with aging muscle, suggesting the presence of deterioration in stress-induced signaling kinase activation. This altered signaling, along with probable epigenetic modifications with age, are likely involved in attenuating the kinetics of mitochondrial adaptations with training. Alternatively, a rise in the expression of age-specific genes, unrelated to physical activity-sensitive genes (126), may participate in suppressing the restorative functions of exercise. One simple approach to the identification of these genes might be to compare age-related changes in the transcriptome within a variety of tissues with high mitochondrial energy requirements (e.g., muscle, kidney, brain), which are not susceptible to exercise-induced changes, to find an age-related expression signature that could shed light on this issue.

The role of mtDNA mutations in mitochondrial dysfunction and sarcopenia. An important role for mtDNA mutations in determining the deterioration of mitochondrial function during aging seems unlikely, given the magnitude of the mutation load (% mutated vs. non-mutated mtDNA) in muscle even in the middle-aged years of life when mitochondrial dysfunction begins (30). However, data on the age of onset of mtDNA mutations, their clonal expansion, and the preferential replication of defective, compared with wild-type, genomes within single fibers seem to suggest that mtDNA mutations could contribute, progressively, to sarcopenia. Table 4 presents an interpretative summary of the rates of changes of "muscle" and "mitochondrial" parameters over the course of time between 50 and 80 years of age. The data confirm the long-held belief that changes in strength exceed the loss of muscle mass, pointing

to a change in the quality of muscle with age. Some of this qualitative difference between young and old muscle could be a result of mitochondrial alterations, since rates of change in organelle function and the accumulation of ETC-abnormal fibers fall within the rates observed for muscle mass decline between 50 and 80 years of age. For example, if each of these ETC abnormal fibers were to undergo cell death, this could contribute, in part, to the sarcopenic rate of muscle loss of 1–2% per year (Table 4). Indeed, further research at the level of individual fibers holds considerable promise in helping to unravel the role of mitochondria in the loss of muscle mass and performance with age.

Focus for the Future

A number of research areas require intensified focus to help us understand the relationship between muscle health and mitochondrial function. These include greater comprehension of *1*) the mitochondrial proteome and the aging transcriptome, *2*) mtDNA mutation load and the effects of different types of exercise training, *3*) the transcriptional regulation of PGC-1α and Tfam, *4*) the role of mitophagy in mitochondrial quality control with training, *5*) mitochondrial dynamics in skeletal muscle, and *6*) possible sex-specific differences in the regulation of mitochondrial content in muscle. ■

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