Training-Induced Changes in Mitochondrial Content and Respiratory Function in Human Skeletal Muscle

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Running Heading: Training-induced mitochondrial adaptations

Abstract:

A sedentary lifestyle has been linked to a number of metabolic disorders that have been associated with sub-optimal mitochondrial characteristics and an increased risk of premature death. Endurance training can induce an increase in mitochondrial content and/or mitochondrial functional qualities, which are associated with improved health and well-being and longer life expectancy. It is therefore important to better define how manipulating key parameters of an endurance training intervention can influence the content and functionality of the mitochondrial pool. This review focuses on mitochondrial changes taking place following a series of exercise sessions (training-induced mitochondrial adaptations), providing an in-depth analysis of the effects of exercise intensity and training volume on changes in mitochondrial protein synthesis, mitochondrial content, and mitochondrial respiratory function. We provide evidence that manipulation of different exercise training variables promotes specific and diverse mitochondrial adaptations. Specifically, we report that training volume may be a critical factor affecting changes in mitochondrial content, whereas relative exercise intensity is an important determinant of changes in mitochondrial respiratory function. As a consequence, a dissociation between training-induced changes in mitochondrial content and mitochondrial respiratory function is often observed. We also provide evidence that exercise-induced changes are not necessarily predictive of training-induced adaptations, we propose possible explanations for the above discrepancies, and we put forward suggestions for future research.

Key points

- Training volume appears to be an important determinant of training-induced increases in mitochondrial content (an effect that may be driven by training duration) whereas exercise intensity appears to be a key factor of training-induced increases in mitochondrial respiration.

- Training-induced changes in mitochondrial content and respiratory function seem to be differentially regulated, and are not necessarily associated to one another.
- High-intensity interval training at a relative exercise intensity ≥ 90% of the maximal power output provides the greatest absolute increase in mass-specific mitochondrial respiration, whereas all-out sprint interval training appears to be the most efficient type of exercise to improve mitochondrial respiratory function in terms of total training volume and/or time.
- Mitochondrial adaptations to exercise training are rapidly reversed following different types of detraining; however, these can be maintained when a sufficient training stimulus is provided.

1 Role of mitochondria and exercise in health and disease

Mitochondria are the primary energy-producing "power houses" of the cell, and they are responsible for the production of ATP - the "energy currency" required to fuel cellular activities. Given the pivotal role of mitochondria in generating ATP, improved mitochondrial characteristics have been associated with greater endurance performance and health [1-4]. Mitochondria also appear to have important roles in ageing and cell pathology [5], and have been implicated in many age-related degenerative diseases [6], as well as a large variety of metabolic disorders [7-9]. These findings underline the importance of a better understanding of the factors that can stimulate mitochondrial adaptations.

Physical inactivity has been reported to contribute to many deadly chronic disorders [10], and represents the fourth leading risk factor for mortality [11]. On the other hand, exercise has been used as a therapeutic tool to fight this epidemic [12, 13]. Since the pioneering work of John Holloszy in the 1960s, it has been known that exercise is a potent stimulus to promote mitochondrial biogenesis (i.e., the making of new components of the mitochondrial reticulum [14]) in skeletal muscle [15]. Mitochondrial adaptations in response to repeated exercise sessions (i.e., exercise training) consist of changes in steady-state protein abundance [16, 17] and mitochondrial content [18, 19], as well as functional adjustments such as improved mitochondrial respiratory function [20-22]. However, little is known about the optimal exercise prescription, and whether these mitochondrial adaptations are altered in the same way by different exercise interventions. Given the role of mitochondria in health and disease [2, 4], it is important to better understand how altering the exercise prescription can modulate the content and the functional qualities of mitochondria.

2 Mitochondrial adaptations to exercise training

Prescribing exercise involves the manipulation of many parameters, with exercise intensity and training volume representing two of the most important [23]. Briefly, exercise intensity can be prescribed in absolute (e.g., 270 W or 19 $km \cdot h^{-1}$) or relative (i.e., as a percentage of the maximal power output [$\dot{W}_{max}$]; e.g., 85% of $\dot{W}_{max}$) terms, whereas training volume can be defined as the product of relative exercise intensity and the duration and number of exercise sessions. Because $\dot{W}_{max}$ is influenced by the protocol chosen [24], a correction factor, based on the bioenergetic model proposed by Morton [25], has been applied to allow for a better comparison between studies (see Electronic Supplementary Material Appendix 1). Relative exercise intensities that have been converted will be reported as a percent of a newly-estimated $\dot{W}_{max}$ value ($\dot{W}_{max'}$). Finally, based on relative exercise intensity, different types of exercise can be prescribed: moderate-intensity continuous exercise or training (MICE or MICT, respectively) when exercise is performed continuously at an exercise intensity of ~50 to 75% of maximal power output ($\dot{W}_{max}$), high-intensity interval exercise or training (HIIE or HIIT, respectively), consisting of intervals performed at an exercise intensity < 100% $\dot{W}_{max}$, and sprint interval exercise or training (SIE or SIT, respectively), when intervals are performed at an intensity ≥ 100% $\dot{W}_{max}$.

An often understudied aspect in exercise physiology relates to the physiological adaptations taking place following a period of detraining (i.e., a reduction in the training stimulus). As a consequence, a strict classification of different detraining protocols is lacking. For the scope of this review, detraining has been classified into three main categories: “taper”, consisting in a reduction of the training volume obtained by manipulating one or more of the key training parameters (e.g., exercise intensity, session duration, training frequency), as well as the overall taper duration and the relative decrease in the training stimulus during each exercise session [26]; “complete detraining”, where no physical activities are undertaken other than those

involved in everyday living [27]; “bed rest” or “unilateral lower limb immobilization”, where an individual is confined to a bed, or her/his lower limbs are immobilized, respectively, resulting in the complete absence of any type of training stimulus [28].

The molecular events triggered by exercise training [29] and detraining [1, 27] lead to a series of adaptations that re-shape the mitochondrial phenotype. Some of these adaptations to training take place despite what appears to be an already adequate capability. For example, it has been reported that mitochondrial respiratory function exceeds oxygen delivery in humans, and is in excess of that required when half or more of the muscle mass is engaged during exercise (e.g., cycling, running) [30-32]. Nonetheless, mitochondrial respiratory function has been reported to increase following exercise training [20, 33, 34], as have both mitochondrial protein synthesis (MitoPS) [35] and mitochondrial content [1, 18, 36], whereas reductions have been reported following different types of detraining [1, 27, 37]. This review will focus on training- and detraining-induced changes in the above parameters, and how these adaptations are influenced by exercise intensity and training volume. Unless specifically mentioned, only cycling interventions investigating training- and detraining-induced changes in the skeletal muscle of healthy humans will be reviewed; studies with diseased or elderly populations, and studies not providing precise and detailed information about the training prescription, have been excluded from figures and Electronic Supplementary Material Tables S1 and S2.

