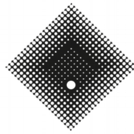

Principles of Exercise Prescription, and How They Influence Exercise-Induced Changes of Transcription Factors and Other Regulators of Mitochondrial Biogenesis

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Title: Principles of exercise prescription, and how they influence exercise-induced changes of transcription factors and other regulators of mitochondrial biogenesis

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Abstract:

Physical inactivity represents the fourth leading risk factor for mortality, and it has been linked with a series of chronic disorders - the treatment of which absorbs ~85% of healthcare costs in developed countries. Conversely, physical activity promotes many health benefits; endurance exercise in particular represents a powerful stimulus to induce mitochondrial biogenesis, and it is routinely used to prevent and treat chronic metabolic disorders linked with sub-optimal mitochondrial characteristics. Given the importance of maintaining a healthy mitochondrial pool, it is vital to better characterize how manipulating the endurance exercise dose affects cellular mechanisms of exercise-induced mitochondrial biogenesis. Herein, we propose a definition of mitochondrial biogenesis and the techniques available to assess it, and we emphasize the importance of standardizing biopsy timing and the determination of relative exercise intensity when comparing different studies. We report an intensity-dependent regulation of exercise-induced increases in nuclear peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) protein content, nuclear phosphorylation of p53 (serine 15), and PGC-1α mRNA, as well as training-induced increases in PGC-1α and p53 protein content. Despite evidence that PGC-1α protein content plateaus within a few exercise sessions, we demonstrate that greater training volumes induce further increases in PGC-1α (and p53) protein content, and that short-term reductions in training volume decrease the content of both proteins, suggesting training volume is still a factor affecting training-induced mitochondrial biogenesis. Finally, training-induced changes in mitochondrial transcription factor A (TFAM) protein content are regulated in a training volume-dependent manner and have been linked with training-induced changes in mitochondrial content.

Key points

- Relative exercise intensity (defined as a percentage of the maximal power output), but not absolute exercise intensity (defined as power [Watts] or speed [km/h]), is an important

determinant of exercise-induced changes in nuclear peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) protein content, the phosphorylation of p53 at serine 15, and PGC-1α messenger RNA (mRNA) content, which modulate early events of mitochondrial biogenesis.

- Exercise-induced changes in the mRNA of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) may begin at the onset of exercise and peak 3 to 6 h from the beginning of an exercise session; this is important when comparing exercise interventions of different durations.
- Whereas both exercise intensity and training volume seem to be important determinants of training-induced changes in PGC-1α and p53 protein content, training-induced changes in mitochondrial transcription factor A (TFAM) protein content seem to be regulated in a training volume-dependent manner.

1 Skeletal muscle and mitochondria

Skeletal muscle is the largest tissue mass in the human body, and it is responsible for movement and bioenergetic homeostasis through a variety of physiological processes. A key component of skeletal muscle are the mitochondria. From a structural standpoint, skeletal muscle mitochondria range in size from 0.1 to 5.0 μm in diameter [1] and are subdivided into subsarcolemmal and intermyofibrillar (~75% of the total content) mitochondria, according to their location [2]. Although traditionally described as an organelle, mitochondria form an integrated reticulum that is constantly remodeled so as to adapt to the cellular requirements [3]. As a consequence, mitochondria undergo continuous changes in both their content and functional qualities.

Mitochondria are responsible for the aerobic production of ATP - the "energy currency" required to fuel cellular activities, and are therefore key regulators of energetic homeostasis [4]. Mitochondria also play a vital role in cell survival and programed cell death [5], and are an important site for the production of reactive oxygen species [6] - which have been linked to mitochondrial damage and retrograde signaling to the rest of the cell [7]. The variety of roles in which mitochondria are involved places them in a central position in both health and disease [8-10]; it is therefore important to better understand the factors that influence improvements in mitochondrial content and functional qualities.

Many factors, such as age [8], sex [11], and health status [12, 13], have been shown to affect the quantity and quality of the mitochondrial pool. Exercise, in particular, represents a powerful stimulus to induce mitochondrial adaptations [14-17], which lead to subsequent increases in mitochondrial content and improved respiratory function [18-22]. Conversely, physical inactivity has been linked with at least 20 of the most life-threatening chronic disorders [12], and currently represents the fourth leading risk factor for mortality [23]. This comes at a high

social and financial cost, as it has been estimated that physical inactivity costs health-care systems 54 billion international dollars (INT$) worldwide per annum, and 25 billion INT$ in the USA alone (equivalent to ~ 85% of the total health-care expenditure) [24]. Given that many of these chronic disorders have also been linked to sub-optimal mitochondrial characteristics [25-27], it is important to better understand and characterize how the exercise prescription influences the molecular events that regulate mitochondrial adaptations to exercise. .

2 Mitochondrial biogenesis

There is debate within the scientific community as to what defines mitochondrial biogenesis [28, 29], and currently there is no widely-accepted definition. Despite its etymology (from the Greek word "*genesis*", meaning: "origin, creation"), mitochondria are not made *ex novo* or *de novo*, but are rather the result of the incorporation of new proteins into pre-existing sub-compartments and protein complexes [30]. Therefore, it seems appropriate to define mitochondrial biogenesis as "the making of new components of the mitochondrial reticulum". However, these synthetic events take place in conjunction with the processes of mitochondrial remodeling (mitochondrial fusion and fission) [31], as well as catabolic events such as mitochondrial protein breakdown (MitoPB), mitophagy [32], and apoptosis [33]. The integration of mitochondrial biogenesis per se with the above processes allows for an elegant regulation of mitochondria that can result in alterations in the content and morphology of the mitochondrial network, an increased mitochondrial functionality, and an improved ability to generate ATP. Understandably, the number of processes involved, together with the absence of a widely-accepted definition, results in a lack of consensus about which technique(s) can best assess mitochondrial biogenesis [28, 29, 34].

From its etymological meaning (i.e., the synthesis of new mitochondrial components), mitochondrial biogenesis can best be assessed by measuring the rate of mitochondrial protein synthesis (MitoPS) using stable isotopic tracers [28, 35]. This technique however, seems to lack the ability, if used in isolation, to provide information regarding mitochondria remodeling, changes in mitochondrial content (the net outcome of MitoPS and MitoPB, with the latter also assessed using stable isotopic tracers [35]), mitochondrial respiratory function, and/or other aspects of mitochondrial quality [29, 34]. Therefore, while it has been argued that only measurements of the synthesis rate of mitochondrial proteins are indicative of mitochondrial biogenesis [28], a more comprehensive assessment of mitochondrial content, structure, quality, and respiratory function is required to put the results of mitochondrial biogenesis in context [29, 36].

Mitochondrial content has often been measured as the best assessment of one of the outcomes of mitochondrial biogenesis, an increase in the mitochondrial reticulum. The gold standard methods are histological, and they include transmission electron microscopy (TEM) and fluorescent microscopy [34]. However, due to the time and financial costs associated with these techniques, a range of biochemical measurements have been proposed as valid biomarkers of mitochondrial content. These include, amongst others, the measurement of: a) the content of key mitochondrial proteins and enzymes (e.g., cytochrome *c*, electron transport system [ETS] complexes, ATP synthase), b) cardiolipin content (an inner mitochondrial membrane phospholipid whose content provides an estimation of the amount of inner mitochondrial membrane and correlates with ETS activity [37]), c) mitochondrial (mt-) DNA content (measured by polymerase chain reaction [PCR], and normalized to nuclear DNA content, as an assessment of mitochondrial content [38]), and d) determination of enzymatic activity [39]. Amongst these, citrate synthase (CS) activity determination is the most widely used; this is due to its strong correlation with baseline mitochondrial content as assessed by TEM in human

skeletal muscle [39]. Furthermore, an excellent agreement between changes in CS activity and changes in mitochondrial content as assessed by TEM has been reported in rabbit skeletal muscle following 28 days of electric stimulation [40].

