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Acute High-Intensity Exercise Impairs Skeletal Muscle Respiratory Capacity

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Abstract

PURPOSE: The impact of an acute bout of exercise, especially high-intensity exercise, on the function of mitochondrial respiratory complexes is not well understood, with potential implications for both the healthy population and patients undergoing exercise based rehabilitation. Therefore, this study sought to comprehensively examine respiratory flux through the different complexes of the electron transport chain (ETC) in skeletal muscle mitochondria before and immediately after high-intensity aerobic exercise.

METHOD: Muscle biopsies of the *vastus lateralis* were obtained at baseline and immediately following a 5 km time-trial performed on a cycle ergometer. Mitochondrial respiratory flux through the complexes of the ETC was measured in permeabilized skeletal muscle fibers by high-resolution respirometry.

RESULTS: Complex I+II state 3 respiration (state 3_{CI+CII}), a measure of oxidative phosphorylation capacity, was diminished immediately after the exercise (PRE: 27±3 ρm.mg^{-1}.s^{-1}; POST: 17±2 ρm.mg^{-1}.s^{-1}, *P*<0.05). This decreased oxidative phosphorylation capacity was predominantly the consequence of attenuated complex-II driven state 3 (state 3_{CII}) respiration (PRE: 17±1 ρm.mg^{-1}.s^{-1}; POST: 9±2 ρm.mg^{-1}.s^{-1}, *P*<0.05). Although Complex-I driven state 3

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(3_{CI}) respiration was also lower (PRE: 20±2 ρm.mg^{-1}.s^{-1}; POST: 14±4 ρm.mg^{-1}.s^{-1}), this did not reach statistical significance (*P*=0.27). In contrast, citrate synthase activity, proton leak (state 2 respiration), and complex IV capacity were not significantly altered immediately after the exercise.

CONCLUSION: These findings reveal that acute high-intensity aerobic exercise significantly inhibits skeletal muscle state 3_{CII} and oxidative phosphorylation capacity. This, likely transient, mitochondrial defect might amplify the exercise-induced development of fatigue and play an important role in initiating exercise-induced mitochondrial adaptations.

Keywords

oxidative phosphorylation capacity; electron transport chain; state 3 respiration; cycling time-trial; mitohormesis

Introduction

Skeletal muscle mitochondria play a critical role in maintaining cellular homeostasis, while also providing the energy required for activities of daily living and exercise. There have been a host of both cross-sectional (1, 2) and longitudinal studies documenting that increased physical activity and chronic endurance exercise enhances muscle mitochondrial content (3, 4) and respiratory capacity (5–7). However, in contrast, the impact of acute exercise on mitochondrial respiratory function is still equivocal, which is interesting in light of the many, potentially deleterious, intracellular perturbations associated with acute exercise, particularly when performed at a high-intensity. Specifically, following acute high-intensity exercise, skeletal muscle mitochondrial oxidative phosphorylation may be hindered by exercise-induced reactive oxygen (O_2) species as well as the accumulation of inorganic phosphate, lactate, and protons (8), all either within or in close proximity to the mitochondria.

Initial animal studies reported that high-intensity exercise to exhaustion induced acute mitochondrial swelling (9) and transiently impaired mitochondrial respiration in skeletal muscle (10–12). However, later studies in humans documented unchanged or even increased complex-I specific ADP-stimulated respiration (State 3_{CI}) and mitochondrial efficiency (P/O) in isolated mitochondria and permeabilized muscle fibers, immediately after prolonged exercise at a moderate intensity (60–75% of maximal oxygen uptake (VO_{2max})) (13–16), or intermittent supramaximal cycling exercise (130% of VO_{2max}) (17). However, a caveat of these prior studies is that the mitochondrial respiration attained during State 3_{CI} yields only 65–75% of the maximal respiratory rate that can be measured without the additional substrates necessary to facilitate convergent electron flow through complex I and II (State 3_{CI+CII}) (18). The tricarboxylic acid (TCA) cycle is, indeed, not complete using NADH-specific substrates (e.g. pyruvate and malate, or glutamate and malate) such that citrate, isocitrate, 2-oxoglutarate and succinate are depleted, which, in turn, prevents both the electron flow through complex-II and the attainment of true maximal muscle respiratory capacity (18). Consequently, the results of these studies do not constitute unequivocal evidence of an uncompromised electron transport chain (ETC) following acute exercise. Furthermore, the acute effects on mitochondrial respiratory function of a short duration high intensity exercise, that ultimately yields VO_{2max} and elicits improvements in muscle aerobic capacity when performed chronically (19, 20), has yet to be assessed.