2.1 Mitochondrial protein synthesis

There is limited information on the effects of endurance exercise on MitoPS. A single session of MICE has been reported to increase MitoPS by 2.5-fold during the first 4 h [35], and by 1.3-fold between 24 and 28 h of recovery [38]. A 1.5-fold increase in MitoPS has also been reported between 2 and 5 h of recovery following 40 min of MICE at 55% $\dot{W}_{max}$ [39]; however,

supplementation with protein was provided to participants immediately post-exercise, making it difficult to separate out the effects of exercise and nutrition (which is known to influence both mixed muscle [40] and myofibrillar [41] protein synthesis). Finally, the only study investigating training-induced changes in MitoPS demonstrated that 36 sessions (12 weeks) of HIIT at 90% of $\dot{W}_{max}$ increased MitoPS by ~1.3-fold in both young and elder volunteers [42].

A study comparing two work-matched exercise sessions observed that while 30 min of MICE at 60% of $\dot{W}_{max}$ increased MitoPS by 1.3-fold 24 to 28 h post-exercise, 60 min of MICE at 30% of $\dot{W}_{max}$ induced no change [38]. It is possible, however, that the very low relative exercise intensity performed by the lower intensity group may have confounded the results due to the lack of activation of important signaling kinases and transcription factors regulating mitochondrial biogenesis [43]. A second study has also observed a 1.3-fold increase in sarcoplasmic protein synthesis (SarcoPS) in elderly individuals following a single session of HIIE at 95% of maximal heart rate (HR_{max}), and no change after MICE at ~55% HR_{max} [44], despite the latter group performing a greater volume of exercise. The authors suggested the increase in SarcoPS may have been driven by increased MitoPS, given that following the sub-fractionation protocol used for protein fractional synthetic rates analysis in this study the majority of skeletal muscle mitochondria are located in the sarcoplasmic (cytosolic) fraction. Finally, MitoPS is similarly increased after a single session of MICE performed at the same relative intensity before and after 10 weeks of training [35]. The above findings highlight the importance of relative exercise intensity to stimulate MitoPS and suggest that if the relative exercise stimulus is maintained MitoPS is upregulated similarly in the untrained and trained state. More research is required to verify these hypotheses; future studies should further characterize training-induced changes in MitoPS and establish if long-term increases in mitochondrial proteins result from the cumulative effect of the adaptations induced by a single session of endurance exercise.

Further considerations in regard to exercise- and training-induced changes in protein synthesis can be drawn from studies investigating changes in mixed-muscle protein synthesis (MPS). While MPS is reduced by ~1.2-fold during exercise [45], mixed-muscle protein breakdown (MPB) is increased by 1.4 to 1.8-fold [46, 47], likely due to the release of amino acids to satisfy the increased energy demand [48]. However, in the hours of recovery following exercise, both MPS [47, 49-51] and MPB [46, 47] are increased (1.2 to 1.8-fold), resulting in greater net muscle protein turnover. The increase in MPS in the recovery period counterbalances the effects of MPB, preventing a loss of muscle mass [47]. Similarly, increased resting MPS (~1.3-fold) [52, 53] and MPB (~1.4-fold) [52] were reported following 4 to 16 weeks of MICT, resulting in greater (~3-fold) muscle protein turnover post-training [52]. These findings suggest endurance exercise training may result in an improved muscle phenotype (as demonstrated by greater endurance performance and a higher maximum rate of oxygen consumption [$\dot{V}O_{2max}$] post-training [52, 53]) due to increased muscle protein turnover, a change that may not necessarily induce an increase in mitochondrial content. More research is required to investigate if endurance exercise is associated with increased MitoPS and MitoPB (similar to mixed MPS and MPB), and if net mitochondrial protein turnover is also increased.

2.2 Mitochondrial content

2.2.1 Training-induced changes in mitochondrial content as assessed by transmission electron microscopy

Transmission electron microscopy (TEM) is routinely used to measure mitochondrial volume density ($Mito_{VD}$) and is widely considered the gold standard technique for the assessment of mitochondrial content [54]. However, only six studies have used TEM to investigate training-induced changes in mitochondrial content in young, healthy participants following a cycle

training intervention [18, 19, 36, 55-57]. These studies demonstrated that exercise training is indeed associated with an increase in $Mito_{VD}$ (1.1- to 1.6 fold), and as little as six exercise sessions are sufficient to increase this parameter [19].

Effect of training volume and exercise intensity. A study comparing five different training volumes (participants completed the same cycle training session either one, two, three, four, or five times per week for six weeks, respectively) reported that training-induced changes in $Mito_{VD}$ were related to training volume, as participants training four or five times per week increased $Mito_{VD}$ to a greater extent than those training only once or twice weekly [19]. However, the lack of significant differences between groups training three, four, or five times per week seems to also indicate the existence of a plateau for changes in $Mito_{VD}$ at greater training volumes; this may relate to participants training at the same absolute exercise intensity for the entire study duration. In this regard, it has been reported that exercise-induced mitochondrial adaptations (e.g., MitoPS) are similar when exercising at the same relative exercise intensity before and after training [35], but are reduced post-training when exercising at the same absolute exercise intensity [58, 59]. This may suggest that the training stimulus associated with the extra exercise sessions performed by the high-volume training groups may have been insufficient to promote further increases in $Mito_{VD,}$ due to the likely reduction in relative exercise intensity as the training intervention progressed and the participants' fitness improved. More research exercising participants at the same relative exercise intensity for the entire training duration is needed to validate the above hypothesis. Finally, plotting results from the same six studies [18, 19, 36, 55-57] seems to support the presence of both a training volume-dependent effect and the existence of a plateau in training-induced changes in $Mito_{VD}$ as assessed by TEM ($R^2 = 0.82$; $P = 0.003$; Fig. 1).

Due to the limited research available, the mix of populations investigated (differences in age and sex), and that a combination of training was often used, no conclusions can be drawn on the effects of different relative exercise intensities on training-induced changes in $Mito_{VD}$. On the other hand, studies investigating training-induced changes in maximal citrate synthase (CS) activity, a valid biomarker of mitochondrial content [60], are plentiful and stronger conclusions can be made.

2.2.2 Training-induced changes in mitochondrial content as assessed by citrate synthase activity

Exercise training is a powerful stimulus to increase CS activity [61-63]; however, a wide range of training-induced changes in this parameter has been reported. While most investigations have observed an increase in CS activity, several studies have reported no change (Electronic Supplementary Material Table S1). These discrepancies may be attributed to participants' characteristics, the timing of muscle sampling, as well as differences in the experimental protocols and methodologies, suggesting it is important to standardize the methods employed to measure maximal CS activity across laboratories.

