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Mitochondrial Biogenesis in Skeletal Muscle: Exercise and Aging

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1. Introduction

Mitochondria are abundantly present in mammalian cells. Their fraction varies from tissue to tissue, ranging from <1% (volume) in white blood cells to 35% in heart muscle cells. However, mitochondria should not be thought of as single entities, but rather a dynamic network that continuously undergoes fission and fusion processes. In skeletal muscle, mitochondria exist as a reticular membrane network. The subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria are located in distinct subcellular regions, and they possess subtle differences in biochemical and functional properties that are characterized by their anatomical locations. SS mitochondria lie directly beneath the sarcolemmal membrane and the IMF mitochondria are located in close contact with the myofibril. Their different properties are likely to influence their capacity for adaptation. SS mitochondria account for 10-15% of the mitochondrial volume and this population has been shown to be more susceptible to adaptation than the IMF mitochondria. However, the IMF mitochondria were found to have higher rates of protein syntheses, enzyme activities and respiration (1).

The mitochondria are equipped with double membranes, crating the intermembrane space between the outer and inner membranes as well as the inner matrix compartment, where most of the metabolic processes take place. The inner membrane is highly folded, forming so-called cristate, to accommodate its large surface area. Embedded in the inner mitochondria membrane are the five complexes that make up the respiratory chain where oxidative phosphorylation takes place. In this process, a proton gradient across the inner membrane is coupled to ATP synthesis at complex V (2). In addition to producing ATP essential for cell survival, the mitochondria are a source for free radical or reactive oxygen species (ROS), production. ROS are small, highly reactive molecules that can be generated by mitochondrial respiration and in active skeletal muscle.

Mitochondria are unique organelles in that they contain their own DNA, which consists of a circular DNA molecule of about 16.6 kb in humans and 16.3 in mice. It encodes 13 of the around 90 proteins that make up the respiratory chain. In addition, mtDNA also encodes 2 ribosomal RNAs (rRNA) and 22 transfer RNAs (tRNA) (3). The presence of mtDNA is explained by the evolutionary origin of mitochondrion as a free-living prokaryotic organism. During the course of time, genes have been transferred to the nuclear genome, and mitochondrial function is highly depended on close coordination between the nuclear and mitochondrial genomes. In mammals, mtDNA is maternally inherited, the paternal mtDNA being destroyed during the first embryonic cell divisions. The individual stands of mtDNA are termed heavy (H) and light (L) stand. Introns are lacking, but there is a long non-coding region, the D loop, which contains control elements for transcription and replication of mtDNA.

The mitochondria are often referred to as the powerhouses of the cell. In turn, It is well stabilised that mitochondria are the site of oxidative energy production in eukaryotic cells and provide the majority of the total ATP required to maintain normal cellular function and homeostasis. Within skeletal muscle, ATP is primarily required for the energy-dependent cross-bridge cycling between actin and myosin, as well as for Ca^{2+} cycling. Within the mitochondrial matrix, enzymes oxidize fatty acids and carbohydrates producing the reducing equivalents, NADH and FADH₂. These reducing equivalents are then used to produce a proton gradient across the inner mitochondrial membrane. Dissipation of this gradient through the F₀F₁-ATPase results in the resynthesis of the ATP that drives every energy-dependent process in the cell. Studies showed Changes in metabolic demand can directly alter the concentration of mitochondria within the cell. Proliferation of mitochondria occurs in muscle in response to endurance exercise training, chronic electrical stimulation and thyroid hormone, while loss of mitochondria is associated with inactivity and aging.

2. Mitochondrial biogenesis – Effects of exercise

Skeletal muscle is a highly malleable tissue, capable of considerable metabolic and morphological adaptations in response to repeated bouts of contractile activity (i.e. exercise). It is well established that chronic contractile activity, in the form of repeated bouts of endurance exercise, usually interspersed with recovery periods, results in the altered expression of a wide variety of gene products, leading to an altered muscle phenotype with improved fatigue resistance. This improved endurance is highly correlated with the increase in muscle mitochondrial density and enzyme activity, referred to as 'mitochondrial biogenesis'. Mitochondrial biogenesis within muscle consists of two possible mutually inclusive alterations: [1] an increase in mitochondrial content per gram of tissue and/or [2] a change in mitochondrial composition, with an alteration in mitochondrial protein-to-lipid ratio (4). Although this phenomenon resulting from exercise has long been established, many of the detailed molecular mechanisms remain to be identified. This has particular relevance for our understanding of the pathophysiology of mitochondrially based diseases, and may improve our understanding of mitochondrial pathways involved in programmed cell death. Additionally, it has been suggested that an age-related accumulation of

dysfunctional mitochondria may result in progressive reactive oxygen species-induced damage, producing a further impairment of oxidative capacity in aged muscle. Moreover, dysfunctional mitochondria have also been implicated in the age-related loss of muscle mass known as sarcopenia. Thus, mitochondrial biogenesis induced by chronic exercise is now recognized to have implications for a broader range of health issues than just the enhancement of endurance performance.

Therefore, the present chapter will highlight important molecular mechanisms that involved in mitochondrial biogenesis and then we will investigate the exercise effects on these mechanisms. In the second Section of this chapter, we examine the effects of aging on mitochondrial content and function and potential role of exercise in attenuation of age-related mitochondrial dysfunction.

2.1. Most important mechanism that involved in mitochondrial biogenesis

2.1.1. Mitochondrial biogenesis requires the cooperation of the nuclear and mitochondrial genomes

One of the most fascinating aspects of mitochondrial synthesis is that it requires the cooperation of the nuclear and mitochondrial genomes (Figure-1). Mitochondria are unique in the fact that they house multiple copies of a small circular DNA molecule (mtDNA) comprising 16,659 nucleotides. As noted above, this mtDNA is minuscule compared with the 3 billion nucleotides found in the nuclear genome, it nonetheless contributes 13 mRNA, 22 tRNA, and 2 rRNA molecules that are essential for mitochondrial function. The thirteen mRNA molecules all encode protein components of the respiratory chain, responsible for electron transport and ATP synthesis.

