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

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

Authors:

David J Bishop[1], Cesare Granata[1], Nir Eynon[1]

Affiliation:

[1]Institute of Sport, Exercise and Active Living (ISEAL), Victoria University, Australia

Corresponding Author:

Professor David J. Bishop
Institute of Sport, Exercise and Active Living (ISEAL),
Victoria University, Melbourne, Australia.
Phone: (03) 9919-9471 International +61-3-9919-9471
Fax: (03) 9919-4891 International +61-3-9919-4891
Room 140, Building P, Footscray Park campus
E-mail: David.Bishop@vu.edu.au

Table of contents:

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.

Key words: Mitochondria, exercise, trainability, mitochondrial biogenesis

Highlights:

1. Physical activity levels appear to affect mitochondrial function more than content.

2. Training intensity may be an important determinant of mitochondrial function.

3. Training volume strongly correlates with changes in mitochondrial volume.

4. Exercise-induced mitochondrial changes are rapidly reversed by detraining.

1. Mitochondria

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 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,

[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.

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 between proteins and ranges from immediate to a few sessions [29]. mRNA is subsequently translated to generate proteins, which can then be transported inside the mitochondria. After translation has taken place, post-translational modification can facilitate protein import, folding or assembly into complexes with subsequent generation of biologically-active proteins. For the 13 mitochondrial-encoded proteins, the process is similar with a mitochondrial transcription factor along with other transcriptional regulators promoting biogenesis from mtDNA. New evidence suggests that PGC-1α and p53, which are typically regarded as nuclear proteins, may translocate inside the mitochondria to help coordinate mtDNA transcription [30, 31]. For a more detailed description of the effects of exercise on the gene expression pathways leading to mitochondrial biogenesis in skeletal muscle, readers are referred to an excellent review on this topic [32].

4. Can we optimise exercise training to improve mitochondrial function and content?

Physical activity increases skeletal muscle energy demand many fold, and it is not surprising that cross-sectional studies suggest an association between physical activity levels and changes in both mitochondria content [33-36] and function [33, 37-40]. Interestingly, analysis of the cross-sectional data published to date indicates that higher physical activity levels are associated with relatively larger values for mitochondrial function (as measured by mitochondrial respiration) than content (as measured by Citrate Synthase activity) (Fig 1). This interpretation contrasts with the results of two individual studies reporting that the ratio between mitochondrial respiration and mitochondrial mass

was not related to physical activity status in humans [41, 42]. However, it should be noted that both of these studies measured mitochondrial respiration in isolated mitochondria, and that the mitochondrial yield in such preparations has been reported to be less than 30% [41]. It is therefore possible that this finding is related to the isolation procedure. Further research, particularly involving training interventions, is required to clarify whether mitochondrial respiration is tightly coupled to mitochondrial content in skeletal muscle.

**** Figure 1***

There is convincing evidence that the maximal oxidative power of human skeletal muscle is in excess of what is required during exercise with large muscle groups (e.g., cycling and running) [23]. Nonetheless, despite this overcapacity, and consistent with the results from cross-sectional studies, increases in both mitochondrial function and content have been reported in humans following endurance training [34, 39, 40, 43-53]. Furthermore, these increases with training are very rapid, with increases in MAPR (46%) and CS activity (16%) reported after only five days of training [53]. These results are consistent with the cross-sectional data summarised in Figure 1, and indicate no clear relationship between the increase in mitochondrial function and content with training. However, an earlier study reported that increases in mitochondrial respiration (measured via MAPR) were directly related to increases in the protein content of the mitochondrial fraction analysed [34]. These inconsistent observations do not appear to be due to the method used to estimate mitochondrial protein content as discordant findings have also been reported in two studies with rats, despite both studies using the same method to measure the total protein content of the mitochondrial fraction [54, 55]. Further research is clearly required to resolve this issue.

While there is agreement that physical activity is a powerful stimulus to increase both mitochondrial function and content, questions still remain regarding the optimal training stimulus. Questions regarding the most effective training stimulus to promote training adaptations are not new. In 1986, Astrand and Rodahl wrote:

> *"It is an important but unresolved question which type of training is most effective: to maintain a level representing 90 percent of the maximum oxygen uptake for 40 minutes, or to exercise at 100 percent of the oxygen uptake capacity for 16 minutes"* [56].

