LEADING ARTICLE

Sports Med 2003; 33 (11): 783-793
0112-1642/03/0011-0783/$30.00/0

Regulation of Mitochondrial Biogenesis in Muscle by Endurance Exercise

Isabella Irrcher,[1] *Peter J. Adhihetty*,[1] *Anna-Maria Joseph*,[2] *Vladimir Ljubicic*[2] and *David A. Hood*[1,2]

1 Department of Biology, York University, Toronto, Ontario, Canada

2 School of Kinesiology and Health Science, York University, Toronto, Ontario, Canada

Abstract

Behavioural and hereditary conditions are known to decrease mitochondrial volume and function within skeletal muscle. This reduces endurance performance, and is manifest both at high- and low-intensity levels of exertion. A programme of regular endurance exercise, undertaken over a number of weeks, produces significant adaptations within skeletal muscle such that noticeable improvements in oxidative capacity are evident, and the related decline in endurance performance can be attenuated. Notwithstanding the important implications that this has for the highly trained endurance athlete, an improvement in mitochondrial volume and function through regular physical activity also endows the previously sedentary and/or aging population with an improved quality of life, and a greater functional independence. An understanding of the molecular and cellular mechanisms that govern the increases in mitochondrial volume with repeated bouts of exercise can provide insights into possible therapeutic interventions to care for those with mitochondrially-based diseases, and those unable to withstand regular physical activity. This review focuses on the recent developments in the molecular aspects of mitochondrial biogenesis in chronically exercising muscle. Specifically, we discuss the initial signalling events triggered by muscle contraction, the activation of transcription factors involved in both nuclear and mitochondrial DNA transcription, as well as the post-translational import mechanisms required for mitochondrial biogenesis. We consider the importance and relevance of chronic physical activity in the induction of mitochondrial biogenesis, with particular emphasis on how an endurance training programme could positively affect the age-related decline in mitochondrial content and delay the progression of age- and physical inactivity-related diseases.

It is a very interesting time to be studying mitochondrial structure and function, particularly in skeletal muscle. Mitochondria are not only vital for cellular energy production, but they are also known to participate in cell signalling events leading to apoptosis (programmed cell death). Moreover, it is now also established that mitochondrial dysfunction, brought about by either nuclear or mitochondrial DNA (mtDNA) mutations, can lead to a wide variety of pathophysiological conditions affecting the nervous system, the heart and/or skeletal muscle. Furthermore, under conditions of skeletal muscle disuse (e.g. microgravity, denervation, immobilisation, sedentary lifestyle, aging), mitochondrial content is diminished. This compromises energy production. Fortunately, skeletal muscle is highly 'plastic', a term popularised by Dirk Pette[1] to describe the remarkable capability of this tissue to alter its gene expression profile and phenotype in response to changes in functional demand imposed by muscle use, disuse, and to developmental or neural influences. This adaptability permits us to be optimistic about the role of training in ameliorating the processes which lead to cell death, and myonuclear decay, or in reversing the pathophysiology of mitochondrial disease in skeletal muscle. To date, however, this still remains to be established. This review will focus on the cellular mechanisms involved in mitochondrial biogenesis as produced by experimental models of endurance exercise. An appreciation of these mechanisms will help us understand the basis for the use of exercise in improving the quality of life in patients with mitochondrial disease.

1. Muscle Fibre Types and Mitochondrial Content

Skeletal muscle is categorised into three classes of fibres on the basis of their metabolic and contractile properties. In humans, the most common method of classification is based on the myosin heavy chain isoform, and consists of the slow-twitch type I fibres, and the fast-twitch type IIa and IIx (formerly classified as type IIb) fibres. Each of these muscle fibre types has varying steady-state mitochondrial contents, which contribute to their endurance capacity. In humans, the type I fibres have the largest volume fraction of mitochondria, followed by the type IIa and type IIx fibres, respectively,[2] although considerable overlap exists.[3] This wide range of mitochondrial content among fibre types clearly indicates the independent regulation of myosin heavy chain expression and mitochondrial biogenesis. Provided that these different muscle fibres are recruited, repeated bouts of exercise in the form of endurance training can induce increases in the mitochondrial content in all three muscle fibres,[2,4,5] resulting in the major functional adaptation which is an improvement in fatigue resistance (figure 1). This occurs without any substantial change in myosin heavy chain composition within the trained muscle.

2. Obligatory Events in Mitochondrial Biogenesis

The expansion of the mitochondrial reticulum in muscle cells as a result of endurance training involves a complex series of events that begins with the first bout of exercise within a given training programme. The morphological manifestation of the accumulation of multiple acute exercise bouts, performed 3–4 times per week over 4–8 weeks, is mitochondrial biogenesis. This can be visualised by electron microscopy as an increase in mitochondrial content in both subsarcolemmal and intermyofibrillar regions of each muscle fibre that has been recruited during the exercise bouts.[8] The intervening time between the onset of the first exercise bout and mitochondrial biogenesis is filled with intermittent, contraction-induced signalling events, which may be attenuated in amplitude[9] as the training period extends. These signals are known to activate protein kinases and phosphatases that modify the activity of transcription factors acting in the nucleus, as well as