Where does the cooperation between the genomes come in? First, these thirteen components comprise only a small fraction of the total respiratory chain proteins. Some act as single protein subunits, but many are combined nuclear-encoded proteins to form multisubunit holoenzymes, like COX or NADH dehydrogenase (Figure-1). The function of these holoenzymes is clearly impaired if contributions from either genome is absent (5). Second, it is known that mtDNA transcription and replication require the import of nuclear gene products, which act as polymerases or transcription factors. Given the diverse promoter regions of nuclear genes encoding mitochondrial proteins, as well as the sequences of the mtDNA promoters, it is not surprising that this coordination can be disrupted. Evidence for this has been presented in cases of thyroid hormone treatment, suggesting that a coordination of gene expression responses leading to strict stoichiometric relationships is not absolutely necessary for the formation of a functional organelle (6).

2.1.2. Exercise effects on cooperation of the nuclear and mitochondrial genomes

A longstanding question has been related to how the two genomes are regulated, or coordinated, in response to a stimulus leading to mitochondrial biogenesis. Williams et al. (7-8) were the first to show that chronic contractile activity led to increases in mRNA levels encoding both nuclear and mitochondrial gene products. Subsequently, this was

demonstrated for subunit mRNAs belonging to the same COX holoenzyme. Because COX contains 10 nuclear encoded and 3 mitochondrial-encoded subunits, this enzyme is a useful model for studying the interactions of the two genomes. The mRNA expression of these subunits is also coordinated across a variety of tissues possessing a wide range of mitochondrial contents. In addition, some evidence for a coordinated regulation of the two genomes was found during the mitochondrial biogenesis induced by cardiac hypertrophy, as well as in human muscle when trained and untrained individuals were compared.

Figure 1. Overall synopsis of mitochondrial biogenesis in a muscle cell. Signals originating at the neuromuscular junction (NMJ) include propagated action potentials and the release of trophic substances, which interact with the postsynaptic membrane. Electrical activity in the sarcolemma is coupled to the release of calcium from the sarcoplasmic reticulum (SR). Calcium acts as a second messenger to activate phosphatases and/or kinases, which are ultimately translocated to the nucleus to affect the activation of transcription factors and which influence the expression of nuclear genes encoding mitochondrial proteins. mRNA produced by transcription is translated into protein in the cytosol, which can either be translocated back to the nucleus (transcription factor) or chaperoned to the protein import machinery and taken up by the organelle. Within mitochondria it may act as a single protein subunit or be combined with other subunits to form a multisubunit holoenzyme (e.g., cytochrome c oxidase). Some subunits of the holoenzyme may be derived from the mitochondrial genome (mtDNA), which also undergoes transcription and translation to synthesize a limited number (13) of proteins that are essential components of the electron transport chain.

2.1.2.1. Protein import machinery (PIM)

The expansion of the mitochondrial reticulum in skeletal muscle is a highly regulated and complex process that appears to require the co-ordinated expression of a large number of genes. Thus, an important aspect of mitochondrial biogenesis is the import machinery regulating the transport of nuclear encoded precursor proteins into the organelle. The vast majority of mitochondrial proteins (>90%) are encoded by nuclear genes and synthesized in the cytosol as preproteins containing a mitochondria import sequence.

Notwithstanding the importance of the mitochondrial genome in contributing proteins to the mitochondrial respiratory chain, it is nevertheless true that most mitochondrial proteins are derived from nuclear DNA. Therefore, a mechanism must exist for targeting these proteins to specific mitochondrial compartments once they have been synthesized in the cytosol. Most proteins are fabricated as “precursor” proteins with a signal sequence, often either located at the NH₂ terminus or as an internal sequence (Figure-2).

Although pathways of protein targeting to the outer membrane, inner membrane, matrix, or intermembrane space differ somewhat from each other (9), the most widely studied path is that of proteins destined for the matrix. In this case, the positively charged NH₂-terminal signal sequence interacts with a cytosolic molecular chaperone that unfolds the precursor and directs it to the outer membrane import receptor complex, termed the translocase of the outer membrane (Tom complex). Cytosolic chaperones include 70-kDa heat shock protein (HSP70) and mitochondrial import stimulating factor (MSF). Precursor proteins can be directed to one of two subcomplexes within the Tom machinery. One of these, consisting of the Tom20 and Tom22 receptors, is the preferential route for HSP70 chaperone precursors.

On the other hand, proteins interacting with MSF are largely directed to the Tom70-Tom37 heterodimer (10). Precursors are then transferred from the Tom receptors to Tom40 and the small Tom proteins 5, 6, and 7, which form an aqueous channel through which the precursor protein passes. Proteins are then sorted to the outer membrane, to the inner membrane, or to the translocase of the inner membrane (Tim), another protein complex that allows movement of precursor proteins to either the matrix or the inner membrane. Those proteins involved in the translocation of the precursor to the matrix are Tim17, Tim23, and Tim44. Tim17 and Tim23 act as integral membrane proteins, spanning the mitochondrial inner membrane and having domains associated with both the matrix and intermembrane space. In a manner similar to the Tom receptor complexes, Tim17 and Tim23 bind the precursor protein, prevent any untimely folding that would inhibit the precursor from translocating into the matrix, and form an aqueous pore through which the precursor can travel. In contrast, Tim44 is a peripheral membrane protein that is secured to the inner face of the inner mitochondrial membrane. Tim44 anchors the matrix chaperone HSP70 (mtHSP70), which acts in a ratchet like manner to pull the precursor into the matrix (Figure-2). Along with these proteins, the inner membrane phospholipid cardiolipin is imperative for protein translocation because it appears to orient the precursor into the correct position for interaction with the Tim44-mtHSP70 complex. The importance of this phospholipid has been shown by studies in which cardiolipin function has been blocked using the drug Adriamycin, resulting in an attenuation of the import of proteins destined for the matrix (11-12).

