REVIEW / SYNTHÈSE

Mechanisms of exercise-induced mitochondrial biogenesis in skeletal muscle[1]

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Abstract: Acute exercise initiates rapid cellular signals, leading to the subsequent activation of proteins that increase gene transcription. The result is a higher level of mRNA expression, often observed during the recovery period following exercise. These molecules are translated into precursor proteins for import into preexisting mitochondria. Once inside the organelle, the protein is processed to its mature form and either activates mitochondrial DNA gene expression, serves as a single subunit enzyme, or is incorporated into multi-subunit complexes of the respiratory chain devoted to electron transport and substrate oxidation. The result of this exercise-induced sequence of events is the expansion of the mitochondrial network within muscle cells and the capacity for aerobic ATP provision. An understanding of the molecular processes involved in this complex pathway of organelle synthesis is important for therapeutic purposes, and is a primary research undertaking in laboratories involved in the study of mitochondrial biogenesis. This pathway in muscle becomes impaired with chronic inactivity and aging, which leads to a reduced muscle aerobic capacity and an increased tendency for mitochondrially mediated apoptosis, a situation that can contribute to muscle atrophy. The resumption, or adoption, of an active lifestyle can ameliorate this metabolic dysfunction, improve endurance, and help maintain muscle mass.

Key words: PGC-1α, mitochondrial transcription factor A, reactive oxygen species, AMP kinase, aging, muscle disuse.

Résumé : Une séance d'exercice déclenche une série rapide de signaux cellulaires aboutissant à l'activation de protéines, ce qui accroît la transcription génique. Il s'ensuit un plus haut degré d'expression d'ARNm, souvent observée au cours de la récupération consécutive à un exercice physique. Ces molécules donnent lieu à des précurseurs protéiques à importer dans la mitochondrie déjà en place. Une fois à l'intérieur de l'organelle, la protéine évolue jusqu'à maturité et stimule l'expression d'un gène dans la mitochondrie ou sert de simple module enzymatique ou est intégré dans un complexe multimodulaire de la chaîne respiratoire dédiée au transport d'électrons et à l'oxydation des substrats. Le résultat de cette séquence d'événements déclenchée par l'exercice physique contribue à augmenter le réseau mitochondrial dans la cellule musculaire et à accroître la production aérobie d'ATP. La compréhension des processus moléculaires impliqués dans la voie complexe de la synthèse d'un organelle est importante au plan thérapeutique et doit faire l'objet d'études fondamentales dans les laboratoires consacrés à l'étude de la biogenèse des mitochondries. Cette voie est entravée par l'inactivité chronique et le vieillissement ce qui entraîne une diminution de la capacité aérobie du muscle et une augmentation de la tendance à l'apoptose médiée par la mitochondrie, d'où la possibilité d'atrophie musculaire. L'adoption ou le retour à un mode de vie actif peut contribuer à contrer cette dysfonction métabolique, à améliorer l'endurance et à maintenir la masse musculaire.

Mots-clés : PGC-1α, facteur A de transcription mitochondriale, espèces oxygénées radicalaires, AMP kinase, vieillissement, inactivité musculaire.

[Traduit par la Rédaction]

Introduction

Regular endurance exercise has a number of health benefits. These benefits include improvements in cardiovascular function and muscle metabolism and increased work capacity. The increase in capacity for sustained work is largely a consequence of greater oxygen extraction by the exercising muscle, which is a direct result of an improved capillary to fiber ratio, as well as a higher mitochondrial content within muscle. The increase in mitochondrial content is a well-established adaptation within the exercised muscle, but the molecular mechanisms underlying this change in muscle phenotype are just beginning to be clarified. The process, re-

