Adaptive mechanisms of mitochondria in response to exercise

Adaptivni mehaniyam mitohondija kod napornog vežbanja

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Introduction

It is known that mitochondria are unique organelles capable of, depending on the physiological stimuli, changing their number and size 1, 2. Physical exercise has proved to be a powerful stimulus to mitochondrial biogenesis in skeletal muscle, which involves the orchestrated expression of the mitochondrial genome and the nuclear genes that encode mitochondrial proteins 3. The human mitochondrial genome consists of approximately 1,500 genes, 37 are encoded mitochondrial DNA (mtDNA), and the rest of the nuclear DNA (nDNA). Mitochondrial DNA (mtDNA) is a small double-stranded circular molecule containing 16,569 pairs of nucleotides. It encodes 13 subunits of complexes involved in oxidative phosphorylation, and components necessary for its own mRNA translation: large and small rRNA and 22 tRNA. The process of oxidative phosphorylation (OXPHOS) is necessary for formation of ATP, which is used for work, heat to maintain body temperature and membrane potential required for ion transport. Mitochondria also generate most of the reactive oxygen species (ROS) and electrons involved in their formation are usually derived from the reduced electron carriers of the respiratory chain. If not neutralized (damaged mitochondria are removed by apoptosis), ROS can damage mitochondrial proteins, lipids and nucleic acids which inhibit oxidative phosphorylation 4, 5. A large number of disorders of oxidative phosphorylation are attributed to mutations, which are more common in mitochondrial DNA (mtDNA) than in the DNA of chromosomes. These mutations are inherited maternally. However, not all mtDNA mutations and variations are deleterious. About 25% of all mtDNA variations are referred to as adaptive, and in some cases may be an important factor in the individual's predisposition to a better physiological condition 6.

This paper is an overview of recent research mitochondrial biogenesis and its adaptive effects to the potential impact of an increase in athlete's endurance.

Exercise-induced mitochondrial biogenesis in skeletal muscle

Skeletal muscles show significant metabolic and morphological adaptations in response to a number of physiological and pathophysiological conditions. One of the major phenotypic changes occurs in mitochondria in response to exercise or chronic contractile activity. In fact, intense exercise leads to significant metabolic changes that may impair mitochondrial function: the formation of reactive oxygen species due to higher rates of oxygen uptake during intense work 7, 8, hydrolysis of creatine phosphate leads to elevated levels of phosphate, which may affect the permeability of mitochondria, increased Ca²⁺ activates pyruvate dehydrogenase, alpha (α)-ketoglutarate dehydrogenase and NAD-linked isocitrate dehydrogenase, and the maximum permeability of the pores can lead to swelling and rupture of the outer membrane of mitochondria leading to autophagia of mitochondria and apoptosis or necrosis of the cells 9. This distortion function of mitochondria in strenuous exercise can cause not only fatigue, but muscle damage. Just under these physiological conditions highly dynamic structure of mitochondria and the appearance of mitochondrial adaptation are expressed. Exercise not only increases mitochondrial ATP synthesis through oxidative phosphorylation but also affects its morphology, increased gene expression of enzymes and proteins and changes the dynamics of fusion and fission, opposing processes that are in balance and are responsible for remodeling mitochondrial network 10, 11. These adaptation changes are most noticeable in low-oxidative white muscle.
Mitochondrial adaptions in muscle are highly specific and dependent on the type of exercise, its frequency, intensity and duration. Prolonged and strenuous training can produce an increase in mitochondrial content of 50–100% for a period of 6 weeks. Experiments in animal models (8 weeks of training on the treadmill, 80% VO_2, 5 days per week) showed an increased mitochondrial function, reflected by an increased activity of mitochondrial enzymes and the maximum speed of ATP synthesis in isolated mitochondria. The physiological meaning of mitochondrial adaptation in muscle is reflected in metabolic changes, which are expressed more in the metabolism of lipids compared to carbohydrates. For example, the formation of lactic acid is reduced, glycogen loss is smaller, the utilization of high-energy phosphates is reduced, as well as muscle fatigue.