Effect of training volume. Four studies observed that greater training volumes were associated with greater increases in CS activity [1, 17, 64, 65], whereas two studies did not [66, 67]. In the first of these two studies, in which participants trained each day for 14 days, CS activity was statistically increased (~1.4-fold) after the third exercise session but did not significantly increase thereafter (showing signs of a possible plateau) [66]. However, participants exercised at the same absolute exercise intensity throughout the study. For the same reasons explained in section 2.2.1, and the fact that mitochondrial adaptations take place within the first few (< 6) exercise sessions [17, 68, 69], it is possible the exercise stimulus in the latter exercise sessions may have been reduced relative to the newly-acquired phenotype. Likewise, in the second of

these two studies, training-induced changes in CS activity (measured per gram of wet weight) recorded after 12 weeks were similar compared to those recorded after six weeks (~1.7 *vs.* ~1.5-fold increase compared to pre-training, respectively; $P > 0.05$; effect size for the difference: 0.40 [95% confidence interval (CI) -0.69, 1.42] [67]. This finding may relate to early changes in CS activity being greater and partially masking the volume effect in studies of longer duration, and/or the small sample size (n = 7). Collectively, the above findings seem to suggest that training volume may be an important determinant of training-induced changes in mitochondrial content, but that it may be important to increase the absolute exercise stimulus to promote continuous adaptations. However, more studies investigating different time points within the one study are required to better define the time course of training-induced changes in CS activity.

Pooling results from 56 training studies further indicates greater training volumes are associated with greater increases in CS activity (r = 0.59 [95% CI 0.41, 0.72]; P < 0.001; Fig. 2a). This association is stronger when removing studies employing SIT (r = 0.71 [95% CI 0.54, 0.83]; $P < 0.001$; Fig. 2b), suggesting that when relative exercise intensity is < 100% of $\dot{W}_{max}$ training volume is indeed an important determinant of training-induced changes in CS activity. Analysis of Fig. 2a, Fig 2b, and results from type I fibers in rat skeletal muscle [70], seems to indicate no plateau for training-induced changes in CS activity. This hypothesis is also supported by the observation that baseline CS activity values increase with greater training status (i.e., sedentary, trained etc.) [70]. Given most of the literature measuring training-induced changes in CS activity investigated relatively low training volumes and short training interventions (i.e., 1 to 12 weeks), future research is required to investigate if CS activity continues to increase even with greater training volumes, or if a plateau is reached. It should also be important to investigate if relative exercise intensity needs to be maintained or increased to achieve further improvements in mitochondrial content as the training volume increases.

The large variability (1- to 1.7-fold change) of training-induced changes in CS activity reported for SIT groups eliciting training volumes of similar magnitude (Fig. 2a) indicates that at relative exercise intensities ≥ 100% $\dot{W}_{max}$ training-induced changes in CS activity may no longer depend on training volume. This is confirmed by the lack of correlation between training volume and training-induced changes in CS activity (r = -0.19 [95% CI -0.58, 0.28]; *P* = 0.433) when pooling only studies employing SIT. A possible reason as to why training volume may differentially regulate changes in CS activity above and below $\dot{W}_{max}$ may relate to the consequent decrease in training volume necessary to elicit very high relative exercise intensities. We conclude that training volume may be an important determinant of training-induced changes in CS activity [70], but this relationship seems limited to relative exercise intensities < 100% $\dot{W}_{max}$.

Training duration is a predominant component of training volume; therefore, it is likely that it may be a dominant factor affecting training-induced changes in CS activity. We are aware of only one study comparing the effects of different training durations (12 h of MICT at 50% of $\dot{W}_{max}$ *vs.* 6 h of SIT at 100% of $\dot{W}_{max}$; the groups were matched for training volume) on training-induced changes in CS activity [71]. In this study, only MICT induced an increase in CS activity, suggesting that training duration may indeed be an important determinant of training-induced changes in CS activity. However, these results may have been affected by the small sample size (n = 3 and 4 for the MICT and SIT groups, respectively). Given the limited evidence available, more studies comparing different training durations matched for training volumes are needed to better characterize the effects of training duration per se on training-induced changes in CS activity.

It has been reported that a correlation exists between estimates of mitochondrial content as assessed by both TEM ($Mito_{VD}$) and CS activity, both pre- and post-training, suggesting CS activity can be used as a valid biomarker of $Mito_{VD}$ in training studies [55]. However, contrary

to the plateau reported when assessing training-induced changes in $Mito_{VD}$ (Fig. 1 and Montero and Lundby [19]), training-induced changes in CS activity do not plateau (Fig. 2a), suggesting a possible dissociation between training-induced changes in mitochondrial content as assessed by $Mito_{VD}$ and CS activity. In addition, no correlation was found between training-induced changes in mitochondrial content as assessed by both $Mito_{VD}$ and CS activity in human skeletal muscle [55]. Therefore, we recommend that future studies should simultaneously investigate training-induced changes in CS activity and $Mito_{VD}$, so as to verify the validity of measuring CS activity as a biomarker of training-induced changes in mitochondrial content in human skeletal muscle.

Effect of exercise intensity. There are contrasting findings on the effects of relative exercise intensity on training-induced changes in CS activity in human skeletal muscle. While one report observed increased CS activity after MICT at 50% $\dot{W}_{max}$, but not after work-matched SIT at 100% $\dot{W}_{max}$ [71], another study reported increased CS activity after HIIT at 65% $\dot{W}_{max}$ and no change after work-matched MICT at 50% $\dot{W}_{max}$ [72]. Two other studies reported similar increases in CS activity following both moderate-intensity MICT (45 to 65% of $\dot{W}_{max}$) and all-out SIT (175 to 205% $\dot{W}_{max,}$ not work-matched) [16, 73], whereas a fifth study reported no change in CS activity after MICT at 55% $\dot{W}_{max'}$, HIIT at 73% $\dot{W}_{max'}$, and all-out SIT (~168% $\dot{W}_{max'}$) (only MICT and HIIT were work-matched) [33]. The above findings indicate that exercise intensity may not be a key determinant of training-induced changes in CS activity. Interestingly, relative exercise intensity has been reported to be an important determinant of exercise-induced changes in several markers of mitochondrial biogenesis [43, 74], but not of training-induced changes in CS activity. This raises the possibility that exercise-induced changes in markers of mitochondrial biogenesis may not always predict or relate to training-induced adaptations, as previously hypothesized [75, 76]. Future research is required to verify these hypotheses.

Pooling results from 49 training studies indicated no significant correlation between relative exercise intensity and training-induced changes in CS activity (r = -0.13 [95% CI -0.37, 0.12]; *P* = 0.315; Fig. 2c), not even when studies employing SIT were removed (r = -0.01 [95% CI -0.31, 0.30]; *P* = 0.971; Fig. 2d), or when the effects of training duration were "partialed out" (i.e., controlled for [77]) ($r_{y1.2}$ = 0.23 [95% CI -0.02, 0.45]; *P* = 0.085). This further suggests exercise intensity may not be a key determinant of training-induced changes in CS activity, consistent with findings from rat type I skeletal muscle fibers [70]. Conversely, relative exercise intensity has been reported to influence changes in CS activity in rat type II skeletal muscle fibers [70]. However, caution is needed when interpreting these results as higher relative exercise intensities were also associated with greater training volumes, which is an important determinant of training-induced changes in CS activity [70].