The outcome of mitochondrial biogenesis can also present as a change in mitochondrial respiratory function [41], an adaptation that does not directly relate to changes in mitochondrial content [42-44]. Measurement of mitochondrial respiratory function in skeletal muscle can be done both invasively and non-invasively [41, 45]. Invasive assessment, which is performed on skeletal muscle biopsy samples, includes measurements of enzyme activity, mitochondrial ATP production rate, or oxygen consumption (mitochondrial respiration). Mitochondrial respiration can be assessed in muscle homogenates, isolated mitochondria, or in permeabilized fibers, and can be expressed relative to gram of tissue (mass-specific mitochondrial respiration), or relative to mitochondrial content (mitochondrial [mt]-specific respiration, obtained by normalizing mass-specific mitochondrial respiration by mitochondrial content, or one of its markers). Mitochondrial respiration in permeabilized fibers is widely considered the gold standard measurement of mitochondrial respiratory function [45-47]. With this technique, a small amount of tissue (5 to 10 mg) enables measurement of mitochondrial respiratory function *in situ* (with minimal disruption of mitochondrial structure and function [47]), and allows an assessment of the contribution of different complexes and the coupling between oxidation and phosphorylation. Non-invasive techniques include phosphorus magnetic resonance spectroscopy and near-infrared spectroscopy [45]. Their advantage is the lack of invasive procedure, and that they take place *in vivo* - within a living organism with an intact biological system. However, the absence of muscle sampling means these techniques do not allow further information to be obtained regarding the molecular mechanisms associated with exercise-induced mitochondrial biogenesis [45].

Although mitochondria possess their own DNA (mtDNA), encoding for 13 polypeptides that assemble as essential subunits in ETS complexes, the vast majority of the ~1500 mitochondrial proteins are encoded by the nuclear genome [48]. Mitochondrial biogenesis is therefore regulated by transcriptional events requiring the concerted integration of both genomes [48]. Although these transcriptional events are important for the activation of mitochondrial biogenesis, they are not by themselves a direct measure of mitochondrial biogenesis [49-52]. Nonetheless, supplementing the invasive assessments of mitochondrial biogenesis described above with measurement of changes in gene expression and protein content of key transcription factors and regulatory proteins modulating transcriptional activity, enables a better understanding of the molecular mechanisms associated with exercise-induced mitochondrial biogenesis. The most common technique used to assess changes in gene expression is real-time PCR (qPCR), whilst immunoblotting is routinely used to determine changes in protein content.

Non-transcriptional pathways are also important determinants of exercise-induced changes in mitochondrial biogenesis. These include, but are not limited to, messenger RNA (mRNA) degradation, micro-RNA (miRNA)-mediated gene expression regulation, epigenetics, ribosome biogenesis, translation efficiency, post-translational modifications, and protein trafficking [49-51]. However, due to the limited research available, no valid conclusions can currently be made on how manipulation of different exercise variables affects these non-transcriptional pathways; therefore, these will not be further discussed in this manuscript. Future research is required to better characterize the role of non-transcriptional events on exercise-induced changes in mitochondrial biogenesis.

Following on from the above considerations, it appears evident that a range of analyses should be used to assess the processes and outcomes of exercise-induced mitochondrial biogenesis. Therefore, we advocate that a comprehensive examination should include assessment of the rate of MitoPS and MitoPB, alongside the analysis of changes in both mitochondrial content

and respiratory function [29, 34]. Furthermore, to expand the knowledge of the mechanisms leading to exercise-induced mitochondrial biogenesis, measurement of changes in gene expression, as well as proteins and transcription factors mediating these molecular processes, should also be considered. Before describing how different types of exercise influence these processes, an overview of the key principles of exercise prescription is provided.

3 Principles of exercise prescription

Exercise training can be defined as the repetition of individual exercise sessions over a period of time. While seemingly simple, the exercise "dose" is as complicated as that of any drug [53]. When prescribing a training intervention several variables need to be considered, such as exercise intensity, duration, and frequency, as well as recovery between sessions, overall duration of the training intervention, and total training volume (the product of the session duration by the exercise intensity by the number of sessions). Different types of endurance exercise (e.g., running, swimming, cycling), the time of the day (i.e., circadian rhythms), nutritional strategies (e.g., exercising in the fed or fasted state, post-exercise nutrition), genetic variants, sex, and many other variables, all influence the outcome of a training intervention. Manipulation of any of the above variables will differentiate the adaptations, providing the ability to tailor-make a training intervention to suit the particular needs of an individual. It is therefore important to better characterize and understand how the variables related to the exercise prescription may influence the exercise-induced adaptations.

While exercise duration and frequency are unambiguous terms, the determination/use of exercise intensity, one of the key variables of a training intervention [54], is surprisingly complex and variable among studies. Exercise intensity can be prescribed in either absolute or relative terms. Absolute exercise intensity refers to a specific value of power (cycling, rowing

etc.) or speed (running, swimming etc.), which is the same for all participants (e.g., 230 W or 17 km·h^{-1}). Conversely, relative exercise intensity is typically determined as a percentage of the maximal power output ($\dot{W}_{max}$) derived from a test consisting of progressive increases in power output[1].

The recommended test to determine $\dot{W}_{max}$ (and the maximum rate of oxygen consumption [$\dot{V}O_{2max}$]) is a short (8 to 12 min) incremental exercise test (IET), where exercise intensity is increased continuously (e.g., 1 W every 2 s) or stepwise (e.g., 30 W every 1 min) [55] (Fig. 1). Another test often used is the graded exercise test (GXT), where exercise intensity is increased using steps of longer durations (e.g., 30 W every ≥ 3 min) to attain steady-state blood lactate concentrations used for the determination of the power at the lactate threshold [55] (Fig. 1). Due to its nature, a GXT is much longer than an IET; therefore, $\dot{W}_{max}$, which is inversely related to the mean ramp slope [56], will be underestimated and is typically referred to as $\dot{W}_{peak}$ ($\dot{V}O_{2max}$ is referred to as $\dot{V}O_{2peak}$ and the associated power output as $\dot{W}_{VO2peak}$).

While the values derived from different tests are often used interchangeably, they can vary considerably depending on the test protocol used (Fig. 1), creating an additional challenge when comparing the outcomes of different studies. For example, reducing the step duration from 180 to 15 seconds increased the value of $\dot{W}_{max}$ from 258 to 428 W; this is an increase of ~75% [56]. In this instance, a relative exercise intensity of 80% of $\dot{W}_{max}$ could range from 206 to 342 W depending on the step duration chosen. This suggests caution is required when comparing studies that have exercised participants at relative exercise intensities based on $\dot{W}_{max}$ values determined via different testing protocols, as this is likely to affect the mitochondrial adaptations observed and the conclusions made.