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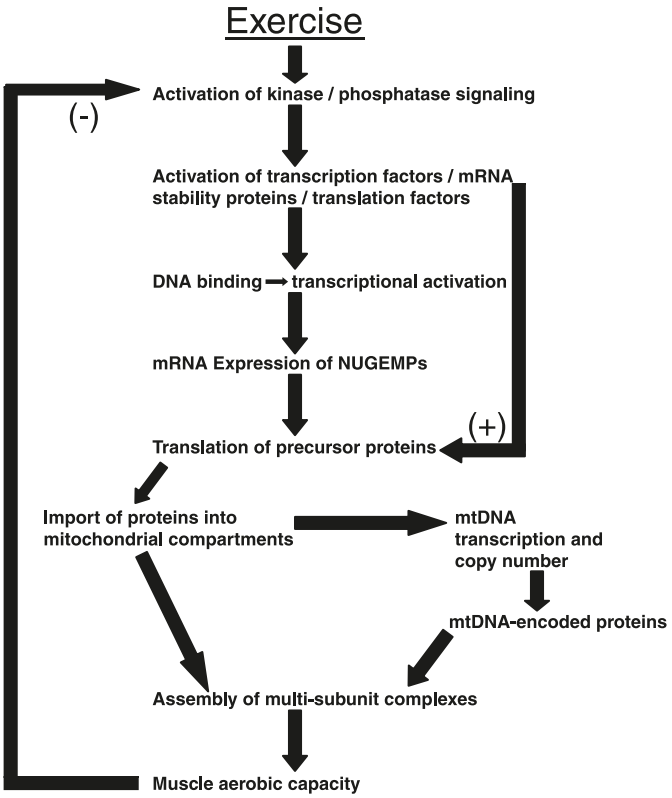
ferred to as mitochondrial biogenesis, is complex because mitochondria are composed of proteins derived from both the nuclear and the mitochondrial genomes. The major steps include exercise-induced activation of signaling reactions, and the subsequent activation of coactivator proteins and transcription factors; the transcriptional regulation of nuclear genes encoding mitochondrial proteins; the stabilization of mRNA transcripts and subsequent mRNA translation into precursor proteins; the import of these precursor proteins into mitochondrial compartments; the expression of mitochondrial DNA (mtDNA); and the assembly of both mitochondrial and nuclear gene products into multi-subunit complexes within an expanding organelle reticulum. A summary of this sequence of events is provided in Fig. 1. The assembly of an entire organelle is a complex process involving lipids, proteins, and DNA. Lessons learned from the synthesis of mitochondria can undoubtedly apply to the synthesis of other organelles, such as peroxisomes, endoplasmic reticulum, or nuclei. Thus, an understanding of this process is vital for our basic comprehension of cell biology.

From a more applied perspective, mammalian physiologists have known for more than 40 years that, when muscle is chronically exercised, mitochondrial content increases, thereby improving the tissue's capacity for oxygen consumption and ATP provision. This phenomenon has a number of health and fitness benefits, not the least of which is an improved muscular endurance and reduced fatiguability during the normal exertional activities of everyday life. Here, we briefly review the major steps involved in the mitochondrial biogenesis resulting from exercise, beginning with the initial signaling events and ending with the posttranslational import of proteins into mitochondria. A number of related reviews have also recently been published on this topic (Arany 2008; Chabi et al. 2005; Hood et al. 2006; Koulmann and Bigard 2006; Yan et al. 2007).

Kinase activation with exercise

The initiation of muscle adaptation begins with the early signals associated with contracting muscle, leading to downstream kinase activation and subsequent gene expression (Fig. 1). The onset of contractile activity evokes a number of rapid events, such as calcium cycling, ATP turnover, reactive oxygen species (ROS) production, and oxygen consumption. The resulting activation of kinases and phosphatases produces the posttranslational modification of proteins (Sakamoto and Goodyear 2002). Although little is known about the activation of phosphatases during exercise, it is well established that many kinases, including AMP-activated protein kinase (AMPK), calcium-calmodulin kinase II, protein kinase B, and the mitogen-activated protein kinase p38, are activated by exercise. The impact of this activation is the phosphorylation of nuclear transcription factors and coactivators that are involved in the regulation of DNA transcription. These kinases increase their phosphorylation in a fashion that is dependent on the intensity and duration of the contractile activity, as well as the fiber-type composition of the muscle (Ljubicic and Hood 2008). Kinase activation affects the phosphorylation and DNA binding activity of transcription factors. This can influence transcriptional activity and modify the steady-state concentration of RNA within the cell. In addition to affecting proteins involved in transcription, kinase activation can alter the phosphorylation of RNA binding proteins, which can influence RNA stability in either a stabilizing or a destabilizing manner. However, much less is known about the regulation of mRNA stability in exercise-induced mitochondrial biogenesis. Indeed, most of the direct protein targets of kinase activation due to exercise remain to be identified. However, it is clear that repeated activation of these signaling cascades during acute bouts of exercise result in phenotypic adaptations such as mitochondrial biogenesis and improved endurance performance.