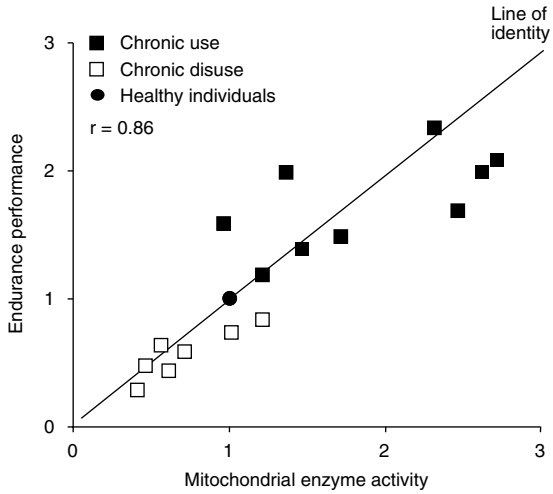

Fig. 1. Relationship of changes in mitochondrial content to alterations in endurance performance. Increases in mitochondrial enzyme activity due to chronic muscle use (e.g. chronic stimulation, endurance training) lead to improvements in endurance performance. Decrements in mitochondrial enzyme activity brought about by chronic disuse (e.g. denervation, immobilisation, physical inactivity) result in decreases in endurance performance. The data indicate that a strong relationship exists over an approximate 6-fold range in enzyme activity.[6,7]

mRNA stability factors acting within the cytosol resulting in an increase in the mRNA expression of nuclear-encoded mitochondrial proteins (NEMPs). The translated NEMPs are then appropriately chaperoned to the mitochondria and imported into the different organelle compartments, such as the matrix space, the inner or outer membranes.

A small subgroup of NEMPs comprises transcription factors that act directly on mtDNA to increase the mRNA expression of mitochondrial gene products, as well as mtDNA copy number. mtDNA is small by genomic standards (16kb), but it is essential because it encodes 13 proteins that are vital for the function of the electron transport chain. Since there are no introns and few non-coding regions in mtDNA, point mutations in mtDNA have severe consequences, leading to a dysfunctional protein (or proteins) involved in mitochondrial respiration.[10] One of the most fascinating aspects of mitochondrial physiology is the fact that biogenesis requires: (i) the integration of a multitude of contraction-induced cellular signals; and (ii) the cooperative and timely interaction between the nuclear and mitochondrial genomes to produce an organelle that is functional in cellular adenosine triphosphate (ATP) provision.

2.1 Contraction-Induced Cell Signalling

The initial cellular signals associated with the onset of muscle activity that ultimately lead to mitochondrial biogenesis are beginning to be defined. These signals are triggered with each bout of contractile activity, and they activate transcription factors that promote the transcription of nuclear and mitochondrial genes (figure 2). There is strong evidence for the participation of intracellular Ca^{2+} and ATP turnover as the initial triggers brought about by exercise. However, the evidence for this is indirect, as the experimental models used to produce mitochondrial biogenesis do not typically involve exercise, but rather utilise other experimental models in which changes in ATP turnover or Ca^{2+} levels can be elicited in a dramatic fashion.

2.1.1 Adenosine Triphosphate Turnover

Exercise increases the rate of ATP turnover within muscle cells. This alters the ATP/free adenosine

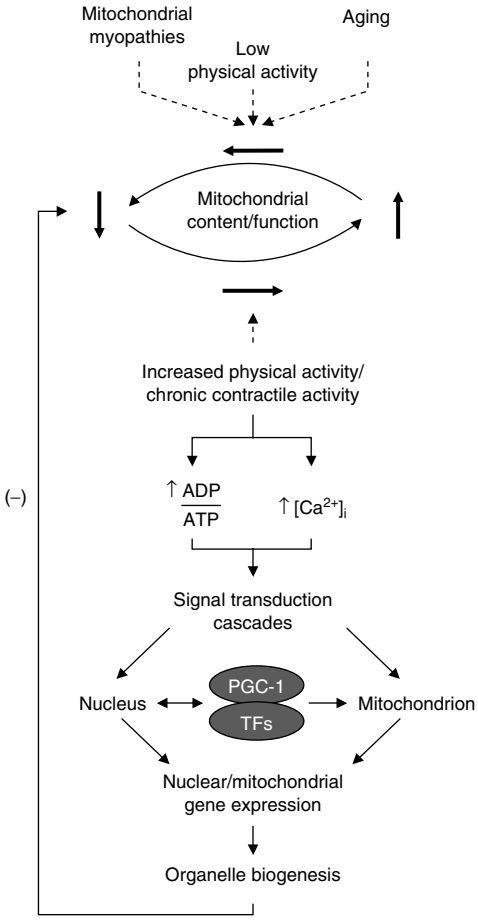

Fig. 2. Mitochondrial content within skeletal muscle can be reduced in mitochondrial myopathies, aging, or by low physical activity. This leads to decreases in endurance performance (see text and figure 1). Time- and intensity-dependent increases in daily physical activity influence mitochondrial volume and function such that deficiencies in energy production can be ameliorated, and increases in endurance performance can be re-established. At the molecular level, the signals that are triggered with each bout of exercise likely arise from a combination of rapid ATP turnover and altered calcium levels that, when sustained for sufficient durations and amplitude result in mitochondrial proliferation. These signals activate signal transduction cascades, resulting in changes in TF activity and the upregulation of diverse genes, including those involved in organelle synthesis and oxidative phosphorylation. The recent identification of PGC-1 has enhanced our understanding of the mechanisms underlying the formation of the organelle. The precise role of PGC-1 in coordinating contractile activity-induced mitochondrial biogenesis is currently under investigation. This enhanced organelle biogenesis provides a negative feedback mechanism to reverse the initial deficit in mitochondrial function and content. **ADP** = adenosine diphosphate; **ATP** = adenosine triphosphate; **PGC-1** = PPAR-γ coactivator-1; **PPAR** = peroxisome proliferator-activated receptor; **TF** = transcription factor.