[1] For interventions employing a mode of exercise for which power is not easily measurable (e.g., running or swimming), the same parameters can be determined, but velocity (v) is used instead of power.

While the majority of studies included in this review have prescribed exercise based on relative exercise intensities determined from a short testing protocol (e.g., a ≤ 12-min IET), some studies have utilized testing protocols of longer durations (e.g., a 30-min GXT). To enable a more accurate comparison between these studies we have applied a correction factor so that values of relative exercise intensity obtained from testing protocols exceeding 12 min in duration have been converted to a newly estimated $\dot{W}_{max}$ value ($\dot{W}_{max'}$), which would be expected from a 12-min IET protocol (see Electronic Supplementary Material Appendix S1). This conversion was made possible based on the bioenergetic model proposed by Morton [57], which was confirmed by Adami et al. [56]. It follows that relative exercise intensities originally reported as percent of $\dot{W}_{peak}$, or $\dot{W}_{VO2peak}$, that were re-estimated based on the above model will be reported in this review as a percent of $\dot{W}_{max'}$.

Based on the relative exercise intensity to be used, both continuous and different types of interval exercise/training can be prescribed. However, although attempts have been made [58], the nomenclature has not been standardized. For the scope of this review, we will refer to: moderate-intensity continuous exercise or training (MICE or MICT, respectively) when exercising continuously at a moderate exercise intensity (e.g., 50 to 75% of $\dot{W}_{max}$), high-intensity interval exercise or training (HIIE or HIIT, respectively), when performing intervals at an exercise intensity < 100% $\dot{W}_{max}$, sprint interval exercise or training (SIE or SIT, respectively), when exercise consists of intervals > 10 s in duration performed at an intensity ≥ 100% $\dot{W}_{max}$, and repeated sprint exercise or training, for all-out repeated sprints lasting ≤ 10 s [59] (Fig. 1).

While it is well accepted that endurance exercise represents a powerful stimulus to increase mitochondrial content and mitochondrial respiratory function [18, 60], it is not yet known which type(s) of exercise training results in the greatest adaptations. In this regard, exercise intensity and training volume have been identified as two of the most important variables [54].

Given that exercise duration is typically inversely related to exercise intensity, and the paucity of studies examining the role of frequency and the overall length of an intervention, this review will focus primarily on the role of exercise intensity and volume and how they affect the molecular events regulating exercise- and training-induced mitochondrial biogenesis. Moreover, considering that the vast majority of the available literature employed cycling as opposed to other types of endurance exercise (e.g., running, swimming, cross-country skiing), only cycling studies will be discussed in this review, as stronger conclusions can be made without the confounding effect of combining different endurance exercise modalities.

4 Literature search

Following the above considerations, two separate literature searches were performed on Scopus® and Web of Science® using the following keywords: “exercise” and “skeletal muscle”, and “PGC-1α” or “p53” or “NRF” or “TFAM”. From the list of returned abstracts, research articles were included in the present manuscript if they met the following criteria: a) they involved cycling (seminal studies involving other endurance exercise types, or where cycling studies were not available or were limited in numbers have been discussed in the text, but have been excluded from the figures); b) they involved young healthy participants (studies with middle-aged or elderly populations were excluded); c) they provided precise and detailed information about the exercise prescription. The reference lists of the included articles and those of other review articles returned by the above searches were also inspected and articles satisfying the above criteria were also included in the present manuscript. Finally, studies using different species (e.g., mouse, rat) have been discussed in those circumstances where no literature was available with human skeletal muscle; these studies have been discussed in the text, but they have been excluded from figures and tables.

5 Cellular mechanisms of exercise-induced mitochondrial biogenesis

At the molecular level, the processes leading to mitochondrial biogenesis are the results of, in chronological order: signaling, transcription, translation, and a host of post-translational events, culminating with protein incorporation into the mitochondria [48]. This sequence of events begins at the onset of contractile activity [61] with a series of homeostatic perturbations (Fig. 2) acting as signals for the activation of sensor proteins such as Ca^{2+}/calmodulin-dependent kinases II, 5' AMP-activated protein kinase (AMPK), p38 mitogen-activated protein kinases (p38 MAPK), and sirtuin 1. These proteins initiate gene transcription by activating transcriptional coactivators (e.g., peroxisome proliferator-activated receptor γ coactivator-1α [PGC-1α] and transcription factors (e.g., p53, nuclear respiratory factor [NRF] 1 and 2 [NRF-1, NRF-2], mitochondrial transcription factor A [TFAM]) by chemical events or by inducing conformational or sub-cellular localization changes, amongst others [48]. Gene transcription and mRNA translation are further regulated by non-transcriptional pathways such as miRNAs and epigenetics. miRNAs are small 19 to 22 nucleotide non-coding RNA molecules involved in post-transcriptional gene expression (both inhibitory and positive regulation) [62, 63], which can be modulated by both a single session of exercise and exercise training in human skeletal muscle [64, 65]. Epigenetic modifications, defined as heritable changes in gene function not affecting a gene’s sequence [66], have also been reported to modulate gene transcription [67]. For example, both a single session of exercise [68, 69] and exercise training [70] decrease DNA methylation (an important epigenetic marker involved in several biological process [67]) of genes affecting oxidative phosphorylation (e.g. PPRGC1A, which encodes for PGC-1α) and more broadly whole-genome DNA methylation, resulting in increased transcriptional activity. The transcriptional and non-transcriptional regulation induced by exercise results in an increase in mRNA content that is dependent on the type, intensity, frequency, duration, and volume of contractile activity [71], and follow different time courses (from the onset [61] up to 24 h and

beyond the termination of exercise [16, 72]). Following mRNA upregulation, the process of translation leads to the generation of proteins, and their import into mitochondria, at which stage, following a host of post-translational modifications, proteins become biologically active [48]. For the 13 proteins encoded by mtDNA the process is similar, and is under the regulation of a series of mitochondrial transcription factors, with TFAM playing the most prominent role [48]. For a comprehensive overview on the molecular mechanisms regulating exercise-induced mitochondrial biogenesis the reader is referred to some excellent reviews [48, 71, 73, 74].

Exercise has been shown to modulate both the mRNA and protein content of key regulators of mitochondrial biogenesis [48]. Thus, a better understanding of how manipulating some of the variables of the exercise prescription regulates changes in these regulatory proteins could have important implications for optimizing the design of exercise training programs aimed at improving the content and functional qualities of skeletal muscle mitochondria. In this review, we have focused on how the exercise prescription affects changes in the mRNA and protein content of PGC-1α, p53, NRF-1, NRF-2, and TFAM, due to their central role in the regulation of exercise-induced mitochondrial biogenesis [75].

5.1 PGC-1α, the "master regulator" of mitochondrial biogenesis

PGC-1α is a key regulator of exercise-induced mitochondrial biogenesis [48], as it modulates cellular processes such as metabolic control, transcriptional activity, mitochondrial respiratory function, and mitochondrial turnover [76, 77]. Through regulation of transcriptional activity, PGC-1α induces gene expression of NRF-1, NRF-2, p53, and TFAM, therefore coordinating the gene expression of both nuclear- and mtDNA-encoded mitochondrial proteins [48]. Although the role of PGC-1α is well established, studies using whole-body PGC-1α knockout

mice have observed that PGC-1α is not mandatory for training-induced mitochondrial biogenesis [78, 79].