Fig. 1. Exercise-induced initiation and propagation of mitochondrial biogenesis in muscle. Acute exercise evokes a unique set of intracellular signaling events involving cytosolic calcium, reactive oxygen species, and ATP turnover. The resultant activation of kinases and phosphatases leads to the covalent modification of proteins involved in transcription, mRNA stability, and translation. Predominantly during the recovery phase, the mRNA expression of nuclear genes encoding mitochondrial proteins (NUGEMPs) is enhanced, and protein synthesis is accelerated. The precursor proteins that are synthesized in the cytosol are rapidly imported into the organelle. These proteins are processed to their mature forms, and act as metabolic enzymes (e.g., in Krebs cycle), form part of multi-subunit electron transport chain complexes, or serve as transcription factors for mtDNA. mtDNA transcription and translation subsequently increases to provide more mtDNA-encoded proteins. These gene products combine with imported nuclear-derived proteins to form multi-subunit complexes of the electron transport chain, thereby increasing the cellular capacity for electron transport, oxygen consumption, and ATP provision. The increased capacity for energy provision can serve to attenuate the initial signaling events brought about by acute contractile activity, in a negative feedback fashion.



PGC-1α and exercise-induced mitochondrial biogenesis

In recent years, peroxisome proliferator activated receptor gamma (PPARγ) coactivator-1α (PGC-1α) has become one of the most widely studied proteins in cellular metabolism. It has been established as an important regulator of a wide variety of metabolic processes, ranging from gluconeogenesis in hepatocytes, brown fat thermogenesis, muscle fiber type specialization in skeletal muscle, and mitochondrial biogenesis in both muscle and heart (Lin et al. 2005). Muscle-specific overexpression of PGC-1α is sufficient to increase mitochondrial content and induce a host of adaptations reminiscent of endurance exercise training, including an increased proportion of type I muscle fibers and a corresponding increase in fatigue resistance (Calvo et al. 2008; Lin et al. 2002).

PGC-1α binds and coactivates DNA binding transcription factors, thus augmenting their activity. Most relevant to the onset of mitochondrial biogenesis is the interaction of PGC-1α with the nuclear respiratory factors NRF-1 and NRF-2. NRF-1 and (or) NRF-2 binding sites are located in the promoters of multiple nuclear genes encoding mitochondrial proteins, including cytochrome *c*, components of the electron transport chain complexes, mitochondrial import proteins, heme biosynthesis proteins, and the mitochondrial transcription factor A (Tfam). Thus, PGC-1α can effectively coordinate the dual genomic regulation of mitochondrial biogenesis. One of the most important upstream kinases involved in PGC-1α activity is p38 mitogen-activated protein kinase. Phosphorylation of p38 activates PGC-1α by mediating de-repression of the protein, and it results in the upregulation of PGC-1α, presumably via promoter activation (Akimoto et al. 2005). In addition, reversible acetylation of PGC-1α also regulates its activity. Deacetylation of PGC-1α by silent information regulator T1 (SIRT1) enhances the ability of PGC-1α to coactivate the transcription of gluconeogenic genes, but has no effect on the coactivation of cytochrome *c* and β-ATP synthase transcription (Rodgers et al. 2005). Thus, posttranslational modifications can alter the ability of PGC-1α to coactivate the transcription of genes involved in mitochondrial biogenesis.