diphosphate ratio and stimulates muscle metabolic rate. A similar change in energy status can also be achieved through the uncoupling of the mitochondrial respiratory chain,[11] by mtDNA depletion,[12] with iron deficiency,[13] by disruption of the creatine phosphokinase system,[14] or with prolonged use of the agent β-guanidinopropionic acid (β-GPA).[15] Changes in cellular energy status associated with these adaptations have been linked to the activation of adenosine monophosphate-activated protein kinase (AMPK).[16] AMPK can be activated physiologically by decreases in phosphocreatine and ATP, and pharmacologically using the adenosine analog 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), which is frequently used to mimic the effects of chronic exercise. AMPK activation via AICAR treatment of animals led to an increase in mitochondrial enzyme activities,[17] while AMPK activation using chronic β-GPA was shown to increase the expression of downstream targets, including cytochrome C and δ-aminolevulinic acid synthase (ALAS).[16] Cytochrome C is an important component of the electron transport chain, while ALAS is the rate-limiting enzyme in the synthesis of heme. This effect was likely mediated by an increase in the binding of the transcription factor nuclear respiratory factor-1 (NRF-1; see section 2.2) to a DNA sequence located within the promoter regions of these genes. Interestingly, increases in NRF-1 DNA binding and its downstream effects were also shown to occur in cells overexpressing uncoupling protein-1.[11] Finally, mtDNA depletion also leads to a reduction in cellular energy status similar to the conditions described above. This provoked an increase in intracellular Ca^{2+}, ultimately leading to the upregulation of nuclear genes including cytochrome C oxidase subunit Vb mRNA.[12]

2.1.2 Calcium

As a result of α-motoneuron-induced depolarisation, Ca^{2+} is released from the sarcoplasmic reticulum. Ca^{2+} then plays a crucial role in mediating

muscle contraction, and it also acts as an important second messenger to couple the initial electrical event to subsequent alterations in gene expression. Changes in intracellular Ca^{2+} levels modify the activities of calcium-dependent protein kinases and phosphatases. These include Ca^{2+}/calmodulin-dependent protein kinases, protein kinase C, and calcineurin, among others.[18] An increase in the mRNA and protein expression of mitochondrial enzymes, as well as the electron transport chain component cytochrome C can be produced in muscle cells using continuous exposure of cells to the calcium ionophore A23187 to artificially elevate intracellular Ca^{2+} concentrations.[19,20] In addition, the intermittent exposure of muscle cells to the Ca^{2+} ionophore ionomycin, or to caffeine, which stimulates the release of Ca^{2+} from the sarcoplasmic reticulum, also produces mitochondrial biogenesis.[21] Finally, in transgenic mice, selectively expressing a constitutively active form of Ca^{2+}/calmodulin-dependent protein kinase IV in muscle, an increase in mtDNA copy number as well as the expression of a wide variety of genes involved in mitochondrial biogenesis occurred. These included gene products directly involved in oxidative phosphorylation, fatty acid metabolism, and also the important coactivator termed peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1 (PGC-1).[22] These data imply that Ca^{2+} released from the sarcoplasmic reticulum during each action potential could serve as an important second messenger leading to mitochondrial biogenesis. However, the amplitude, duration and temporal pattern of the Ca^{2+} signals needed to provoke such changes need to be established in the physiological setting of muscle contraction.

In summary, the work to date suggests that alterations in the energy status of the cell brought about by contractile activity can simultaneously activate AMPK, as well as modify intracellular Ca^{2+} levels. It seems likely that both of these signalling pathways are instrumental in producing mitochondrial biogenesis.

2.2 Transcription Factors Involved in Mitochondrial Biogenesis

A subsequent effect of the contractile activity-mediated activation of signal transduction cascades involves the post-translational phosphorylation/dephosphorylation of transcription factors. This can then mediate the upregulation of nuclear and mitochondrial gene expression (figure 2). A number of transcription factors have been implicated in mitochondrial biogenesis. These include NRF-1 and -2, PPAR-α and -γ, specificity protein-1 (Sp1) and the products of the immediate early genes, *c-jun* and *c-fos*.[18,23] This variety of transcription factors is important given the large differences in regulatory DNA sequences that are found within the promoter regions of nuclear genes encoding mitochondrial proteins.[24] It therefore stands to reason that a single transcription factor could not function unilaterally to induce mitochondrial biogenesis. Nonetheless, evidence to date has implicated NRF-1 as a particularly important component in the process of contractile activity-induced mitochondrial biogenesis.[23] This is mainly because NRF-1 expression increases in response to contractile activity in cell culture[25] and as a result of exercise *in vivo*.[26] Moreover, its induction precedes the expression of cytochrome C, an NRF-1 target gene. Finally, NRF-1 also transcriptionally activates mitochondrial transcription factor A (Tfam),[23] a DNA binding protein largely responsible for regulating mtDNA transcription and replication (see next paragraph). However, while NRF-1 appears to play an important role, not all nuclear genes encoding mitochondrial proteins contain putative NRF-1 DNA binding sites. Thus, further work led to the discovery of PGC-1.[27] This protein has emerged as an important regulator of fibre type determination[28] and mitochondrial biogenesis in skeletal muscle (figure 2). PGC-1 mRNA

is increased in muscle by swimming exercise,[29] and PGC-1 protein is increased by contractile activity, as well as thyroid hormone treatment,[30] consistent with a role for this coactivator in mediating mitochondrial biogenesis.

