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Muscling in on Mitochondria in Exercise, Health and Disease

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Skeletal muscle is a remarkably plastic tissue. One of the best examples is the ability of muscle to adapt to the stress of regular endurance exercise by increasing mitochondrial content. Regular exercise is also one of the most effective therapies in minimising muscle atrophy due to disease. Understanding the molecular regulation of these processes has only emerged in the past 15 years. Key regulators in these processes are the transcriptional co-activators, peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α and -1β (PGC-1α and PGC-1β, respectively). These transcriptional co-activators are known to positively regulate mitochondrial content and attenuate muscle atrophy pathways (1). PGC-1α and PGC-1β regulate mitochondrial content and function by the activation of transcription factors including the nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2), peroxisome proliferator-activated receptor-α (PPARα) and estrogen-related receptor-α (ERRα) (1) (**Fig. 1**). These transcription factors transcribe genes that encode proteins important for oxidative enzymes, mitochondrial fusion and mitochondrial DNA (mtDNA) transcription, such as cytochrome oxidase (COX), mitofusins-1 (Mfn1) and -2 (Mfn2) and mitochondrial transcription factor A (Tfam), respectively.

Exercise Increases Mitochondrial Biogenesis

Endurance exercise potently stimulates increases in skeletal muscle mitochondrial volume; the increased mitochondrial biogenesis following exercise training is thought to be largely attributed to the cumulative effects of each acute bout of exercise (2). Key steps in the exercise-induced mitochondrial biogenesis pathway in skeletal muscle following acute exercise involve p38 MAPK phosphorylating and activating transcription factor-2 (ATF-2), allowing the latter to bind to the cAMP-response element-binding protein (CREB) site on the PGC-1α promoter and induce PGC-1α gene expression (3). Several hours following acute exercise, the expression of the skeletal muscle genes encoding PGC-1α, NRF-1, NRF-2 and Tfam is increased in each case, as also occurs for genes encoding NRF-1 and -2, DNA-binding proteins which are also involved in coordinating the exercise training response (3,4). Acute exercise also activates AMP-activated protein kinase (AMPK) (4), which is known to phosphorylate and activate PGC-1α (2). Additional regulation by PGC-1 also appears to involve its subcellular localisation. In skeletal muscle under resting conditions, most PGC-1α protein is localised in the cytosol; however, following endurance exercise nuclear PGC-1α protein content increases,

Fig. 1. A schematic example of several key factors that contribute to the exercise-induced increase in mitochondrial biogenesis and function.

The exercise-induced increase in cytosolic Ca^{2+}, AMP, reactive oxygen species (ROS) and possibly NAD^+ activate several signaling proteins, such as p38 MAPK, which phosphorylates and activates transcription factor-2 (ATF-2) resulting in an increase in PGC-1α gene expression; there are also increases in AMPK and SIRT-1 that phosphorylate and deacetylate PGC-1α, respectively, resulting in an increase in PGC-1α activity. PGC-1α increases the transcription and the activation of NRF-1, NRF-2, PPARα and ERRα, which are transcription factors that transcribe genes such as COX2, COX4, Mfn1, Mfn2 and Tfam; each representing a target that controls cellular respiration, mitochondrial fusion and mtDNA transcription.
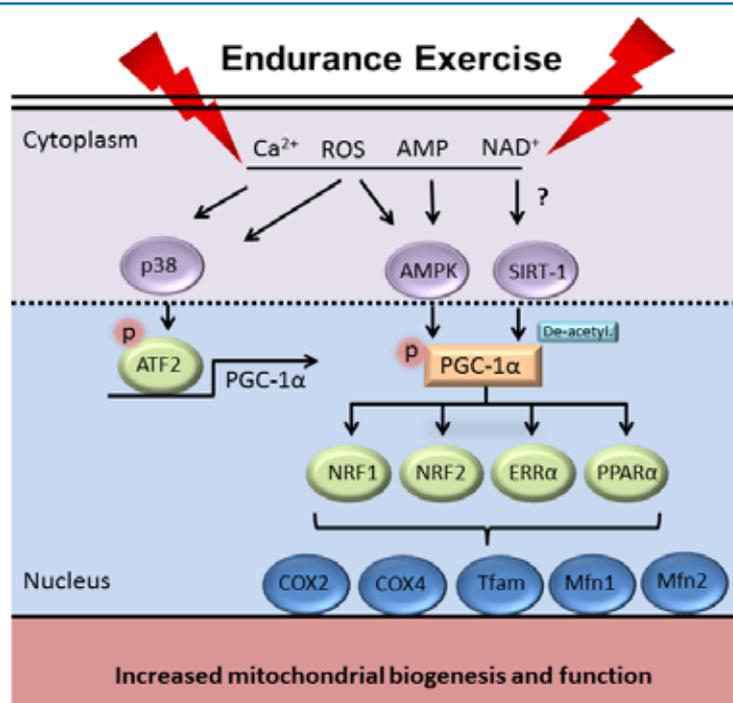
probably by translocation from the cytosol (2,5). Recent evidence also suggests that following endurance exercise there is a translocation of PGC-1α to the mitochondria for co-activation of Tfam (2). Further post-translational modification of PGC-1α can be achieved following its deacetylation by sirtuin-1 (SIRT-1), which is activated by increased NAD^+ levels (2) (**Fig. 1**).