These mitochondrial adaptations in response to exercise are generally referred to as mitochondrial biogenesis, as a synonym for metabolic plasticity. It is a complex process that involves increasing in the mitochondrial content per gram of tissue and changes in the mitochondrial composition, with an alteration in mitochondrial protein-to-lipid ratio. This sequence of molecular events that initiate mitochondrial biogenesis begins with an increase in intracellular Ca²⁺, which is a mediator of interaction actin and myosin, which then activates the kinase, for example, Ca²⁺ calmodulin kinase (CaMK) and phosphatase, which trigger a signaling cascade and increase gene expression of transcription factors. Specifically, muscle contraction leads to an increase in the maximum capacity of muscle to generate ATP via oxidative phosphorylation. Repeated muscle contractions lead to reducing the concentration of ATP and increasing the concentration of free ADP, thus causing activation of creatine phosphokinase (CPK), formation of ATP and creatine. ADP is also a substrate and allosteric activator of the glycolytic pathway and control mitochondrial respiration. These adaptations, along with increased activities of mitochondrial β-oxidation enzymes, lead to a greater lipid and less carbohydrate oxidation during exercise and enhance endurance performance. As a result of increased mitochondria, oxygen consumption and ATP production per mitochondrion are less at the same submaximal work rate in trained compared to untrained muscle. This means that with more mitochondrial respiratory chains, the rate of electron transport per respiratory chain will be “turned on” to a lower level to achieve the same rates of oxygen utilization and ATP production per gram of muscle at the same work rate in the trained compared to the untrained state. Consequently, the concentration of ATP and PC decreases less, and ADP, AMP and inorganic P increase to lower “steady state” levels, while glycogenolysis and glycolysis are turned on to a lower degree in the trained compared to the untrained state in response to the same submaximal work.

The literature supports the fact that adaptive responses to exercise are manifested during the recovery phase that follows the exercise period. This happens because stoppage the exercise, reduces the energy required for the processes such as gene expression and protein synthesis from serving contractile activity purposes to those that are more anabolic. Holloszy and Winder showed that Δ-aminolevulinic acid synthase (ALAs), enzyme involved in determining the functional content of mitochondrial cytochromes of respiratory chain, was increased several hours after the exercise bout. Similar results were observed in heart muscle postexercise. It suggests that the recovery period is an important component of the adaptation phase of the genes necessary for the proliferation of mitochondria in muscle.

However, research shows that chronic muscle disuse, as limb immobilization, denervation or bed rest, decreases mitochondrial content and the whole oxidative capacity. Chronic muscle inactivity disrupts the expression of both nuclear and mitochondrial genomes and inhibits mitochondrial biogenesis, increases apoptotic susceptibility contributing to a greater degree of apoptosis and a resultant increase in muscle atrophy.

Mitochondrial dynamic structure is also reflected in the ability to constantly fuse and divide in response to various physiology and pathological stimuli. They are able to change their shape through fission and fusion events, opposing processes that exist in equilibrium, leading to continuous remodeling of the mitochondrial network. If fusion predominates, mitochondria become more interconnected and networked. In contrast, excessive fission leads to mitochondrial network breakdown, the loss of mtDNA, an increase in ROS production and respiratory defects. Recent studies show that these processes have important consequences for the morphology, function and distribution of mitochondria. First, fusion and fission control the shape, length and number of mitochondria. Second, fusion and fission allow mitochondria to exchange lipid membranes and intramitochondrial content. Third, the shape of mitochondria affects the ability of cells to distribute their mitochondria to specific subcellular locations, and finally, mitochondrial fission facilitates apoptosis, which has consequences for development and disease.

Despite the fact that the exact mechanisms responsible for mitochondrial fission and fusion events have not been identified, a significant progress has been made in recognizing some genes and proteins involved in this process – mitofusin 1 and 2 (Mfn1 and Mfn2) and dynamin-related GTPase (OPA). The mechanisms of mitochondrial fission are still poorly understood, but there are dynamin-related protein 1 (Drp1) and mitochondrial fission protein (Fis1), which regulates this process. A recent study has demonstrated an increase in Mfn1 and Mfn2 mRNA levels in human skeletal muscle 24 h postexercise, but the regulation of the expression of these mitofusin isoforms have not yet been investigated. This remains an important area for future investigation in the study of mitochondrial structure and function in muscle.

