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REVIEW ARTICLE

Citius, Maius, Melius: Mitochondria and Endurance Performance

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Abstract

Endurance performance relies on a range of physiological adaptations classified as central or peripheral depending on whether the remodeling occurs in the cardiovascular system or in the skeletal muscle, respectively. One of the goals of these adaptations is to enhance oxygen transport and its utilization by mitochondria to sustain ATP resynthesis. While a link between skeletal muscle mitochondrial characteristics and endurance performance may seem obvious, there is no consensus on whether mitochondrial characteristics are key determinants of endurance performance. In this narrative review, we examine cross-sectional, correlational, and intervention studies conducted in humans that support or challenge the role of mitochondria in endurance performance. Cross-section studies suggest that individuals with superior endurance performance exhibit greater mitochondrial content and respiratory function than those with lower fitness levels. Correlation studies have shown positive associations between multiple mitochondrial characteristics and markers of endurance performance. However, a lack of correlation between training-induced changes in mitochondrial characteristics and endurance performance has also been reported. Intervention studies indicate that changes in mitochondrial characteristics following training, phlebotomy, or detraining are often associated with changes in markers of endurance performance. Conversely, increasing oxygen delivery to the working muscle (i.e., via increasing oxygen concentration) has been shown to improve performance markers, suggesting these improvements are not limited by mitochondrial characteristics. In conclusion, while substantial evidence associates mitochondrial characteristics with endurance performance, this relationship is not universal; central factors display an equally strong influence independently of mitochondrial characteristics. We propose that enhanced mitochondrial characteristics represent an important and often necessary, but not sufficient, adaptation that is required to support endurance performance.

Key Points

The role of skeletal muscle mitochondria in determining endurance performance remains unclear.

Cross-sectional, correlation, and intervention studies show a consistent association between mitochondrial characteristics and endurance performance

Better mitochondrial characteristics seem important but not necessary for greater endurance performance.

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1 Introduction

In 490 BCE, *Pheidippides,* an Athenian herald, completed one of the first recorded human endurance journeys when he embarked on his last run of ~40 km from a battlefield

near Marathon to Athens to announce the Greek victory in the Battle of Marathon. This event inspired the founders of the modern Olympic Games and paved the way for the first modern endurance event, the marathon. Since the beginning of the modern Olympic Games, and supported by the motto "*Citius, Altius, Fortius*", athletes have tried, time and time again, to improve upon historical endurance performances over many events. These remarkable performances have motivated scientists to better understand the intricacies of endurance performance [1–4], and, in turn, the growth of sport science in the last century has facilitated many breakthrough sporting achievements [5]. In doing so, athletes, coaches, and scientists have worked together to synergistically spur improvements in human endurance performance and increase the scientific knowledge of its underlying biological and physiological determinants.

Endurance performance is context-dependent and exists along a continuum of events differing in duration and distance [6–8]. Accordingly, the relative and integrated contributions of the underlying physiological systems, including the cardiovascular, respiratory, metabolic, neuromuscular, and thermoregulatory systems, vary across this continuum [7]. Hence, to provide a consistent framework for the present review, endurance performance has been defined as the maximum average velocity (i.e., distance/time) that an individual can achieve in a given continuous event ranging from approximately 10 min to several hours [9].

Although endurance performance is typically determined in competitive events, laboratory tests have been developed as relevant and practical proxies of performance. Among the many laboratory tests available, time-trial (TT) time is regarded as the most valid laboratory-based measure of endurance performance [10]. Therefore, in the present review, TT time will be considered the primary indirect measure (or marker) of endurance performance.

In addition, maximal aerobic power ($\dot{W}_{max}$), obtained via an incremental exercise test, has been shown to strongly predict TT time in trained cyclists [11–14] and runners [15, 16], with correlation coefficients ranging from -0.87 to -0.97 (all $p < 0.05$). Moreover, $\dot{W}_{max}$ may provide a stronger prediction of endurance performance than single physiological variables linked to endurance performance, such as maximal oxygen uptake ($\dot{V}O_{2max}$), $\dot{V}O_2$ at the lactate threshold ($\dot{V}O_{2LT}$), and running economy [16]. This likely reflects the ability of $\dot{W}_{max}$ to integrate the combined influence of $\dot{V}O_{2max}$, $\dot{V}O_{2LT}$, exercise efficiency/economy, and fatigue resistance more effectively than any single physiological variable alone [16, 17]. Therefore, $\dot{W}_{max}$ will be considered as an integrative primary marker of endurance performance in this review.

From a strictly physiological standpoint, endurance performance is determined by the combination of an individual's $\dot{V}O_{2max}$, submaximal anchors or metabolic thresholds such as ventilatory (VT) or lactate (LT) thresholds, and economy of movement—defined as the oxygen consumption required to maintain a specific speed or power output [18] (Fig. 1). Among these, submaximal anchors, such as power at the ventilatory ($\dot{W}_{VT}$) and lactate ($\dot{W}_{LT}$) thresholds, the maximal lactate steady state (MLSS) and critical power (CP) [19–22]), as well as $\dot{V}O_{2max}$ [23], have been shown to correlate, with various degrees of strength, with laboratory and/or TT performance in populations ranging from active individuals to elite athletes. Therefore, these physiological metrics will be used in this review as secondary indirect markers of endurance performance.

The physiological characteristics of an individual reflect a multitude of underlying biological factors that, if modified, could ultimately improve the capacity to transport and utilize oxygen (O_2) [24] and support the energetic cost of skeletal muscle contraction during exercise. These factors are often separated into central and peripheral components (Fig. 1; reviewed elsewhere [25]), with the O_2 exchange from the circulation to the working muscle as the demarcating point. The final fate of O_2 occurs in the mitochondria—the organelles responsible for resynthesizing ATP through oxidative phosphorylation (OXPHOS [26, 27]). The path completed by O_2 molecules, from inspiration all the way to the mitochondria, is referred to as the oxygen cascade [28]. Each step in the oxygen cascade is thought to represent a possible limitation to O_2 diffusion, potentially becoming a limiting factor in O_2 utilization during exercise [25, 29]. Given the role of mitochondria in utilizing O_2 to resynthesize ATP, a critical step to sustain skeletal muscle contraction during exercise, it has been speculated that mitochondria play a pivotal role in determining endurance performance [30–32].

Mitochondria are multifaceted organelles that are involved in many critical functions [33]. As a consequence, they play a central role in health and disease, and mitochondrial alterations have been shown to underpin many pathologies [34, 35]. Mitochondria present with several characteristics [33, 36]; however, the present review will focus on the characteristics most often reported in the exercise literature. These are mitochondrial content, best assessed by transmission electron microscopy (TEM) or any of its validated biomarkers [37, 38], and mitochondrial respiratory function in the maximal coupled state, which best represents the mitochondria's ability to utilize O_2 for ATP resynthesis and is commonly assessed by *ex vivo* high-resolution respirometry [38, 39]. It is important to acknowledge the presence of other integrative measures of mitochondrial respiratory function, such as near-infrared spectroscopy (NIRS), magnetic resonance spectroscopy (MRS), and a-vO_2diff (reviewed elsewhere [40–43]). However, these approaches will not be considered in detail, as they do not readily allow the specific contribution of mitochondria to be separated from that of central (e.g., O_2 delivery) or other peripheral (e.g., capillary

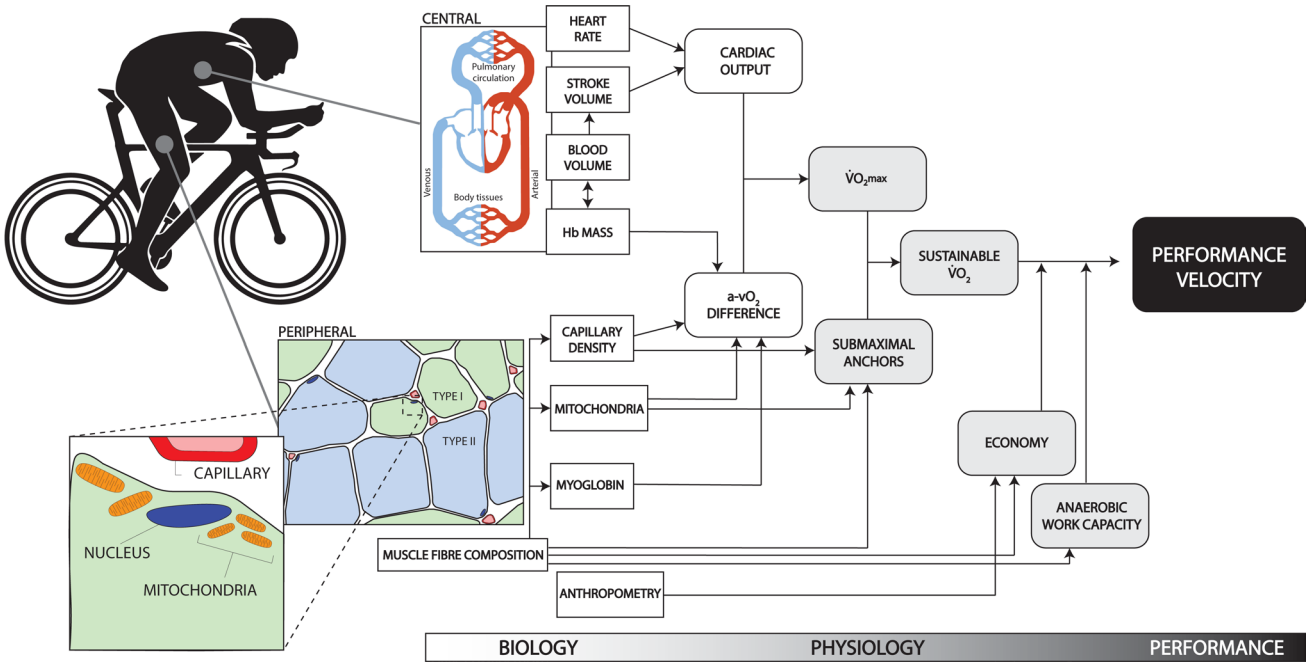


Fig. 1 Schematic framework of the biological and physiological factors underlying endurance performance. *a-vO*$_2$ arterio-venous oxygen, *Hb* hemoglobin, $\dot{V}O_2$ volume of oxygen consumption, $\dot{V}O_{2max}$ maximal rate of oxygen consumption

density and distribution) factors and are therefore outside the specific scope of this review.