Overexpression studies in animals and in cell culture have shown that PGC-1 induces the expression of a variety of genes within the nuclear genome. This effect appears to be mediated by its strong coactivation of NRF-1, and possibly other transcription factors, which leads to increased levels of several NEMPs, including Tfam. The effect on Tfam is important, since this protein regulates the transcription and replication of mtDNA, leading to mitochondrial biogenesis and an increase in cellular oxygen consumption in muscle,[31] heart[32] and adipocytes.[27] However, this function of Tfam on mtDNA transcription and replication is also dependent on its import into the mitochondrion, another important step in the regulation of mitochondrial biogenesis.

2.3 Mitochondrial Protein Import

Given the limited coding capacity of mtDNA (i.e. 13 proteins), most of the proteins found within mitochondria are derived from the nuclear genome. Mitochondrial biogenesis, therefore, is dependent not only on the import of Tfam, but also on the incorporation of many nuclear-encoded matrix and membrane precursor proteins into the organelle. This occurs via the multi-subunit complex known as the protein import machinery, which is comprised of the translocases of the outer membrane (Toms) and the translocases of the inner membrane (Tims) [figure 3].[18] Mitochondrially-destined proteins are targeted to the organelle import site via the molecular chaperones cytosolic heat shock protein 70 (HSP70), mitochondrial import stimulation factor[33] and possibly Tom34.[34] Protein import into the mitochondria is initiated by receptors found in the Tom complex, which then transfers the precursor protein to the Tim proteins. The precursor protein is then guided by the mtHSP70/Tim44 complex, where mtHSP70 acts as a molecular ratchet, to pull proteins into the matrix.[35] This is followed by cleavage of the N-terminal targeting presequence by the matrix processing peptidase, followed by the refolding of the mature form by HSP60 and chaperonin 10 kDa (cpn10). The mature protein is then integrated to its appropriate site within the organelle.

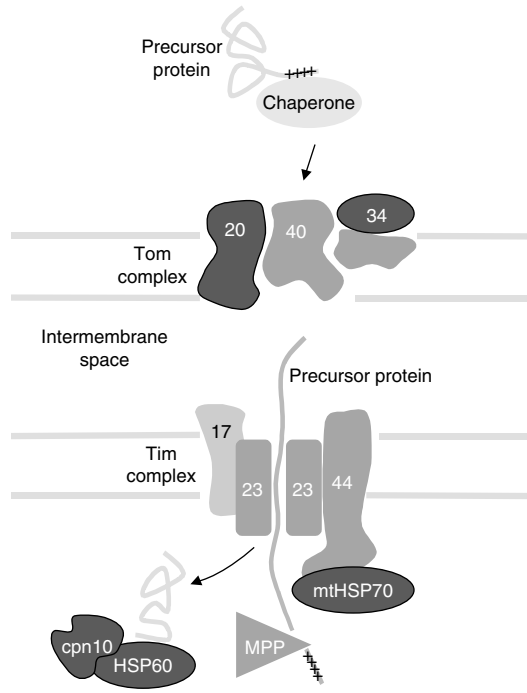

Fig. 3. Overview of the mitochondrial protein import pathway and the targeting of a matrix protein. Precursor proteins synthesised in the cytosol during translation are chaperoned to the mitochondrial Tom complex. Following ATP-dependent unfolding, the proteins are guided through the outer membrane Tom complex to the Tim complex and pulled into the matrix space by the action of mtHSP70. Matrix processing peptidase cleaves the N-terminal targeting sequence, and the resulting mature protein is refolded by HSP60/cpn10. Numbers indicate the molecular mass in kDa. Chronic contractile activity is known to increase the rate of protein import into the matrix, mediated by an increase in the expression of components of this import machinery. Those proteins which are known to be induced by contractile activity are hatched and shaded. Only a subset of proteins within the entire import machinery is shown. **ATP** = adenosine triphosphate; **cpn10** = chaperonin 10 kDa; **HSP** = heat shock protein; **MMP** = matrix processing peptidase; **mt** = mitochondrial; **Tim** = translocation of the inner membrane; **Tom** = translocation of the outer membrane.

Skeletal muscle contains distinct subfractions of mitochondria that are found within different cellular compartments. Subsarcolemmal mitochondria are located immediately below the surface of the sarcolemma, while intermyofibrillar mitochondria are found intermingled within the myofibrils.[8,36] It is surmised that these subfractions have different functional roles within the muscle cell, likely due to differences in subcellular location, and also due to their distinct biochemical differences. These can be attributed, in part, to differential rates of protein import.[37] Protein import into mitochondrial subfractions is closely related to their distinct rates of oxygen consumption and ATP synthesis, the latter of which is important for protein unfolding in the cytosol, translocation across the membrane, and the final refolding of the protein within the matrix.

Chronic exercise affects the rate of import into mitochondria (figure 3). Using a model of chronic electrical stimulation, it has been shown that contractile activity induces increases in the expression of multiple protein import machinery components.[6,38,39] Most importantly, these include the outer membrane receptor Tom20, the inner membrane phospholipid cardiolipin, the matrix mtHSP70, which pulls the precursor protein inside, and the cytosolic chaperones HSP70 and mitochondrial import stimulation factor. Accelerations in the rate of protein import into the matrix occurred coincident with these changes. Thus, the adaptive response of protein import machinery components appears to be an important aspect of mitochondrial biogenesis that occurs with contractile activity. This is physiologically relevant, because it implies that the import process will exhibit a greater sensitivity for import at any given concentration of cytosolic precursor protein produced by translation. A similar adaptation in protein import occurs with thyroid hormone-induced mitochondrial biogenesis in the heart.[40] These adaptations are particularly relevant now, given recent studies demonstrating that defects in protein import can lead to a number of pathological conditions.[41,42] Therefore, these studies indicate the potential of regular exercise (or thyroid hormone treatment) to ameliorate defects that may arise in the protein import process.