Figure 2. Left: mitochondrial transcription factor A (Tfam) is a nuclear-encoded transcription factor that is synthesized in the cytosol as a larger, “precursor” protein with a positively charged NH₂-terminal presequence (blue). It must interact with the protein import machinery to enter the organelle. Once inside the matrix, mature Tfam will bind within the D-loop region of the circular (not shown) mtDNA on the heavy-strand (HSP) and light-strand promoters (LSP) and stimulate the transcription and replication of mtDNA. Right: enlarged view of the components of the protein import machinery. A typical matrix-destined precursor like Tfam is unfolded and directed to the import machinery by a cytosolic chaperone, either cytosolic 70-kDa heat shock protein (cHSP70) or mitochondrial import stimulating factor (MSF). On interaction with the translocase of the outer membrane (Tom complex), it is correctly oriented by interacting with the inner membrane phospholipid cardiolipin (not shown) before being transferred to the translocase of the inner membrane (Tim complex). The matrix chaperone mtHSP70 pulls in the precursor, and the signal sequence is cleaved by the mitochondrial processing peptidase (MPP). Subsequently, the mature protein is refolded by matrix chaperonins HSP60 and Cpn10. ATP is required at multiple steps during the import process. The number within each import machinery component refers to its size in kDa.

Two other elements are required for correct import of precursor proteins into the matrix. These are 1) the presence of an inner membrane potential (DC, negative inside) across the inner membrane to help pull the positively charged presequence into the matrix and 2) the availability of ATP both in the cytosol and in the matrix. Uncoupling agents that dissipate DC reduce protein import, whereas ATP depletion prevents the unfolding of the precursor in the cytosol and/or the action of mtHSP70 in the matrix. Thus reductions in cellular ATP levels such as that produced by severe contractile activity or defects in ATP production as might be encountered in cells with mtDNA mutations could affect the rate of import into mitochondria.

After its arrival in the matrix, the NH₂-terminal signal sequence is cleaved by a mitochondrial processing peptidase (MPP) to form the mature protein. It is then refolded into its active conformation by a mitochondrial chaperonin system consisting in part of 60-kDa heat shock protein (HSP60) and 10-kDa chaperonin (Cpn10). The vast majority of work that defines the components of the protein import machinery, as well as their cellular function, has been done in *Saccharomyces cerevisiae* and *Neurospora crassa*. This is now being extended to mammalian cells. For example, the kinetics of matrix precursor protein that import into skeletal muscle SS and IMF mitochondrial fractions, the ATP and cardiolipin dependence of the process, and the relationship to mitochondrial respiration have all been defined (13). IMF mitochondria import precursor proteins more rapidly than SS mitochondria, and there is a direct relationship between the capacity for mitochondrial respiration (and thus ATP production) and the rate of protein import. It has also been shown that a number of protein import machinery components are induced in response to chronic contractile activity. These include the chaperones MSF, cytosolic HSP70 (cHSP70), mtHSP70, HSP60, Cpn10, as well as the import receptor Tom20. Coincident with these increases are contractile activity-induced increases in the rate of import into the matrix but not into the outer membrane. This differential effect on protein targeting to mitochondrial compartments provides an example of how contractile activity can result in an altered mitochondrial protein stoichiometry. The accelerated rate of protein import into the matrix can be reproduced in cardiac mitochondria obtained from animals treated with thyroid hormone. Thus the effect is not a unique response to contractile activity but appears to be common to stimuli that increase mitochondrial biogenesis. To more easily define the role of specific components of the import pathway in determining the kinetics of import, measurement of import in intact cells can be employed. When C2C12 cells were incubated with [³⁵S] methionine and the import of radiolabeled MDH into mitochondria was measured, a greater rate of import was found during the progress of mitochondrial biogenesis occurring coincident with muscle differentiation. As expected, thyroid hormone accelerated the rate of import and induced the expression of Tom20. To evaluate the role of Tom20 alone in mediating the accelerated import rate, forced overexpression of Tom20 in these cells using a mammalian expression construct was used. Parallel increases in the rate of import and the magnitude of overexpression were observed. Conversely, inhibition of Tom20 expression using specific antisense oligonucleotides led to equivalent decreases in MDH import. These data suggest that the import of matrix-destined proteins is controlled, at least in part, by the expression of Tom20. The protein import pathway represents an example of intracellular trafficking that is important for organelle biogenesis, and it may, under some conditions, determine the increase in mitochondrial content as a result of chronic exercise. For this to be the case, it must be shown that it is inducible and that it operates at a rate that limits the overall pathway under some conditions (i.e., chronic exercise). If the import rate was slow enough to limit mitochondrial biogenesis, then a pool of precursor proteins in the cell cytosol would be measurable. In the absence of such a pool, the assumption is that newly synthesized precursor proteins are rapidly taken up by mitochondria, and the kinetics does not limit the synthesis of the organelle as a whole. This has yet to be rigorously tested in a cellular system in which any other fates of the precursor (i.e., cytosolic degradation) are blocked. It is possible that the import of proteins might become limiting under conditions

of chronic contractile activity if upstream steps (i.e., transcription, translation) are accelerated such that a saturating abundance of precursor proteins are presented to the import machinery.