Despite the large increase in research investigating mitochondrial adaptations to cardiorespiratory exercise and training in the last few decades (Fig. 2A), and the obvious link between mitochondrial characteristics and the fate of inspired O_2, there remains inconclusive evidence on whether mitochondrial characteristics play a critical role in endurance performance. Hence, the aim of this narrative review is to appraise the role of skeletal muscle mitochondria as potential determinants of endurance performance.

2 Historical Perspective of Mitochondria and Endurance Performance

The field of mitochondria, exercise, and skeletal muscle research spans over 80 years (Fig. 2B). The first known study was published in 1939 [44, 55] and reported that 14–21 days of skeletal muscle contractions in rabbits resulted in an ~90% increase in the activity of succinate dehydrogenase (SDH)—a key enzyme of the OXPHOS system and a validated biomarker of mitochondrial content [37]. In 1967, the work of Holloszy [45] demonstrated that following 12 weeks of training, multiple mitochondrial characteristics were concurrently improved in rat skeletal muscle; these included an ~100% increase in mitochondrial respiratory function, an ~60–80% increase in the activities of SDH and cytochrome *c* oxidase (COX, another key enzyme of the OXPHOS system and validated biomarker of mitochondrial content [37]), and an ~60% increase in total mitochondrial protein content. In 1971, the work by Morgan et al. [47] was the first to report skeletal muscle mitochondrial adaptations following cardiorespiratory[1] exercise training in humans. One month of daily, single-leg, 2-hour cycling sessions led to significant increases in multiple mitochondrial characteristics, such as mitochondrial protein content (~25%), phospholipid content (~45%), SDH and COX activity (~30% and ~60%, respectively), mitochondrial respiratory function (~45%), mitochondrial volume density ($mito_{VD}$; ~55%), and mitochondrial size—as assessed in electron micrographs (~25%).

Following these initial publications showing that repeated muscle contraction (e.g., exercise training) led to improvements in skeletal muscle mitochondrial characteristics across mammalian species, researchers started to explore whether these mitochondrial adaptations could contribute to the observed training-induced improvements in primary

[1] *Cardiorespiratory exercise*: a mode of exercise that requires the circulatory, respiratory, and muscular systems to work coordinately to support the metabolic demands required for skeletal muscles and other engaged organs. It is preferred over terms such as aerobic exercise (which describes the predominant energy system used) or endurance exercise (which describes the ability to sustain the cyclic movement). It includes activities such as walking, running, swimming, and cycling [56].

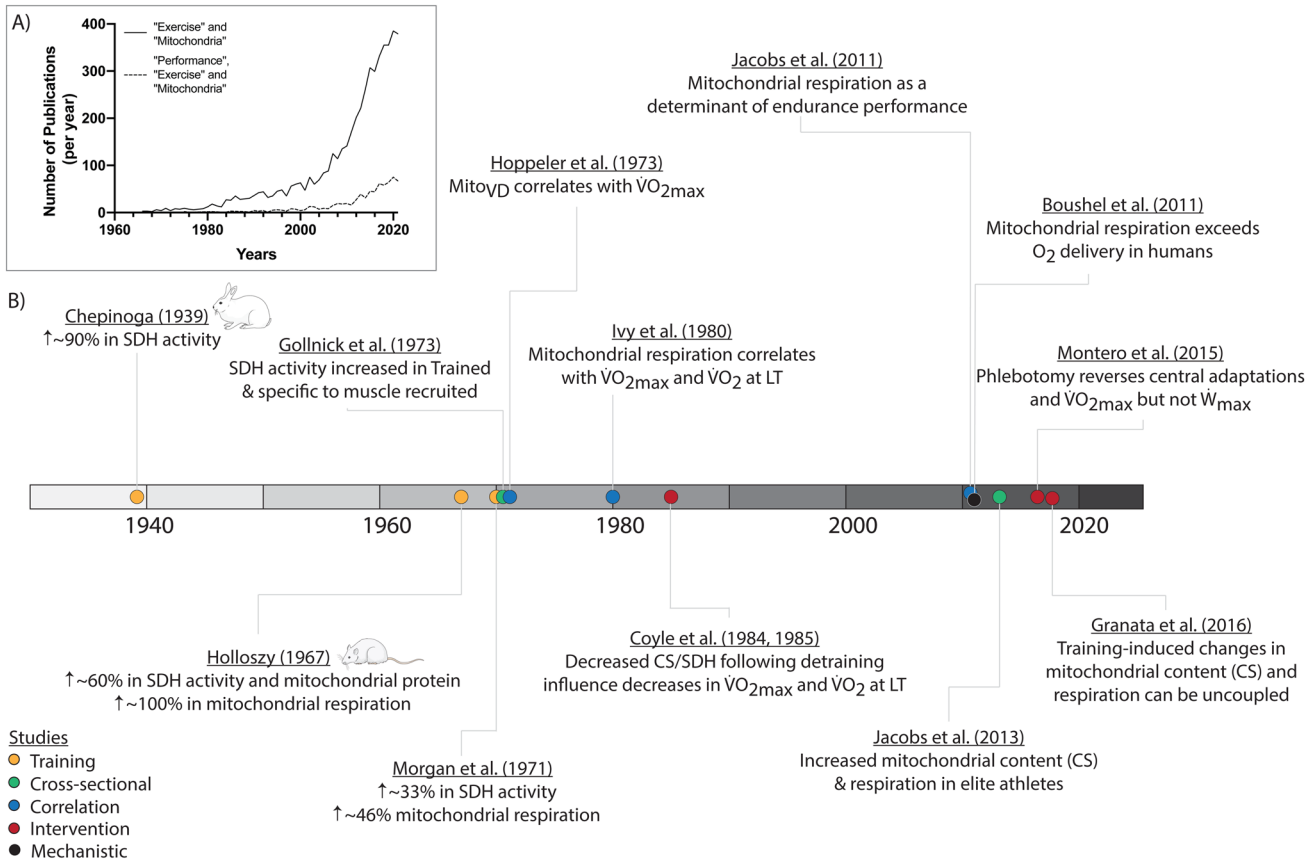


Fig. 2 Historical perspective and milestones of exercise training research. **A** Evolution of the annual number of published scientific research in the area of exercise, performance, and mitochondria. The data was obtained by retrieving the number of times the terms 'exercise' and 'mitochondria' with or without 'performance' appeared in scientific publications listed in PubMed by year. **B** Timeline of key publications that have improved our understanding of the involvement of skeletal muscle mitochondria in endurance performance. Publications involving non-human animal studies include a drawing of the used animal model. *CS* citrate synthase activity, *LT* lactate threshold, $mito_{VD}$ mitochondrial volume density, *SDH* succinate dehydrogenase activity, $\dot{V}O_2$ volume of oxygen consumption, $\dot{V}O_{2max}$ maximal rate of oxygen consumption, $\dot{W}_{max}$ maximal aerobic power. Studies included are [30, 31, 44–54]

and secondary markers of endurance performance. To the best of our knowledge, the study led by Saltin in 1976 [57] was the first to explore the relationship between changes in mitochondrial characteristics and markers of endurance performance following training. This study showed that, following 4–5 sessions per week, for 4 weeks, of single-leg sprint interval or moderate-intensity continuous training (SIT and MICT, respectively), improvements in the trained leg were associated with peripheral adaptations. Increases in SDH activity were reported following MICT (+33%) and SIT (+19%); similarly, single-leg $\dot{V}O_{2max}$ improved following both MICT (+20–24%) and SIT (+11–15%). When the groups were combined, there was a significant correlation between changes in SDH activity and $\dot{V}O_{2max}$. The authors concluded that "*This [adaptation] focuses attention on peripheral factors as being at least as essential for the cardiovascular performance during exercise as any central factors*". This study initiated the search for a better understanding of the physiological (both central and peripheral [Fig. 1]) determinants of endurance performance [58–60].

Historically, the search for physiological determinants of endurance performance is best exemplified by a series of articles discussing the limits of $\dot{V}O_{2max}$ and endurance performance that were published in 1985. Saltin [61], in contrast to his earlier publication in 1976, argued in favor of a central limitation to $\dot{V}O_{2max}$ on the basis that the metabolic potential of skeletal muscle is in excess of the oxygen available during exercise. This work also distinguished between changes in $\dot{V}O_{2max}$ and endurance performance as being functional and performance adaptations, respectively. It further suggested that improved peripheral adaptations following endurance training would facilitate a slower blood mean transit time through the blood capillaries and improve the metabolic response to exercise; for example, by increasing mitochondrial fatty acid oxidation.