2.4 mtDNA and Chronic Contractile Activity

As noted at the beginning of section 2, mitochondria contain their own circular 16 kilobase mtDNA encoding 13 protein, 22 tRNA and two rRNA genes. In order to transcribe and replicate this genome, a process that is vital for mitochondrial biogenesis, the import of nuclear-encoded mtDNA maintenance proteins is required. These proteins include Tfam, as well as mtRNA polymerase, DNA polymerase, single-stranded binding protein (mtSSB), RNA processing enzymes[43] and mitochondrial transcription factor B.[44] Chronic contractile activity is known to induce an increase in mtDNA copy number, as well as an increase in the transcription of mtDNA genes.[45,46] This is facilitated by the rapid and early increase in Tfam mRNA and protein expression, followed by an accelerated rate of import and Tfam/mtDNA binding.[47] An increase in Tfam protein has also been observed in response to endurance training in humans.[48] In addition, contractile activity also increases the expression of RNA processing enzyme subunits[49] and mtSSB, but not DNA polymerase.[50] These changes are likely responsible for the observed coordination between nuclear and mitochondrial mRNA responses to chronic contractile activity.[46,51] This may be important to maintain the correct stoichiometry between nuclear- and mitochondrially-encoded subunits during the assembly of protein complexes within mitochondria. These changes may conceivably represent the most important contractile activity-induced adaptations within the organelle, since no effect on intramitochondrial protein synthesis or degradation have been observed,[52] although protein

synthesis is a definite requirement for the mitochondrial adaptations to contractile activity.[53]

The extent to which mitochondrial biogenesis will occur in human muscle depends on the intensity, duration and frequency of the training programme, and perhaps the initial mitochondrial content within the muscle, leading to significant improvements in endurance capacity. The functional consequences of physical activity become even more important when we consider: (i) the societal tendency towards inactivity; (ii) the age-related decline in oxidative capacity; and (iii) the involvement of mitochondria in the progression of disease.

3. Mitochondrial Biogenesis, Aging and Disease

A significant loss in the number of muscle fibres occurs with age. This is defined as sarcopenia, and along with significant muscle atrophy, demonstrable biochemical and morphological abnormalities also occur.[54] Some of these age-related alterations could be attributable to mitochondrial dysfunction. Mitochondrial enzyme activities and rates of intra-organelle protein synthesis are known to decline with age.[55,56] Aged skeletal muscle tissue from a variety of species show a greater number of fibres with cytochrome C oxidase deficiency, along with increased succinate dehydrogenase activity.[57-60] These histological changes are similar to the 'ragged red fibres' typically observed in mitochondrial myopathy patients,[61,62] which result from an abnormal proliferation of subsarcolemmal mitochondria. This represents the most common morphological change associated with impaired oxidative phosphorylation[63,64] as a result of mtDNA defects. While it is known that endurance training can ameliorate the decline in enzyme activities evident in older individuals,[65-67] the effect of exercise-induced mitochondrial biogenesis on age-related changes in mtDNA content, mitochondrial protein synthesis, reactive oxygen species (ROS) production or apoptosis, is currently unknown.

A decline in the oxidative capacity of muscle with age could be related to the augmented production of ROS. Approximately 1–2% of the oxygen within a cell is converted to ROS, the basal production of which increases with aging in tissues of many mammals and humans.[68] ROS compromise the integrity of macromolecules such as mtDNA housed within the mitochondria, and lead to organelle dysfunction. mtDNA is particularly vulnerable to ROS because: (i) mtDNA is located in close proximity to the electron transport chain; (ii) mtDNA lacks the protective sheath of histones compared with nuclear DNA; and (iii) mitochondria have an insufficient repair system for mtDNA mutations. This can develop into a feed-forward cycle of progressive deterioration, since defects of oxidative phosphorylation brought about by mtDNA mutations will further exacerbate ROS production. Interestingly, coincident increases in mtDNA content, the frequency of mtDNA mutations, and elevated levels of Tfam have been reported as a function of age in healthy humans.[69,70]

The age-related increases in ROS production have the potential to activate programmed cell death, or apoptosis,[71,72] a process that results from a cascade of events initiated by the release of cytochrome C and other pro-apoptotic factors from mitochondria. An increased incidence of apoptosis within aged rat skeletal muscle has recently been documented,[73] although it remains to be determined whether this is ROS-mediated. In addition, while it is established that various forms of exercise training can produce an increase in mitochondrial content in aged individuals,[65-67,74] it is not known whether this can exert a beneficial, or detrimental effect on the progression of aged muscle toward apoptosis or myonuclear destruction.

Very limited research has involved studying the effect of training in patients with known mitochon-

drial diseases; however, the results are promising. Noticeable improvements in levels of respiratory enzymes in mitochondrial myopathy patients with mtDNA defects were a consequence of aerobic training.[75] This change led to an improvement in work capacity. Further, increases in the relative proportion of wild type mtDNA copy number were observed in a case study as a result of resistance training.[76] These promising outcomes warrant further investigation, particularly when corrected for age. However, the compensatory events involved in the training adaptation, in the face of a mutant mtDNA fraction, remain to be determined. Studies using different training regimens in a variety of patients are required to establish regular exercise as a valuable approach to the treatment of patients with mitochondrial disorders.