While there are many training variables that can be manipulated (e.g., intensity, duration, volume, frequency, length of intervention etc), two of the most important variables, as reflected by the above quote, are intensity and volume of training. In the following section, we will review the small number of studies that have investigated the impact of varying training intensity and volume on changes in mitochondrial function and content. Other training variables have not been reviewed as there are not enough studies to make robust conclusions.

4.1 Training intensity and mitochondrial function and content

4.1.1 Mitochondrial function

We are aware of only six studies (including our unpublished work) that have investigated exercise-induced changes in mitochondrial function in humans (using a variety of substrate combinations) [34, 39, 40, 52, 53, 57] (see Table 1). These include studies that have assessed mitochondrial function via MAPR [34, 53], oxygen consumption in isolated mitochondria [39], or oxygen consumption in

permeabilised fibres [40, 52, 57]. Interpreting the influence of training intensity is further complicated by the fact that three of these studies used "mixed training" (i.e., a combination of continuous, moderate-intensity training, and high-intensity interval training), and only one study directly compared two different training intensities [52]. This latter study reported that maximal, ADP-stimulated respiration (V_{max}), measured in permeabilised skeletal muscle fibres, was only significantly increased after high-intensity interval training (36%, $P<0.05$), and not following work-matched, moderate-intensity, continuous training (18%, $P>0.05$) [58]. While these authors suggested that repeated fluctuations of O_2 consumption (e.g., high-intensity interval training) seem necessary to increase mitochondrial function, large increases (50%) in mitochondrial respiration (assessed via MAPR) have been reported following moderate-intensity continuous exercise [34].

*****Table 1******

The hypothesis that training intensity may be an important determinant of improvements in mitochondrial respiration is consistent with the cross-sectional data (see Figure 1), and also three studies involving young, healthy rats (note: studies involving voluntary running, or aged or diseased rats were not included) [59-61]. While once again the number of studies is small, and there are many differences between the studies (e.g., type and sex of rat), there is a trend for greater increases in mitochondrial respiration with higher training intensities (Fig 2). Further studies comparing different training intensities within the same study are needed to clarify the influence of training intensity on changes in mitochondrial respiration.

****Figure 2****

4.1.2 Mitochondrial content

Citrate synthase is an enzyme that is exclusively located in the mitochondria [41], whose activity is strongly correlated with mitochondrial content [4], and which is routinely measured in training studies. It is therefore an ideal candidate to assess if changes in mitochondrial content are related to training intensity when the results of individual studies are pooled. As can be seen in Figure 3, there appears to be no relationship between training intensity and training-induced changes in CS activity in humans. A similar result can be observed for the type I, oxidative, muscle fibres of rat (Figure 4a,c). It appears that there may be an effect of training intensity on exercise-induced changes in CS activity in type II, glycolytic, skeletal muscle fibres (Figure 4e). This however, needs to be interpreted with caution as the higher training intensities were also associated with a higher volume of training (Fig 4f), which may confound this interpretation. Nonetheless, these findings suggest that mitochondrial content is more strongly related to training volume, while mitochondrial function may be more dependent on training intensity. Further research examining this hypothesis, and potential underlying mechanisms, is warranted.

****Figure 3****

****Figure 4****

4.2 Training volume and mitochondrial function and content

4.2.1 Mitochondrial function

For all of the reasons mentioned previously (e.g., small number of studies, mixed types of training, different measures of mitochondrial function), it is very difficult to make strong conclusions about the influence of training volume on mitochondrial function. Furthermore, we are aware of only one study that has directly compared changes in mitochondrial respiration in response to two different training programs matched for training volume [52]. These authors observed that V_{max} was only significantly

increased (36%, $P<0.05$) after high-intensity interval training, and not moderate-intensity continuous training ($P>0.05$), despite the same total amount of work being performed; participants trained three times per week for eight weeks (24 sessions). Our recent research, in which participants performed a much higher volume of high-intensity interval training, but only had a similar increase in V_{max} compared to the above study, also suggests that improvements in mitochondrial respiration are not proportional to training volume in humans [57]. This conclusion is supported by the limited number of studies that have been performed in rats (Figure 2b).

4.2.2 Mitochondrial content

In contrast to the results observed for training intensity, it appears that training volume may be an important determinant of exercise-induced improvements in mitochondrial content (as assessed by CS activity). In both humans (Figure 3b) and rats (Figure 4b,d,f), there is a strong correlation between training volume and changes in skeletal muscle CS activity. While the consistent observations in both rats and humans help to strengthen this conclusion, caution is obviously warranted given the many small methodological differences between these pooled studies. Further research, particularly in humans, comparing different training volumes within the same study, are needed to clarify the influence of training volume on changes in mitochondrial content. While CS activity is a useful biomarker, which exhibits a strong association with mitochondrial content [4], it would also be informative for future studies to assess mitochondrial content via transmission electron microscopy - regarded as the gold standard for measuring mitochondrial content.