It is not known if a greater relative exercise intensity is required to increase CS activity in human type II skeletal muscle fibers. In human skeletal muscle it has been reported that at exercise intensities < $\dot{W}_{max}$ slow twitch type I fibers are recruited earlier [78] and to a greater extent [79, 80] than fast twitch type II fibers, whereas at relative exercise intensities > $\dot{W}_{max}$ both fiber types are fully recruited from the early stages of exercise [78]. However, subsequent research reported no difference in fiber-specific glycogen depletion patterns following MICE (30 min at ~65% $\dot{W}_{max}$) and SIE (8 x 20 s at ~170% of $\dot{W}_{max}$), suggesting that fiber recruitment was similar between MICE and SIE protocols [81]. These contrasting findings highlight that more research is required to investigate the effects of relative exercise intensity on both fiber recruitment and subsequent fiber-specific changes in mitochondrial content.

Effect of training status and aerobic fitness levels. Seven weeks of MICT at 60 to 90% of HR_{max} increased (1- to 6-fold) the CS activity of sedentary, but not previously-trained participants [37], suggesting training status may influence training-induced changes in CS activity.

Moreover, no change in CS activity was reported for highly-trained endurance athletes after cycling for 3211 km over 21 days (~170 km/day) [82], or following seven weeks of a combination of SIT and HIIT [83]. This may be explained by findings from cross-sectional studies observing that greater training status is associated with greater CS activity [2, 37, 84-90], and the possibility that there may exist an upper limit above which CS activity does not increase further. Regardless, these findings suggest training-induced changes in CS activity may be influenced by a participant's training status, consistent with the notion that training adaptations are reduced as training status increases [91-93], and that homogenous groups should be chosen when investigating changes in this parameter.

Pooling data from Electronic Supplementary Material Table S1 indicated no significant correlation between baseline $\dot{V}O_{2max}$ (representative of an individual aerobic fitness level) and training-induced changes in CS activity (r = -0.18 [95% CI -0.42, 0.08]; *P* = 0.167). No correlation was found even when the effects of training volume were "partialed out" ($r_{y1.2}$ = -0.24 [95% CI -0.47, 0.02]; *P* = 0.065), suggesting baseline $\dot{V}O_{2max}$ does not strongly influence training-induced changes in CS activity. Due to the large variability of absolute CS activity values presented in different studies, it was not possible to conduct a valid analysis of the effects of baseline CS activity values on training-induced changes in CS activity. It would be useful if the scientific community could agree on a standard methodology for the determination of CS activity, so as to enable better comparisons across different studies.

2.2.3 Effect of detraining - reversibility of mitochondrial adaptations

With the exception of one study, reporting no change in CS activity following three weeks of complete detraining [27], the majority of studies indicated that complete detraining is associated with a significant decrease in CS activity. Studies have reported a 1.1- to 1.3-fold reduction in this parameters after as little as 7 to 10 days of complete detraining [84, 94], and

greater reductions (1.4- to 1.5-fold) following prolonged (3 to 12 weeks) complete detraining [37, 95]. However, following a taper lasting one to four weeks, previous training-induced gains in CS activity were preserved [1, 94, 96, 97], and were increased further if the reduction in training volume was accompanied by an increase in exercise intensity [94]. Future research is needed to better characterize the effects of different types of detraining on mitochondrial content and $Mito_{VD}$.

2.3 Mitochondrial respiratory function

Cross-sectional studies demonstrate that greater training status is associated with greater mass-specific mitochondrial respiration (i.e., the rate of oxygen consumption per gram of tissue) [2, 85, 90, 98], suggesting exercise training induces an increase in this parameter (Fig. 3, Electronic Supplementary Material Table S2). Mitochondrial respiratory function can also be assessed by measuring mitochondrial ATP production rate (MAPR) in isolated mitochondria [27, 99], with greater training-induced improvements being observed with MAPR compared with mass-specific mitochondrial respiration. These differences may originate from improved coupling between oxidation and phosphorylation, and a greater amount of ATP generated per molecule of oxygen consumed (P/O ratio). However, despite suggestions that training may induce a greater energy efficiency due to a lower degree of uncoupling [100], no change in P/O ratio has been observed following training [22]. Another possibility is that more mitochondria are isolated post-training due to improved mitochondrial content and quality. Nonetheless, further research is needed to explain these discrepancies. As measurement of mass-specific mitochondrial respiration in permeabilized muscle fibers is considered the gold standard technique for the assessment of mitochondrial respiratory function [31, 101, 102], this review will focus on studies utilizing this technique.

2.3.1 Training-induced changes in mass-specific mitochondrial respiration

Effect of training volume. Previous research suggests training volume is not a primary determinant of training-induced changes in mass-specific mitochondrial respiration [70]. This is supported by the lack of change in mass-specific mitochondrial respiration reported following HIIT or MICT, even though the training volume for these groups was three times greater than that of a SIT group that did have a significant increase in mass-specific mitochondrial respiration [33]. Similarly, when directly comparing training groups matched for total work only the group exercising at the higher relative exercise intensity increased mass-specific mitochondrial respiration [20, 72]. Moreover, analysis of Fig. 3 indicates that training groups eliciting some of the lower training volumes were still associated with a significant increase in mass-specific mitochondrial respiration [33, 68, 103, 107], whereas groups associated with some of the greater training volumes did not [20, 36, 55, 104]. Conversely, the only study directly comparing different training volumes (while maintaining similar relative exercise intensities) observed that only the group eliciting the greater training volume induced an increase in mass-specific mitochondrial respiration [1]. The authors suggested the increase in mass-specific mitochondrial respiration was likely driven by an increase in mitochondrial content, rather than by improved mitochondrial efficiency (a concept discussed in section 2.4). This indicates that while training volume may not be a primary determinant of changes in mass-specific mitochondrial respiration, it represents an important factor when the relative exercise intensity is similar.

Comparisons of studies employing MAPR further confirm training volume is not a primary determinant of training-induced changes in mitochondrial respiratory function. Starritt et al. [99] reported a larger increase in MAPR than Wibom et al. [27] (2.6- *vs.* 1.9-fold, respectively), despite assessing respiration with the same substrates and employing a training volume that was approximately half. A major difference was that about half of the training in the former

study was performed at 95% of $\dot{W}_{max}$, whereas in the latter all training took place at 70% of $\dot{W}_{max}$.

Effect of exercise intensity. Two separate studies directly comparing two groups matched for total work reported that only HIIT at 90% of $\dot{W}_{max}$, but not MICT at 61% of $\dot{W}_{max}$ [20], and only single-leg cycling HIIT at 65% of $\dot{W}_{max}$, but not MICT at 50% of $\dot{W}_{max}$ [72], induced a significant increase in maximal mass-specific mitochondrial respiration. Consistent with these findings, a third study observed that while SIT at ~168% of $\dot{W}_{max'}$ significantly increased mass-specific mitochondrial respiration, HIIT at 73% of $\dot{W}_{max'}$ and MICT at 55% of $\dot{W}_{max'}$ resulted in no change despite an ~3-fold greater training volume [33]. Collectively, the above observations indicate that relative exercise intensity is an important determinant of changes in mass-specific mitochondrial respiration.