4. Conclusions

Initial cellular disturbances that are triggered by contractile activity appear to involve changes in intracellular Ca^{2+} concentrations and accelerations of ATP turnover. These changes result in the activation of signal transduction cascades, the activation of important transcription factors, followed by the increased expression of nuclear and mitochondrial gene expression. The coordination of these responses results in mitochondrial biogenesis. While the precise details of the specific pathways involved in mitochondrial biogenesis in skeletal muscle are not yet clearly understood, the benefits of regular exercise are undisputed.

Chronic exercise is known to induce increases in mitochondrial volume and function, from which we derive a greater work capacity and an improved resistance to fatigue. Furthermore, chronic muscle use can also be used to reverse the trend toward the decrease in mitochondrial content brought about by disuse (figure 1 and figure 2). Thus, under circumstances in which mitochondrial content is compromised, such as in some mitochondrial myopathies or in age- and physical inactivity-related diseases, the plasticity of mitochondrial phenotype and content within muscle by exercise can likely be viewed as a highly favourable consequence. Further elucidation of the molecular mechanisms involved in mitochondrial biogenesis may be important in the future to identify potential alternative therapeutic interventions in patients in which even small amounts of physical activity cannot be tolerated. It is clear, however, that the full spectrum of exercise-associated physiological (i.e. central and peripheral cardiovascular, metabolic, muscular and hormonal) and psychological benefits will never be acquired via treatments other than exercise itself.

Acknowledgements

Work in the authors' laboratory is funded by the Natural Science and Engineering Research Council (NSERC) of Canada and by the Canadian Institutes for Health Research. Peter J. Adhihetty is the recipient of a Heart and Stroke Foundation of Canada Doctoral Fellowship. Isabella Irrcher and Vladimir Ljubicic are recipients of NSERC Postgraduate Scholarships. David A. Hood is the holder of a Canada Research Chair in Cell Physiology. The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

References

1. Pette D, editor. Plasticity of skeletal muscle. Berlin: Walter de Gruyter, 1980
2. Howald H, Hoppeler H, Claassen H, et al. Influences of endurance training on the ultrastructural composition of the different muscle fiber types in humans. Pflugers Arch 1985; 403: 369-76
3. Reichmann H, Pette D. A comparative microphotometric study of succinate dehydrogenase activity levels in type I, IIA, and IIB fibres of mammalian and human muscles. Histochemistry 1982; 74: 27-41
4. Holloszy JO. Biochemical adaptations in muscle: effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J Biol Chem 1967; 242: 2278-82
5. Baldwin KM, Klinkerfuss GH, Terjung RL, et al. Respiratory capacity of white, red, and intermediate muscle: adaptive response to exercise. Am J Physiol 1972; 222: 373-8
6. Takahashi M, Hood DA. Chronic stimulation-induced changes in mitochondria and performance in rat skeletal muscle. J Appl Physiol 1993; 74: 934-41