PGC-1α expression is dynamically regulated by altered patterns of physical activity. In response to a single bout of exercise, PGC-1α mRNA and protein are significantly elevated in mice, rats, and humans (Akimoto et al. 2005; Baar et al. 2002; Norrbom et al. 2004; Pilegaard et al. 2003). This increase in gene expression is evident as early as 2 h post exercise. Interestingly, PGC-1α protein has been shown to increase progressively over the course of a long-term training program in rats (Taylor et al. 2005), with repeated bouts of chronic low-frequency stimulation in animals and in C2C12 cells electrically stimulated in culture (Irrcher et al. 2003). These studies illustrate that contractile activity is a main stimulus for exercise-induced PGC-1α upregulation.

How changes in muscle activity are transduced to produce alterations in gene transcription and subsequent phenotypic adaptations is an important unresolved question in exercise physiology. In view of the important role of PGC-1α in mitochondrial biogenesis, the exercise-induced signals that regulate PGC-1α expression have been the subject of much investigation. The increase in PGC-1α mRNA following acute exercise is at least partly due to an increase in transcription (Pilegaard et al. 2003), and the major exercise-induced signals appear to act on PGC-1α expression at this level. Calcium-calmodulin kinase and p38 mitogen-activated protein kinase increase PGC-1α promoter activity through the activation of cAMP response element-binding protein and activating transcription factor 2, respectively (Akimoto et al. 2005; Handschin et al. 2003). In addition, the PGC-1α promoter contains a binding site for myocyte enhancer factor 2, a transcription factor that is activated by both calcium-calmodulin kinase and p38. Electrical stimulation of skeletal muscle in mice activates the PGC-1α promoter, and this effect is abolished when either the myocyte enhancer factor 2 or cAMP response element binding site is mutated (Akimoto et al. 2004). Interestingly, PGC-1α activates its own promoter by coactivating myocyte enhancer factor 2, an effect that is augmented by the Ca^{2+}-dependent phosphatase calcineurin. Finally, PGC-1α protein levels are reduced in muscle from p53 knockout animals (Saleem et al. 2009). A p53 binding site is found within the PGC-1α promoter (Irrcher et al. 2008), which suggests that p53 may play a role in regulating the steady-state level of this protein. Taken together, these studies point to the cooperative action of myocyte enhancer factor 2, cAMP response element-binding protein, activating transcription factor 2, and possibly p53 transcription factors in altering PGC-1α transcription in response to multiple exercise-induced signals.

It is evident that PGC-1α is sufficient to induce mitochondrial biogenesis. However, whether it is necessary for exercise-induced mitochondrial biogenesis is not fully resolved. Mitochondrial volume is lower in the skeletal muscle of PGC-1α knockout (PGC-1α$^{-/-}$) mice than in wild-type controls, with a concomitantly reduced expression of Tfam, cytochrome *c*, and cytochrome oxidase subunit IV (COXIV) (Leone et al. 2005). PGC-1α$^{-/-}$ mice suffer a reduced capacity to increase work output to match an increase in metabolic demand in slow-twitch muscle. Specifically, PGC-1α$^{-/-}$ mice display a diminished capacity for endurance exercise and fatigue resistance, and isolated mitochondria from these animals display a reduced capacity for ADP-stimulated respiration (Adhihetty, Uguccioni, and Hood, unpublished observations). Thus, it is clear that PGC-1α plays a vital role in the maintenance of mitochondrial content and function in muscle. However, it is also evident that the absence of PGC-1α does not abolish the effect of endurance exercise training on mitochondrial biogenesis (Leick et al. 2008), because similar increases in protein markers are evident with training, even in PGC-1α null animals. This suggests that alternative transcription factors can substitute for PGC-1α in its absence, to coordinate an exercise-induced increase in mitochondrial content.