Di Prampero [62] modelled the different steps of oxygen transport and concluded that 75–80% of the $\dot{V}O_{2max}$ relies on central factors, while the remaining 20–25% depends on peripheral adaptations. This model would suggest that large peripheral adaptations are required to drive small/modest improvements in $\dot{V}O_{2max}$ (this aligns with the magnitude of changes in mitochondrial adaptations [large] and $\dot{V}O_{2max}$ [small/modest] commonly observed following exercise training [52]). Hoppeler and Lindstedt [63] approached this question from an evolutionary perspective and demonstrated that, across a variety of animal species studied, skeletal muscle was capable of metabolizing a similar volume of oxygen per unit of mitochondria. Therefore, they argued that the amount of skeletal muscle mitochondria had a direct influence on $\dot{V}O_{2max}$. Lastly, Gollnick et al. [64] argued in favor of mitochondria being key players, suggesting that enhanced mitochondrial characteristics in skeletal muscle would translate into an increased fatty acid utilization at a given $\dot{V}O_2$, which in turn would increase the submaximal anchors and delay glycogen utilization—both of which are important factors for endurance performance [65–67]. However, despite the increase in research over the last decades, our current understanding of this topic remains under intense debate, is still largely incomplete, and requires further research to better clarify the biological factors underlying endurance performance.

3 The Role of Mitochondrial Characteristics in Endurance Performance

The following sections will break down the available evidence 'in favor of' or 'against' the role of mitochondria as key determinants of endurance performance, as provided by three different types of studies: (i) cross-sectional, (ii) correlational, and (iii) intervention/longitudinal. We will focus on studies performed in humans to avoid the influence of experimental confounding factors when comparing results across mammalian species.

3.1 Cross-Sectional Studies

Cross-sectional studies, in which heterogeneous groups of participants are investigated, usually provide the initial evidence of support for a potential causal relationship. These types of studies allow researchers to highlight the putative importance of a given biological characteristic. In cross-sectional studies, grouping can be obtained by dividing participants based on their performance level (e.g., TT time), cardiorespiratory fitness (e.g., $\dot{V}O_{2max}$, $\dot{W}_{max}$), as well as training (e.g., sedentary, active, highly trained) or health (e.g., heart failure, obesity, diabetes, healthy controls) status [68]. For the scope of this section, studies will be only included if valid markers of endurance performance (e.g., TT time, $\dot{W}_{max}$, $\dot{V}O_{2max}$) are provided for the different groups. Improved mitochondrial characteristics at increasing levels of cardiorespiratory fitness (e.g., lower TT time, or greater $\dot{V}O_{2max}$) will be considered preliminary evidence in favor of their role as determinants of endurance performance. These characteristics may include greater $mito_{VD}$ or citrate synthase (CS) activity, a mitochondrial enzyme involved in the tricarboxylic acid cycle and a validated biomarker of mitochondrial content in skeletal muscle [37], as well as greater mitochondrial respiration. The opposite pattern will represent evidence against this interpretation.

3.1.1 Evidence in Favor

To date, no study has investigated endurance performance and skeletal muscle mitochondrial characteristics in humans grouped cross-sectionally according to markers of endurance performance (e.g., TT time, $\dot{W}_{max}$, $\dot{V}O_{2max}$). However, by pooling the results from two published training studies from the same research group [54, 69], we divided 53 young healthy humans into tertiles based on their pre-training 20-km TT performance: low, medium, and high ($n = 18$, 18, and 17, respectively), whereby a higher TT completion time was categorized as a low performance. By design, the three groups were significantly different from each other (all $p < 0.05$, Fig. 3A). We observed a significantly greater skeletal muscle CS activity in the high compared with the low group ($p = 0.034$; Fig. 3B). Due to differences in the mitochondrial respiration protocols used in the two aforementioned studies, it was not possible to pool the results for evaluating mitochondrial respiratory function. Therefore, we grouped into tertiles only the 30 participants from one of these studies [54]. By design, the three groups differed significantly for pre-training 20-km TT times (Low, Medium, and High; all $n = 10$; all $p < 0.05$; Fig. 3C). The ensuing analysis demonstrated a greater maximal coupled mitochondrial respiration in the High compared with the Low group ($p = 0.046$; Fig. 3D). Similarly, when grouping the 30 participants of the same published study [54] into tertiles by $\dot{W}_{max}$ ($n = 9$, 11, and 10 for the Low, Medium, and High, respectively; all $p < 0.05$; Fig. 3E) or by $\dot{V}O_{2max}$ (Low, Medium, and High; all $n = 10$; all $p < 0.05$; Fig. 3G), we observed a greater mitochondrial respiration in the High compared with the Low $\dot{W}_{max}$ group ($p = 0.016$; Fig. 3F), as well as the High compared with the Low $\dot{V}O_{2max}$ group ($p = 0.013$; Fig. 3H). This cross-sectional evidence highlights the potential importance of different mitochondrial characteristics as determinants of endurance performance.

Many other cross-sectional studies have grouped participants based on training status (e.g., sedentary, active, highly trained) and have repeatedly demonstrated greater $mito_{VD}$, CS activity, and/or mitochondrial cristae density [51,

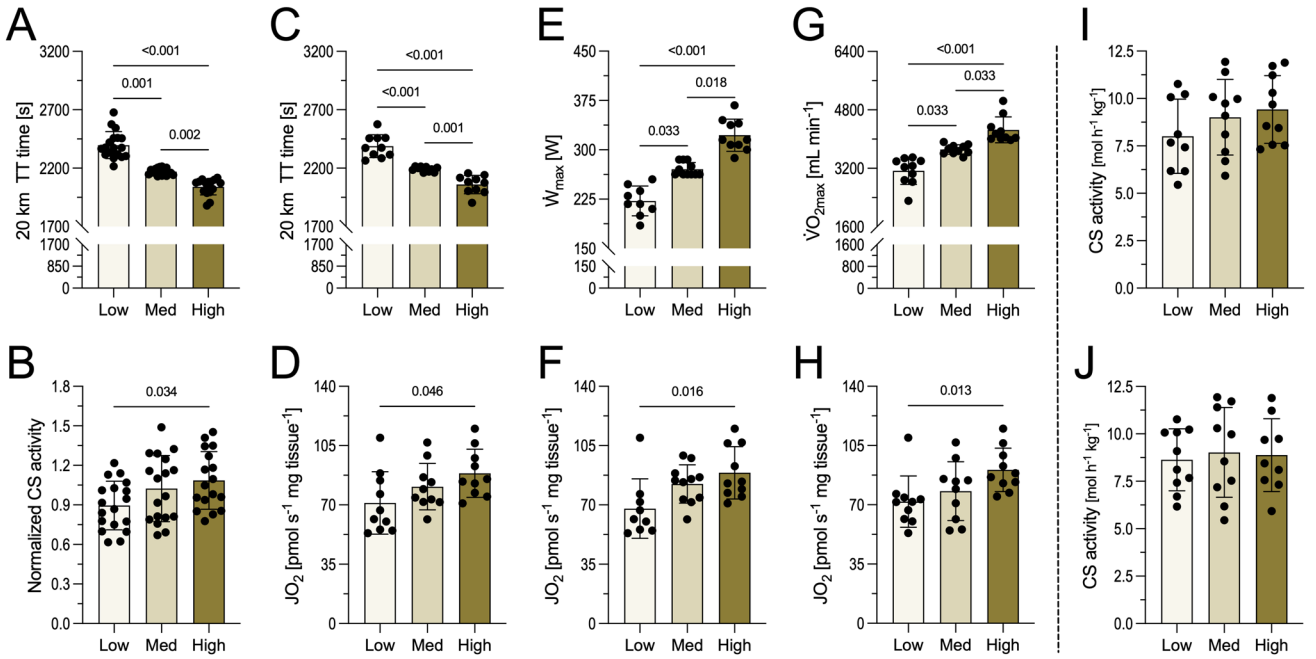


Fig. 3 Evidence from cross-sectional studies in favor of and against the importance of mitochondrial characteristics as determinants of endurance performance. Group differences in pre-training **A** 20-km time trial (TT) time and **B** normalized citrate synthase (CS) activity from 53 young healthy humans grouped in tertiles based on their pre-training 20-km TT time from two published studies [54, 69]. CS activity data in each study were normalized by each study's CS activity average to account for technical differences between studies. Group differences in pre-training **C** 20-km TT time and **D** maximal coupled mitochondrial respiration (JO_2) with convergent electron input via the NADH (N) and succinate (S) (NS) pathway from 30 young healthy humans grouped in tertiles based on their pre-training 20-km TT time from one published study [54]. Group differences in pre-training **E** maximal aerobic power ($\dot{W}_{max}$) and **F** JO_2 with convergent electron input via the NS pathway from 30 young healthy humans grouped in tertiles based on their pre-training $\dot{W}_{max}$ from one published study [54]. In panel (**E**), because six participants (ranked 10th to 15th) at the border between the Low to Med (medium) group and three participants (ranked 18th to 20th) at the border between the Med to High group presented with identical $\dot{W}_{max}$ values, we split tertiles into 9, 11, and 10, for Low, Med, and High, respectively, to avoid participants with identical $\dot{W}_{max}$ values being spread across two separate groups, and to maintain the most similar sample size amongst groups. As a consequence, grouping in **F** followed the same split. Group differences in pre-training **G** maximal oxygen uptake ($\dot{V}O_{2max}$) and **H** JO_2 with convergent electron input via the NS pathway from 30 young healthy humans grouped in tertiles based on their pre-training $\dot{V}O_{2max}$ from one published study [54]. Group differences in pre-training CS activity from 30 young healthy humans from one published study [54] grouped in tertiles based on their pre-training **I** $\dot{W}_{max}$ and **J** $\dot{V}O_{2max}$. Group differences in $\dot{W}_{max}$ and $\dot{V}O_{2max}$ are presented in panels **E** and **G**, respectively. Grouping in **I** followed the same split as in panel **E**; grouping in **J** followed the same split as in panel **G**. **A**, **E**, **F**, **G**, and **H**: Kruskal–Wallis test followed by Dunn's multiple comparisons test. **B**–**D**, **I**, and **J**: ordinary one-way ANOVA test followed by Tukey's multiple comparisons test. Significant difference was set at $p < 0.05$ for all tests. $n = 18$, 18, and 17 for the Low, Medium, and High groups, respectively, in **A** and **B**; $n = 10$ for all groups in **C**, **D**, **G**, and **H**; $n = 9$, 11, and 10 for the Low, Medium, and High groups, respectively in **E** and **F**; $n = 9$, 10, and 10 for the Low, Medium, and High groups in **I**; $n = 10$, 10, and 9 for the Low, Medium, and High groups in **J**. Data are mean ± SD