7. Wicks KL, Hood DA. Mitochondrial adaptations in denervated muscle: relationship to muscle performance. Am J Physiol 1991; 260: C841-50
8. Hoppeler H. Exercise-induced ultrastructural changes in skeletal muscle. Int J Sports Med 1986; 7: 187-204
9. Durante PE, Mustard KJ, Park S-H, et al. Effects of endurance training on activity and expression of AMP-activated protein kinase isoforms in rat muscles. Am J Physiol 2002; 283: E178-86
10. Wallace DC. Mitochondrial diseases in man and mouse. Science 1999; 283: 1482-8
11. Li B, Holloszy JO, Semenkovich CF. Respiratory uncoupling induces delta-aminolevulinate synthase expression through a nuclear respiratory factor-1-dependent mechanism in HeLa cells. J Biol Chem 1999; 274: 17534-40
12. Biswas G, Adebanjo OA, Freedman BD, et al. Retrograde Ca^{2+} signaling in C2C12 skeletal myocytes in response to mitochondrial genetic and metabolic stress: a novel mode of inter-organelle crosstalk. EMBO J 1999; 18: 522-33
13. Ohira Y, Cartier LJ, Chen M, et al. Induction of an increase in mitochondrial matrix enzymes in muscle or iron-deficient rats. Am J Physiol 1987; 253: C639-44
14. Van Deurson J, Heerschap A, Oerlemans F, et al. Skeletal muscles of mice deficient in muscle creatine kinase lack burst activity. Cell 1993; 74: 621-31
15. Shoubridge EA, Challiss RA, Hayes DJ, et al. Biochemical adaptation in the skeletal muscle of rats depleted of creatine with the substrate analogue beta-guanidinopropionic acid. Biochem J 1985; 232: 125-31
16. Bergeron R, Ren JM, Cadman KS, et al. Chronic activation of AMP kinase results in NRF-1 activation and mitochondrial biogenesis. Am J Physiol 2001; 281: E1340-6
17. Winder WW, Holmes BF, Rubink DS, et al. Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. J Appl Physiol 2000; 88: 2219-26
18. Hood DA. Invited review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. J Appl Physiol 2001; 90: 1137-57
19. Lawrence Jr JC, Salsgiver WJ. Levels of enzymes of energy metabolism are controlled by activity of cultured rat myotubes. Am J Physiol 1983; 244: C348-55
20. Freyssenet D, Di Carlo M, Hood DA. Calcium-dependent regulation of cytochrome C gene expression in skeletal muscle cells. J Biol Chem 1999; 274: 9305-11
21. Ojuka EO, Jones TE, Han H-H, et al. Intermittent increases in cytosolic Ca^{2+} stimulate mitochondrial biogenesis in muscle cells. Am J Physiol 2002; 283: E1040-5
22. Wu H, Kanatous SB, Thurmond FA, et al. Regulation of mitochondrial biogenesis in skeletal muscle by CaMK. Science 2002; 296: 349-52
23. Scarpulla RC. Transcriptional activators and coactivators in the nuclear control of mitochondrial function in mammalian cells. Gene 2002; 286: 81-9
24. Nelson BD, Luciakova K, Li R, et al. The role of thyroid hormone and promoter diversity in the regulation of nuclear encoded mitochondrial proteins. Biochim Biophys Acta 1995; 1271: 85-91
25. Xia Y, Buja LM, McMillin JB. Activation of the cytochrome C gene by electrical stimulation in neonatal rat cardiac myocytes: role of NRF-1 and c-Jun. J Biol Chem 1998; 273: 12593-8
26. Murakami T, Shimomura Y, Yoshimura A, et al. Induction of nuclear respiratory factor-1 expression by an acute bout of exercise in rat muscle. Biochim Biophys Acta 1998; 1381: 113-22
27. Puigserver P, Wu Z, Park CW, et al. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 1998; 92: 829-39
28. Lin J, Wu H, Tarr PT, et al. Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. Nature 2002; 418: 797-801
29. Terada S, Goto M, Kato M, et al. Effects of low-intensity exercise on PGC-1 mRNA expression in rat epitrochlearis muscle. Biochem Biophys Res Commun 2002; 296: 350-4
30. Irrcher I, Adhihetty PJ, Sheehan T, et al. PPARγ coactivator-1alpha expression during thyroid hormone and contractile activity-induced mitochondrial adaptations. Am J Physiol 2003; 284 (6): C1669-77
31. Wu Z, Puigserver P, Andersson U, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 1999; 98: 115-24
32. Lehman JJ, Barger PM, Kovacs A, et al. Peroxisome proliferator-activated receptor γ coactivator-1 promotes cardiac mitochondrial biogenesis. J Clin Invest 2000; 106: 847-56
33. Mihara K, Omura T. Cytosolic factors in mitochondrial import. Experientia 1996; 52: 1063-8
34. Chewawiwat N, Yano M, Terada K, et al. Characterization of the novel mitochondrial protein import component, Tom34, in mammalian cells. J Biochem 1999; 125: 721-7
35. Schneider HC, Berthold J, Bauer MF, et al. Mitochondrial Hsp70/MIM44 complex facilitates protein import. Nature 1994; 371: 768-74
36. Cogswell AM, Stevens RJ, Hood DA. Properties of skeletal muscle mitochondria isolated from subsarcolemmal and intermyofibrillar regions. Am J Physiol 1993; 264: C383-9
37. Takahashi M, Hood DA. Protein import into subsarcolemmal and intermyofibrillar skeletal muscle mitochondria: differential import regulation in distinct subcellular regions. J Biol Chem 1996; 271: 27285-91
38. Ornatsky OI, Connor MK, Hood DA. Expression of stress proteins and mitochondrial chaperonins in chronically stimulated skeletal muscle. Biochem J 1995; 311: 119-23
39. Takahashi M, Chesley A, Freyssenet D, et al. Contractile activity-induced adaptations in the mitochondrial protein import system. Am J Physiol 1998; 274: C1380-7
40. Craig EE, Chesley A, Hood DA. Thyroid hormone modifies mitochondrial phenotype by increasing protein import without altering degradation. Am J Physiol 1998; 275: C1508-15
41. Koehler CM, Leuenberger D, Merchant S, et al. Human deafness dystonia syndrome is a mitochondrial disease. Proc Natl Acad Sci U S A 1999; 96: 2141-6
42. Tranebjaerg L, Hamel BC, Gabreels FJ, et al. A de novo missense mutation in a critical domain of the X-linked DDP gene causes the typical deafness-dystonia-optic atrophy syndrome. Eur J Hum Genet 2000; 8: 464-7