With the exception of the HIIT group in Granata et al. [1], for which the very large training volume may have contributed, results from Fig. 3 indicate there is a clear increase in mass-specific mitochondrial respiration (i.e., confidence interval not crossing the dotted line) only when at least part of a short-duration (< 10 weeks) training intervention is performed at a relative exercise intensity ≥ 90% of $\dot{W}_{max}$. Groups sitting at the suggested cut-off point performed at least one third [105], or half [22, 108], of the training at a relative exercise intensity ≥ 90% of $\dot{W}_{max}$. This suggests that to increase mass-specific mitochondrial respiration a relative exercise intensity threshold (~90% of $\dot{W}_{max}$) may exist. This could relate to a possible earlier and greater recruitment of type II fibers (alongside that of type I fibers) at relative exercise intensities ≥ 90% of $\dot{W}_{max}$ compared to lower relative exercise intensities [78, 80], although another study has questioned whether there are differences in fiber recruitment between MICE and SIE [81]. Further research is required to determine the effects of relative exercise intensity on fiber recruitment and subsequent fiber-specific changes in mitochondrial respiratory function.

The results summarized in Fig. 3 also indicate training-induced changes in mass-specific mitochondrial respiration may be differentially regulated at relative exercise intensities > 100% of $\dot{W}_{max}$. The smaller increases recorded at supramaximal relative exercise intensities compared with those immediately below 100% of $\dot{W}_{max}$ may relate to the substantial reduction in training volume necessary to elicit these intensities. Therefore, it is plausible that to maximize improvements in mass-specific mitochondrial respiration an individual needs to exercise at the highest relative exercise intensity that allows a large volume of training to be performed (e.g., ~90 to 95% of $\dot{W}_{max}$). This is consistent with previous recommendations regarding the type of training required to maximize improvements in $\dot{V}O_{2max}$ and endurance performance [110].

Although HIIT induces greater changes in mass-specific mitochondrial respiration compared with SIT (Fig. 3), analysis of Fig. 4 (studies employing single-leg cycling are not included here, as explained later in this section) indicates SIT may be a more efficient form of exercise [111]. When training-induced changes in mass-specific mitochondrial respiration are normalized per unit of training volume (Fig. 4a), or per unit of total training time (Fig. 4b), the biggest gains are obtained following SIT. The efficacy of exercising at supramaximal relative exercise intensities is further highlighted when training-induced changes in mass-specific mitochondrial respiration are normalized per unit of effective training time (i.e., the actual time spent exercising, exclusive of the recovery periods used in HIIT- and SIT-like protocols) (Fig. 4c). This shows an exponential-like increase in this parameter at relative exercise intensities > 100% of $\dot{W}_{max}$, and that performing all-out SIT may help to maximize improvements in mass-specific mitochondrial respiration when time availability is limited.

The above findings highlight the specificity of different training protocols on mitochondrial adaptations; while exercising at relative exercise intensities of ~90 to 95% of $\dot{W}_{max}$ provides the greatest absolute increase in mass-specific mitochondrial respiration (Fig. 3), exercising at

relative exercise intensities > 100% of $\dot{W}_{max}$ returns the most benefits per unit of time (Fig. 4c). For example, while professional athletes can devote as much time as required to improve mitochondrial respiratory function, individuals with limited time availability could obtain the best returns by engaging in SIT-like training protocols. When prescribing exercise to different individuals, however, an important, yet understudied, factor is the inter-individual variability of training-induced mitochondrial adaptations. Future large scale randomized controlled trials are required to better characterize the individual responses to different training protocols both between individuals within the same population, and between different populations.

Two final observations can be made. Training-induced changes in mass-specific mitochondrial respiration via fatty acid oxidation (ETF_P) may occur earlier (i.e., after fewer exercise sessions) compared with those via complex I (CI_P) or complex I+II ($CI+II_P$) [103], as also observed in unpublished data from our lab (ETF_P increased significantly following 6 exercise sessions, whereas $CI+II_P$ did not). The second observation stems from the study by MacInnis et al. [72], where participants performed single-leg cycling; the HIIT group in this study had similar or greater increases in mass-specific mitochondrial respiration compared with studies eliciting similar relative exercise intensities employing double-leg cycling [20, 33, 36, 104, 106], despite a markedly lower training volume. This indicates single-leg cycling may represent a more powerful stimulus than double-leg cycling to improve mass-specific mitochondrial respiration, as previously suggested [112].

2.3.2 Reversibility of mitochondrial respiratory function following detraining

One week of complete detraining induced a 1.5-fold decrease in mitochondrial respiration measured in deltoid muscle homogenates of elite swimmers, with no further changes for the following three weeks [113]. Similarly, three weeks of complete detraining resulted in a ~1.3-fold decrease in MAPR measured with different substrate combinations in the vastus lateralis

muscle of active men [27]; the lower decrease in the latter study may relate to the different muscle investigated (non-weight-bearing, *vs.* weight-bearing, respectively). A two-week taper reduced (~1.2-fold) mass-specific mitochondrial respiration in permeabilized muscle fibers of the vastus lateralis of moderately-trained men [1], whereas a one-week taper resulted in no change in the same parameter (unpublished data from our laboratory). These findings indicate that mitochondrial respiratory function is rapidly reversed following inactivity or a substantial reduction in training volume, and they highlight the importance of maintaining the training stimulus to help reduce or prevent this loss. More research is required to better characterize the effect of different types of detraining on mitochondrial respiratory function.

2.4 Dissociation between training-induced changes in mitochondrial content and mitochondrial respiratory function

Although a concomitant change in mitochondrial content and mitochondrial respiration is typically reported, a dissociation between these two parameters has been observed in both mice [114] and humans [1, 2, 33, 36, 55]. This is supported by findings indicating that in human skeletal muscle higher training statuses are associated with relatively larger values of mass-specific mitochondrial respiration than CS activity [70]. Thus, it appears improvements in mitochondrial respiratory function in human skeletal muscle can take place independently of changes in mitochondrial content.

This dissociation results in training-induced changes in mitochondrial (mt)-specific respiration (i.e., mass-specific mitochondrial respiration normalized to mitochondrial content, or one of its markers). Four weeks of SIT induced an increase in mass-specific respiration despite no change in mitochondrial content (as assessed by CS activity), resulting in a ~1.2-fold significant increase in mt-specific respiration [33]. Conversely, despite an increase in mitochondrial

content (as assessed by TEM), six weeks of MICT did not change mass-specific mitochondrial respiration, resulting in an ~1.2-fold significant decrease in mt-specific respiration [36, 55]. This dissociation has also been demonstrated in a cross-sectional study that reported elite athletes have significantly greater mt-specific respiration than individuals of lower training status [2].