In any event, the physiological value of the observed contractile activity-induced increases in mitochondrial protein import is that mitochondria are more sensitive to changes in precursor protein concentration, a situation that would be advantageous for mitochondrial biogenesis at any given upstream production rate of cytosolic precursor proteins. Progress in the area of protein import will advance substantially as additional mammalian homologues of the import machinery are identified. Recently, the first disease that can solely be attributed to a mutation in a protein component of the import machinery has been identified. A mutation in deafness dystonia protein (DPP) results in a neurodegenerative disorder characterized by muscle dystonia, sensorineural deafness, and blindness. DPP has been shown to be a mitochondrial protein that closely resembles Tim8p, a protein of the intermembrane space involved in the import process. In addition, mutations in the import receptor Tom70 have been shown to produce mtDNA rearrangements in the fungus *Podospora anserina*, presumably because of defective import of a component involved in mtDNA maintenance. The cloning of Tom22, as well as members of the Tim machinery, will be of help in elucidating the functional roles of individual import machinery components in the import process and the relevance of import in mitochondrially based diseases and in organelle biogenesis.

2.1.2.2. Exercise effects on PIM

As noted above, exercise has been shown to induce the expression of several protein import machinery components, occurring coincident with an increased rate of translocation into the mitochondria. In turn, activity-induced changes have been observed in Tom20, Hsp60 and mtHSP70 protein and cpn10 mRNA levels, as well as cytosolic concentrations of Hsp70 and MSF (13-15). Coincident with these changes is acceleration in the rate of protein import into the matrix. Thus, the upregulation of protein import machinery components appears to be an important aspect of mitochondrial biogenesis which occurs with contractile activity. This greater capacity for protein import is physiologically relevant because it means that a greater rate of translocation into the organelle will occur at any given concentration of cytosolic protein produced by translation.

2.1.2.3. Transcription factors that involved in mitochondrial biogenesis

Expression of genes promoting mitochondrial biogenesis is predominantly controlled by the global principles of gene regulation, that is, transcription initiation and interaction at the gene promoter. Therefore, transcription factors and transcriptional co-activators represent critical regulators of mitochondrial biogenesis.

Numerous transcription factors have been implicated in mediating the physiological and metabolic adaptations associated with expression of genes involved in mitochondrial biogenesis. While no single transcription factor has been found to be responsible for the coordination of mitochondrial gene expression, several candidates appear to be important for mitochondrial biogenesis. These include two nuclear respiratory factors (NRF-1 and NRF-2), two peroxisome proliferator-activated receptors (PPAR- γ and PPAR- α), specificity protein 1

(Sp1), mitochondrial transcription factor A (Tfam), early growth response gene-1 (Egr-1) and the products of the immediate early genes, c-jun and c-fos. This diversity is important given that the characterization of an assortment of upstream promoter regions of genes encoding mitochondrial proteins has revealed considerable variability in their composition.

NRF-1 and NRF-2 are implicated in the transcriptional control of multiple mitochondrial genes including mitochondrial transcription factor A (Tfam) and identified mitochondrial transcription specificity factors TFB1M and TFB2M, while Egr-1 is associated with promoting transcription of the electron transport chain protein cytochrome C oxidase (COX). The peroxisome proliferator receptor gamma co-activator-1 alpha (PGC-1 α) has been established as an important regulator of mitochondria content in skeletal muscle due to its apparent co-activation of multiple mitochondrial transcription factors. Indeed, PGC-1 α is the founding member of a family of transcriptional co-activators that has been proposed as a potential “master regulator” of mitochondrial biogenesis (16). In support of this contention, Lin and co-workers (2002) over expressed PGC-1 α in mice skeletal muscle and observed increased proportions of type I fibers and increased resistance to fatigue (17). In addition, the biogenesis and maintenance of mitochondrial architecture is controlled by altered rates of mitochondrial protein fusion and fission, a role for which mitofusin (Mfn) 1/2 proteins have been strongly implicated (18).

Similarly, PGC-1 α also mediates Tfam activation, a key component in mitochondrial DNA replication and transcription. The NRF-1 transcription factor has been shown to activate Tfam which enhances the capacity for assembly of protein complexes within the mitochondria. Therefore, as a co-activator of NRF-1 transcription PGC-1 α is involved in regulating Tfam function. Importantly, Tfam activity appears to increase in response to contractile activity and exercise suggesting enhanced mitochondrial protein assembly with endurance training. Most notably, PGC-1 α is the co-activator of the peroxisome proliferator activated receptor (PPAR) family (19). The three PPAR sub-types α , γ and δ have distinct functions but all appear to regulate lipid homeostasis via expression of genes involved in mitochondrial fatty acid oxidation. The initial cellular perturbations associated with the onset of muscle activity leading to the activation of these transcription factors are beginning to be defined (Figure-3).

2.1.2.4. Exercise effects on transcription factors

Researchers showed NRF-1, Tfam and PPAR- γ (has emerged as a potential master regulator of mitochondrial biogenesis) mRNA in response to contractile activity in cell cutlers and endurance exercise in vivo is increased. In turn, studies have been shown that PGC-1 α mediates a regulatory pathway involving estrogen-related receptor alpha (ERR α) and Mfn1/2, and this pathway has been shown to be up-regulated following a 10-km cycling time trial (20). Also, this suggests that a PGC-1 α activated pathway promotes an increase in mitochondrial content in response to endurance exercise through enhanced mitochondrial protein fusion. This provokes an increase in mtDNA transcription and replication. The result is that PGC-1 overexpression can produce an overall increase in cellular oxygen consumption and subsequently, increases the aerobic capacity in endurance activities. The physiological significance of increased PGC-1 α -PPAR activated gene expression with

endurance training is an enhanced capacity for fat utilisation during prolonged exercise, and may also be related to fast-to-slow fibre type conversion (21). Indeed, this was highlighted by Wang and colleagues (2004) who generated transgenic mice over expressing PPAR δ that resulted in a 2.3-fold increase in mitochondrial DNA content, significant type I fibre transformation and a 90% increase in running performance (22).

Figure 3. Transcription factors and mitochondrial biogenesis

The small numbers of studies investigating PPAR activation following exercise support these findings where both acute (21, 23-24) and chronic (25-27) endurance exercise induces PPAR transcription. The initial cellular perturbations associated with the onset of muscle activity leading to the activation and increment of these transcription factors are beginning to be defined. A considerable amount of evidence implicates alterations of intracellular Ca^{2+} (28-29) and ATP (30-31) turnover as the initial triggers eliciting the activation of signalling cascades which provoke changes in these gene expressions, as noted above.