From the pooled data, two additional observations can be made. The first is that in type I, oxidative, rat skeletal muscle (e.g., the soleus and red vastus), there is no sign of a plateau; greater training volumes

are associated with greater increases in CS activity (Figure 4b,d,f). A similar observation can be made with respect to the pooled results of the human studies performed to date (analyses performed on the mixed fibre-type vastus lateralis muscle) (Figure 3b). Further research is required to investigate if there is a point where further increases in training volume do not lead to further increases in CS activity. In contrast, there does appear to be a plateau in type II, glycolytic muscles of rats (e.g., white vastus), such that further increases in training volume do not result in further increases in CS activity (Figure 4f). Given that a decrease in training intensity is typically required to increase training volume, and that there is a correlation between training intensity and changes in CS activity in this muscle (Figure 4e), it is difficult to determine whether this latter observation can be attributed to an accompanying decrease in training intensity or to an effect of training volume per se. As fibre recruitment depends on exercise intensity [62], it may be that training volume is an important determinant of changes in CS activity in red, oxidative skeletal muscle (recruited at low intensities), while training intensity is a more important determinant of changes in CS activity in white, glycolytic skeletal muscle (only recruited at higher intensities). In mixed, human skeletal muscle (i.e., the vastus lateralis), training volume appears to be the more important determinant of changes in CS activity (Figure 3b).

4.3 Reversibility and mitochondrial function and content

The above discussion has highlighted that skeletal muscle is a remarkably plastic tissue that responds rapidly to an increase in the amount of physical activity. However, an important corollary is that skeletal muscle is also highly responsive to decreases in the amount of physical activity, such that mitochondrial adaptations are quickly reversed following a reduction or cessation of physical activity [34, 57, 63]. This has important implications, as it could mean that one of the most important considerations when prescribing exercise training is that it is maintained.

It has been reported that there is a 12-18 percent decrease in MAPR in isolated mitochondria following a three-week period of detraining (although this decrease was not significant for all substrate combinations) [34]. This is consistent with our observation of a similar reduction in mitochondrial respiration (in permeabilised fibres) following a marked decrease in training volume (Figure 5) [57]. In addition, there are indications that the majority of this decrease occurs soon after the cessation of training. It has been reported that mitochondrial respiration (measured in crude homogenates of biopsy samples taken from the posterior deltoid muscle of swimmers) decreased by 50% after only one week of inactivity (with no further changes over the next 3 weeks) [63].

**** Figure 5***

While it is clear that mitochondria rapidly respond to decreases in contractile activity (i.e., exercise), further research is required to confirm these findings and to determine if the mitochondrial sub-populations respond in the same way. There is consensus regarding the existence of two populations of mitochondria in skeletal muscle: sarcolemmal (SS, located just below the sarcolemma) and intermyofibrillar (IMF, encased between myofibrils). It appears that these two respond differently to a decrease in contractile activity. For example, it was reported that two days of hind-limb immobilisation in rats resulted in a 37% decrease in the V_{max} of SS, without a change in IMF mitochondria isolated from the gastrocnemius muscle [64]. In contrast, four weeks of hind-limb suspension was associated with an 18% decrease in the V_{max} of IMF mitochondria, without a change in SS mitochondria [65]. These differences can potentially be explained by the different types and durations of reduced contractile activity, but need to be clarified by additional studies. Further research is also required to

determine if population-specific changes in mitochondria also occur in response to reduced physical activity in humans.

The decrease in mitochondrial function with training is accompanied by decreases in mitochondrial enzymes [34, 63, 66-68]. Interestingly, it appears that mitochondria do not turn over as a unit, as originally suggested [69], but that the rates of regression differ between enzymes (Figure 6). For example, in response to a period of detraining, the estimated half-life of cytochrome c oxidase (COX) is 5-8 days [67, 70], while it is approximately 2-3 times longer for CS [68] and succinate dehydrogenase (SDH) [67, 71]. These different half-lives are somewhat surprising given that all three enzymes are located on the inner mitochondrial membrane and therefore might be expected to turnover with similar half-lives [72]. It should be acknowledged however, that there have been relatively few studies and that further research is required to better establish the rates of regression of different mitochondrial enzymes in response to a decrease in physical activity.