Signaling to mitochondrial biogenesis

Reactive oxygen species

Exercise produces an increase in oxygen consumption. A number of studies have demonstrated a connection between this increase in oxygen utilization and the formation of ROS. This oxidative stress contributes to the accumulation

of somatic mutations and oxidative damage to mtDNA, as well as to the damage of macromolecular structures within the cell. This has been apparent in mitochondrial diseases, tumorgenesis aging, degenerative diseases, and diabetes (Ames et al. 1993). Under normal conditions, the majority of ROS formation originates from the mitochondrial respiratory chain. The measured percent of oxygen converted to ROS is approximately 1% to 4% of that which incompletely passes through the electron transport chain. However, during exercise, the increase in ROS production may also be generated by alternative sources. McArdle et al. (2004) showed that ROS are also released into the extracellular fluid of the muscle following bouts of contractile activity. Indeed, it has been proposed that the flavoprotein oxidoreductase system, located at the plasma membrane, is a predominant generator of extracellular superoxide during contractile activity (Pattwell et al. 2004).

Although considerable focus has been placed on the damage created by the production of ROS, it also known that ROS can activate signaling pathways involved in phenotypic adaptations. ROS have been demonstrated to induce mitochondrial network branching and elongation. mtDNA copy number has also been shown to increase with rising levels of ROS in aging skeletal muscle (Pesce et al. 2005). The increase in mtDNA was accompanied by an induction in mitochondrial mass. This response appeared to be mediated by PGC-1α and NRF-1, because the expression of both increased following exogenous ROS treatment (Suliman et al. 2003). Recently, we have demonstrated that ROS can induce an increase in PGC-1α promoter activity and expression via both AMPK-dependent and AMPK-independent pathways (Irrcher et al. 2009). These pathways likely account, in part, for the increase in mitochondrial biogenesis observed in the presence of ROS.

AMPK activation

AMPK is an energy-sensing enzyme that is activated by a high AMP:ATP ratio, such as that which occurs during exercise, and by phosphorylation mediated by an upstream kinase. The enzyme is a heterotrimer that consists of a catalytic α subunit and 2 regulatory subunits, β and γ. Skeletal muscle expresses both an α1 and α2 isoform of the catalytic subunit, and the α2 isoform is highly activated by exercise (Stephens et al. 2002). Activation of α2 AMPK also occurs with 5-aminoimidazole-4-carboxamide riboside treatment. 5-aminoimidazole-4-carboxamide riboside is taken up by cells and phosphorylated to AICAR monophosphate (ZMP), an analog of AMP. Pharmacological activation of AMPK by 5-aminoimidazole-4-carboxamide riboside increases PGC-1α mRNA and protein (Irrcher et al. 2003). This is likely mediated by transcriptional activation, because AMPK activation leads to enhanced PGC-1α promoter activity (Irrcher et al. 2008). The upregulation of PGC-1α transcription and translation is accompanied by the increased DNA binding activity of NRF-1, an important transcriptional regulator of proteins involved in mitochondrial biogenesis. Moreover, mice genetically engineered to lack AMPK activity do not display an increase in PGC-1α or mitochondrial content in response to an increased AMP:ATP ratio in skeletal muscle during energy deprivation (Zong et al. 2002). In addition, chronic activation of AMPK using 5-aminoimidazole-4-carboxamide riboside has resulted in increases in mitochondrial enzymes such as cytochrome *c*, citrate synthase, and malate dehydrogenase in skeletal muscle (Winder et al. 2000). Thus, AMPK activation is another important regulator of mitochondrial biogenesis under conditions of energy supply–demand imbalance in muscle cells.