A single exercise session activates signaling kinases (e.g., AMPK, p38 MAPK) and deacetylases (e.g., sirtuin 1), which, via phosphorylation or deacetylation, increase PGC-1α protein stability [80, 81] and PGC-1α mRNA and protein content [14, 15, 82-86]. This activation takes place immediately post-exercise [84, 85, 87] (ceasing approximately within two hours [87]), and takes place in an exercise intensity-dependent manner [14, 84], suggesting high-intensity exercise may be a potent stimulus to increase mitochondrial biogenesis. Subcellular localization also plays a key role in the modulation of PGC-1α functional activity [88, 89]. A single session of exercise induces an increase in both nuclear [89, 90] and mitochondrial [90] PGC-1α protein content in rodent skeletal muscle, indicating that the PGC-1α protein coactivates both nuclear and mtDNA transcription and coordinates their cross-talk [89, 90]. Finally, research in both mouse [89] and human [14, 15, 86] skeletal muscle observed that the increase in nuclear PGC-1α protein content post-exercise precedes the increase in the cytosol, highlighting the importance of assessing changes in PGC-1α protein in subcellular compartments. Considering the central role of PGC-1α as a prominent regulator of mitochondrial biogenesis [48], it is important to characterize how it is affected by manipulation of different variables of the exercise prescription.

5.1.1 Exercise-induced changes in nuclear PGC-1α protein content

Research in human skeletal muscle demonstrates the exercise-induced accumulation of PGC-1α protein in the nucleus takes place before increases in the cytosol [14, 15, 86, 91]. Studies have attributed this increase to increased PGC-1α protein stability mediated by p38 MAPK and/or AMPK [14, 15, 86], and/or greater nuclear import from, or decreased nuclear export to, the cytosol of existing PGC-1α protein [15, 86, 91]. Regardless, the early accumulation of

PGC-1α protein in the nucleus has been linked with an increase in the activity of existing PGC-1α protein, an event that may mediate the initial phase of exercise-induced mitochondrial biogenesis [14, 15, 89].

While no study has investigated the effects of exercise volume on exercise-induced accumulation of PGC-1α protein in the nucleus, one study has directly compared the effects of different relative exercise intensities [14]. These authors reported that only all-out SIE (~168% of $\dot{W}_{max}$'), but not MICE (55% of $\dot{W}_{max}$', associated with a much greater exercise volume), induced a significant increase in nuclear PGC-1α protein content. It was concluded that exercise intensity, rather than exercise volume, may be an important factor affecting exercise-induced changes in nuclear PGC-1α protein content. These suggestions are confirmed when pooling results from the available literature, which indicates that a single session of MICE at ~55 to 75% of $\dot{W}_{max}$ does not induce a large increase (~1 to 1.5-fold) in nuclear PGC-1α protein content [14, 86, 91-93], whereas all-out SIE is associated with larger increases (~1.7 to 2.3-fold), despite much lower exercise volumes [14, 15]. Further research is required to confirm these hypotheses and to characterize the effects of exercise volume on PGC-1α subcellular localization.

5.1.2 Exercise-induced changes in PGC-1α mRNA content

A single session of exercise also induces an increase (~2 to 15-fold) in PGC-1α mRNA, which peaks ~2 to 5 h post-exercise (Fig. 3a, Electronic Supplementary Material Table S1). While the majority of research suggests this increase does not reach significance until ~1 h after the termination of exercise, some studies have reported an increase immediately post-exercise [91, 94-99]. A likely explanation for this apparent discrepancy is that the timing of PGC-1α mRNA upregulation is normally reported based on the time elapsed from the termination of exercise (Fig. 3a). However, given that exercise-induced signaling responses have been reported to

begin with the onset of exercise [61], it appears more appropriate to report changes in PGC-1α mRNA based on the time elapsed from the beginning of exercise (Fig. 3b). In this regard, all cycling studies measuring PGC-1α mRNA immediately post-exercise, and for which the exercise session lasted 90 min or more, reported a significant increase in PGC-1α mRNA at this time point [94, 95, 97-99]. Conversely, in the cycling studies assessing PGC-1α mRNA immediately post-exercise, and for which the session lasted less than 90 min [14, 15, 87, 91, 96, 100-104], only two exercise groups induced a significant increase in PGC-1α mRNA [91, 96]. These findings suggest that the exercise-induced upregulation of PGC-1α mRNA may begin at the onset of exercise - an observation that warrants further investigation. Accordingly, the timing of biopsy sampling should be carefully considered when planning a study design (e.g., a delay of 3 to 6 hours after the onset of exercise should be chosen if investigating the maximal exercise-induced changes in PGC-1α mRNA), or when directly comparing exercise sessions of different durations. Two other factors that may affect PGC-1α mRNA content are the presence of different PGC-1α transcript variants that are differentially regulated by exercise [105], and the fact that upregulation of PGC-1α mRNA may be temperature sensitive [106-108]. Finally, the exercise-induced upregulation of PGC-1α mRNA is transient [16], with most studies indicating PGC-1α mRNA returns to baseline values within 16 to 24 h from the end of the exercise session [15, 16, 95, 100, 103, 109, 110].

Effect of exercise volume. No research has directly compared the effect of different exercise volumes on increases in PGC-1α mRNA, while controlling for exercise intensity. In the absence of rigorously controlled direct comparison studies, separate studies were pooled to determine a possible correlation between exercise-induced changes in PGC-1α mRNA and exercise volume. Given that exercise-induced increases in PGC-1α mRNA peak between 3 to 6 h from the onset of exercise, only findings from studies investigating this time frame were pooled. Results from 53 exercise groups suggest there is no correlation between exercise

volume and exercise-induced changes in PGC-1α mRNA (r = 0.18 [-0.10, 0.43]; $P = 0.206$; the values in square brackets represent the upper and lower limit of the 95% confidence interval; Fig. 4a). However, given the many methodological differences between studies caution must be used when interpreting these results. Well-designed training studies directly comparing different exercise volumes at the same relative exercise intensity are required to better define the role of exercise volume on changes in PGC-1a mRNA content.

<u>Effect of exercise intensity</u>. There is emerging evidence that the exercise-induced increase in PGC-1α mRNA is regulated in an exercise intensity-dependent manner, but only at submaximal exercise intensities (Fig. 4b). A study investigating work-matched HIIE sessions at 73%, 100%, and 133% of $\dot{W}_{max}$ observed a greater increase in PGC-1α mRNA content after the session at 100% of $\dot{W}_{max}$, compared with that obtained after sessions at either 73% or 133% of $\dot{W}_{max}$ - which induced similar increases [111]. The similar increase in PGC-1α mRNA reported following MICE at 62% of $\dot{W}_{max}$, and work-matched SIE at 112% of $\dot{W}_{max}$, is in agreement with the above conclusions [112]. The greater increase in PGC-1α mRNA reported after a session of MICE at 80% of $\dot{W}_{max}$ compared to a work-matched session of MICE at 40% of $\dot{W}_{max}$ indicates that the exercise-induced increase in PGC-1α mRNA below $\dot{W}_{max}$ may indeed be regulated in an exercise intensity-dependent manner [84]. Similarly, another study reported a greater increase in PGC-1α mRNA following a session of HIIE at 70% of $\dot{W}_{max}$, compared with a work-matched session of MICE at 54% of $\dot{W}_{max}$ [113]. Finally, the significant correlation between relative exercise intensity below $\dot{W}_{max}$ and exercise-induced changes in PGC-1α mRNA (r = 0.38 [0.06, 0.64]; P = 0.023; Fig. 4b) seems to further validate this hypothesis.