3. Mitochondria and aging

3.1. Involment of mitochondria in the aging process

Mitochondria are cited regularly as the main site of superoxide generation that contributes to the majority of reactive oxygen species (ROS) to the cell, although other sites of ROS production within the cell are documented. The potential for ROS to induce oxidative damage has significant implications for the cellular integrity of highly metabolic, long-lived and post-mitotic tissues such as brain, heart, and skeletal muscle. In addition, the effect of ROS is exacerbated by its potential to induce mutations in mtDNA, which is located in close proximity to the source of ROS generation. mtDNA has no protective histones and has substantially less repair mechanisms than nuclear DNA. Thus, ROS-induced accumulations in faulty proteins, oxidized fatty acids, and mtDNA mutations would result in a progressive, feed-forward, and irreversible

cycle of cellular dysfunction that leads to the onset of phenotypes associated with aging. These observations are the major features of the mitochondrial theory of aging, which was first proposed, and then refined, by Denham Harman (32-33), suggesting that changes to mitochondrial integrity, content, and function could have a determining role on the rate at which we age. The role of mitochondria in promoting sarcopenia was uncovered by studies showing that muscle fibers containing dysfunctional mitochondria were atrophied compared to fibers that did not. As well, these authors and other groups (34-36) have reported that histochemical analyses of skeletal muscle fibers revealed an increase in the number of ragged red fibers, characterized by elevated levels of succinate dehydrogenase and a deficiency in COX activity. An in-depth description on the involvement of ROS in mitochondrial dysfunction associated with aging is provided in a later section.

Along with their role in ROS production, mitochondria play a critical role in maintaining cellular integrity through the regulation of programmed cell death, also termed apoptosis. Within mitochondria reside proteins, which upon release from the organelle, can initiate a cascade of proteolytic events that converge onto the nucleus leading to the fragmentation of DNA. This compromises cell viability and ultimately leads to cell death (37). The release of these apoptotic proteins, such as cytochrome C (cytoC), endonuclease G (EndoG) and apoptosis-inducing factor (AIF), through either the mitochondrial permeability transition pore (mtPTP) or the homo-oligomeric BAX pores in the outer membrane, occurs in response to cellular stressors such as reactive oxygen species (ROS), chronic elevations in intracellular Ca^{2+} concentration, or gamma irradiation. Thus, the intimate connection between mitochondrial function and the viability of skeletal muscle suggests that this organelle plays a significant role in the progression of aging. Indeed, it is evident that in skeletal muscle of aged individuals, the induction of apoptosis is greater when compared with younger subjects. The increase in cytoC and EndoG release from the mitochondria of aged individuals is paralleled by an increase in caspase-3 cleavage, and p53 mediated apoptosis. The result of apoptosis is a loss in myonuclear number, resulting in a reduction in myofiber diameter to maintain a constant myonuclear domain size. Alternatively, a consequence of fiber atrophy may be the initial activation of apoptotic events that lead to a decrease in myonuclear number. Irrespective of the mechanism involved, mitochondria appear to have an involvement in the progression of sarcopenia. A discussion of the importance of apoptotic signalling during the development of age-related phenotypes caused by mtDNA mutations follows below.

3.2. Alternation in mitochondrial content and morphology with aging

Electron microscopic (EM) analyses reveal that the volume of mitochondria within skeletal muscle declines by 66% with age when compared with younger counterparts (38). Similar EM findings are documented in a human study, revealing a 25% decrease in the density of mitochondria within the vastus lateralis muscle of males and females aged greater than 60 years (39). Related to mitochondrial content is the level of cardiolipin found within skeletal muscle. Cardiolipin is a fatty acid that is exclusively found within the inner membrane of mitochondria, and it is linked to the optimal function and structure of the multitude of enzymes and respiratory complexes. The proximity of cardiolipin to the sites of ROS production makes it

particularly vulnerable to oxidative damage. Numerous studies have investigated whether aging has an effect on cardiolipin content or oxidation in cardiac muscle. Some results have indicated that cardiolipin content is decreased along with an increased degree of peroxidation (40). This is linked to decreased activities of COX, ANT, and carrier complexes. However, other reports have failed to indicate a decline in cardiolipin content or its peroxidation within either SS or IMF mitochondria with age. One study in skeletal muscle has illustrated that cardiolipin content in 36-month-old rats is not decreased when compared with 6-month-old rats in isolated SS and IMF mitochondria (41). However, whether cardiolipin is oxidatively modified with age in skeletal muscle remains to be determined. The morphology of mitochondria may also be altered with age in skeletal muscle, in that a proportion of the organelles are enlarged, depolarized, and non-functional. When compared with the elongated morphology of mitochondria in skeletal muscles of young animals, mitochondria tend to be more rounded in shape within aged skeletal muscle, suggesting that mitochondrial fusion events may be impaired in skeletal muscle. Indeed, decreased OPA1 protein expression has been documented in experimentally-generated, giant mitochondria which may have physiological relevance to the morphology of mitochondria seen in aged individuals (42). Mitochondria have also been shown to undergo significant swelling with age because of the increased retention of calcium. EM has also identified losses in mitochondrial cristae formation, leading to homogenization of the materials found within the mitochondrial Compartments.