70–73], as well as greater mitochondrial respiratory function (normalized to either mg of tissue or mitochondrial content) [51, 71, 73–75] in the skeletal muscle of human groups presenting with greater $\dot{W}_{max}$ or $\dot{V}O_{2max}$. Moreover, it has been shown that while most differences between groups of different training statuses relate to simple quantitative changes in mitochondrial content, there are also qualitative mitochondrial improvements [51]. In this regard, a recent study suggests that skeletal muscle mitochondria demonstrate similar respiratory function per cristae surface area across different training statuses [73], suggesting that qualitative mitochondrial differences may be driven by differences in cristae density. However, these results must be interpreted with caution, as the design of these studies does not allow to isolate the confounding effects of exercise training, which has been demonstrated to increase both mitochondrial content and respiratory function [39]. Similarly, cross-sectional studies grouping participants based on health or age status (e.g., heart failure, obesity, diabetes, aged individuals) have also demonstrated improved skeletal muscle mitochondrial characteristics in the human groups with greater $\dot{V}O_{2max}$ [76–82]. Once again, the validity of these results remains limited in this context, as previous research demonstrates that health status, as well as the physical activity patterns within these populations [79–82], affects the mitochondrial phenotype and mitochondrial characteristics in humans [34].

Taken together, the aforementioned findings support the notion that mitochondrial adaptations may indeed play a role in determining endurance performance. However, it is important to underline that cross-sectional studies only provide a snapshot of variables at a single point and do not establish a cause-and-effect relationship. Therefore, this evidence should be used with caution and considered only as preliminary evidence.

3.1.2 Evidence Against

To the best of our knowledge, no published cross-sectional study has reported a lack of difference in mitochondrial characteristics across populations with differences in cardiorespiratory fitness. However, it is important to acknowledge that negative results are difficult to publish [83], which could contribute to the current evidence gap. Due to differences in the protocols used to determine $\dot{W}_{max}$ or $\dot{V}O_{2max}$ in the two aforementioned studies [54, 69], it was not possible to pool the results to evaluate CS activity. Nonetheless, when the 30 participants from the study with greater statistical power [54] were grouped into tertiles by $\dot{W}_{max}$ or $\dot{V}O_{2max}$ (Fig. 3E and G, respectively), no differences in CS activity were observed between groups (all $p > 0.05$; Fig. 3I and J, respectively). Although these findings seem to downplay the importance of changes in mitochondrial content as determinants of endurance performance, they may also result from the relatively low sample size, a narrower range of $\dot{V}O_{2max}$ values, and the use of surrogate mitochondrial markers. Therefore, adequately powered studies directly linking TT performance with the gold-standard assessment of mitochondrial content ($mito_{VD}$) and respiratory function (mitochondrial respiration in permeabilized muscle fibers) [39] are needed to more clearly define the contribution of mitochondrial characteristics to endurance performance.

3.2 Correlational Studies

Correlational studies provide a means of linking two variables and allow the identification of potential relationships (positive or negative) between them. While these studies offer weak scientific evidence, as correlation does not necessarily imply causation, and observed relationships may be influenced by confounding variables, they can be useful when direct manipulation is impractical or unethical.

3.2.1 Evidence in Favor

Jacobs et al. [50] investigated the best predictors of 26-km TT performance in a homogeneous group of 16 elite cyclists (mean $\dot{V}O_{2max} = 70.5$ mL·min^{-1}·kg^{-1}). The single parameter that best explained TT performance (expressed by mean power) was maximal mitochondrial respiratory function ($R = 0.68$; $p = 0.004$). Similarly, Batterson et al. [84] demonstrated that NIRS-derived mitochondrial function was a better predictor of cycling endurance performance (26-km TT) in 24 trained participants than even the best combination of traditional markers, such as $\dot{V}O_{2max}$, $\dot{V}O_{LT}$, and economy. These findings suggest that mitochondrial characteristics may also be linked with, and represent, a factor that determines endurance performance within a single homogeneous group, such as the elite athletes or the trained individuals investigated in these studies, and not solely across groups with heterogeneous training status, as shown in previous sections. In line with this, we have previously shown that maximal mitochondrial respiratory function correlates with the time to complete a 20-km TT in 29 moderately trained males (mean $\dot{V}O_{2max} = 46.2$ mL·min^{-1}·kg^{-1}; $R = -0.65$; $p < 0.001$) [54]. A similar relationship was found between CS activity and time to complete a 20-km TT pre-training in the same study cohort ($R = -0.47$; $p = 0.012$, unpublished data). Our group has also shown significant correlations between maximal mitochondrial respiratory function and $\dot{W}_{max}$ ($R = 0.67$; $p < 0.001$) in the same aforementioned cohort of 29 moderately trained male individuals [54]. Finally, the relative $\dot{W}_{max}$ has been shown to correlate with $mito_{VD}$ in a sample that included untrained and highly trained participants ($R = 0.80$; $n = 18$; $p < 0.001$ [85]).

In addition to the aforementioned studies investigating correlations between mitochondrial characteristics and primary markers of endurance performance, there are other studies correlating mitochondrial characteristics with secondary markers of endurance performance. Mitochondrial content, as measured by $mito_{VD}$, has been shown to correlate with $\dot{V}O_{2max}$ ($R = 0.91$; p-value not reported; Fig. 4A extracted from [85, 86]) in a group that included untrained and highly trained participants. Similarly, a correlation has been observed between COX activity and $\dot{V}O_{2max}$ (mean $\dot{V}O_{2max} = 51.9$ mL·min^{-1}·kg^{-1}; $R = 0.75$; $p < 0.05$) in a sample of nine healthy males [87]. SDH activity has also been reported to correlate with $\dot{V}O_{2max}$ ($R = 0.79$; p-value not reported) in a mixed-sex cohort (34 males and 27 females) combining untrained individuals and highly trained track athletes across various events (e.g., distance running, shot-put, etc.) [88]. CS activity has been shown to correlate with CP in a cohort of 15 recreationally active to competitive athletes (mean $\dot{V}O_{2max} = 52.2$ mL·min^{-1}·kg^{-1}; $R = 0.75$ and 0.88 for absolute and relative CP, respectively; both $p \leq 0.001$) [89]. Finally, a systematic review combining data from 70 publications found that training-induced changes in CS activity correlate with training-induced changes in $\dot{V}O_{2max}$ ($r = 0.45$; $p < 0.001$; $n = 97$) [90]. Beyond the large sample size from which these findings were drawn, they may hold greater significance as correlations between training-induced changes in these variables may provide more physiologically relevant evidence than correlations with baseline

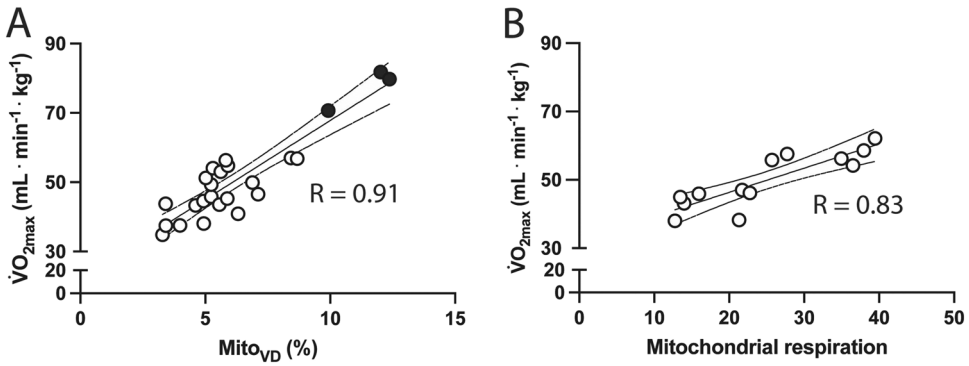


Fig. 4 Correlational evidence in favor of the importance of mitochondrial characteristics as determinants of endurance performance. Correlation between maximal oxygen uptake ($\dot{V}O_{2max}$) and **A** mitochondrial volume density (Mito$_{VD}$) (adapted from [85, 86]), as well as **B** mitochondrial respiration (adapted from [31]). *Black dots* represent samples obtained from highly trained individuals. For both panels, data were extracted with WebPlotDigitizer, and Pearson correlation coefficient R was either calculated from the extracted values or obtained from the respective manuscripts. No *p*-values were reported in those manuscripts

values. This is because training-induced adaptations are more directly driven by skeletal muscle contraction-related processes, whereas baseline measurements may be more strongly influenced by genetic factors. Further evidence of strong correlations between mitochondrial and endurance performance markers has been frequently reported [7, 30, 54, 71, 78, 91–93]. Finally, mitochondrial respiratory function has also been shown to significantly correlate with $\dot{V}O_{2max}$ ($R = 0.83$; $p < 0.01$; Fig. 4B) in a group of 13 male participants (mean $\dot{V}O_{2max} = 50.6$ mL·min^{-1}·kg^{-1}) [31].

In summary, many studies have shown that multiple mitochondrial characteristics, including mitochondrial content and respiratory function, correlate well with endurance performance and its associated markers in untrained to highly trained individuals and elite athletes. Despite the caution needed when interpreting correlational studies, this adds to the evidence suggesting that mitochondrial adaptations may be an important factor underlying improved endurance performance.