Analysis of the available research also suggests that relative exercise intensity is a more important determinant of the exercise-induced increase in PGC-1α mRNA content than absolute exercise intensity. A study comparing trained and untrained individuals observed

similar increases in PGC-1α mRNA when the two groups exercised at the same relative exercise intensity (same percent of $\dot{W}_{max}$), whereas a smaller increase in PGC-1α mRNA was reported for the trained group when they exercised at the same absolute exercise intensity (i.e., a lower relative exercise intensity) as the untrained group [96]. Similarly, three studies (including unpublished research from our group) comparing exercise sessions repeated at the same absolute intensity both before and after a period of training observed a significantly lower increase in PGC-1α mRNA post-training, when the relative exercise intensity was lower compared to pre-training [114, 115]. Finally, a study investigating the effects of seven HIIE sessions on mitochondrial adaptations observed a progressively smaller exercise-induced increase in PGC-1α mRNA with every subsequent session performed at the same relative exercise intensity, suggesting the transcriptional stimulus is reduced as the training intervention progresses [16]. However, the adjustments in relative exercise intensity were made based on the maintenance of a similar heart rate between sessions, and not on improvements assessed via an IET. Further research, strictly determining changes in relative exercise intensity via an IET, is needed to determine if relative exercise intensity remains an important determinant of exercise-induced increases in PGC-1α mRNA content as the training progresses.

5.1.3 Exercise-induced changes in whole-muscle PGC-1α protein content

Following transcription and mRNA upregulation, protein synthesis begins via a process called translation; thus, it is expected that greater amounts of PGC-1α protein will be observed in the subsequent hours following exercise-induced increases in PGC-1α mRNA content (Electronic Supplementary Material Table S1). The majority of research has reported no changes in PGC-1α protein content for the first 3 to 4 h post-exercise [15, 16, 82, 85, 86, 92, 99, 102, 116]. However, three studies have reported an increase within this time frame [95, 115, 117]; these discrepancies likely relate to the type of exercise, nutritional interventions during the recovery

period, and/or differences in the immunoblotting protocol. If the exercise stimulus is sufficient, there seems to be consensus that 16 to 24 h from the cessation of exercise is necessary to observe an increase in PGC-1α protein content; reported increases at these time points range from 1.2 to 1.6 fold [15, 16, 95, 109]. No study has investigated the effects of exercise volume, or exercise intensity, on changes in PGC-1α protein content; therefore, no conclusions can be made. More research is needed to determine if intensity and volume affect exercise-induced changes in PGC-1α protein content similarly to PGC-1α mRNA.

5.1.4 Training-induced changes in whole-muscle PGC-1α protein content

Studies indicate that PGC-1α protein content increases as the training intervention progresses, reaching maximum values after 5 to 7 sessions [16, 109]. With the exception of four studies that did not observe a significant change [118-121], the majority of the published research has observed that PGC-1α protein content increases ~1.2- to 2-fold following a training intervention [16, 18, 42, 109, 115, 122-128] (Electronic Supplementary Material Table S2).

Effect of training volume. Despite using different training protocols, resulting in slightly different training volumes, two studies have reported that increases in PGC-1α protein content reach an apparent plateau following 5 to 7 exercise sessions (~1.4 to 1.9-fold increase) [16, 109]. This suggests training volume may not be a key determinant of changes in PGC-1α protein content. However, while Granata et al. [18] observed a non-significant 1.4-fold increase in PGC-1α protein content after 12 HIIT sessions, they reported a 1.7-fold significant increase compared to pre-training after a further 40 HIIT sessions performed at a similar relative exercise intensity. Thus, in some circumstances, it may be possible to further increase PGC-1α protein content by increasing the duration or volume of training. Future research is required to better assess the role of training volume on the time-course of changes in PGC-1α protein content.

Effect of exercise intensity. It has been reported that six weeks of MICT (65% of $\dot{W}_{max}$) or all-out SIT (~176% of $\dot{W}_{max}$) increased PGC-1α protein content to a similar extent (~2-fold) [122]. However, a subsequent study comparing four weeks of MICT (55% of $\dot{W}_{max'}$), HIIT (73% $\dot{W}_{max'}$), and all-out SIT (~168% of $\dot{W}_{max'}$), reported that only SIT increased PGC-1α protein content (~1.6-fold), concluding that PGC-1α protein content may be sensitive to exercise intensity [42]. Moreover, three out of four studies employing all-out SIT reported an increase in PGC-1α protein content [42, 122, 127], with only the fourth study observing no change [119]; no change in PGC-1α protein content has also been reported by two studies employing non all-out SIT (i.e., 100% [120] and 120% [121] of $\dot{W}_{max}$). These findings suggest that SIT may be a potent stimulus to increase PGC-1α protein content, but only when performed all-out. However, training volume may have been a confounder as studies reporting increased PGC-1α protein content involved ~10 to 25 exercise sessions [42, 122, 127], whereas those reporting no change only involved ~6 to 8 [119-121]. This finding adds to our previous observation that training volume may be an important factor regulating training-induced changes in PGC-1α protein content. Future studies are required to validate these hypotheses; the effects of exercise intensity should also be investigated in single fibers to elucidate any fiber-specific adaptations.

5.1.5 Reversibility of training-induced changes in PGC-1α protein content

After a significant increase following 52 HIIT sessions in seven weeks, two weeks of reduced-volume training (5 HIIT sessions) resulted in a non-significant ~1.1-fold decrease in PGC-1α protein content; however, PGC-1α protein content at this time point was not significantly different compared to pre-study values [18]. It was concluded that PGC-1α protein content is sensitive to short-term reductions in training volume, and that human skeletal muscle rapidly adapts to the new metabolic and energy requirements. Future research is required to investigate

the effects of complete detraining and shorter training volume reductions on changes in PGC-1α protein content, both in mixed and single skeletal muscle fibers.

5.2 p53, "guardian of the genome" and metabolic regulator

The tumor suppressor protein p53 [129-131] is widely regarded as the "guardian of the genome" as it regulates processes such as cell cycle arrest, senescence, apoptosis, autophagy, DNA-damage and repair, and tumor suppression [129]. Moreover, p53 is necessary for the activation of exercise-induced mitochondrial biogenesis [132], as deletion of the p53 gene in mice is associated with reduced mitochondrial content, decreased mitochondrial respiratory function, and impaired exercise capacity [133-135]. p53 regulates mitochondrial respiratory function via transcriptional control of several components of the ETS [133, 136-138], and by modulating the balance between glycolytic and oxidative pathways [133]. In addition, p53 can regulate mitochondrial remodeling [139-141], as well as the transcription of PGC-1α [142] and TFAM [134], and is therefore emerging as an important regulator of mitochondrial biogenesis (for some excellent reviews, the reader is referred to Saleem et al. [75], Oren [130], Vousden, Ryan [131], and Bartlett et al. [143]). Despite the above evidence, a study utilizing muscle-specific p53 knockout mice reported that p53 is not required to develop or maintain baseline mitochondrial content and/or enzyme activity in mouse skeletal muscle [144].