**** Figure 6***

While a decrease in physical activity is associated with a reduced activity of oxidative enzymes in mixed fibre types, this decrease has been reported to be much slower in type II, glycolytic than in type I, oxidative fibres [66, 68]. Following 12 weeks of detraining, mitochondrial enzyme activity (CS, SDH) was reported to be 50-80% above control values in type II fibres, but very similar to control values in type I fibres [66]. This observation needs to be further investigated, and may have implications for the maintenance of mitochondrial enzyme activities in participants with different proportions of type I and type II fibres.

The decreases in CS activity discussed above indicate that detraining leads to a rapid decrease in mitochondrial content. However, while this has consistently been observed in humans, it has not always been observed in rats, with some studies reporting an increase in CS activity [73], or a tendency for an increase in total mitochondrial volume density [74], in response to hind-limb suspension. It has been suggested that these findings could be reconciled if the non-mitochondrial cell protein decreased to a greater extent than the mitochondrial fraction [73]. However, decreases in myofibril volume density are quite small (< 10%) and seem unlikely to explain why hind-limb suspension induced an almost doubling of CS activity in type I oxidative rat skeletal muscle fibres [73], while detraining leads to CS activity returning close to control values in human type I fibres [66]. A more likely explanation is that these conflicting findings can be explained by species (rats vs. humans) or protocol (inactivity vs. hind-limb suspension) differences. Nonetheless, further studies are warranted to investigate the effects of detraining on mitochondrial content in human skeletal muscle fibres (both type I and type II fibres). Interestingly, when a decrease in training volume is accompanied by an increase in training intensity, this has been reported to increase CS activity in humans [75]. This observation needs to be further investigated as it has important implications for optimising training-induced changes in mitochondrial content. Nonetheless, it is clear that when seeking to optimise mitochondrial adaptations it is important to also consider how best to structure any periods of reduced physical activity.

4.4 Trainability – role of genes

It is apparent from figure 5 that there is individual variability for changes in mitochondrial function (i.e., mitochondrial respiration) in response to high-intensity exercise training and de-training [57]. The concept of individual differences in the response to exercise training, or trainability, was first

investigated in the early 1970s [76]. It was shown that some people are 'non-responders' (not improving their fitness following exercise training), while others respond well (responders), or even very-well (high-responders) to a similar exercise training program [76]. These observations, together with twin and family studies, suggest that some of the variable response to exercise training can be explained by genetic factors.

The first studies to investigate the genetic influence on human trainability used twin models, comparing the variation in trainability between monozygotic (MZ) and dizygotic (DZ) twins. Using 25 pairs of twins (15 MZ and 10 DZ preadolescent boys), it was reported that there was a 93.4% heritability for maximal oxygen uptake (VO_2 max) measured during an incremental exercise test [77]. A genome-wide linkage scan for athletic status reported a heritability of roughly 66% for athletic status in 700 British female dizygotic twin pairs [78]. Finally, data from the Health, Risk factors, Training and Genetics (HERITAGE) family study suggested that the heritability of changes in VO_2 max with exercise training is ~47% in sedentary subjects [79]. Collectively, these studies support the notion that a large portion of the inter-individual variability in training-induced changes in aerobic characteristics can be attributed to genetics.

This concept of comparing twins and family members was an important milestone, revealing the genetic basis of trainability. However, the research focus has now shifted to continuously-developing, molecular-based, laboratory methods (e.g., candidate genetic variations analysis, direct sequencing) designed to directly test the interaction between genetic and environmental factors, not only in family or twin studies, but also in studies involving populations of interest [80]. The hypothesis, shared by

many scientists, is that common genetic variants (i.e., polymorphisms), which are present throughout the human genome, may cause biological changes that influence mitochondrial adaptations [81, 82].

4.4.1 Mitochondrial-related genetic variants and their association with trainability

Some of the important genes involved in mitochondrial biogenesis include Peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1α protein, encoded by *PPARGC1A* gene), peroxisome proliferator-activated receptor δ (PPARδ protein, encoded by *PPARD* gene), Nuclear respiratory factors 1 and 2 (*NRF*-1 and 2), and Transcription factor A, mitochondrial (*TFAM)*. It has therefore been suggested that specific genetic variants within these genes may influence responsiveness to aerobic exercise training (e.g., trainability) [81, 83]. While evidence, mainly derived from observational studies, suggests that genetic variants within the *PPARGC1A, PPARD, NRF,* and *TFAM* are associated with elite athletic performance [76], little is known on the effect of these variants on the response to exercise training. The G allele of the *PPARD* registered single nucleotide polymorphism (rs)2267668 and the ser482 allele of the Gly482Ser genetic variant in *PPARGC1A* gene were independently associated with smaller increases in the individual anaerobic threshold following nine months of endurance exercise training [83]. Two *NRF-1* genetic variations have been shown to be associated with the training responsiveness of submaximal endurance capacity (expressed as running economy) [84]. Genetic variations in the *NRF-2* gene have also been shown to influence the response to endurance training [77].