43. Moraes CT. What regulates mitochondrial DNA copy number in animal cells. Trends Genet 2001; 4: 199-205
44. McCulloch V, Seidel-Rogol BL, Shadel GS. A human mitochondrial transcription factor is related to RNA adenine methyltransferases and binds S-adenosylmethionine. Mol Cell Biol 2002; 22: 1116-25
45. Williams RS. Mitochondrial gene expression in mammalian striated muscle: evidence that variation in gene dosage is the major regulatory element. J Biol Chem 1986; 261: 12390-4
46. Hood DA, Zak R, Pette D. Chronic stimulation of rat skeletal muscle induces coordinate increases in mitochondrial and nuclear mRNAs of cytochrome-c-oxidase subunits. Eur J Biochem 1989; 179: 275-80
47. Gordon JW, Rungi AA, Inagaki H, et al. Effects of contractile activity on mitochondrial transcription factor A expression in skeletal muscle. J Appl Physiol 2001; 90: 389-96
48. Bengtsson J, Gustafsson T, Widegren U, et al. Mitochondrial transcription factor A and respiratory complex IV increase in response to exercise training in humans. Pflugers Arch 2001; 443: 61-6
49. Ordway GA, Li K, Hand GA, et al. RNA subunit of mitochondrial RNA-processing enzyme is induced by contractile activity in striated muscle. Am J Physiol 1993; 265: C1511-6
50. Schultz RA, Swoap SJ, McDaniel LD, et al. Differential expression of mitochondrial DNA replication factors in mammalian tissues. J Biol Chem 1998; 273: 3447-51
51. Puntschart A, Claassen H, Jostarndt K, et al. mRNAs of enzymes involved in energy metabolism and mtDNA are increased in endurance-trained athletes. Am J Physiol 1995; 269: C619-25
52. Connor MK, Bezborodova O, Escobar CP, et al. Effect of contractile activity on protein turnover in skeletal muscle mitochondrial subfractions. J Appl Physiol 2000; 88: 1601-6
53. Williams RS, Harlan W. Effects of inhibition of mitochondrial protein synthesis in skeletal muscle. Am J Physiol 1987; 253: C866-71
54. Evans W. Functional and metabolic consequences of sarcopenia. J Nutr Suppl 1997; 127: 998S-1003S
55. Conley KE, Jubria SA, Esselman PC. Oxidative capacity and ageing in human muscle. J Physiol 2000; 526: 203-10
56. Rooyackers OE, Adey DB, Ades PA, et al. Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle. Proc Natl Acad Sci U S A 1996; 93: 15364-9
57. Aspnes LE, Lee CM, Weindruch R, et al. Caloric restriction reduces fiber loss and mitochondrial abnormalities in aged rat muscle. FASEB J 1997; 11: 573-81
58. Lee CM, Aspnes LE, Chung SS, et al. Influences of caloric restriction on age-associated skeletal muscle fiber characteristics and mitochondrial changes in rats and mice. Ann N Y Acad Sci 1998; 854: 182-91
59. Lopez M, Van Zeeland N, Dahl D, et al. Cellular phenotypes of age-associated skeletal muscle mitochondrial abnormalities in rhesus monkeys. Mutat Res 2000; 452: 123-38
60. Wanagat J, Cao Z, Pathare P, et al. Mitochondrial DNA deletion mutations colocalize with segmental electron transport system abnormalities, muscle fiber atrophy, fibre splitting and oxidative damage in sarcopenia. FASEB J 2001; 15: 322-32
61. Muller-Hocker J. Cytochrome C oxidase deficient fibres in the limb muscle and diaphragm of man without muscular disease: an age-related alteration. J Neurol Sci 1990, 21
62. Rifai Z, Welle S, Kamp C, et al. Ragged red fibers in normal aging and inflammatory myopathy. Ann Neurol 1995; 37: 24-9
63. Bresolin N, Moggio M, Bet L, et al. Progressive cytochrome C oxidase deficiency in a case of Kearns-Sayre syndrome: morphological, immunological and biochemical studies in muscle biopsies and autopsy tissues. Ann Neurol 1987; 21: 564-72
64. Bua EA, McKiernan SH, Wanagat J, et al. Mitochondrial abnormalities are more frequent in muscles undergoing sarcopenia. J Appl Physiol 2002; 92: 2617-24
65. Orlander J, Aniansson A. Effect of physical training on skeletal muscle metabolism and ultrastructure in 70- to 75-year-old men. Acta Physiol Scand 1980; 109: 149-54
66. Proctor DN, Sinning WE, Walro JM, et al. Oxidative capacity of human muscle fiber types: effects of age and training status. J Appl Physiol 1995; 78: 2033-8
67. Young JC, Chen M, Holloszy JO. Maintenance of the adaptation of skeletal muscle mitochondria to exercise in old rats. Med Sci Sports Exerc 1983; 15: 243-6
68. Sohal RS, Sohal BH. Hydrogen peroxide release by mitochondria increases during aging. Mech Ageing Dev 1991; 57: 187-202
69. Lezza AM, Pesce V, Cormio A, et al. Increased expression of mitochondrial transcription factor A and nuclear respiratory factor-1 in skeletal muscle from aged human subjects. FEBS Lett 2001; 501: 74-8
70. Pesce V, Cormio A, Fracasso F, et al. Age-related mitochondrial genotypic and phenotypic alterations in human skeletal muscle. Free Radic Biol Med 2001; 30: 1223-33
71. Cook SA, Sugden PH, Clerk A. Regulation of bcl-2 family proteins during development and in response to oxidative stress in cardiac myocytes: association with changes in mitochondrial membrane potential. Circ Res 1999; 85: 940-9
72. von Harsdorf R, Li PF, Dietz R. Signaling pathways in reactive oxygen species-induced cardiomyocyte apoptosis. Circulation 1999; 99: 2934-41
73. Dirks A, Leeuwenburgh C. Apoptosis in skeletal muscle with aging. Am J Physiol 2001; 282: R519-27
74. Jubrias SA, Esselman PC, Price LB, et al. Large energetic adaptations of elderly muscle to resistance and endurance training. J Appl Physiol 2001; 90: 1663-70
75. Taivassalo T, Shoubridge EA, Chen J, et al. Aerobic conditioning in patients with mitochondrial myopathies: physiological, biochemical, and genetic effects. Ann Neurol 2001; 50: 133-41
76. Taivassalo T, Fu K, Johns T, et al. Gene shifting: a novel therapy for mitochondrial myopathy. Hum Mol Genet 1999; 8: 1047-52

Correspondence and offprints: Dr *David A. Hood*, School of Kinesiology and Health Science, York University, Ontario, 4700 Keele Street, Toronto, M3J 1P3, Canada .
E-mail: dhood@yorku.ca