Post-translational modifications and subcellular localization of p53 appear to be important determinants of p53 activity. Phosphorylation of p53 at serine 15 (p-p53^{Ser15}) increases p53 stability and activity [145], whereas cellular stress is associated with an increase in p53 protein content in the nucleus [130, 146] - where p53 exerts the majority of its transcriptional and biochemical activity [130]. For these reasons, it is important to better understand exercise-induced changes in nuclear p53 protein content and p-p53^{Ser15}. Following exercise, p53 content

in the mitochondria has been reported to increase [147] or decrease [148] in mice, and remain unchanged in humans [93]. However, given the limited research on this topic, this will not be discussed further in this review. Nonetheless, it is clear further research is required to understand how exercise affects subcellular localization of the p53 protein.

5.2.1 Exercise- and training-induced changes in p53 protein content, p-p53^{Ser15}, and p53 mRNA

A single session of exercise in humans has been reported to increase the protein content of p53 in the nucleus immediately post-exercise (~1.7-fold) [14], or after three hours of recovery (~1.5-fold) [93], consistent with the notion that cellular stress induces nuclear p53 protein accumulation [130, 146]. The increase in nuclear p53 protein content in human skeletal muscle is in agreement with the majority of findings observed in rodent skeletal muscle [148-150]. Exercise-induced increases in nuclear p53 protein content following MICE at 55% $\dot{W}_{max}$, and all-out SIE (~168% $\dot{W}_{max}$) were not significantly different (1.6- and 2.5-fold, respectively) [14], suggesting exercise intensity may not have a large influence on these changes. No study has investigated the effects of different exercise volumes in human skeletal muscle.

The content of p-p53^{Ser15} in the nucleus is increased following a single session of all-out SIE both immediately (3.1-fold) and three hours (2.1-fold) post-exercise; conversely, no significant change in p-p53^{Ser15} was reported following MICE at 55% $\dot{W}_{max}$, at the same two time points (1.5 and 1.2-fold respectively) [14]. It was concluded that relative exercise intensity may be an important factor affecting exercise-induced changes in nuclear p-p53^{Ser15}. No research has investigated the effect of different exercise volumes on p-p53^{Ser15}, and no conclusions can be made in this regard. Exercise-induced increases in p-p53^{Ser15} in human skeletal whole-muscle fractions take place only after three hours of recovery [82, 151]. The earlier increase in p-p53^{Ser15} in nuclear compared to whole-muscle fractions suggests that increases in nuclear p-

$p53^{Ser15}$ may represent an early event of the exercise-induced mitochondrial biogenesis regulated by p53 [14].

In human skeletal muscle, a single session of HIIE (73, 100, and 133% of $\dot{W}_{max}$) induced a small ~1.3-fold increase in p53 mRNA [111], whereas no significant change was reported after MICE at 55% of $\dot{W}_{max}$, or SIE at ~168% of $\dot{W}_{max}$ [14], within three hours post-exercise. Conversely, an ~2.0 and ~2.5-fold increase was reported 4.5 and 7.5 h after high-intensity interval running at 85% $\dot{W}_{max}$, respectively [152]. Possible confounders in this study were the use of eccentric exercise (running), as well as the feeding of participants and a second exercise session (continuous running at 70% $\dot{W}_{max}$) before the post-exercise biopsies were obtained. No research has investigated how exercise volume affects exercise-induced changes in p53 mRNA; therefore, no conclusions can be made in this regard. No exercise intensity effect on changes in p53 mRNA was reported by two studies comparing different relative exercise intensities [14, 111], suggesting exercise intensity may not be an important determinant of exercise-induced changes in p53 mRNA. However, unpublished research from our laboratory suggests that exercise-induced increases in p53 mRNA content peak ~24 to 48 hours post-exercise, suggesting that significant exercise-induced increases in p53 mRNA may have taken place after the last time point investigated in the above studies (3 h post-exercise). More research investigating longer recovery periods is required to determine the role of exercise intensity (and exercise volume) on the regulation of p53 mRNA.

Only two studies have investigated the effects of exercise training on p53 protein content in human skeletal whole-muscle fractions. The first study observed that p53 protein content did not change significantly (1.7-fold) after 12 sessions (4 weeks) of HIIT, whereas a significant 2.7-fold increase from pre-training was reported after a further 40 sessions (3 weeks) of HIIT [18]. These findings suggest that training-induced changes in p53 protein content may depend on training volume or duration. A 1.2-fold non-significant decrease was reported following a

subsequent reduction in training volume (5 HIIT sessions in 2 weeks), at which time there was no significant difference with the pre-study values. This indicates the protein content of p53 may be sensitive to short-term reductions in the training stimulus, and this suggests once again that human skeletal muscle rapidly adapts to the new metabolic and energy requirements. The second study reported an exercise-intensity effect on training-induced changes in p53 protein content, as only all-out SIT (at ~168% of $\dot{W}_{max'}$), but not HIIT (73% of $\dot{W}_{max'}$) nor MICT (55% of $\dot{W}_{max'}$), induced an increase (1.9-fold) in p53 protein content after 12 training sessions [42]. Thus, while further research is required, it appears that both exercise intensity and training volume can influence training-induced changes in p53 protein content.

5.3 NRFs, the nuclear respiratory factors

NRFs are DNA-binding nuclear transcription factors modulating mitochondrial biogenesis [153]. More specifically, NRF-1 is a positive transcriptional regulator activating the expression of key metabolic genes regulating cellular growth, genes encoding subunits of the ETS, mitochondrial transcription factors (e.g., Tfam), genes involved in heme biosynthesis, and mitochondrial DNA transcription and replication [154]. Similarly, NRF-2 is a multi-subunit transcriptional activator involved in the expression of cytochrome oxidase and mitochondrial protein import complexes [154]. Together, the NRFs exert a direct role over a vast array of nuclear genes required for respiratory chain expression and function [153]. PGC-1α coactivates both NRF-1 and NRF-2, increasing their transcriptional activity and inducing mitochondrial biogenesis [155]. Given that exercise induces PGC-1α activation [156], it is important to investigate the effects of exercise on NRF-1 and NRF-2 mRNA and protein content.

5.3.1 Exercise- and training-induced changes in NRF-1 mRNA and protein content

The vast majority of research (including unpublished data from our group) has reported no change in NRF-1 mRNA content within the first four hours following a single session of exercise [65, 91, 100, 104, 107, 112, 156-158]. Two studies however, reported a change within this time frame; the first observed a 1.7-fold increase in NRF-1 mRNA content 30 min after a single session of HIIE at a mix of 70 and ~80% of $\dot{W}_{max}$ [117], whereas the second observed a significant decrease in NRF-1 mRNA content following four hours of MICE at 56% of $\dot{W}_{max}$, [159]. In addition, two studies reported a 1.5-fold increase in NRF-1 mRNA content five hours after HIIE at 70-80% of $\dot{W}_{max}$, [117], and 16 hours after MICE at 80% of $\dot{W}_{max}$ [109], raising the possibility that longer times are required to increase NRF-1 mRNA content. Given the limited research available, and that a lack of change has often been reported, no valid conclusions can be made on the effects of relative exercise intensity and exercise volume on exercise-induced changes in NRF-1 mRNA.