The dissociation between training-induced changes in mitochondrial content and mitochondrial respiratory function should not come as a surprise, as we have revealed that relative exercise intensity appears to be a primary determinant of training-induced changes in mitochondrial respiration, whereas training volume seems to be a primary determinant of training-induced changes in mitochondrial content. This highlights the specificity of different training interventions, and it suggests these two parameters can be manipulated to induce the desired mitochondrial adaptation (Fig. 5). For example, mitochondrial respiratory function can be improved as a consequence of increased mitochondrial content (i.e., increased mass-specific and unchanged mt-specific respiration) [1, 68], by improving mt-specific respiration (i.e., increased mass-specific respiration and unchanged mitochondrial content) [33], or through any combination of these (Fig. 5).

The origin of this dissociation is not readily apparent, and it may have many explanations. For example, mitochondrial remodeling [115] and autophagy/mitophagy [116, 117] constitute important physiological responses to exercise. Hence, an increase in mitochondrial respiratory function may stem from improved mitochondrial morphology, or selective mitochondrial degradation, resulting in more efficient mitochondria following autophagy [118], and may not necessarily translate into increased mitochondrial content [119]. In section 2.1 we highlighted that MPS, MPB and net muscle protein turnover all have been reported to increase after training [52, 53]. Similarly, if exercise-induced increases in MitoPS [35, 38] were accompanied by increased MitoPB (a response that may be expected as exercise-induced increases in MPB have

been reported [47]), mitochondrial content would not change despite increased protein turnover.

Nevertheless, replacement of old proteins with newly synthesized ones would likely result in improved mitochondrial respiratory function [119]. In addition, mitochondrial cristae density, and surface area of mitochondrial cristae per muscle volume, are increased with greater training status [120]. Cristae shape has been observed to determine mitochondrial respiratory efficiency via regulation of the assembly and stability of respiratory chain supercomplexes [121] - the formation of which is enhanced following exercise training in human skeletal muscle [122]. Therefore, it is tempting to speculate that exercise training may improve mitochondrial respiratory efficiency through increased mitochondrial cristae density and tightening, which increases supercomplexes assembly and stability [121, 123]. These findings suggest another possible mechanism that may improve mitochondrial respiratory function independent of changes in mitochondrial content. Finally, metabolic regulation could also play a part, as both peroxisome proliferator-activated receptor γ coactivator-1α; (PGC-1α) [124] and p53 [125] promote a switch from glycolytic to oxidative energy provision. This indicates that modulation of metabolic regulation may improve mitochondrial respiratory function independent of changes in mitochondrial content. In conclusion, mitochondrial remodeling, autophagy/mitophagy, mitochondrial protein turnover, changes in mitochondrial ultrastructure, and metabolic regulation, are all potential mechanisms that may contribute to the dissociation between training-induced changes in mitochondrial content and mitochondrial respiratory function. Further research is required to investigate the effects of altering the training prescription on mitochondrial ultrastructure and the mitochondrial quality control processes described above.

3 Relationship between exercise- and training-induced mitochondrial adaptations

It has been suggested that training-induced mitochondrial adaptations are the result of the cumulative effects of transient changes in protein localization [126] and messenger RNA (mRNA) expression [17] after each exercise session. This leads to the concept that exercise-induced changes can provide information about factors affecting training-induced adaptations. While previous research has reported both a relationship [17, 127] and a lack of relationship [17, 75] between these parameters, results from Fig. 6 indicate a generally poor correlation. Therefore, caution must be used when predicting mitochondrial adaptations to training based on the assessment of a limited set of parameters measured after a single exercise session. This lack of a relationship between exercise- and training-induced mitochondrial adaptations may relate to methodological shortcomings such as the arbitrary and limited number of time points of post-exercise muscle sampling, the limited range of candidate genes and proteins investigated, and the inability to control for confounders such as circadian rhythm, previous training history, genetic variations, pre-biopsy nutrition, etc. Moreover, it has been reported mRNA content can only predict ~40% of the variability in protein levels in mammalian cells [128], indicating assessment of gene transcription alone may not predict changes in protein content and biological function (for an interesting debate on this topic readers are referred to an excellent viewpoint article and its related counterpoints [76, 129, 130]). However, even though there is not strong evidence supporting a relationship between exercise- and training-induced mitochondrial adaptations, we still advocate assessing exercise-induced changes in the mRNA expression and protein content and localization of key transcriptional regulators of mitochondrial biogenesis as this enhances our understanding of the molecular mechanisms associated with mitochondrial adaptations. Moreover, although with our current knowledge we are not able to predict training-induced mitochondrial adaptations based on exercise-induced responses, the value in being able to do so makes this a very important area for future study.

4 Conclusions and Future Research

We have demonstrated that training volume seems to be an important determinant of training-induced changes in mitochondrial content (as assessed by CS activity, Fig. 2a), an effect seemingly restricted to training interventions employing relative exercise intensities < 100% of $\dot{W}_{max}$. When training-induced changes in $Mito_{VD}$ are assessed by TEM, a plateau in this parameter has been reported (Fig. 1). Conversely, relative exercise intensity does not seem to be a key determinant of training-induced changes in mitochondrial content (as assessed by CS activity, Fig. 2c). Finally, stagnation may occur if the training stimulus is not maintained (e.g., exercising at the same absolute exercise intensity), and training-induced increases in CS activity are rapidly reversed following detraining.

We have also demonstrated relative exercise intensity appears to be a key determinant of training-induced changes in mass-specific mitochondrial respiration (Fig. 3) (and MitoPS), whereas training volume is only of secondary importance (i.e., when relative exercise intensity is similar greater training volumes result in greater increases in mass-specific mitochondrial respiration). A relative exercise intensity threshold of ~90% of $\dot{W}_{max}$ may exist to increase mass-specific mitochondrial respiration, whereas at relative exercise intensities > 100% of $\dot{W}_{max}$ changes in this parameter may be differentially regulated (Fig. 3). We also report that SIT represents a more efficient form of exercise to improve mass-specific mitochondrial respiration both on a per-time or per-training volume basis (Fig. 4). Similar to mitochondrial content, a reduction in the training stimulus, or complete detraining, induces a rapid loss of mitochondrial respiratory function. These findings suggest the training stimulus needs to be maintained to preserve previous training-induced gains in mitochondrial respiratory function.