3.2.2 Evidence Against

Only a handful of studies have reported a lack of correlation between mitochondrial characteristics and primary or secondary markers of endurance performance. In a study investigating a homogeneous cohort of 15 highly trained cyclists (mean $\dot{V}O_{2max} = 69.5$ mL·min^{-1}·kg^{-1}), it was observed that CS activity did not correlate with either primary (40-km TT time) or secondary ($\dot{V}O_{2max}$) markers of endurance performance ($R = 0.46$ and 0.17, respectively; both $p > 0.05$) [94]. Our group has also reported no correlation between training-induced changes in the time to complete a 20-km TT and training-induced changes in mitochondrial respiratory function in a cohort of 29 moderately trained males (mean $\dot{V}O_{2max} = 46.2$ mL·min^{-1}·kg^{-1}; $R = 0.21$; $p = 0.279$) [54]. Moreover, the previously mentioned correlation between mitochondrial respiratory function and 20-km TT performance found pre-training ($R = -0.65$; $p < 0.001$) was abolished after a 4-week exercise training intervention ($R = -0.26$; $p = 0.180$) [54]. In 15 recreationally active to competitive athletes (mean $\dot{V}O_{2max} = 52.2$ mL·min^{-1}·kg^{-1}) [89], mitochondrial respiratory function was not significantly correlated with CP ($R = 0.30$ and 0.55 for absolute and relative CP, respectively; both $p > 0.05$). Similarly, no correlations were found between mitochondrial-specific respiration (i.e., mitochondrial respiration normalized by mitochondrial content) and CP in the same cohort ($R = -0.21$ and 0.15 for absolute and relative CP, respectively; both $p > 0.05$).

The reasons for the discrepant results observed in correlational studies may stem from the relatively small sample size of most studies, differences in participants' characteristics, experimental conditions, and variability of the measurements [38, 90], as well as differences in sex, age, and cultural background, amongst others. In addition, it must be noted again that publishing null or negative results is often difficult, raising the possibility of publication bias.

3.3 Intervention and Longitudinal Studies

The most conclusive scientific findings usually come from longitudinal and intervention studies where mitochondrial changes are examined in conjunction with physiological and performance alterations. A similar or different change of a given variable over time (longitudinal) or following an intervention can be used as evidence of any potential causal relationship between two variables. The studies presented in the following subsections have explored how physiological (e.g., exercise training, detraining, graded exercise tests) and

non-physiological (e.g., phlebotomy) interventions affect mitochondrial characteristics and endurance performance.

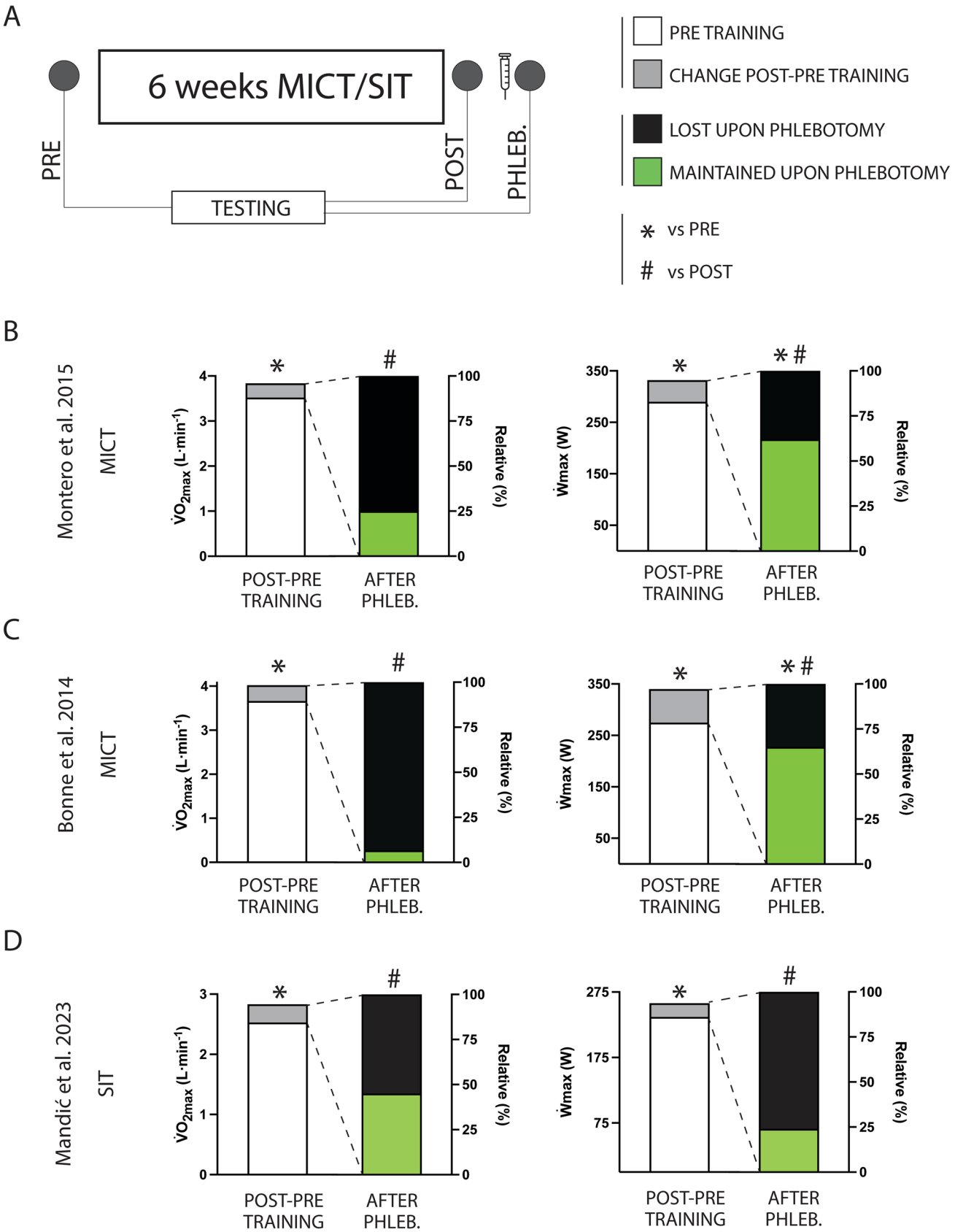
3.3.1 Evidence in Favor

Due to the obvious ethical restrictions on interventions allowed with human participants, there is no best or easiest way to isolate the direct contributions of central and peripheral adaptations to endurance performance. One appropriate study design would involve performing a short training intervention (e.g., 2 weeks) that leads to rapid peripheral adaptations without obvious central adaptations. Another approach would be to perform a training intervention that is long enough to induce both central and peripheral adaptations, but short enough to avoid structural cardiac adaptations (i.e., an intervention lasting 4–6 weeks [52, 95, 96]), followed by the immediate removal of one of the two types of adaptations while maintaining the other. This can be achieved either by phlebotomy (the process of blood withdrawal), to remove central adaptations, or by training reduction/detraining, to remove mitochondrial peripheral adaptations while maintaining capillary density and central adaptations. However, both approaches come with caveats that could influence performance: following the former, other cardiac or autonomic adaptations may remain, whereas following the latter, some loss of central adaptations may occur. Lastly, previous research in rats has linked changes in mitochondrial characteristics with endurance performance independently of changes in central components, over periods of iron deficiency and repletion [97, 98]. A similar study design could be informative in humans, where ethically feasible.

A study by Jacobs et al. [99] showed that 2 weeks of high-intensity interval training was sufficient to induce adaptations in mitochondrial respiratory function and in COX activity, but not in central adaptations (maximal cardiac output, total hemoglobin, plasma volume, or total blood volume). These findings suggest that the observed improvements in primary (TT time) and secondary ($\dot{V}O_{2max}$ and $\dot{W}_{max}$) markers of endurance performance were mediated, at least in part, by improved mitochondrial characteristics, thereby supporting their physiological relevance to endurance performance.

We are aware of four studies employing phlebotomy after training. One of these studies reported structural cardiac adaptations after 10 weeks of cardiorespiratory exercise training [100]; as these adaptations were not reversed by phlebotomy, we refrained from using these findings. From the three remaining studies, Montero et al. [52] reported that 6 weeks of MICT (study design shown in Fig. 5A) significantly increased blood volume, maximal cardiac output ($\dot{Q}_{max}$), and $\dot{V}O_{2max}$ (Fig. 5B, left panel). These improvements were subsequently abolished, and all values returned to pre-training levels when blood volume was re-established to baseline levels via phlebotomy. Crucially, no structural cardiac adaptations were observed. Despite the abrogation of the training-induced improvements in $\dot{V}O_{2max}$ after phlebotomy (Fig. 5B, left panel), $\dot{W}_{max}$, which was also increased post-training, remained elevated (+9%) when compared with pre-training levels and maintained ~60% of the training-induced improvements (Fig. 5B, right panel). These findings were confirmed in a study by Bonne et al. [95], who utilized an identical study design (Fig. 5A) to also show increased blood volume, $\dot{Q}_{max}$, $\dot{V}O_{2max}$, and $\dot{W}_{max}$ post-training. Consistent with the results of Montero et al. [52], all central markers assessed and $\dot{V}O_{2max}$ returned to baseline values when blood parameters were re-established to pre-training levels via phlebotomy (Fig. 5C, left panel), while $\dot{W}_{max}$ remained elevated (+17%) compared with pre-training levels, maintaining ~65% of the training-induced improvements (Fig. 5C, right panel). Importantly, the study from Montero et al. [52] assessed a series of peripheral adaptations showing enhanced capillary-to-fiber ratio (+17.6%; $p < 0.05$) and $mito_{VD}$ (+42.7%; $p < 0.05$), and a non-significant change in both the a-vO_2 difference (+2.2%) and mitochondrial respiratory function (+10.5%) post-training. Although these parameters were not reassessed after phlebotomy, one can argue that these peripheral adaptations (primarily mitochondrial content) would be maintained in the short time span (i.e., 2–3 days) between post-training and post-phlebotomy testing, as previous literature has shown maintained mitochondrial protein content following 3 days of single-limb immobilization [101]. If this holds true, it is tempting to speculate that the maintained portion of $\dot{W}_{max}$ post-phlebotomy may be associated with the increased peripheral adaptations (including mitochondrial content).