3.3. Mitochondrial dysfunction within skeletal muscle of aged individuals

Upstream of the synthesis of ATP, the activities of the metabolic enzymes in Krebs' cycle and those involved in lipid oxidation are altered with age. Citrate synthase activity is significantly decreased with age and the activities of β -hydroxyacyl-CoA dehydrogenase (β -HAD) and succinate dehydrogenase are also reduced with age (43). Oxidation of lipids is also impaired within skeletal muscle of aged individuals. Aged muscle also exhibits characteristics of decreased mitochondrial respiratory capacity and ETC enzyme activities. Functional analyses reveal decreased activities of complex I and IV. In line with these alterations, the activity of COX has been shown to decrease with age and the activities of complexes I, II, III, and IV decrease by 28–43%. Reduced oxidative capacity of approximately 30% has also been reported per mitochondrion (44). As a result of decreased enzyme and complex activities, ATP synthesis and content within aged skeletal muscle is reduced. Thus, there is an increased probability of affecting cellular processes reliant on a constant supply of ATP, such as muscle contractions, protein turnover, and the maintenance of membrane potential.

Skeletal muscle oxidative capacity is a reflection of the ability of working muscle to regenerate ATP through aerobic metabolism. Studies support that whole body maximal oxygen consumption ($\text{VO}_{2\text{max}}$) declines with age and there is reduced aerobic capacity per kilogram of muscle in late-middle aged individuals. Oxidative phosphorylation capacity decreased by 50% in 70-year-old human subjects, evaluated using in vivo measurements (39). ATP production rates were decreased by 50% in the gastrocnemius of aged animals (45). Assessments of mitochondrial respiration that was stimulated with a variety of substrates in the presence of ADP revealed that this parameter decreased in aged skeletal

muscle. At rest, muscle ATP synthesis was reduced in 30-month, compared with 7-month-old mice (46). In addition, the ATP content in aged gastrocnemius muscle is 50% lower when compared with that found in young animals (45), and a lower ATP/ADP ratio in 30-month-old mice has been illustrated as well (46).

Despite this evidence, numerous studies have also demonstrated that the oxidative capacity of skeletal muscle does not change with age and discrepancies in results can arise for a number of reasons. One is the lack of consistency of the ages used to make comparisons. Studies may pool together subjects in their late teenage years with middle-aged subjects to represent an “adult” group, whereas the “old” group could encompass subjects ranging from 40 to 90 years of age. Another variable between aging studies is the differences in the species used, which can range from rats, mice, monkeys, yeast, flies, worms, and humans. The selection of muscle studied, and the method of preparation are also not standardized, such that measurements have been made using either whole muscle homogenates or isolated mitochondrial populations. Related to this, many studies have ignored the potential biochemical differences between the SS and IMF mitochondria and report their findings on mixed mitochondrial samples. It is very possible that these skeletal muscle mitochondrial populations are affected differentially by the aging process. Finally, many studies fail to control for physical activity levels in their subjects, and there is evidence that the majority of age-related declines in mitochondrial oxidative capacity disappear after accounting for this variable (47). Thus, it is controversial whether mitochondrial dysfunction is due to aging per se, or whether the lack of regular physical activity is the major reason for the divergent age-related phenotypes of skeletal muscle. Then again, a reduced oxidative capacity was observed in aged subjects even after accounting for physical activity and fat-free mass. Thus, more research is needed to fully clarify these important issues.

3.4. Causes for the alternations in mitochondrial biogenesis associated with aged skeletal muscle

The impairment in mitochondrial biogenesis may be due to a plethora of causes that lead to the propagation of mitochondrial dysfunction. As discussed below, a change in the content of mitochondria may be due to a decrease in the expression of genes coding for mitochondrial proteins, and/or alterations in the control of protein turnover that occur with aging. In addition, alterations in mitochondrial function may be due to oxidative modifications resulting from an increase of ROS, an elevation of mtDNA mutations, or increased uncoupling of oxidative phosphorylation with age.

3.4.1. Dysregulated experssion of mitochondrial genes

Declines in mitochondrial content and function may be related to the altered expression of nuclear genes encoding mitochondrial proteins (NUGEMPS) in skeletal muscle of the elderly. The huge reliance of mitochondria on the nuclear genome suggests that impaired protein synthesis rates could lead to the decline in mitochondrial biogenesis that is observed with old age, especially if the transcription of NUGEMPS is decreased with age. An

interesting study by Zahn et al. revealed that expression of mitochondrial ETC transcripts decreased, whereas cytosolic ribosomal transcripts were increased in skeletal muscle with age (48). This increased expression of ribosomal subunits may represent a compensatory response for decreased translational efficiency, particularly because protein synthesis has been illustrated to decrease with age. Deficits in ETC enzyme activities have been observed in number of studies and may be linked to a reduction in the transcription of genes located within mtDNA, or to a reduction in the content of mtDNA with age. However, in response to the decline in mitochondrial respiratory function, compensatory increases in mtDNA content in tissues such as skeletal muscle, kidney, and cardiac muscle have been observed. Conversely, the preponderance of evidence seems to suggest that mitochondrial mRNA content is reduced with age. Mitochondrial DNA copy number and mtDNA transcript levels of COX I and COX III have been shown to decrease in 27-month aged animals versus 6-month young animals (49). Similarly in humans, mtDNA content was significantly decreased in muscle biopsies obtained from 67-year-old subjects (50), whereas Welle et al. revealed that mRNA transcripts of components of the respiratory complexes also decrease in their abundance in aged skeletal muscle (51). It has been illustrated that in skeletal muscle of aged humans the rate of mitochondrial protein synthesis is decreased and this may have contributed to the decrease in COX and CS activities observed.