The above sections have provided an extensive review on the current knowledge regarding training-induced mitochondrial adaptations (Fig. 7). To date, very few studies have measured

many of the parameters associated with exercise- and training-induced mitochondrial adaptations simultaneously. The importance of mitochondrial remodeling events [131, 132] and autophagic processes influencing mitochondrial turnover [119, 133], as well as mitochondrial ultrastructure processes regulating cristae density [120] and shape [121], and supercomplexes formation [122], is only starting to become apparent. New evidence is continuing to emerge regarding the role of transcription factors such as PGC-1α, p53, TFAM, and transcription factor EB (TFEB) in the regulation of exercise-induced mitochondrial biogenesis [29, 124, 134, 135]. We have also shown training-induced changes in mitochondrial content and mitochondrial respiratory function are not necessarily linked, and they should be differentiated and measured in parallel. Therefore, we propose future research investigating exercise- and training-induced mitochondrial adaptations should take a more holistic and integrative approach and measure changes in the above parameters simultaneously. It is also important to underline that most of the conclusions put forth in this review (and in this field of research in general) are based on comparisons across a number of studies performed in different laboratories using different populations and often employing a small sample size. Therefore, to address some of the questions that remain to be answered we suggest that new research should be conducted as part of large scale multi-site randomized controlled trials (so as to increase the sample size), and that factors such as the population investigated and the methodology utilized should be tightly and rigorously controlled, with the aim to strengthen the validity of the conclusions made. These studies should incorporate multiple sampling time-points to capture dynamic responses to exercise training and to better define the time course of training-induced mitochondrial adaptations. In addition, classic techniques (e.g. TEM, enzyme activity, mitochondrial respiration) should be integrated with "omics" technologies (e.g.; transcriptomics, proteomics, metabolomics) and bioinformatics [136]. The emergence of these technologies provides a new and exciting discovery potential to understand the complex and

integrative nature of exercise in skeletal muscle, as well as inter-organ crosstalk [137, 138]. This approach would allow a deeper understanding of the processes involved in the regulation of training-induced mitochondrial adaptations, increasing our knowledge of the events leading to skeletal muscle phenotype remodeling. This would also provide physicians and practitioners with greater information to better design exercise training programs aimed at improving health and performance.

Compliance with Ethical Standards

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

Author contributions

Cesare Granata conducted the literature searches. Cesare Granata, Nicholas Jamnick and David Bishop analysed and interpreted the data. Cesare Granata wrote the manuscript. Cesare Granata, Nicholas Jamnick and David Bishop critically revised and contributed to the manuscript. Cesare Granata and David Bishop have primary responsibility for final content. Data analysis took place at Victoria University. All authors read and approved the final manuscript.

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Figure Captions

Fig. 1 The relationship between training-induced changes in mitochondrial volume density ($Mito_{VD}$) as assessed by transmission electron microscopy [TEM] and training volume in the vastus lateralis muscle of healthy human participants. A second order polynomial regression (solid line), and 95% confidence bands (dotted lines) are shown, as well as the coefficient of determination R^2 and the *P* value. Training volume was calculated by multiplying the exercise intensity relative to $\dot{W}_{max}$ (or $\dot{W}_{max'}$) by the duration of exercise training (in minutes) and by the total number of training sessions. Only studies using a cycling intervention were used; studies with middle-aged, elderly, or diseased (e.g., chronic heart failure, diabetics, obese) populations, studies investigating a single session of exercise, and studies not providing precise and detailed information about the training prescription were excluded. Data were obtained by pooling results from the following studies: □ [18], ○ [55], ● [19], ◊ [36], ■ [56], ▼ [57]. A second order polynomial regression analysis was used to calculate the coefficient of determination (R^2) between variables (R statistical software). The level of statistical significance was set at $P < 0.05$. a.u.: arbitrary units; $\dot{W}_{max}$: maximal power output; $\dot{W}_{max'}$: estimated $\dot{W}_{max}$ based on the bioenergetic model [25]

Fig. 2 The relationship between (a) training volume or (c) relative exercise intensity, and training-induced changes in citrate synthase (CS) activity measured in the vastus lateralis muscle of healthy human participants. The same two correlations are presented in panel (b) and (d), respectively, after removing all the studies employing SIT. Linear correlation (solid line), and 95% confidence bands (dotted lines) are shown, as well as the correlation coefficient r [with 95% confidence intervals] and the *P* value. Exercise intensity was expressed relative to $\dot{W}_{max}$ (or $\dot{W}_{max'}$), whereas training volume was calculated by multiplying the exercise intensity relative to $\dot{W}_{max}$ (or $\dot{W}_{max'}$) by the duration of exercise training (in minutes) and by the total number of training sessions. Only studies using a cycling intervention were used; studies with middle-aged, elderly, or diseased (e.g., chronic heart failure, diabetics, obese) populations, studies investigating a single session of exercise, and studies not providing precise and detailed information about the training prescription were excluded. Relative exercise intensity for studies employing sprint interval training (SIT) is often not provided; for these studies, a value of relative exercise intensity was used based on that attained by participants of similar fitness completing the same number of repetitions in a training study from our group [33]. These ranged from ~181% to ~165% of $\dot{W}_{max}$ for four to ten 30-s all-out bouts, respectively. (a) was

obtained by pooling results from the following studies: [1, 16, 17, 22, 27, 33, 61-67, 71-73, 99, 103, 107, 109, 139-173]. (c) was obtained by pooling results only from studies using a single relative exercise intensity: [1, 16, 17, 27, 33, 61-67, 71-73, 103, 107, 109, 139-153, 155-161, 163-170, 173], whereas (b) and (d) where obtained by pooling results from the same studies used to generate (a) and (c), respectively, with the exclusion of SIT studies (Electronic Supplementary Material Table S1). A linear correlation analysis was used to calculate the correlation coefficient between variables in each of the four panels, according to Pearson's product moment (r) (SigmaStat software; Jandel Scientific, San Rafael, CA, USA). The level of statistical significance was set at $P < 0.05$. MICT: moderate-intensity continuous training; HIIT: high-intensity interval training; SIT: sprint interval training; Mixed: mixed type of training (e.g. MICT+HIIT, MICT+SIT); a.u.: arbitrary units; $\dot{W}_{max}$: maximal power output; $\dot{W}_{max'}$: estimated $\dot{W}_{max}$ based on the bioenergetic model [25]

Fig. 3 Forest plot representation of training-induced fold-changes in maximal mass-specific mitochondrial respiration in the vastus lateralis of human skeletal muscle of healthy participants, following a cycle training intervention ranked (greatest to lowest) by relative exercise intensity (values in bracket on the left Y axis). For studies employing a combination of different relative exercise intensities, a mean value is presented; recovery periods for which the relative exercise intensity was either not reported, or was reported as 30 W, or when it was performed at a relative exercise intensity ≤ 50% $\dot{W}_{max}$ (or $\dot{W}_{max'}$), were not included in the mean. Training volume (values presented on the right Y axis) was calculated by multiplying the exercise intensity relative to $\dot{W}_{max}$ (or $\dot{W}_{max'}$) by the effective duration of the exercise session (in minutes) and by the total number of sessions. Studies with middle-aged, elderly, or diseased (e.g., chronic heart failure, diabetics, and obese) populations, studies investigating a single session of exercise, and studies not providing precise and detailed information about the training prescription, were excluded. The means of the individual changes and the 95% confidence intervals reported in this plot are those relative to the maximum value of coupled mass-specific mitochondrial respiration reported in each individual investigation, and were kindly provided by the corresponding authors of the following studies: [1, 20, 22, 33, 36, 55, 68, 72, 103-108]. We were not able to obtain results from Christensen et al. [103] and Robach et al. [106]; therefore, only the mean change (with no confidence interval) estimated by the results in the manuscript is presented. Results from Irving et al. [104] are relative to the young group only. Results from Robach et al. [106] are relative to the normoxic training group only. Unpublished data for the "Vincent et al. [107] - no training" group were kindly provided by