In the third study, by Mandić et al. [96], participants underwent a 6-week SIT intervention, followed by phlebotomy, as shown in Fig. 5A. In agreement with the prior two studies, 6 weeks of SIT led to increases in blood volume, $\dot{Q}_{max}$, $\dot{V}O_{2max}$, and $\dot{W}_{max}$. Following phlebotomy, all central markers assessed and $\dot{V}O_{2max}$ returned to baseline levels, consistent with the two previous studies (Fig. 5D, left panel). However, unlike the two previous studies, $\dot{W}_{max}$ reverted close to baseline levels, losing ~75% of the training-induced improvements (Fig. 5D, right panel). The main difference between this and the previous two studies is the type of training performed. A possible explanation may relate to the divergent effects of different exercise intensities on mitochondrial adaptations [69]. In this regard, MICT, used in the previous two studies, has been shown to increase mitochondrial content, whereas SIT, which was used in the study by Mandić et al. [96], primarily enhances mitochondrial respiratory function [39, 54, 69]. Based on this knowledge, we hypothesize that the increase in mitochondrial content associated with MICT may be the determining factor in maintaining $\dot{W}_{max}$ in the studies by Montero et al. [52] and Bonne


A
6 weeks MICT/SIT
PRE
POST
PHLEB.
TESTING
PRE TRAINING
CHANGE POST-PRE TRAINING
LOST UPON PHLEBOTOMY
MAINTAINED UPON PHLEBOTOMY
* vs PRE
# vs POST
B
Montero et al. 2015
MICT
C
Bonne et al. 2014
MICT
D
Mandić et al. 2023
SIT
V̇O2max (L·min-1)
Ẇmax (W)
Relative (%)
POST-PRE TRAINING
AFTER PHLEB.

◂**Fig. 5** Results from available training studies in humans involving phlebotomy. **A** Study design utilized in the studies described in panels **B**–**D**. Schematic representation of the effects of reversing training-induced central adaptations via phlebotomy on the training-induced increases in determinants of endurance performance ($\dot{V}O_{2max}$ and $\dot{W}_{max}$), adapted from the studies of **B** Montero et al. [52], **C** Bonne et al. [95], and **D** Mandić et al. [96]. Although the *black/green bars* depict relative changes occurring upon phlebotomy, the significant symbols on top of these bars relate to the changes in absolute values reported by the three respective papers. $^{*}p < 0.05$ vs pre-training values; $^{\#}p < 0.05$ vs post-training values. *MICT* moderate-intensity continuous training, *Phleb* phlebotomy, *SIT* sprint-interval training, $\dot{V}O_{2max}$ maximal oxygen uptake, $\dot{W}_{max}$ maximal aerobic power

et al. [95] (Fig. 5B and C). This would also help to explain the decrease in $\dot{W}_{max}$ observed in the study by Mandić et al. [96] (Fig. 5D), given that participants engaged in SIT, which has not been consistently associated with increases in mitochondrial content [39, 60, 69]. In line with this, we have observed significant increases in markers of mitochondrial content following 8 weeks of MICT, but not SIT, which were also associated with significantly greater improvements in $\dot{W}_{max}$ [69]. However, it is important to acknowledge that a recent systematic review found that SIT and MICT can both increase mitochondrial content [102]. Taken together, the results from the three studies discussed highlight that there is a component of improved endurance performance that cannot be explained by central adaptations and that may be linked to mitochondrial content.

Results from two studies examining reductions in training volume further highlight the potential role of mitochondrial content as an important determinant of endurance performance. The design of both studies was similar and involved 5–7 weeks of high-intensity interval training (HIIT) consisting of a combined normal- and high-volume training segment; this was followed by a 1- to 2-week reduction in HIIT volume [103, 104] (Fig. 6A). In both studies, the significant enhancements in mitochondrial content and respiratory function post-training mirrored the significant improvements in 20-km TT time, although mitochondrial changes were more pronounced than those in endurance performance (Fig. 6B and C). Following the reduction in HIIT volume, endurance performance and mitochondrial content did not change and remained elevated compared with pre-training values in both studies. Conversely, mitochondrial respiratory function was maintained and remained elevated compared with pre-training values in one study (−4%; $p > 0.05$) [103] (Fig. 6B), but decreased significantly (−17%; $p < 0.05$), so that no significant difference was reported compared with pre-training values, in the other [104] (Fig. 6C). As suggested by the authors, mitochondrial respiratory function is more rapidly reversed following a reduction in the training stimulus than mitochondrial content, a finding that is in line with other studies [101]. Therefore, the longer duration (2 vs 1 week) and the overall lower training volume (0.6 vs 3.1 MJ) of the reduced-volume training phase in the second [104] versus the first [103] study represented a stimulus that was too weak to preserve mitochondrial respiration, although the gains in endurance performance were maintained and these were mirrored by the maintenance of mitochondrial content. Collectively, these findings support the notion that mitochondrial content may be a more important determinant of endurance performance than maximal coupled mitochondrial respiratory function in humans.

In two landmark papers, Coyle et al. [48, 49] conducted a comprehensive study with moderately trained participants (mean $\dot{V}O_{2max} = 46.0$ mL·min^{-1}·kg^{-1}) who underwent an 84-day (12-week) detraining period. The multiple time points used during the detraining period allowed the authors to distinguish between the contributions of central and peripheral factors to changes in $\dot{V}O_{2max}$. Following 12–21 days of detraining, changes in both central (Fig. 7A) and peripheral (Fig. 7B) factors mirrored the reductions in $\dot{V}O_{2max}$, suggesting a similar contribution of central and peripheral factors in the early stage of detraining. Later, from day 21 to 56 and/or 84 of detraining, there were no further reductions in any of the central factors (Fig. 7A). However, a further reduction in $\dot{V}O_{2max}$ was observed, which was mirrored by the reduction in the activity of mitochondrial enzymes (i.e., CS, SDH) at both 56 and 84 days of detraining (Fig. 7B). This was also accompanied by a decrease in the a-vO_2 difference (an indirect measure of oxygen utilization by skeletal muscle mitochondria) after 84 days (Fig. 7B), which could possibly be related to the decrease in mitochondrial content, as previously suggested [57]. Given that capillary density and myoglobin content were not further reduced following 56–84 days of detraining, it was suggested that mitochondrial content adaptations were the primary drivers of the observed late changes in $\dot{V}O_{2max}$.

Taken together, the results from human phlebotomy and detraining studies presented in this section indicate that mitochondrial characteristics are an important determinant of endurance performance, as they often match and underpin the longitudinal changes observed in secondary markers of endurance performance.

3.3.2 Evidence Against

We are not aware of any studies that have reported the absence of a mechanistic link between mitochondrial characteristics and primary markers of endurance performance. However, one longitudinal study [105] examined the time-course of changes in $\dot{V}O_{2max}$ and markers of mitochondrial content (SDH and COX activity) following training (8 weeks) and detraining (6 weeks). While during the training phase both $\dot{V}O_{2max}$ (+19%) and markers of mitochondrial content (+32–35%) increased, during the detraining phase there was a rapid and large decline in markers