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Consequently, the purpose of this study was to comprehensively examine respiratory flux through the complexes of the ETC in skeletal muscle mitochondria, both before and immediately after high-intensity aerobic exercise. We used a 5 km cycling time-trial, an exercise modality that increases aerobic metabolism to VO_{2max} for several minutes and induces considerable intracellular metabolic perturbations and fatigue (8). Based upon the large intracellular perturbations associated with this form of exercise (8) and the apparently preserved complex-I specific ADP-stimulated respiration immediately after exercise (13–16), we hypothesized that state 3_{CI+CII} respiration, assessed in permeabilized muscle fibers obtained from the *vastus lateralis*, would be significantly diminished immediately after exercise, as a consequence of compromised electron flow through complex II.

Methods

Subjects:

Following informed consent procedures, 8 recreationally active, healthy, non-medicated, and non-smoking males participated in this study (mean ± SD: weight, 83 ± 14 kg; height, 180 ± 6 cm; age, 26 ± 2 years). Intramuscular metabolites and neuromuscular function results have previously been published for these subjects (8). Subjects were instructed to refrain from exercise for 48 h and caffeine for 24 h before each trial. The study was approved by the Institutional Review Boards of both the University of Utah and the Salt Lake City Veterans Affairs Medical Center.

Exercise protocol:

During preliminary visits, all participants performed 2–3 practice 5 km cycling time-trials and a maximal incremental exercise test to exhaustion to determine VO_{2peak} on a computer-controlled electromagnetically braked cycle ergometer (Velotron, Elite Model, Racer Mate, Seattle, WA, USA). On the day of the experiment, with the subject lying on a bed, a percutaneous biopsy of the *vastus lateralis* muscle, approximately 3.5 cm deep, 15 cm proximal to the knee and slightly distal to the ventral mid-line of the muscle, was obtained from the right leg prior to exercise. The 5 mm diameter biopsy needle (Bergstrom) was attached to sterile tubing and a syringe to apply a negative pressure to assist in the muscle sample collection (21). Immediately after the muscle sample (~100 mg) was removed from the leg, a portion of the sample (~20 mg) was immersed in ice-cold biopsy preservation fluid BIOPS (in mM: 2.77 CaK^2EGTA, 7.23 K_2EGTA, 20 imidazole, 50 K^+MES, 20 Taurine, 0.5 dithiothreitol, 6.56 $MgCl_2$, 5.77 ATP, 15 phosphocreatine, pH 7.1) for respiratory analysis while the remaining sample was immediately frozen and stored at −80°C for later analysis (8). Subjects then moved to the cycle ergometer to complete a 5 km cycling time trial with freedom to alter power output by changing the gear ratio and/or pedalling frequency (8). Immediately following the exercise, a cuff, placed on the upper part of the thigh, was rapidly inflated to supra-systolic pressure (250 mmHg) to clamp the metabolic milieu until muscle sampling was complete (< 30 s after exercise cessation). Given the relatively large O_2 store of the muscle (22, 23) and the relatively short duration of the ischemia in comparison to the time documented to induce mitochondrial defects (24), it is unlikely that this procedure elicited an anoxic state and, in turn, altered mitochondrial respiratory capacity. The right leg was used for baseline sampling while the left leg was used for post-exercise sampling.

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Pulmonary gas exchange measurements

At rest and throughout exercise, pulmonary gas exchange and ventilation were measured continuously using an open circuit calorimetry system (Parvo Medics, True Max 2400, Salt Lake City, UT).

Preparation of permeabilized muscle fibers and mitochondrial respiration measurements