Nuclear receptor peroxisome proliferator-activated receptor delta (PPAR-δ) and coactivator peroxisome proliferator-activated receptor-γ coactivator1-γ (PGC1γ) are considered as important regulators of many metabolic processes, including mitochondrial biogenesis in muscle and heart. PGC-1γ binds and coactivate DNA binding transcription factors and increases their activity, or binding for many nu-

clear receptors increases transcriptional activity of their target genes. In addition to increased mitochondrial content, this coactivator induces other adaptations related to the strenuous training, including an increased proportion of muscle type I fibers and an increase in resistance to fatigue.\textsuperscript{27,28}

Expression of PGC-1\(\gamma\) is dynamically regulated by altered types of physical activity. In response to a single bout of exercise, PGC1\(\gamma\) mRNA and protein are significantly elevated in mice, rats and humans.\textsuperscript{29} This increase in gene expression is present as early as two hours after exercise. The same increase is also present in the repeated exercise,\textsuperscript{30} which indicates that the contractile activity is a main stimulus for exercise-induced PGC-1\(\gamma\) upregulation. It is evident that this coactivator plays an important role in the maintenance of mitochondrial content and function in muscle, but the literature data show that its absence does not abolish the effect of endurance exercise on mitochondrial biogenesis, which was confirmed by increasing the protein markers, and concludes that there is a substitution of alternative transcription factors in the coordination of increased mitochondrial content.\textsuperscript{32}

Thus, it is clear that exercise can lead to changes in the expression of numerous transcription factors involved in mitochondrial biogenesis. The progressive increase in the accumulation of these proteins and coactivating factors in response to exercise indicates their important role in the mediation of mitochondrial adaptation to exercise, but the mechanisms by which this expression is regulated remain unclear.

**Mitochondrial mutations and physical performance**

Since the majority of mitochondrial proteins are encoded by nuclear genes, inheritance of mitochondrial disorder is autosomal recessive. In contrast, the disorders caused by mutations in mtDNA show great variability due to the phenomenon of heteroplasmy (intracellular mixture of mutant and normal mtDNA), because when the heteroplasmic cell divides, it is just a matter of coincidence which mitochondria and thus mtDNA will be distributed into the daughter cells. There is a combination of neurological and myopathic symptoms (MELAS, Leigh disease, Barth syndrome, Leber hereditary neuropathy of opticus etc.). On the other hand in many mitochondrial mutations declines of the energy output or energy deficit are present.\textsuperscript{33} All mtDNA variations are usually classified into deleterious mutations present in maternally inherited disease, ancient polymorphisms, the characteristic of our ancestors to adapt to new environmental conditions and somatic, that occur with aging (they provide the aging clock).\textsuperscript{34} However, some variations of mtDNA appeared to have a positive effect and led to a functional mitochondrial adaptation. For example, the mtDNA variant of adaptation to warm climates results in more tightly coupled oxidative phosphorylation, with maximum ATP output and minimizing heat production. These changes in mtDNA permit maximum muscle performance, but these people are predisposed to obesity, diabetes, excessive ROS production, degenerative diseases and premature aging. Partially uncoupled mitochondria generate more heat, but at the expense of efficiency in ATP production. Individuals with these variants are more tolerant to cold, and less susceptible to obesity, they generate less ROS and are more resistant to aging and degenerative diseases, but have reduced endurance.

Based on the fact that the mitochondrial genome has 37 genes, alleles in some places define nine haplogroups.\textsuperscript{35} The different versions of mtDNA within a population can be defined by distinct sets of polymorphisms called as haplogroups. Haplogroups serve as markers of genetic as well as geographic clusters. Castro et al.\textsuperscript{36} were among the first to study the correlation of each haplogroup with elite athletic performance. Analysis of the Spanish long-distance runners, professional cyclists and rowers, revealed that the haplogroup T is less frequent in these athletes compared with the control, and athletes carrying this haplotype are clearly at a genetic disadvantage for performance in endurance sports. Scott et al.\textsuperscript{37} compared the frequencies of mtDNA haplogroups found in elite Kenyan endurance athletes with those in the general Kenyan population. National Kenyan athletes, international Kenyan athletes and members of the general population of Kenya were compared and results showed that the haplogroup distribution of national and international athletes differed significantly from controls, and mtDNA haplogroup of international athletes were different from the general Kenyan population. The definitive conclusions of other studies are not relevant because of a small number of athletes, and because this one points out the complexity of comparing results from athletes of different ethnic groups.\textsuperscript{38}

Since mitochondrial metabolic and genetic therapies used to treat mitochondrial disease, it may become the subject of use by healthy people who want to change their energetic phenotype changing their mtDNA genotype and enhancing their physical performance. For example, changing a single mtDNA nucleotide of elite athletes to increase mitochondrial ATP production through altered oxidative phosphorylation coupling could increase physical performance by several percent. Such a substitution could not be detected by standard anti-doping tests.