Regulators of Exercise-induced Mitochondrial Biogenesis

During exercise there is an increase in several molecular 'stress' signals in skeletal muscle that appear to be responsible, at least in part, for the initial activation of mitochondrial biogenesis after exercise. These molecular signals include elevated levels of cytosolic Ca^{2+} (6,7), AMP (6), reactive oxygen species (ROS) (8) and possibly NAD^+ (2) (**Fig. 1**). Increasing cytosolic Ca^{2+} levels in L6 muscle cells via caffeine treatment activates Ca^{2+}-calmodulin kinase (CAMK) and increases markers of mitochondrial biogenesis, which include PGC-1α, Tfam, COX and citrate synthase (6,7); conversely, inhibitors of Ca2+ release abolish these caffeine effects (7). The activation of muscle AMPK by 5'aminoimidazole-4-carboxyamide-ribonucleoside (AICAR) in L6 muscle cells also increases many of these mitochondrial biogenesis markers (6). Increasing the ROS levels in skeletal muscle cells activates the redox sensitive kinases, AMPK and p38 MAPK, resulting in elevated PGC-1α (8); these ROS effects are blocked by co-treatment with antioxidants (8).

Type 2 Diabetes

Skeletal muscle accounts for approximately 80% of insulin-stimulated glucose uptake and a key defect in the aetiology of type 2 diabetes (T2D) is the development of insulin resistance in skeletal muscle. It has been well documented that skeletal muscle of people with T2D have impaired mitochondrial function, due to reduced mitochondrial content (9). Furthermore, many of the regulatory components involved in mitochondrial biogenesis, such as PGC-1α and NRF-1, are also known to be reduced in the skeletal muscle of people with T2D (9). This led to the popular hypothesis that the reduction in mitochondrial content in T2D was causative in the development of insulin resistance in skeletal muscle, via the reduced capacity for lipid oxidation and subsequent accumulation of lipids that may impair insulin-stimulated glucose uptake (10). However, it has been shown that the majority of studies who find reduced mitochondrial content in T2D are confounded by several factors, such as not controlling for reduced physical activity, which strongly impacts mitochondrial content (10). Therefore it appears that mitochondrial dysfunction, at least in humans with T2D, is secondary to the development of insulin resistance in skeletal muscle.

Although mitochondrial dysfunction may not be causative in the development of insulin resistance in skeletal muscle, it is still quite likely that improving skeletal muscle mitochondrial content and function could be causative in improving insulin resistance. Indeed, people with T2D respond to endurance training with normal increases in insulin sensitivity and skeletal muscle mitochondrial proteins (10). Furthermore, studies that mimic the exercise training response via electrotransfection of PGC-1α into

rat skeletal muscle result in increased PGC-1α protein content, mtDNA and mitochondrial enzyme activities with concomitant improvements in insulin sensitivity in skeletal muscle (11).

Motor Neuron Disease: Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a severe motor neuron disorder resulting in the progressive degeneration of upper and lower motor neurons, a decline in strength, severe muscle atrophy and respiratory insufficiency. It is the most common of the five motor neuron diseases. There is no cure for ALS and death occurs within 3–5 years after diagnosis. The general consensus is that ALS is caused by motor neuron death and that perturbations in mitochondrial function are an important component in ALS pathogenesis. Skeletal muscle of ALS patients has considerable mitochondrial disruption and dysfunction, indicated by NADH:CoQ oxidoreductase and cytochrome c oxidase deficiency, reduced mtDNA and reduced levels of mitochondrial Mn-SOD (12). Neuronal degeneration in an animal model of ALS (transgenic mice expressing SOD1G93A) is preceded by the degeneration of the neuromuscular junction (13) and muscle atrophy and degeneration (14), suggesting that skeletal muscle degeneration may contribute to neurodegeneration and play a key role in the cause and/or progression of ALS. It is therefore logical to hypothesise that the pathogenesis and progression of ALS may involve perturbations in signalling molecules that are normally required for the healthy maintenance of mitochondrial biogenesis and function in skeletal muscle, as well as muscle mass overall. A significant reduction in PGC-1α and PGC-1β, as well as in key downstream targets such as ERRα, NRF-1, MFN1, Mfn2 and COXIV, has been observed in ALS patients, suggesting that perturbations in these molecular signalling proteins may play a role in the onset or severity of the disease.