Professor Anthony J. R. Hickey and were collected during the study presented in Vincent et al. [107] (Electronic Supplementary Material Table S2). Results from Larsen et al. [109] have not been included as the authors only provided values of mitochondrial-specific respiration. Filled diamonds (♦): studies in permeabilized muscle fibers; open circles (○): studies in isolated mitochondria; open squares (□): studies using single leg cycling (and permeabilized muscle fibers); CI_P: oxidative phosphorylation capacity (*P*) through complex I; $CI+II_P$; oxidative phosphorylation capacity (*P*) through complex I and II combined; MICT: continuous training; HIIT: high-intensity interval training; SIT: sprint interval training; a.u.: arbitrary units; $\dot{W}_{max}$: maximal power output; $\dot{W}_{max'}$: estimated $\dot{W}_{max}$ based on the bioenergetic model [25]

Fig. 4 Values of maximal mass-specific mitochondrial respiration expressed as percent change and normalized (a) per unit of training volume, (b) per unit of total training time, and (c) per unit of effective training time (± SEM, where available) measured in the vastus lateralis of human skeletal muscle of healthy participants following a cycle training intervention. Studies are ranked left to right by increasing relative exercise intensity elicited during the training. The vertical dotted line represents $\dot{W}_{max}$. For studies employing a combination of relative exercise intensities, a mean value is presented; recovery periods for which the relative exercise intensity was either not reported, or was reported as 30 W, or when the recovery was performed at a relative exercise intensity ≤ 50% $\dot{W}_{max}$ (or $\dot{W}_{max'}$), were not included in the calculation of the mean relative exercise intensity. Training volume was calculated by multiplying the exercise intensity relative to $\dot{W}_{max}$ (or $\dot{W}_{max'}$) by the effective duration of the exercise session (in minutes) and by the total number of sessions. Effective training time represents the effective time spent cycling and does not include recovery periods for which the exercise intensity was either not reported, or was reported as 30 W, or when recovery was performed at a relative exercise intensity ≤ 50% $\dot{W}_{max}$ (or $\dot{W}_{max'}$). Studies with middle-aged, elderly, or diseased (e.g., chronic heart failure, diabetics, and obese) populations, studies investigating a single session of exercise, and studies not providing precise and detailed information about the training prescription, were excluded. The means of the individual changes used to generate the data presented in this figure are those relative to the maximum value of coupled mass-specific mitochondrial respiration reported in each individual investigation, and were kindly provided by the corresponding authors of the following studies: [1, 20, 22, 33, 36, 55, 68, 103-106, 108]. We were not able to obtain results from Christensen et al. [103] and Robach et al. [106]; therefore, only the percent change (with no SEM) estimated by the results in the manuscript is presented. Results from Irving et al. [104] are relative to the young group only. Results from

Robach et al. [106] are relative to the normoxic training group only. Results from Larsen et al. [109] have not been included as the authors only provided values of mitochondrial-specific respiration, Results from in MacInnis et al. [72] and Vincent et al. [107] have not been included as these studies employed single-leg cycling. CI_P: oxidative phosphorylation capacity (*P*) through complex I; $CI+II_P$; oxidative phosphorylation capacity (*P*) through complex I and II combined; MICT: continuous training; HIIT: high-intensity interval training; SIT: sprint interval training; SEM: standard error of the mean; $\dot{W}_{max}$: maximal power output; $\dot{W}_{max'}$: estimated $\dot{W}_{max}$ based on the bioenergetic model [25]

Fig. 5 Schematic representation of predicted training-induced changes in mitochondrial content (mt-C; empty arrows), mass-specific mitochondrial respiration (ms-R; filled arrows), and mitochondrial-specific respiration (mt-R; empty/filled arrows) at different relative exercise intensities (Ex-Int) and training volumes (Tr-Vol), based on the results from the available literature. Exercise intensity is considered relative to $\dot{W}_{max}$ determined with an 8 to 12 min incremental exercise test. Training volume is obtained by multiplying the exercise intensity relative to $\dot{W}_{max}$ (or $\dot{W}_{max'}$), by the effective duration of the exercise session (in minutes), and by the total number of sessions. Arrows represent no change (horizontal), an increase (upward), or a decrease (downward). Arrow thickness is representative of the magnitude of the predicted change. n/a: not applicable; a.u.: arbitrary units; $\dot{W}_{max}$: maximal power output; $\dot{W}_{max'}$: estimated $\dot{W}_{max}$ based on the bioenergetic model [25]

Fig. 6 Correlations between exercise- and training-induced (12 exercise sessions) mitochondrial adaptations following a cycling intervention in the vastus lateralis muscle of healthy humans. Linear correlation analyses were used to calculate the correlation coefficient between exercise- and training-induced mitochondrial adaptations, according to Pearson's product moment (r) (SigmaStat software; Jandel Scientific, San Rafael, CA, USA); 95% confidence intervals (CI) for the r values are reported in square brackets. The level of statistical significance was set at $P < 0.05$. Unpublished results from our laboratory. PGC-1α: Peroxisome proliferator-activated receptor γ coactivator 1-α; p.c.: protein content; CS: citrate synthase; $CI+II_P$: maximal mass-specific mitochondrial respiration through complex I and II combined; $CI+II_P$/CS activity: maximal mt-specific respiration through complex I and II combined; p-$p53^{Ser15}$: p53 phosphorylation at serine 15. mRNA: messenger RNA

Fig. 7 Schematic representation of the magnitude and timing of training-induced mitochondrial adaptations measured in the vastus lateralis of human skeletal muscle of healthy participants,

following a cycle exercise or training intervention, respectively. Results for PGC-1α mRNA were generated based on findings from [17] and unpublished research from our laboratory; results for PGC-1α protein content were generated based on findings from [1, 16, 17, 33, 59, 66, 104, 107, 109, 150, 151, 158, 174-176] and unpublished research from our laboratory; results for CS activity were generated based on findings from [17, 66] and unpublished research from our laboratory; results for mitochondrial respiration were generated based on findings from [1, 20, 22, 33, 36, 68, 72, 103-105, 107, 108] and unpublished research from our laboratory. The mRNA and protein content of PGC-1α were chosen for this figure as these represent some of the most widely studied exercise- and training-induced adaptations, and because PGC-1α is widely considered the master regulator of mitochondrial biogenesis [69]. PGC-1α: peroxisome proliferator-activated receptor γ coactivator-1α; mRNA: messenger RNA; CS: citrate synthase