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Review

Can we optimise the exercise training prescription to maximise improvements in mitochondria function and content?☆

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ABSTRACT

Background: While there is agreement that exercise is a powerful stimulus to increase both mitochondrial function and content, we do not know the optimal training stimulus to maximise improvements in mitochondrial biogenesis.

Scope of review: This review will focus predominantly on the effects of exercise on mitochondrial function and content, as there is a greater volume of published research on these adaptations and stronger conclusions can be made.

Major conclusions: The results of cross-sectional studies, as well as training studies involving rats and humans, suggest that training intensity may be an important determinant of improvements in mitochondrial function (as determined by mitochondrial respiration), but not mitochondrial content (as assessed by citrate synthase activity). In contrast, it appears that training volume, rather than training intensity, may be an important determinant of exercise-induced improvements in mitochondrial content. Exercise-induced mitochondrial adaptations are quickly reversed following a reduction or cessation of physical activity, highlighting that skeletal muscle is a remarkably plastic tissue. Due to the small number of studies, more research is required to verify the trends highlighted in this review, and further studies are required to investigate the effects of different types of training on the mitochondrial sub-populations and also mitochondrial adaptations in different fibre types. Further research is also required to better understand how genetic variants influence the large individual variability for exercise-induced changes in mitochondrial biogenesis.

General significance: The importance of mitochondria for both athletic performance and health underlines the importance of better understanding the factors that regulate exercise-induced changes in mitochondrial biogenesis. This article is part of a Special Issue entitled Frontiers of Mitochondrial Research.

1. Introduction

Mitochondria are membrane-enclosed organelles found in most eukaryotic cells. They typically range from 0.5 to 1.0 μm in diameter [1], and are composed of five compartments that carry out specialised functions: the outer mitochondrial membrane, the inter-membrane space (the space between the outer and inner membranes), the inner mitochondrial membrane, the cristae (formed by infoldings of the inner membrane), and the matrix (space within the inner membrane). In skeletal muscle, mitochondria are organized in a reticulum and one of their main roles is the production of Adenosine Triphosphate (ATP) — the energy currency of living organisms. The production of ATP takes place during the reactions of the tricarboxylic acid (TCA) cycle, located within the matrix, and via the electron transport system (ETS), located along the inner mitochondrial membrane (IMM). The ETS consists of 5 multi-polypeptide complexes (complexes I to V) embedded in the inner mitochondrial membrane that receive electrons from the reduced forms of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2), generated mainly in the TCA cycle. During the initial step, electrons are transferred along complexes I to IV of the ETS, with O_2 serving as the final acceptor at complex IV [2]. During this process, protons are pumped out of the matrix into the inter-membrane space generating an electrochemical gradient that represents the driving force enabling Complex V to generate ATP by phosphorylation of adenosine diphosphate (ADP). The combination of these last two processes is described as oxidative phosphorylation (OXPHOS).

Given the pivotal role of mitochondria in providing the energy required for activities of daily life, it is not surprising that mitochondrial adaptations have been associated with endurance performance [3]. However, while early studies focussed on changes in mitochondrial enzymes, such as citrate synthase (CS) activity (an indicator of mitochondrial content [4,5]) [6], subsequent studies have suggested that mitochondrial function (e.g., mitochondrial respiration) is a more important determinant of endurance performance than mitochondrial content [3,7]. The mitochondria also appear to have an important role

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in ageing and cell pathology [8], and have been implicated in many age-related degenerative diseases such as Parkinson's, Alzheimer's and Huntington's diseases, atherosclerosis and cardiomyopathies [9], as well as a large variety of metabolic disorders such as obesity [10,11], insulin resistance [12] and type 2 diabetes [13]. For example, both mitochondrial content (as assessed by CS activity) and function (as determined by mitochondrial respiration) have been reported to be lower in patients with type 2 diabetes [13,14]. The above findings underline the importance of a better understanding of the factors that regulate both mitochondrial content and function. Exercise is one such factor that has been shown to provide a powerful stimulus for mitochondrial biogenesis [15,16], yet little is known about the optimal exercise prescription, and whether mitochondrial content and function are altered by the same or different exercise prescription.

2. Mitochondrial biogenesis

Exercise is a potent stimulus for mitochondrial biogenesis. However, while mitochondrial biogenesis is sometimes used in reference to the formation of new mitochondria, it is important to note that the mitochondrial reticulum is not made ex-novo or de-novo[1]. Instead, the mitochondrial reticulum recruits new proteins to the organelle with subsequent continuous remodelling of the mitochondrial network following the interplay of the fission process with fusion [17]. Thus, mitochondrial biogenesis (from the Greek word "*genesis*", meaning "origin" or "coming into being of something"), more accurately refers to the generation of new mitochondrial components. Despite this seemingly simple definition, researchers have used the term "mitochondrial biogenesis" in many different ways and there is a lack of agreement on the best methods to assess mitochondrial biogenesis [18–20].