3.4.2. Impaired regulation of protein degradation

Mitochondrial function and morphology depend on the balance between protein synthesis and assembly, and the clearance of damaged or improperly assembled proteins. A reduced ability of degradation pathways to remove whole or damaged compartments of mitochondria could lead to impaired organelle bioenergetics. These effects likely manifest as decreased ATP synthesis, increased ROS generation, accumulated mtDNA mutations and cell death, characteristics which are observed in skeletal muscle of aging individuals. The major pathways that contribute to mitochondrial protein quality control include intramitochondrial proteases and autophagy. Studies have illustrated that with increasing age, the activity and expression of the intramitochondrial Lon protease is reduced, reflected by an accumulation of dysfunctional aconitase (52). Decreased activity of the Lon protease is likely due to oxidative modifications by elevated ROS levels within the mitochondrial matrix. In the cytosolic environment, lipofuscin has been implicated in contributing to the progressive decline in mitochondrial protein turnover and the onset of dysfunction that occurs with age. Lipofuscin, referred to as the aging pigment, is a non-degradable protein that is the product of incomplete autophagic degradation followed by the peroxidation of remaining contents within the lysosome by reactive oxygen species. Lipofuscin localizes within vesicles throughout tissues in aged individuals, which may reduce the availability of vesicles to form autophagosomes to remove damaged and dysfunctional mitochondria (53). Thus, it appears that the activities of these housekeeping pathways related to protein quality control are altered with aging, resulting in the accumulation of damaged mitochondria and cellular dysfunction. More research is required in this area with skeletal muscle as a function of age.

3.4.3. Elevated damage to macromolecules by ROS

Research unequivocally indicates that ROS production increases in aging skeletal muscle (54). Chabi et al. observed that the generation of ROS is elevated in both the SS and IMF mitochondrial pools of fast-twitch muscles isolated from senescent animals (41). One consequence of increased aberrant ROS production is oxidative damage to complex V leading to a decrease in ATP synthesis and content within skeletal muscle. Additionally, increases in oxidative modifications in DNA occur with age, reflected by higher levels of 8-oxodeoxyguanosine, (8-oxoG) and the corresponding repair enzyme, 8-oxoguanine-DNA glycosylase 1 (OGG1) in skeletal muscle. Increased levels of protein carbonyls have also been associated with aging skeletal muscle. It is well known that slower respiration rates increase the likelihood of the donation of electrons to oxygen at complexes I or III (55), and this may be a feature of mitochondrial respiration in aged individuals. It has also been hypothesized that during aging, there is increased dysfunction of these two complexes, leading to increased ROS generation.

Antioxidant enzymes have evolved to buffer the deleterious, effects of ROS. Enzymes such as manganese superoxide dismutase (MnSOD), catalase (CAT), and glutathione peroxidase (GPX), can ultimately reduce ROS to hydrogen peroxide (H₂O₂), and then finally into water. The role of ROS in limiting lifespan was elucidated in an elegant study, in which human CAT was targeted to the mitochondria. This resulted in improved aconitase activity, decreased mtDNA mutations, and increased mean lifespan (56). Conversely, transgenic mice lacking copper/zinc SOD (CuZnSOD) showed rapid aging and muscle atrophy similar to sarcopenia in concert with elevated oxidative modifications in proteins, lipids, and DNA, when compared with wild-type animals (57). However, whether the activity or content of these antioxidant enzymes is truly altered with age remains an equivocal issue. A number of studies have illustrated that there is an increase in antioxidant activities with age, as this would be the intuitive hypothesis in response to the elevated ROS generation that occurs during aging (58-59). However, other studies suggest that CuZnSOD, MnSOD, CAT, and GPX activities decrease with age in skeletal muscle, even though the protein and mRNA content of these enzymes were either unchanged or decreased with age (60-61). To add more complexity to this issue, it remains to be determined whether antioxidant enzyme activities are differentially affected by age in the two mitochondrial subfractions. One study suggests that there is no change in the content of MnSOD in SS and IMF mitochondria from tibialis anterior and extensor digitorum longus muscles of aged, compared with young animals. In cardiac muscle, IMF mitochondria exhibit increased levels of GPX, CAT, and MnSOD with age, whereas SS mitochondria exhibit increased levels of GPX and MnSOD and a decrease in CAT activity (62). Because it is clear that oxidative modifications to mitochondrial macromolecules are indeed occurring in skeletal muscle with age, it is likely that the increased ROS production overwhelms the buffering capacity of the antioxidant enzymes that are available. This suggests that other means to reduce ROS, independent of antioxidant enzyme activities, would be beneficial in reducing cellular oxidative damage.

3.4.4. *Elevated mutation in mtDNA*

An important component of the free radical theory of aging is that mitochondrial dysfunction is a result of accumulated oxidative damage to mtDNA, leading to mutations in coding regions for ETC proteins. The last point is especially critical because mtDNA contains no introns or spacer regions (63), thus even point mutations could lead to the expression of faulty proteins. It is accepted that ROS generation by skeletal muscle mitochondria increases with age and is accompanied by an increase in mtDNA mutations, impaired energy production, mitochondrial dysfunction, and a greater susceptibility to undergo apoptotic signalling that results in the downfall of skeletal muscle function. The most common mtDNA mutation associated with aging has a frequency rate of 30–35%, is found within the D-loop region, and is a deletion mutation that affects the expression of seven of the 13 proteins encoded by mtDNA (64–65). In addition, mtDNA deletion mutations appear to be highly localized in small regions of muscle fibers in mosaic patterns, rather than distributed ubiquitously throughout aged skeletal muscle.

Interestingly, research has illustrated that mtDNA mutations may be an important contributor to the aging process. Genetically altered mice lacking DNA polymerase gamma (Polg) activity exhibited an elevated accumulation of random mtDNA point mutations, in conjunction with a severe deficiency in ATP synthesis and the early onset of aging-related phenotypes. However, these occurred in the absence of increased ROS production, protein carbonylation or mtDNA damage (66). Although there was no evidence for increased oxidative stress in this study, apoptotic signalling was significantly elevated in the Polg mice, and it is conceivable that areas of the cell with accumulated oxidative modifications may have been cleared away by cell death and subsequent autophagy processes. In future experiments, it would be interesting to determine whether the enhanced expression of Polg activity could result in extended lifespan in normal animals. A definite role for ROS in producing mutations and mitochondrial dysfunction was illustrated in a mouse model with compromised MnSOD activity and content. Age-related alterations observed included 25% decreases in complex I and V activities, a 50% increase in basal ROS generation and a 45% increase in 8-oxoG content (67). However, both the mean and maximum lifespan were not altered. As a result of this, there is considerable debate regarding the validity of the mitochondrial theory of aging. As Conley et al. reviewed, mitochondrial dysfunction can be observed in skeletal muscle before the detection of mtDNA mutations (68). In addition, the theory postulates that mitochondrial dysfunction is irreversible; however, much evidence exists to contradict this point. Clearly, more research is required, with a focus on when and how mtDNA mutations are involved with aging. Despite this, the associations between dysfunctional mitochondria, mtDNA mutations, and apoptosis remain strong themes in the description of mechanisms that may be causative to the aging process.