Mitochondrial protein import

Mitochondria are particularly interesting because they contain their own DNA (mtDNA) that is distinct from that found in the nucleus. mtDNA is small and compact and has a limited coding capacity for proteins. It encodes only 13 proteins, all of which are found within the electron transport chain complexes of the mitochondrial inner membrane. Proteomic studies of mitochondria reveal that the organelle contains approximately 1500 proteins. Thus, the remaining vast majority of proteins that reside within the organelle must be transcribed from nuclear DNA and synthesized within the cytoplasm (see Bolender et al. 2008; Neupert and Herrmann 2007 for reviews). Subsequently, they are imported into preexisting mitochondria via a complex transport process involving the protein import machinery. Thus, the mitochondrial reticulum expansion that accompanies mitochondrial biogenesis requires an accelerated incorporation of hundreds of proteins into submitochondrial compartments. The protein import machinery responsible for this consists of a series of multi-subunit complexes. The protein import machinery is composed primarily of the translocases of the outer membrane (TOM complex) and the translocases of the inner membrane (TIM complex). The TOM complex includes receptor proteins such as TOM20, TOM22, and TOM70 that accept precursor proteins from cytosolic chaperones and usher them to the general import pore of ~400 kDa. TOM40 is the main component of this pore, along with smaller components such as TOM5, TOM6, and TOM7.

The TIM machinery is composed of a group of proteins that assist in targeting precursor proteins to the intermembrane space, inner membrane, and matrix. This translocation requires both a mitochondrial membrane potential and ATP. The main component of the TIM pathway is the TIM23 complex, which contains a channel made up of TIM17, TIM50, and TIM21. This pathway is involved in the translocation of precursor proteins to the matrix compartment. The TIM22 complex, the second complex of the TIM pathway, is responsible for inserting proteins with internal targeting signals into the inner membrane.

The mitochondrial protein import pathway is not static but can respond to energy perturbations within the cell. For example, the expression of several key protein import machinery components is increased in response to chronic contractile activity. These include the intramitochondrial chaperone mitochondrial heat shock protein 70 (mtHSP70), the outer membrane receptor TOM20, and the cytosolic chaperone mitochondrial import stimulation factor. The coordinated upregulation of chaperone proteins, as well as TOM and TIM proteins, directly results in the greater import of matrix precursor proteins into mitochondria (Takahashi et al. 1998). More work is required to determine whether contractile activity alters the import of proteins into the inner or outer membrane compartments. However, the acceleration

of protein import into the matrix brought about by contractile activity suggests that defects in import could be ameliorated by regular exercise. Reduced import capacity could be implicated during conditions of chronic muscle inactivity. Indeed, we have found that matrix protein import is impaired during denervation-induced muscle disuse (Singh and Hood, unpublished observations).

All the proteins that regulate the replication and transcription of mtDNA are nuclear encoded and require import into the organelle. Arguably the most important of these regulatory proteins is Tfam. The importance of Tfam is evident from the phenotype exhibited by Tfam knockout mice. Tfam knockout is embryonic lethal, and mtDNA copy number and respiratory chain complex activities are reduced in heterozygous Tfam knockout animals (Larsson et al. 1998). Exercise is known to increase the expression and function of Tfam in muscle in both animals and humans. One week of chronic contractile activity of rat muscle led to an increase in Tfam mRNA level after 4 days, an accelerated protein import into the matrix, an increase in Tfam–mtDNA binding and elevated mtDNA transcript levels encoding cytochrome *c* oxidase (COX) subunit III, and a higher COX enzyme activity by day 7 (Gordon et al. 2001). A similar increase in Tfam expression has been found following endurance training in humans (Bengtsson et al. 2001). Thus, the increase in Tfam expression during the progression of exercise training contributes substantially to mtDNA expression, the synthesis of protein subunits, and their subsequent incorporation into electron transport chain respiratory complexes in skeletal muscle.