As biogenesis is by definition "the making of new", it has been suggested that only measurements of the synthesis rates of mitochondrial proteins are indicative of mitochondrial biogenesis [18]. In support of this, it has been noted that changes in the abundance of transcription factors for mitochondrial genes, or the mRNAs encoding mitochondrial proteins, are not by themselves sufficient as measurements of mitochondrial biogenesis [18]. Furthermore, changes in mitochondrial content (described below) may be due to changes in both synthesis and degradation rates, and therefore are not solely indicative of mitochondrial biogenesis (i.e., the making of new mitochondrial components).

Although the measurement of the synthesis rates of mitochondrial proteins may be the best assessment of mitochondrial biogenesis, it has also been argued that multiple methods are required to understand the complex mechanisms underlying mitochondrial biogenesis, and that associated outcomes (e.g., changes in mitochondrial content and function) are required to put mitochondrial synthesis results in context [19,20]. Even though it is well established that mitochondria exist in a three-dimensional network [21], two-dimensional imaging using transmission electron microscopy (TEM) is still considered the gold standard for measuring mitochondrial content [4]. Since the TEM technique is time consuming, and is not available in many laboratories, researchers often measure indirect markers of mitochondrial content (e.g., cardiolipin content, mitochondrial DNA content, activities of mitochondrial complexes and enzymes). In particular, CS activity is a commonly-used biomarker in exercise training studies, and has been strongly associated with mitochondrial content (as measured by TEM) [4].

As mitochondrial biogenesis can be associated with either a gain of function or pathological responses, the assessment of mitochondrial biogenesis (e.g., mitochondrial protein synthesis) and evidence of mitochondrial biogenesis (e.g., mitochondrial content) should be coupled with measurements of mitochondrial function to more accurately determine whether the observed changes are adaptive or maladaptive [22]. The most commonly used methods for assessing mitochondrial function are the measurement of mitochondrial respiration, with an O_2-sensitive electrode, in either isolated or permeabilised muscle fibres, or the measurement of the rate of ATP production (MAPR) in isolated mitochondria using chemiluminescence [23]. A potential limitation of MAPR is that the information that can be obtained from this technique is limited to ATP production. Furthermore, due to the relatively low yield, large muscle samples are usually required for the measurement of both MAPR and mitochondrial respiration in isolated mitochondria; there is also the potential for mitochondria to be damaged during the isolation procedure [24]. The main advantages of using permeabilised muscle fibres are that only small muscle samples are required (<10 mg), and that the mitochondria may be studied in situ inside the fibres — allowing the structure and function of mitochondria less likely to be affected during preparation.

In summary, due to the complex nature of mitochondria, it is recommended to use multiple parameters to assess both the presence of mitochondrial biogenesis and associated outcomes. Therefore, a thorough analysis of mitochondrial biogenesis should include a range of measurements assessing protein synthesis rate, as well as mitochondrial content and function [25]. Measurement of changes in transcription factors and key regulatory proteins should also be considered to understand the mechanisms underlying mitochondrial biogenesis. However, while we acknowledge the importance of understanding the underlying genetic and transcription pathways, and that mitochondrial protein synthesis rate may be the best measure of mitochondrial biogenesis per se, there are very few studies that have investigated the effects of different types of exercise training on these two parameters. This review will therefore focus predominantly on exercise-induced changes in mitochondrial function and content, as there is a greater volume of published research on these adaptations and stronger conclusions can be made.

3. Overview of exercise-induced mitochondrial biogenesis

Although most of the DNA in humans is packaged in chromosomes within the nucleus, mitochondria also possess their own circular DNA, usually referred to as mitochondrial DNA (mtDNA). The 16,569-base pair (bp) human mtDNA contains 37 genes encoding for 13 polypeptides involved in the mitochondrial oxidative phosphorylation process, as well as 2 ribosomal RNA (rRNA) and 22 transfer RNA (tRNA) genes that are essential for protein synthesis within the mitochondria [26,27]. All the other proteins required for the correct functioning of mitochondria are encoded by the nuclear genome [28]. Thus, mitochondrial biogenesis requires a complex interconnected system of interactions and the concerted integration of the mitochondrial and nuclear genome.

Mitochondrial biogenesis is the result of signalling, transcription, translation, the import of precursor proteins into the mitochondria, and the co-ordinated incorporation of both mitochondrial and nuclear gene products into an expanding mitochondrial reticulum. At the onset of contractile activity, the cascade of events leading to mitochondrial biogenesis begins with the activation of signalling proteins such as kinases, deacetylases and others. Amongst the most important signalling events generated during contractile activity are: calcium (Ca^{2+}) release, and changes in the AMP:ATP ratio, the cellular redox state, and the production of ROS. These signalling events initiate the process of exercise-induced mitochondrial biogenesis by altering the conformation, content, activity or sub-cellular localization of sensor enzymes such as transcription factors, coactivators and regulators. These events trigger an increase in the messenger RNA (mRNA) of such enzymes and that of downstream proteins, hence activating the transcription process. The timing of these events varies considerably

[1] Novo is a Latin word meaning "new/fresh". Generally, ex novo and de novo differ in the fact that ex novo indicates an event or object made from scratch, while de novo is an event or object made from scratch again.

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