Ageing

In cells of individuals of advanced age, mitochondria often appear abnormally rounded. They tend to be lower in density and a reduced mtDNA content is evident. In ageing, there are reduced activities of some enzymes, impaired respiration and there is evidence of increased oxidative stress. It has been suggested that the increased production of reactive oxygen species is a key factor contributing to the perturbations in mitochondrial morphology and DNA content and that this is a major contributor to the aging process. Correlations between mtDNA mutations, impaired biogenetics and muscle wasting have been observed (15). From a molecular perspective, AMPK activation is reduced, as are levels of PGC-1α and Mfn2, while Fission 1 (Fis1) is increased in muscle from the elderly. A reduction in these signalling molecules is consistent with impaired mitochondrial biogenesis and function, and would also contribute to the age-related decline in muscle mass. Several of these observations can be influenced by a reduction in physical activity; thus, when levels of such activity are considered, age-related impairment in mitochondrial activities is not present. Because exercise increases mitochondrial biogenesis and function in young and older subjects, the maintenance of physical activities throughout life may delay the onset of age-related mitochondrial decline.

Mitochondria and Muscle Wasting

The complex and interconnected mitochondrial network involves the dynamic processes of mitochondria fission (division) and mitochondrial fusion (joining). The equilibrium between fission and fusion is responsible for the mitochondrial shape, size and networking within mitochondria. Major proteins regulating fission include dynamin related-protein 1 (Drp1) and Fis1, while fusion is controlled by Mfn1, Mfn2 and optic atrophy 1 (Opa1). Mitochondrial fusion involves the fusion of both mitochondrial membranes and is required to maintain mitochondrial morphology and metabolism (2). Mitochondrial fusion is controlled by Mfn1 and Mfn2 that are located on the outer mitochondrial membrane. Recent studies have shown that disruption of regulatory components of the mitochondrial network influences the balance of skeletal muscle atrophy programs. Deletion of both Mfn1 and Mfn2 in mouse skeletal muscle causes mitochondrial dysfunction, compensatory mitochondrial proliferation, mtDNA depletion, high levels of mtDNA mutations and muscle atrophy (16). Additionally, under catabolic conditions it was shown that mitochondria are removed via autophagy and that this was caused by the activation of AMPK and mitochondrial fission (2). The inhibition of fission abrogated muscle wasting under fasting conditions. PGC-1α and PGC-1β regulate Mfn1 and Mfn2 in an ERRα-dependent manner in C2C12 myotubes (17). In mice the double deletion of skeletal muscle PGC-1α and PGC-1β results in a 60-70% reduction in *Mfn1* and *Mfn2* mRNA levels and also induces mitochondrial dysfunction (18). Skeletal muscle wasting is also associated with an increase in the mitochondrial E3 ubiquitin-ligase 1 (Mul1). Mul1 is a FoxO-regulated gene that increases mitochondrial fission via the ubiquitination and degradation of Mfn2. The suppression of Mul1 protects against mitochondrial fission and was found to partially rescue muscle wasting (19). Nuclear factor 90 (NF90) is involved in transcription and translation. Mice transgenically overexpressing NF90 have skeletal muscle atrophy, with mitochondrial vacuolation caused by autophagocytosis (20). This was accompanied by reduced expression of PGC-1α, PGC-1β and NFR-1. Additionally, COX-4 and COX-2 mRNA levels were reduced. Whether NF90 acts as a direct transcriptional suppressor of PGC-1α/β remains to be established.

Conclusion

Mitochondria play a vital role in maintaining health over the lifespan of the organism. They regulate substrate utilisation, energy production and, consequently, muscle size and function. It is clear that perturbation in muscle mitochondrial biogenesis can significantly contribute to the onset and or severity of various chronic diseases and the numerous health issues our ageing population faces. Mitochondria display high sensitivity to contraction-initiated signals and demonstrate rapid plasticity. Therefore, physical activity and exercise play important roles in promoting the biogenesis and function of these organelles and, as a consequence, maintains cellular and whole body health.

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