Two studies have investigated changes in NRF-1 protein content. The first study observed a 1.5-fold increase in NRF-1 protein content three hours after a 60-min session of MICE at 70% of $\dot{W}_{max}$, but no change after ten sessions of a combination of MICT and HIIT cycling [65]. The second study also reported no change in NRF-1 protein content after 24 sessions of continuous cycling at ~ 80 to 85% of maximal heart rate [160]. Given the paucity of available literature, more research is needed to define the effects of relative exercise intensity and training volume on exercise- and training-induced changes in NRF-1 mRNA and protein content; studies should also investigate these changes after longer recovery times.

5.3.2 Exercise- and training-induced changes in NRF-2 mRNA and protein content

Similar to NRF-1, the majority of research (including unpublished data from our group) observed no change in NRF-2 mRNA content within the first four hours following a single session of exercise [107, 115, 117, 156, 158, 161, 162]. However, two studies reported an ~2- to 3-fold increase in NRF-2 mRNA content two hours after a 10-km cycling uphill time trial (~46 min at ~75% $\dot{W}_{max}$) [100], or three hours after 90 min of MICE at ~62% $\dot{W}_{max}$ [112]. An increase in NRF-2 mRNA content has also been reported 16 [109] and 24 [100] hours post MICE at 75-80% $\dot{W}_{max}$, suggesting longer time frames may be necessary to observe significant changes in NRF-2 mRNA. Finally, research has demonstrated that exercising at a lower relative exercise intensity may be more beneficial to increase NRF-2 mRNA content as only MICE at 62% $\dot{W}_{max}$ and not SIE at 112% of $\dot{W}_{max}$ (sessions were matched for total work and total exercise time) increased this parameter three hours post-exercise [112].

Three studies have investigated changes in NRF-2 protein content. Whereas no change was reported after 14 sessions of MICE at 80% $\dot{W}_{max}$ [109], or following 24 sessions of MICE at ~ 80-85% of maximal heart rate [160], an increase in NRF-2 protein content was reported after the third and seventh session of a 7-session HIIT program at ~90% of $\dot{W}_{max}$ [16]. Due to the limited research available, no conclusions can be made on the effects of exercise intensity and training volume on changes in NRF-2 protein content. Future research directly comparing the effects of different relative exercise intensities and different training volumes on exercise- and training-induced changes in NRF-2 mRNA and protein content is required.

5.4 TFAM, the mitochondrial transcription factor

TFAM is a nuclear-encoded transcription factor regulating mtDNA expression and the transcription of 13 mtDNA-encoded subunits of the ETS [48], which is transcriptionally

regulated by PGC-1α [155]. TFAM overexpression [163] and ablation [164] studies suggest TFAM is necessary for mtDNA maintenance and transcription. In this regard, exercise increases TFAM binding to the promoter region of mtDNA [165], and induces the formation of TFAM-PGC-1α [90] and TFAM-p53 [147] complexes, which drive mtDNA transcriptional activity. Despite the limited amount of available research, some conclusions can still be made.

5.4.1 Exercise-induced changes in TFAM mRNA and protein content

There are inconsistent findings about the effects of a single exercise session on changes in the content of TFAM mRNA. While some studies have reported no change 2 to 6 hours post-exercise [91, 112, 113, 115, 158, 162], other studies observed a 1.2- to 3-fold increase within the same time frame [16, 112, 113, 152, 156, 162, 166]; there does not appear to be any clear reason for these divergent findings. No study has investigated the effects of exercise volume on changes in the content of TFAM mRNA; however, the effects of relative exercise intensity are controversial. While two studies have observed an exercise intensity-dependent upregulation of TFAM mRNA content (MICE at 59% of $\dot{W}_{max}$, *vs.* SIE at ~125% of $\dot{W}_{max}$, [162], MICE at 54% of $\dot{W}_{max}$ *vs.* HIIE at 70% of $\dot{W}_{max}$ [113]), a third study reported that only MICE at 62% of $\dot{W}_{max}$, and not SIE at 112% of $\dot{W}_{max}$, increased TFAM mRNA content [112]. Finally, no change in TFAM protein content has been observed within 24 h of the completion of a single session of exercise [16, 109].

5.4.2 Training-induced changes in TFAM protein content

There are also inconsistent findings regarding training-induced changes in TFAM protein content, as studies involving MICT, HIIT, and SIT have been reported to not change [16, 42, 109, 123] or to increase [18, 114, 120, 167] TFAM protein content. More specifically, it has been reported that TFAM protein content did not change significantly (1.2-fold) after 12

sessions (4 weeks) of HIIT, but was significantly increased from pre-training (1.8-fold) after a further 40 sessions (3 weeks) of similar HIIT, and decreased significantly (2.0-fold) after a subsequent 2-week reduction in training volume (5 HIIT sessions) [18]. The authors concluded that training-induced changes in TFAM protein content may be related to training volume and may be associated with changes in mitochondrial content. Conversely, no changes in TFAM protein content have been reported following four weeks of MICT at 55% of $\dot{W}_{max}$, HIIT at 73% of $\dot{W}_{max}$, or SIT at ~168% of $\dot{W}_{max}$ [42]. This suggests that exercise intensity is unlikely to be an important determinant of training-induced changes in TFAM protein content. The paucity of available research, and the often-divergent findings reported in the literature, suggest more research is required to better characterize the exercise- and training-induced modulation of TFAM.

6 Conclusions

The above sections have provided an extensive review on the current knowledge regarding some of the key molecular events associated with mitochondrial adaptations that occur in response to exercise and training (Fig. 5). We have reported an exercise intensity-dependent regulation for events such as the activation of signaling kinases (e.g., AMPK, p38 MAPK) and deacetylases (e.g., sirtuin 1), and increases in nuclear PGC-1α protein content, which are all early events regulating mitochondrial biogenesis. Upregulation of PGC-1α mRNA content also seems to be regulated in an exercise intensity-dependent manner, although this is limited to submaximal exercise intensities. The importance of exercise intensity is further highlighted by the greater training-induced increase in PGC-1α protein content reported following four weeks of all-out SIT compared with same duration HIIT or MICT protocols. The role of training volume is less well characterized. However, despite the lack of correlation between exercise

volume and exercise-induced changes in PGC-1α mRNA, and despite a plateau in PGC-1α protein content having been reported within the first five to seven sessions, greater increases in PGC-1α protein content still take place when the training volume is greatly increased (up to 40 sessions). This, together with the finding that PGC-1α protein content is decreased following a short-term reduction in training volume, seems to indicate that training volume, although not of primary importance, may still be a factor affecting training-induced mitochondrial biogenesis driven by PGC-1α.

Although evidence is still limited, regulation of mitochondrial biogenesis by p53 seems to also take place in an exercise intensity-dependent manner - as demonstrated by greater exercise-induced phosphorylation of p53 at serine 15 with greater exercise intensities, and by the greater training-induced increase in p53 protein content reported following four weeks of SIT compared with HIIT or MICT protocols employing lower exercise intensities. The role of training volume on p53-regulated mitochondrial biogenesis is still largely unknown. However, studies have demonstrated that training-induced changes in p53 protein content may depend on training volume or duration, and that the protein content of p53 is sensitive to short-term reductions in training volume, underlining the importance of maintaining the training stimulus.