**Mitochondrial nutrient supplementation**

Mitochondrial nutrients are a group of micronutrients that are either mitochondrial components or those which metabolites influence the structure and function of mitochondria.\textsuperscript{39} They protect mitochondria from oxidative damage and eliminate oxidative stress, increase the antioxidant defense, enhance mitochondrial metabolism by repairing of mitochondria or by increasing mitochondrial biogenesis, protects mitochondrial enzymes and stimulate mitochondrial enzyme activity by elevating substrate and cofactor levels.\textsuperscript{40} Well-known mitochondrial nutrients or prosthetic groups are: R-alpha lipoic acid, acetyl-L-carnitine, coenzyme Q10, B vitamins, creatine, resveratrol, vitamin E, etc. Their individual effects in reducing oxidative stress and tissue damage and improved mitochondrial function in strenuous exercise have been demonstrated both in animal and human studies.\textsuperscript{41}
but more positive effects of combined supplements have been pointed out because of their synergistic action 42. Special attention is paid to their role in stimulating transcription of genes involved not only in mitochondrial biogenesis but also in mitochondrial fusion in skeletal muscle, resulting in the increase in mitochondria function and better antioxidant defense, and thus leading to enhancement of physical performance and of fatigue recovery. Mitochondrial nutrients are selected based on their characteristics, the target of action and possible synergistic interactions, such as a group of antioxidants (coenzyme Q10, lipoic acid and glutathione), the energy enhancers (creatine, pyruvate, choline) or their precursors and cofactors (lipoic acid, coenzyme Q10, B vitamins). Some nutrients may have multiple functions, and some combinations may possess unique functions, quite different from their individual effects. B vitamins (riboflavin, piridoxin, biotin and nicotinamide) are used for cellular repair and production, and are particularly important for the protection of mitochondrial and other enzymes, because they are their precursors and cofactors. It is found that athletes with a lack of vitamin B have a reduced high-intensity exercise performance and are less able to repair damaged muscles 43. Lipoic acid is a coenzyme involved in mitochondrial metabolism, it recycles vitamins C and E, raises intracellular glutathione and chelates iron and copper, and in coadministration with creatine and acetyl-L-carnitine shows synergistic effects in improving mitochondrial function 44. Coenzyme Q10 affects the synthesis of ATP, thus increasing mitochondrial function and capacity and defining the phenotype of elite athletes. The purpose of these changes in mitochondria is not only the process of energy supplying for muscle work, but they are part of a well-tailored mechanism of metabolic adaptation that reduces exercise-induced stress and maintains the physiological balance. Individual effects of numerous transcription factors that are part of mitochondrial biogenesis cascade and mitochondrial network remodeling, are not exactly specified, as well as their interactions, and they need further study and definition of sites, roles and possible activation outside in the form of natural, dietary or pharmacological activators. Better understanding of mitochondrial variation can contribute to more detailed introduction with the differences in aerobic capacity and defining the phenotype of elite athletes.

Mitochondrial nutrient supplementation enhances the physical performance of endurance exercise, decreases oxidative stress and fatigue and stimulates mitochondrial biogenesis. Future directions include their identifications and investigation using modern technology of nutrigenomics for optimal effects and combinations.

**Conclusion**

Exercise is a powerful stimulus to mitochondrial biogenesis in skeletal muscle. The results of mitochondrial biogenesis are increased mitochondrial content, improved aerobic capacity and better ATP output, thus improving muscular endurance, reducing the predisposition to fatigue and increasing the effectiveness of physical exercise. The purpose of these changes in mitochondria is not only the process of energy supplying for muscle work, but they are part of a well-tailored mechanism of metabolic adaptation that reduces exercise-induced stress and maintains the physiological balance. Individual effects of numerous transcription factors that are part of mitochondrial biogenesis cascade and mitochondrial network remodeling, are not exactly specified, as well as their interactions, and they need further study and definition of sites, roles and possible activation outside in the form of natural, dietary or pharmacological activators. Better understanding of mitochondrial variation can contribute to more detailed introduction with the differences in aerobic capacity and defining the phenotype of elite athletes.

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