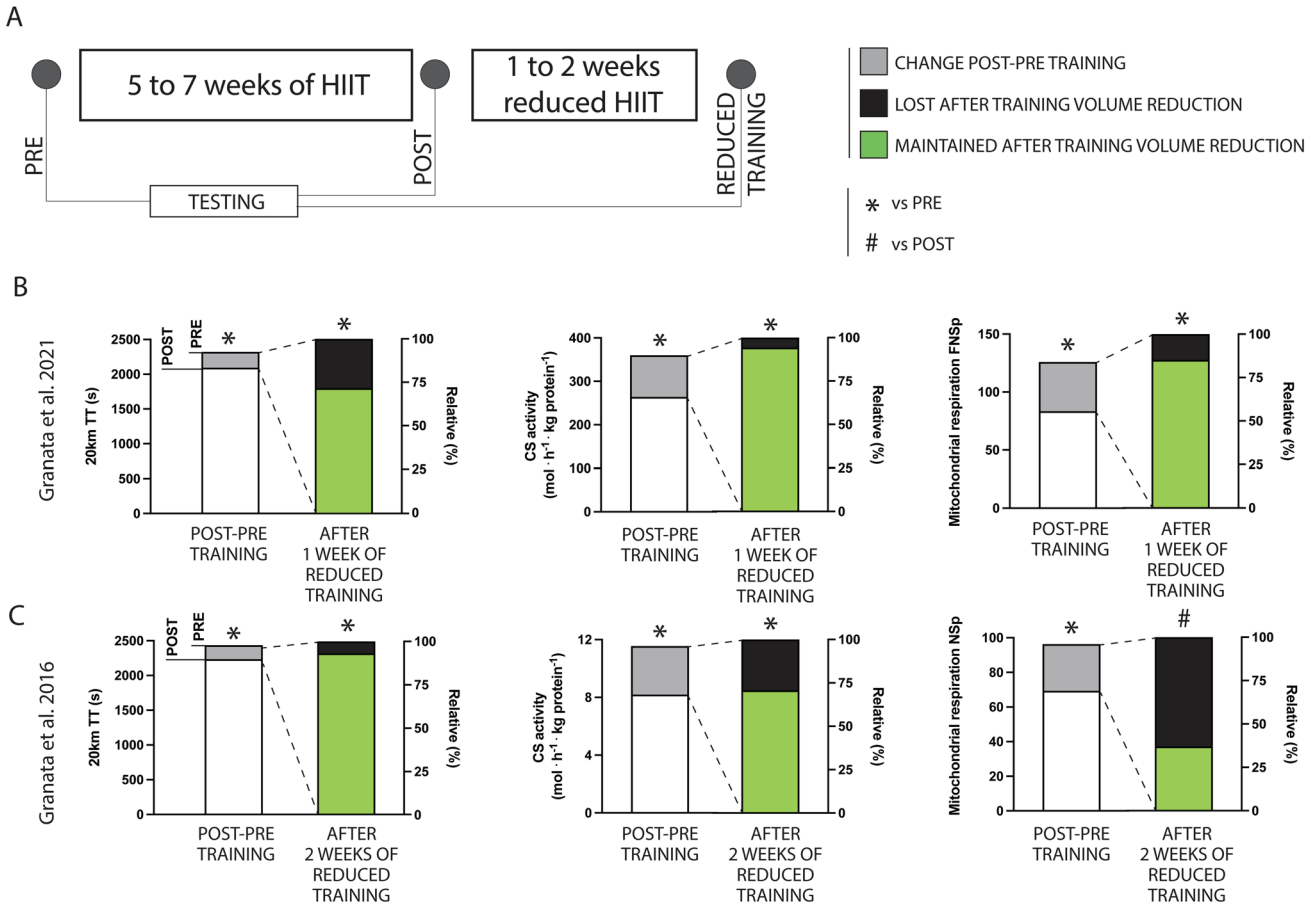


Fig. 6 **A** Study design utilized in the studies described in panel B and C. Schematic representation of the effects of reversing training-induced adaptations via a reduction in training volume on the training-induced increases in endurance performance (20k-TT time) and mitochondrial characteristics (CS activity and maximal coupled mitochondrial respiration) adapted from the studies of **B** Granata et al. [103] and (**C**) Granata et al. [104]. Although the *black/green bars* depict relative changes occurring upon reductions in training volume, the significant symbols on top of these bars relate to changes in the absolute values reported by the two respective papers. $^{*}p < 0.05$ vs pre-training values, $^{\#}p < 0.05$ vs post-training values. *20k-TT* 20-km time trial, *CS* citrate synthase, *F* fatty acid oxidation pathway, *HIIT* high-intensity interval training, *N* NADH pathway, *OXPHOS* oxidative phosphorylation, $_{P}$ phosphorylation (i.e., coupled) respiration state, *S* succinate pathway

of mitochondrial content (returning to baseline 2–4 weeks post-training) that was not mirrored by decreases in $\dot{V}O_{2max}$. The authors suggested that enhancements in mitochondrial content may not be necessary to increase $\dot{V}O_{2max}$. They also added that exercise training is a powerful stimulus to enhance the underlying factors mediating improvements in $\dot{V}O_{2max}$ and mitochondrial characteristics, but that these processes occur independently due to their differing kinetics during training and detraining.

Another study investigating adaptations in humans following a 42-day cross-country ski expedition reported decreased mitochondrial respiratory function (−22%) and CS activity (−15%), despite maintained peak pulmonary and leg $\dot{V}O_2$ and $\dot{W}_{max}$ [106]. The authors concluded that $\dot{V}O_{2max}$ can be sustained despite substantial reductions in OXPHOS capacity, given that skeletal muscle mitochondrial respiratory function is submaximal at $\dot{V}O_{2max}$. This suggests that, in these specific conditions, mitochondrial characteristics may not be primary determinants of secondary markers of endurance performance.

A substantial body of literature supports the important contribution of central hemodynamic adaptations to improvements in secondary markers of endurance performance ($\dot{V}O_{2max}$) [52, 95, 96]. Intervention studies from Boushel and colleagues have investigated the rates of oxygen utilization ($\dot{V}O_2$) in vivo (from a graded exercise test) and ex vivo (from maximal mitochondrial respiratory function) to determine whether the in-vivo demands match the ex-vivo 'capacity' during exercise, and vice versa [53, 107]. These studies showed that the in-vivo $\dot{V}O_2$ (~4.5 mmol·kg^{-1}·min^{-1}) during maximal exercise involving a relatively small muscle

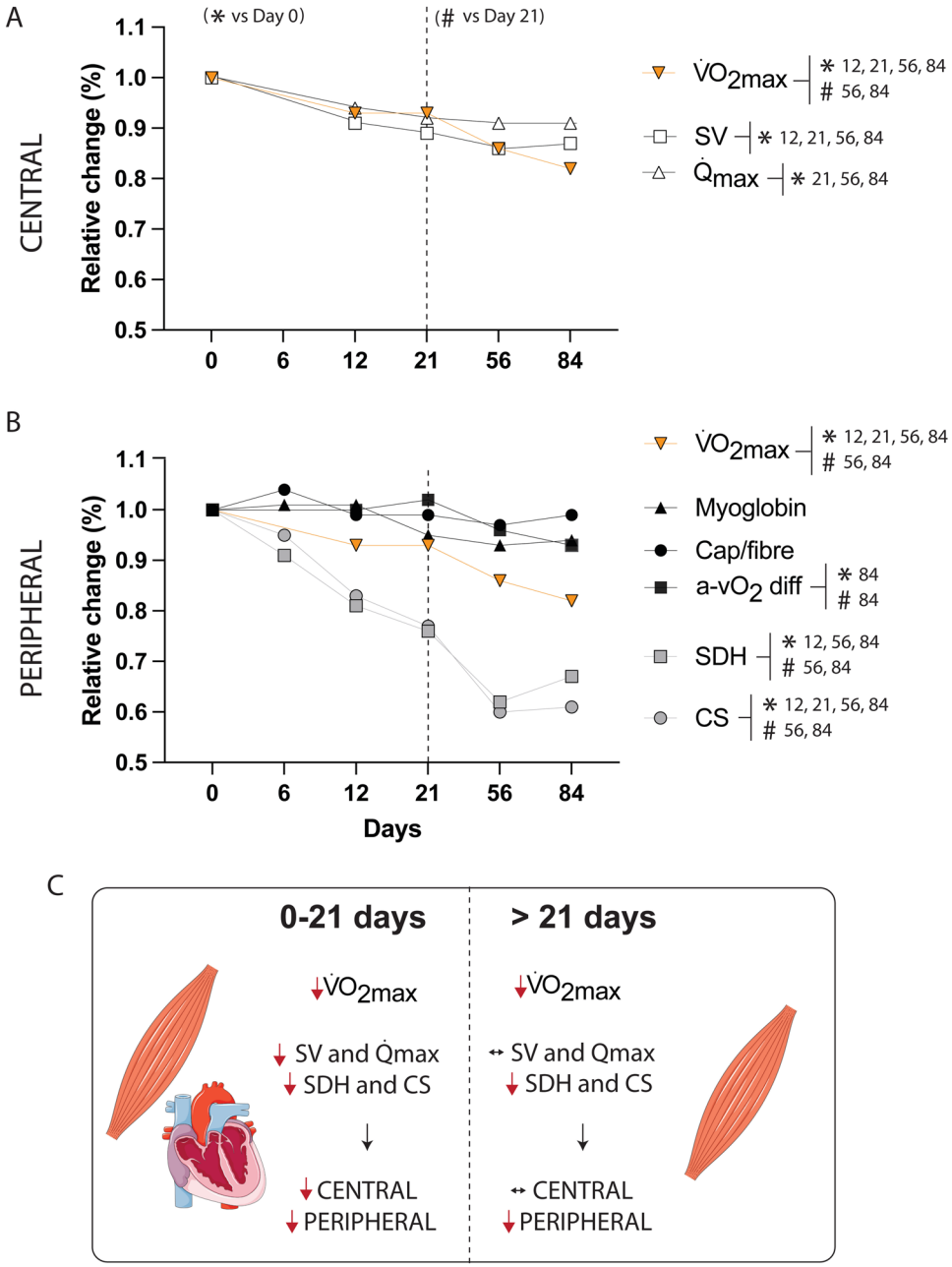


Fig. 7 Time-course of changes in **A** central and **B** peripheral physiological parameters alongside secondary markers of endurance performance following an 84-day detraining period (adapted from [48, 49]), **C** with the summarized central and peripheral changes observed. *a-vO_2 diff* arterio-venous oxygen difference, *Cap/fiber* number of capillaries per fiber, *CS* citrate synthase activity, $\dot{Q}_{max}$ maximal cardiac output, *SDH* succinate dehydrogenase, *SV* stroke volume, $\dot{V}O_{2max}$ maximal oxygen uptake,* significant change from day 0; # significant change from day 21

mass, such as arm cranking or single-leg extension, matches the ex-vivo $\dot{V}O_2$. This suggests that during exercise involving a relatively small muscle mass, muscle mitochondrial respiratory function tightly matches the requirements of maximal exercise in untrained participants. However, when a greater muscle mass is engaged, such as during two-leg cycling, the in-vivo $\dot{V}O_2$ (~5 mmol·kg^{-1}·min^{-1}) is lower than the ex-vivo $\dot{V}O_2$ (~6.9 mmol·kg^{-1}·min^{-1}). This suggests the presence of an ~35% excess in skeletal muscle mitochondrial respiratory function during whole-body exercise in the population investigated. Therefore, when more than half of the body muscle mass is engaged in exercise, the skeletal muscle mitochondrial OXPHOS capacity exceeds maximal oxygen delivery. The authors concluded that in the cascade defining maximal oxidative rate in humans there is an excess skeletal muscle mitochondrial respiratory capacity over O_2 delivery by the circulation. It must be noted that previous studies had shown that the in-vivo $\dot{V}O_2$ exceeds the ex-vivo muscle mitochondrial OXPHOS capacity measured in isolated mitochondria, whereby the latter accounted for only ~60–80% of the measured $\dot{V}O_2$ in vivo [108, 109]. However, it was suggested that damaged mitochondria from the isolation procedure may have influenced the results [107, 108], which is in agreement with technical reports [110].