3.4.5. *Uncoupling of oxidative phosphorylation*

Coupling of the energy generated from electron transfer through the respiratory complexes to the synthesis of ATP is a major function of the mitochondrial network. However, the flow of

protons through complex V can be bypassed and redirected through protein channels which serve to uncouple respiration. The result of uncoupling is a decrease in ATP synthesis, despite increased oxygen consumption and respiratory rates (69). There is evidence which suggests that coupling is reduced with age. When compared with young individuals, coupling was lower by 50% in 30-month-old mice, resulting in decreased ATP production per O₂ consumed (46). Another study supplemented this finding with the observation that uncoupling occurs in human skeletal muscle of subjects greater than 65 year of age that was accompanied with reduced ATP content (70). In the same study, it was determined that uncoupling affects muscles with a high type II fibre composition, compared with those that are composed of predominately type I fibers (41). Ghabi et al. also observed 21 and 40% decreases in the coupling of the IMF and SS mitochondria, respectively, in 36-month-old animals when compared with their younger counterparts (41). Potential causes for uncoupling of oxidative phosphorylation occurring with age may involve the increased activity and expression of uncoupling protein 3 (UCP3) that can be stimulated by oxidative stress. An increased activity of UCP3 has been proposed to lend protection to the cell, in response to increased oxidative stress that occurs with age. Indeed, mitochondria from UCP3 null mice demonstrated elevated levels of ROS production and oxidative modifications to cellular components. Whether the expression of uncoupling proteins in skeletal muscle is altered with aging is not well established. Some studies have observed a trend for increased UCP3 content (43), whereas others have suggested there is an age-related decrease or no change in this protein content (46, 71). Thus, if UCP3 content is not increased with age, it is likely that a greater proton leak with age could occur through increased permeability of the inner membrane by ROS-induced oxidative modifications of the lipid bilayer.

3.5. Potential of exercise to attenuate age-related mitochondrial dysfunction

Although it has long been established that exercise training increases, and muscle disuse decreases, the activity of mitochondrial oxidative enzymes in skeletal muscle, a lack of consideration of this notion in aging studies has led to discrepancies in our overall understanding of the effect of aging on muscle mitochondrial function. Indeed, some of the age-associated alterations found in mitochondrial activity can be the result of a reduction in the level of voluntary physical activity as individuals age (31). In this regard, it is notable that the adaptation to exercise is not limited to young individuals, because older athletes can increase the activity of mitochondrial oxidative enzymes as a result of training (72). This likely happens through increases in expression of the coactivator PGC-1 α and the specific transcription factors NRF-1 and Tfam, the main regulators of organelle biogenesis and protein expression. One can assume that if mitochondrial function deteriorates with age, organelle biogenesis induced by exercise may attenuate this age-related decline, and therefore may have a protective role. However, despite the fact that exercise-induced increases in enzyme activities and mitochondrial content have been reported in aging individuals, less is known about the effects of exercise on the expansion of mtDNA mutations, ROS balance, and apoptosis in aged skeletal muscle. For example, in patients suffering from mitochondrial diseases due to mtDNA mutations, the introduction of an exercise program to improve muscle oxidative capacity and

mitochondrial function has been approached with caution. In those patients, exercise induced mitochondrial biogenesis but also increased both wild-type and mutant mtDNA, worsening the heteroplasmy ratio in muscle fibers (73). Thus, one might expect that this phenomenon could also occur in older individuals. However, in view of the evidence that chronic exercise can attenuate proapoptotic protein release from mitochondria in young animals, and reduce ROS production in intermyofibrillar mitochondria, it is worth investigating whether exercise can attenuate the enhanced apoptotic susceptibility evident in muscle from aged individuals.

Several lines of evidence support the fact that exercise may be beneficial in attenuating an aging-induced ROS imbalance. Old animals that were submitted to an 8-week treadmill exercise program, or 1 year of swimming, were found to have reduced oxidative damage compared with untrained old rats, notably due to alterations in antioxidant defences (74). At the mitochondrial level, recent work has revealed a 10% decrease in mitochondrial hydrogen peroxide production in animals as a result of lifelong voluntary wheel running (75). This may occur through the exercise-induced increase in mitochondrial content, a better redistribution of electrons through the electron transport chain, and (or) a better coupling between oxygen consumption and ATP synthesis in the exercised muscle of old animals. The precise mechanism for this effect remains to be determined.

4. Conclusion

Skeletal muscle is a remarkably adaptive tissue that is capable of changing its morphological, physiological, and biochemical properties in response to various perturbations. One of the most profound changes in skeletal muscle is mitochondrial biogenesis. Mitochondrial biogenesis is a very complex cellular process that requires the coordination of several mechanisms involving nuclear-mitochondrial corporation, mitochondrial protein expression and import, mtDNA gene expression, transcription factors activity, assembly of multisubunit enzyme complexes, regulation of mitochondrial fission and fusion as well as mitochondrial turnover. In turn, it seems with recognition of variant component of mitochondria of skeletal muscle; we can understand precisely the function of these component in mitochondrial biogenesis process and effects of many interventions (e.g. Aging and diseases) on them. Also, we can comprehend the uncountable positive effects of exercise on these components. But, many vast and precise researches are needed to fully clarify these important issues.

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