4.4.2 Mitochondrial haplogroups and their association with trainability

Owing to a lack of histone-mediated protection, the mtDNA is more exposed to oxidative damage and its mutation rate is ten times higher than the mutation rate in nuclear DNA [85]. Some characteristic clusters of tightly-linked mtDNA mutations form a series of population–specific lineages known as mtDNA *haplotypes* or *haplogroups*. The type of mtDNA haplogroups varies among different populations around the globe. Mitochondrial haplogroup L3 is proposed to be the ancestor of all non-African populations, European haplogroups (H, I, J, K, S, T, U, V, W, etc.) belong to macrohaplogroup N, whereas Asian haplogroups belong to both macrohaplogroups N and M (haplogroups A, B, F, and N9a to macrohaplogroup N; and haplogroups M7a, M7b, M8, D, and G to macrohaplogroup M) [86, 87]. It has been suggested that mitochondrial haplogroups have the potential to modulate mitochondrial metabolism (leading to mild differences in OXPHOS activity), and hence may affect the training response [88].

Unlike nuclear DNA, mtDNA is maternally inherited. Evidence of an association between mtDNA genes and exercise phenotypes arises from familial studies, suggesting that aerobic capacity has a stronger maternal inheritance than paternal [89]. Dionne et al. [90] were the first to study specific mtDNA polymorphisms in relation to training adaptation. Carriers of three mtDNA polymorphisms, two in subunit 5 of the NADH dehydrogenase gene (*MTDN5*) and one in the tRNA for threonine, had a higher base-line (pre-training) VO_2max, compared to non-carriers. Furthermore, a smaller increase in VO_2max in response to 20 weeks of standardised exercise training was observed for carriers of *MTDN5* subunit 5 variant. Subsequently, Rivera et al. [91] reported no association between elite endurance athletic status and three mtDNA polymorphisms in *MTND5* gene (MTND5-BamHI at bp

13,470, MTND5-NciI at bp 13,364, and MTND5-HincII at bp 12,406) and one in the D-loop region (D-loop-KpnI at bp 16,133).

In summary, the above-mentioned studies, along with other reports (reviewed by Eynon et al. [92]), suggest that mitochondrial-related genetic variants (within the nucleus), and mtDNA haplogroups (within the mitochondria) influence training-induced changes in mitochondrial biogenesis. These genetic variants may help to explain the individual variability for exercise-induced mitochondrial adaptations that we and others have observed. We recommend that future studies use high through put technologies to sequence large portions of human mitochondrial and mitochondrial-related genes. This will allow the identification of more genetic variants that have the potential to influence trainability. We also suggest that future studies will focus on using high-intensity training methods to create appropriate muscle stimuli, and thus enhance the accuracy of the muscle phenotype. Such studies can highlight the inter-subject response to similar exercise training. Indeed, our unpublished results (Figure 5) demonstrate that tightly-controlled, high-intensity interval training markedly alters mitochondrial function, and hence enhances the accuracy of the mitochondrial-related phenotype. Applying such studies can confirm the gene–exercise interactions derived from observational studies.

The primary limiting factor in gene-training studies, however, is the need to recruit large groups of individuals, to undertake supervised exercise training for a few weeks, to overcome the obvious barrier of large sample size for detecting genetic associations. To address this, large multi-site collaborations, and data sharing between researchers, will be necessary to ensure sufficient statistical power is obtained. Improving cohort numbers and advances in molecular technologies will also enable researchers to apply genome-wide association (GWAS) approach by analysing 100 000 to several

millions of genetic variants across the entire genome without any previous hypotheses about potential mechanisms. Indeed, recent GWAS data from the HERITAGE family study revealed novel variants associated with trainability; three of the four novel candidate variants that predicted VO_2 max changes in response to exercise training where further validated by mRNA expression [93]. It is to note that in these studies sequencing of the human mitochondrial genome was not performed and, as such, genomic markers within the mitochondria that can predict the response to training remains to be elucidated.