The view that O_2 delivery to the working muscle is the limiting factor determining $\dot{V}O_{2max}$ has been partially challenged by a study in which untrained and trained participants engaged in maximal knee-extension exercise (i.e., using relatively small muscle mass) [111]. This study showed that only trained, but not untrained, individuals possess a large mitochondrial respiratory function 'reserve', even during exercise engaging a small muscle mass. The authors concluded that, in untrained individuals, $\dot{V}O_{2max}$ is limited by skeletal muscle OXPHOS capacity, whereas in trained participants, oxygen supply to the mitochondria is the limiting factor. In line with these results, another study in previously untrained humans showed that 8 weeks of cardiorespiratory exercise training shifted the limitation of muscle $\dot{V}O_{2max}$ from mitochondrial O_2 utilization capacity to O_2 delivery capacity to the mitochondria [112]. A possible explanation for the excess in skeletal muscle mitochondrial respiratory function in trained individuals may be to maintain a low mitochondrial O_2 affinity to efficiently sustain oxygen extraction [106]. However, it is not fully understood why an excess of mitochondrial respiratory function, or content, may occur in trained individuals, given the large energy cost (due to protein synthesis and turnover [113]) that is required to maintain the 'excess' mitochondria.

Lastly, scientists have investigated the mismatch between ex-vivo and in-vivo $\dot{V}O_2$ during maximal exercise by also manipulating the levels of oxygen availability (e.g., by increasing the inspired O_2 fraction) (extensively reviewed previously; [114, 115]). Briefly, studies have shown that increasing the inspired fraction of oxygen by breathing pure oxygen enhances $\dot{V}O_{2max}$ by an average of 8.1% [116]. It has also been shown that linear increases in the inspired oxygen fraction (21%, 40%, 60%, 80%, 100% of O_2 fraction) result in a linear improvements ranging from 18 to 38% in the time to exhaustion at 110% of peak aerobic speed [117]. These findings have been replicated [118, 119] and are now widely accepted in the field, suggesting that central components play a crucial role in determining endurance performance.

Collectively, the results presented in this last section suggest that mitochondrial adaptations do not systematically match the longitudinal changes in secondary performance markers and may not be key determinants of endurance performance. Intriguingly, the ability of skeletal muscle mitochondria to operate at higher rates when provided with increased oxygen raises the question of whether this 'excess' mitochondrial OXPHOS capacity is important for other aspects of endurance performance that remain to be fully elucidated.

4 Further Considerations, Limitations, and Open Research Questions

While the interrogation of skeletal muscle mitochondrial respiratory function has shown a mismatch between ex-vivo and in-vivo $\dot{V}O_2$ during maximal exercise in humans [53, 107], the underlying biological mechanisms and their importance remain unexplained. Most evidence has been obtained by sampling skeletal muscle during resting conditions and assumes that mitochondrial characteristics remain constant and are maintained throughout an endurance competition. However, during a 4-min bout of maximal exercise, oxidative ATP resynthesis declines by ~ 15% in the last 2 min; similarly, the ADP-specific ATP resynthesis declines by up to ~ 25% at the end of the same 4-min bout [120]. These impairments in oxidative bioenergetics have been shown to be mediated by increased mitochondrial uncoupling [120]. Therefore, the 'excess' mitochondrial OXPHOS capacity reported in the abovementioned studies may represent a compensatory mechanism to cater for the decline in the mitochondria's ability to resynthesize ATP via OXPHOS during maximal cardiorespiratory exercise, which one could hypothesize would be exacerbated for events of longer durations. In line with this hypothesis, new evidence suggests that the ability to attenuate the decline in power output after accumulated work (termed durability [121, 122]) is an emerging critical component of endurance performance in elite cyclists. Therefore, having a mitochondrial 'excess' may ensure sufficient resources to sustain power output when a substantial decline in ATP resynthesis efficiency is expected. In addition, mitochondria provide the majority of the ATP required for multiple cellular functions (e.g., transcription, protein folding) [35]; hence, an excess mitochondrial OXPHOS capacity may represent a necessary adaptation to ensure sufficient resources are available for vital cellular processes during repeated muscle contraction in endurance events.

Another technical factor to consider is that most research investigating the mitochondria's ability to resynthesize ATP assesses oxygen consumption in a high-resolution respirometer rather than by directly measuring ATP resynthesis. This implies that the efficiency of mitochondrial ATP resynthesis during OXPHOS, expressed as the P/O ratio, remains constant. Although the P/O ratio remained unchanged after a 6-week MICT+HIIT training intervention [123], or immediately after the termination of a HIIT session [124], it has been shown to decline during all-out [120] or after prolonged (24 h) ultra-endurance [125] exercise. A model of metabolic bypass of mitochondrial complex I has been reported to increase the ATP resynthesis rate per gram of protein compared with the classic OXPHOS pathway [126], and has been suggested to partially rescue the decline in ATP

resynthesis efficiency [127]. Despite this elegant rescuing mechanism, the overbuilt mitochondrial OXPHOS capacity reported by Boushel et al. [53, 107] may once again be necessary to prevent the decline in ATP resynthesis efficiency during endurance competitions.

The concept of excess mitochondrial OXPHOS capacity and its potential implications for endurance performance warrants further consideration. Given the extremely high energy costs of mitochondrial biogenesis [113], it seems unlikely that this overbuilt mitochondrial capacity would be irrelevant to the skeletal muscle mitochondrial pool. This aligns with the concept of symmorphosis, which proposes that a biological system's structural design is matched to its functional requirements [29]. Additionally, the widely accepted view that mitochondrial structure is tailored to meet its specific functional demands [29, 128, 129] supports the idea that this 'over-adaptation' may serve to fine-tune its biological efficiency and performance. However, whether this extra capacity directly contributes to endurance performance remains unknown.

Although we have provided some evidence that mitochondrial content may be more important than mitochondrial respiratory function in supporting improved endurance performance, the debate over which specific mitochondrial characteristic is more important is far from settled. Despite the continuous publication of newer research and the use of more advanced molecular techniques and bioinformatics, we are only starting to understand the complexity of mitochondrial adaptations to endurance training and their importance for performance as well as health [69, 102, 103, 130–132]. Whether other mitochondrial characteristics, such as mitochondrial dynamics and reactive oxygen species production, play an important role in endurance performance remains unknown. Future research should therefore explore whether these training-specific mitochondrial adaptations may underlie diverse cellular requirements that may, or may not, enhance endurance performance.

In addition to the limitations discussed in the sections above, several other important limitations warrant consideration. Due to the laborious and costly nature of TEM, and the subsequent paucity of available literature linking TEM findings with endurance performance, most studies have utilized CS activity as a proxy of mitochondrial content. Although validated in human skeletal muscle [37], CS activity remains an indirect marker and results can be affected by several methodological and biological factors. Similarly, mitochondrial respiration assays are performed under standardized laboratory conditions and in the presence of excess substrate availability, which is not truly representative of in-vivo conditions. Moreover, these assays are commonly performed at 37 °C (and in some cases at 30 °C), despite evidence suggesting that, in vivo, mitochondria could operate at temperatures approaching 50 °C [133]. These factors, together with the mitochondrial redox state, are tightly regulated in vivo and can substantially influence mitochondrial respiratory function, potentially leading to inaccurate interpretation. Finally, the interpretation of enzymatic assays and ex-vivo measurements should consider several context-specific limitations. These include methodological variability, potential disturbances to the mitochondrial network and bioenergetics during biopsy sampling, the difficulty of extrapolating findings to other skeletal muscle types, and differences among distinct population groups, such as sex differences, diseased state, aging, and/or training status [38, 79, 134].

5 Conclusions

A strong relationship between enhanced mitochondrial characteristics and endurance performance has been demonstrated through cross-sectional, correlational, and intervention studies. For example, differences in mitochondrial characteristics have been reported between groups of significantly different performance levels, and mitochondrial respiratory function has emerged as the single parameter that best explains performance differences within a homogeneous group of elite cyclists. Taken together, these findings suggest that enhanced mitochondrial characteristics may be a defining hallmark of superior endurance performance. However, while this review provides multiple lines of evidence suggesting that mitochondrial characteristics are an important biological determinant of endurance performance, research indicates that central factors have an equally strong influence independent of mitochondrial characteristics and/or adaptations. The debate over whether central or peripheral factors are the more critical determinants of endurance performance remains unsettled and would likely benefit from acknowledging the importance of both. Future research should aim to determine whether enhanced mitochondrial characteristics may play a critical role in distinct and specific aspects of endurance performance that have been overlooked in the literature, and whether current ex-vivo methods accurately reflect in-vivo mitochondrial behavior to mechanistically resolve this ongoing debate.

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Declarations

Conflict of Interest The authors have no conflicts of interest to declare.

Availability of Data and Material The raw data generated for this review, together with data extracted from prior studies, are available from the corresponding authors upon request.

Author Contributions JB, DJB, and CG conducted the literature searches, analyzed and interpreted the data. JB and CG wrote the manu-

script. DJB and CG critically revised the manuscript and supervised the work. JB, DJB, and CG have primary responsibility for final content. All authors read and approved the final manuscript.

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