Only limited conclusions can be made on the effects of different exercise parameters on TFAM and the NRFs. We have reported that training volume and not training intensity may be an important determinant of training-induced changes in TFAM protein content, and that these changes are associated with changes in mitochondrial content. In contrast to previous findings related to the other transcription factors and coactivators, it has been reported that exercising at a lower exercise intensity may be more beneficial to increase NRF-2 mRNA content; however, due to the limited number of studies available, more research is needed to verify these hypotheses.

This review has also emphasized the importance of standardizing other methodological aspects (e.g., biopsy timing, and how exercise intensity is determined) that need to be controlled when designing and interpreting findings from different studies, and has demonstrated that relative, rather than absolute, exercise intensity is a more important determinant of exercise-induced mitochondrial biogenesis. Finally, we have provided a thorough analysis of how relative exercise intensity and training volume can be manipulated to differentially modulate the early events of mitochondrial biogenesis, and how these may be used to prescribe exercise programs aimed at improving mitochondrial characteristics. It is important to note however, that exercise-induced changes in the mRNA and protein content, and protein phosphorylation and localization of key transcription factors modulating mitochondrial biogenesis, may not necessarily predict training-induced mitochondrial adaptations (e.g., changes in mitochondrial density, the content of mitochondrial proteins, and/or mitochondrial respiratory function). In this respect, both a relationship [16], and a lack of relationship [16, 83, 107] have been reported. It is important for future research to establish if a relationship is indeed present, and between which parameters. It would also be useful to determine an array of biomarkers predicting training-induced mitochondrial adaptations that can be measured after a single exercise session.

Compliance with Ethical Standards

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Conflicts of Interest

Cesare Granata, Nicholas Jamnick and David Bishop declare that they have no conflicts of interest relevant to the content of this review.

Author Contributions

Cesare Granata conducted the literature searches. Cesare Granata, Nicholas Jamnick and David Bishop analysed and interpreted the data. Cesare Granata wrote the manuscript. Cesare Granata, Nicholas Jamnick and David Bishop critically revised and contributed to the manuscript. Cesare Granata and David Bishop have primary responsibility for the final content. Data analysis took place at Victoria University. All persons designated as authors qualify for authorship, and all those qualifying for authorship are listed. All authors read and approved the final manuscript.

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Figure Captions

Fig. 1 Relationship between oxygen consumption measured during an incremental exercise test (IET) with continuous (shorter duration) ramp, or with a graded exercise test (GXT) with stepwise (longer duration) power increases, and cycling power on a cycle ergometer. A typical lactate threshold (LT) curve, and typical exercise intensity zones for different exercise modalities (MICE: moderate-intensity continuous exercise; HIIE: high-intensity interval exercise; SIE: sprint interval exercise; RSE: repeated sprint exercise), are also presented. $\dot{V}O_{2max}$: maximal oxygen uptake; $\dot{V}O_{2peak}$: peak oxygen uptake; $\dot{W}_{LI}$: power associated with the lactate inflection point; $\dot{W}_{LT}$: power associated with the lactate threshold; $\dot{W}_{max}$: maximal power output; $\dot{W}_{peak}$: peak power output; $\dot{W}_{VO2max}$: power associated with the $\dot{V}O_{2max}$; [La^-]: blood lactate concentration

Fig. 2 Schematic of transcriptional activity leading to exercise-induced mitochondrial biogenesis. NADH: Nicotinamide adenine dinucleotide; AMP: adenosine monophosphate; ROS: reactive oxygen species; Ca^{2+}: calcium; SIRT1: silent mating type information regulation 2 homolog 1; AMPK: 5' AMP-activated protein kinase; p38 MAPK: p38 mitogen-activated protein kinase; ERK1/2: extracellular-regulated kinase 1 and 2; JNK: *c-jun* N-terminal kinase; CaMKII: Ca^{2+}/calmodulin-dependent protein kinase II; PGC-1α: peroxisome proliferator-activated receptor γ coactivator-1α; CREB: cAMP response element binding protein; HDAC: Histone deacetylase; ATF2: activating transcription factor 2; MEF2: myocyte enhancer factor-2. *(*Adapted from Egan, Zierath [71], with permission*)*

Fig. 3 Timing of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) messenger RNA (mRNA) upregulation relative to the time elapsed from the end (a), or the onset (b) of exercise measured following a single cycling session in the vastus lateralis muscle of healthy human participants. Studies with middle-aged, elderly, or diseased (e.g., chronic heart failure, diabetics, and obese) populations were excluded. Results were obtained from references 14-16, 83-85, 87, 91, 94-104, 109-118, 158, 161, 162, 166, 168-171 (Electronic Supplementary Material Table S1). All values are mean ± standard error of the mean.

Fig. 4 The relationship between exercise-induced increases in peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) messenger RNA (mRNA) and (a) exercise volume, and (b) relative exercise intensity, measured in the vastus lateralis muscle of healthy human participants. Linear correlation line (solid line), and 95% confidence bands (dotted

lines) are shown, as well as the correlation coefficient r [with 95% confidence intervals] and the *P* value. Exercise volume was calculated by multiplying the exercise intensity relative to maximal power output ($\dot{W}_{max}$) or estimated maximal power output ($\dot{W}_{max'}$) by the duration of exercise session (in minutes). Only studies involving cycling that measured exercise-induced changes in PGC-1a mRNA 3 to 6 h after the onset of exercise were included. Studies with diseased populations (e.g., chronic heart failure, diabetics, obese), and studies not providing precise and detailed information about the exercise prescription, were excluded. Relative exercise intensity for studies employing sprint interval exercise (SIE) is often not provided; for these studies, a value of relative exercise intensity was used based on that attained by participants of similar fitness completing the same number of repetitions in a training study from our group [42]. These ranged from ~181% to ~165% of $\dot{W}_{max}$ for four to ten 30-s all-out bouts, respectively. (a) was obtained by pooling results from references 14-16, 83-85, 87, 91, 94-97, 99, 101, 102, 104, 111-118, 158, 161, 162, 166, 168-171. (b) was obtained by pooling results from the same studies used to generate (a), with the exception of studies using a mix of relative exercise intensities, and studies using a relative exercise intensity ≥ 100% $\dot{W}_{max}$ (or $\dot{W}_{max'}$) (Electronic Supplementary Material Table S1). MICE: moderate-intensity continuous exercise; HIIE: high-intensity interval exercise; SIE: sprint interval exercise, Mixed: combination between two or more of MICE, HIIE and SIE. A linear correlation analysis was used to calculate the correlation coefficient between variables, according to Pearson's product moment (r) (SigmaStat software; Jandel Scientific, San Rafael, CA, USA). The level of statistical significance was set at $P < 0.05$. a.u.: arbitrary units

Fig. 5 Schematic representation of the magnitude and timing of exercise-induced mitochondrial adaptations measured in the vastus lateralis of human skeletal muscle of healthy participants, following a single cycle exercise session. This figure was generated by pooling results from the studies reviewed in this manuscript, as well as unpublished research from our group. The shaded grey area represents a hypothetical exercise session. PGC-1α: peroxisome proliferator-activated receptor γ coactivator-1α; mRNA: messenger RNA