5. **Conclusions**

Skeletal muscle is a remarkably plastic tissue, with mitochondria responding rapidly to increases or decreases in the amount of physical activity (exercise). This review has highlighted that training intensity appears to be an important determinant of improvements in mitochondrial function (as assessed by mitochondrial respiration), but not mitochondrial content (as assessed by citrate synthase activity). In contrast, training volume seems to be an important determinant of training-induced improvements in mitochondrial content, but not function. Further research, directly comparing different training intensities and volumes within the same study are required to verify these observations. Further studies investigating the potential underlying mechanisms is also warranted. Due to the small number of studies conducted to date, more research is required to determine if fibre-type-specific, and population-specific (i.e., SS or IMM), mitochondrial changes occur in response to increased and decreased levels of physical activity. Finally, researchers have only recently begun to investigate how genetic variants influence trainability; this research needs to be expanded to understand the genetic influence on exercise-induced changes in mitochondrial biogenesis.

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Table 1. Changes in mitochondrial respiration in humans with training.

Study	Participants	Training Program	Method and substrates	Change
[53]	Untrained (M&F)	1x/d for 10 d 60 min @ 75% VO_{2max} or 6 x 5 min @ 95% VO_{2max}	MAPR - PPKM - PCM	 ↑ 136 % ↑ 161 %
[34]	Untrained (M)	4x/wk for 6 wk 36 min @ 75% VO_{2max}	MAPR - PPKM - PCM - PM	 ↑ 70 % ↑ 92 % ↑ 50 %
[39]	Untrained (M&F)	4x/wk for 6 wk [30 min @ 70% VO_{2max} + 5 x 2 min @ 100% VO_{2max}]	Isolated mitochondria - PM	 ↑ 40 %
[52]	Untrained (M&F)	3x/wk for 8 wk IT: 4 min@VT1: 1 min@90% VO_{2max} CON: 28 min @ 61% VO_{2max}	Permeabilised fibres - GM - GM	 ↑ 40 % ↑ 18 %
[40]	Untrained (M&F)	4x/wk for 6 wk [30 min @ 70% VO_{2max} + 5 x 2 min @ 100% VO_{2max}]	Permeabilised fibres - PM	 ↑ 38 %

M = Males, F = Females, VT1 = first ventilator threshold, VO_{2max} = maximal oxygen uptake, MAPR = Maximal ATP Production Rate, PPKM = pyruvate + palmitoyl-L-carnitine + α-ketoglutarate + malate, PCM = palmitoyl-L-carnitine + malate, PM = pyruvate + malate, GM = glutamate + malate

Figure Captions:

Figure 1. Mitochondrial Respiration and Citrate Synthase activity in humans of differing training status [33-40]. SED = sedentary, ACT = active, TRA = trained, MT = moderately-trained, WT = well-trained, and HT = highly-trained.

Figure 2. The relationship between A) training intensity and B) training volume and training-induced changes in mitochondrial respiration in rats [59-61]. Studies were excluded if they did not provide precise information about the training prescription or if they used "mixed training" (i.e., a combination of continuous, moderate-intensity training, and high-intensity interval training).

Figure 3. The relationship between A) training intensity and B) training volume and training-induced changes in citrate synthase activity in humans [34, 43-51]. Training volume was calculated by multiplying the training intensity (%VO2max) by the duration (minutes of exercise) by the number of training sessions per week. Studies were excluded if they did not provide precise information about the training prescription or if they used "mixed training" (i.e., a combination of continuous, moderate-intensity training, and high-intensity interval training).

Figure 4. The relationship between training intensity and training volume and training-induced changes in citrate synthase activity of rats in the red soleus (A and B respectively), the red vastus (C and D respectively), and the white vastus (E and F respectively) [55, 59, 61, 64, 94-104]. Studies were excluded if they did not provide precise information about the training prescription or if they used "mixed training" (i.e., a combination of continuous, moderate-intensity training, and high-intensity interval training).

Figure 5. Individual changes in mitochondrial respiration during training and de-training [57]. Pre_IT = pre interval training, Post_IT = post interval training, Post_DT = post de-training.

Figure 6. Changes in the activities of cytochrome c oxidase (COX), citrate synthase (CS) and succinate dehydrogenase (SDH) during training cessation in humans. Values are based on results from the few studies that have measured changes in enzyme activity during the cessation of training [67, 68, 70, 71]. Values on the y-axis are percent of pre-training values.