Mitochondrial biogenesis during chronic muscle disuse

There is strong evidence to suggest that chronic muscle disuse, in the form of space flight, limb immobilization, denervation, or bed rest, decreases mitochondrial content and whole muscle oxidative capacity. Chronic muscle inactivity disrupts the expression of both the nuclear and the mitochondrial genomes (Wicks and Hood 1991) and inhibits mitochondrial biogenesis. For example, prolonged muscle disuse has been shown to decrease cytochrome *c* mRNA in both slow-twitch and fast-twitch muscles, as well as the enzymatic activities of cytochrome *c* oxidase, succinate dehydrogenase, citrate synthase, and malate dehydrogenase (Babij and Booth 1988; Rifenberick et al. 1973; Wicks and Hood 1991). As a consequence, disused skeletal muscle displays a decreased ability to generate ATP aerobically, and becomes more dependent on glycolytic pathways for ATP production. Muscle disuse brings about a rapid decline in subsarcolemmal mitochondrial content, and compromises its ability to generate ATP within 48 h of disuse (Krieger et al. 1980). Conversely, intermyofibrillar mitochondria exhibit a slower, more gradual decrease in response to reductions in muscular activity. As a result of these adaptation differences, intermyofibrillar mitochondria constitute a greater proportion of the total mitochondrial content during muscle disuse. Because intermyofibrillar mitochondria are more susceptible to the release of proapoptotic proteins than subsarcolemmal mitochondria (Adhihetty et al. 2005), it is evident that apoptotic susceptibility increases with muscle disuse, contributing to a greater degree of apoptosis and a resultant increase in muscle atrophy.

Mitochondrial content and function during aging

Considerable debate exists regarding the status of mitochondrial function and content within skeletal muscle during the aging process (cf. Huang and Hood 2009; Conley et al. 2007; Kent-Braun 2009) and the consequent effects on muscle endurance performance. Results depend to some degree on the species studied, the age of the individuals, the amount of physical activity of the subjects, and whether or not specific fiber types have been considered in the analyses. Several studies have shown that skeletal muscle from older humans and animals displays reduced activities of several complexes of the electron transport chain and citrate synthase, as well as decreases in oxygen consumption and ATP production. In addition, an assortment of mtDNA mutations (large-scale deletions and point mutations) previously identified in mitochondrial diseases have been shown to accumulate in aging muscle. Even though these mutations accumulate exponentially with advancing age, it has been argued that they occur after the onset of mitochondrial dysfunction in aging humans (Conley et al. 2007). Further, within single fibers, mtDNA mutations can lead to an increase in the number of ragged-red fibers, which can invoke muscle fiber loss, contributing to the sarcopenia of aging (Bua et al. 2002). There is little doubt that, when present in sufficient quantities, mtDNA mutations can lead to dysfunctional electron transport chain function and the formation of ROS. ROS production has generally been shown to be elevated in aged skeletal muscle and in isolated mitochondria (Chabi et al. 2008), and these would produce cellular damage, particularly if the antioxidant defenses are not appropriately upregulated. Because ROS are potent activators of the mitochondrial apoptotic pathway, an imbalance in ROS production in defective mitochondria may increase the potential to trigger apoptosis and myonuclear decay in aged skeletal muscle. In senescent animals, this precise scenario exists. Mitochondria produce more ROS and release more apoptotic proteins, leading to higher rates of DNA fragmentation (Chabi et al. 2008). In addition, the expression of the important transcriptional coactivator PGC-1α is reduced in aging muscle (Chabi et al. 2008). This contributes to a reduced transcriptional drive for mitochondrial synthesis in the muscle of aging animals.

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 (Brierley et al. 1996). 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 (Coggan et al. 1992; Orlander and Aniansson 1980). This likely happens through increases in expres-

sion of the coactivator PGC-1α and the specific transcription factors NRF-1 and Tfam, the main regulators of organelle biogenesis and protein expression (Short et al. 2003). 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 (Taivassalo et al. 2001). 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 (Adhihetty et al. 2007), 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 defenses (Radak et al. 2002). 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 (Judge et al. 2005). 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.

Conclusions

An appreciation of the mechanisms of mitochondrial biogenesis is now recognized as relevant to an understanding of a large number of cellular pathological conditions evident in many tissues. In skeletal muscle, exercise can play a significant role in accelerating the rate of mitochondrial biogenesis. This can serve to attenuate the possible mitochondrial dysfunction that arises during aging and conditions of muscle disuse, thereby improving work performance and the quality of life.

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