The tissue preparation and respiration measurement techniques were adapted from established methods (25, 26) and have been previously described (27). Briefly, BIOPS-immersed fibers were carefully separated with fine-tip forceps and subsequently bathed in a BIOPS-based saponin solution (50 μg saponin.ml^{-1} BIOPS) for 30 minutes. Following saponin treatment, muscle fibers were rinsed twice in ice-cold mitochondrial respiration fluid (MIR05, in mM: 110 Sucrose, 0.5 EGTA, 3 $MgCl_2$, 60 K-lactobionate, 20 taurine, 10 KH_2PO_4, 20 HEPES, BSA 1 g.L^{-1}, pH 7.1) for 10 minutes each. After the muscle sample was gently dabbed with a paper towel to remove excess fluid, the wet weight of the sample was measured using a standard, calibrated scale (2–4 mg). The muscle fibers were then placed in the respiration chamber (Oxytherm, Hansatech Instruments, UK) with 2 ml of MIR05 solution and warmed to 37°C. After allowing the permeabilized muscle sample to equilibrate for 5 minutes, mitochondrial respiratory function was assessed in duplicate. To assess the function of each mitochondrial complex, O_2 consumption was assessed with the addition of a series of respiratory substrates and inhibitors in the following order and final concentrations in the chamber: glutamate-malate (10 and 2 mM), ADP (5 mM), succinate (10 mM), cytochrome *c* (10 μM), rotenone (0.5 μM), antimycin-A (2.5 μM), oligomycin (2 μM), and *N*,*N*,*N*,*N*-tetramethyl- *p*-phenylenediamine (TMPD)-ascorbate (2 and 0.5 mM). Pilot studies indicated that the concentration of the substrates and inhibitors used were at saturating levels (27). This allowed the determination of *1*) state 2 respiration, the nonphosphorylating resting state, which provides an index of proton leak, assessed in the presence of malate + glutamate; *2*) complex-I driven state 3 respiration (State 3_{CI}), the ADP-activated state of oxidative phosphorylation, assessed in the presence of glutamate + malate + ADP; *3*) complex I + II driven state 3 respiration (state 3_{CI+CII}), assessed in the presence of glutamate + malate + ADP + succinate; 4) complex II driven state 3 respiration (state 3_{CII}), assessed in the presence of glutamate + malate + ADP + succinate + rotenone, and *4*) $uncoupled_{CIV}$ respiration, where the link between the electron transport chain and ATP synthesis has been abolished, assessed by inhibiting complex III (antimycin A) and complex V (oligomycin) followed by the addition of TMPD + ascorbate.

Respirometry data analysis

Only one sample demonstrated impaired mitochondrial membrane integrity (more than a 10% increase in respiration in response to cytochrome c) and was therefore excluded from the analysis. In each condition, the respiration rate was recorded for at least 3 min until a steady state was reached, and the average of the last minute was used for data analysis. Inhibition of respiration with combined antimycin A and oligomycin allowed for the determination and correction for residual O_2 consumption, indicative of non-mitochondrial O_2 consumption (i.e., from chemical and instrument-related respiration in the chamber). Given the within-subject comparison design, the rate of O_2 consumption was simply

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expressed relative to muscle sample mass (in $pmol \cdot s^{-1} \cdot mg$ wet wt^{-1}) as mitochondrial content is unlikely to change within such a short time frame (28). The leak control ratio (i.e. the ratio between state 2 and $uncoupled_{CIV}$ respiration) was calculated as an index of uncoupling due to electron leak across the membrane or slip of protons in the respiratory chain (25). The ratio between State 3CI+CII and uncoupledCIV respiration was also calculated as an index of the excess capacity of cytochrome-c oxidase (25, 29, 30).

CS and succinate dehydrogenase activity

Following the respiration measurements, the same muscle samples (2–4 mg wet weight) were homogenized with homogenization buffer containing 250 mM sucrose, 40 mM KCl, 2 mM EGTA, and 20 mM Tris·HCl (Qiagen, Hilden, Germany). The CS activity assay was performed as previously described (27) using a spectrophotometer with light absorbance set at 412 nm (Synergy 4, Biotek Instruments, Winooski, VT). Complex II enzyme activity, succinate dehydrogenase (SDH) of homogenized tissue sample was measured using a microplate assay kit and performed according to the manufacturer's recommendations (Abcam ab109908). In this assay, the production of ubiquinol by complex II is coupled to the reduction of the dye DCPIP (2,6-diclorophenolindophenol) and SDH activity was measured using a spectrophotometer with light absorbance set at 600 nm.

Statistical Analysis:

Due to the limited sample size, differences between baseline and post-exercise were evaluated with a nonparametric Wilcoxon test (Statsoft, version 5.5; Statistica, Tulsa, Oklahoma). Statistical significance was accepted at $P < 0.05$. Results are presented as mean ± SD in figures.

Results

VO_{2peak} and time- trial performance:

During the maximal and graded cycling exercise, the group mean VO_{2peak} was 3.6 ± 0.5 $L.min^{-1}$ corresponding to 43.8 ± 6.8 $ml.min^{-1}.kg^{-1}$, which was reached at 296 ± 37 W. The mean power output over the entire 5 km time-trial was 220 ± 24 W for an exercise duration of 8.75 ± 0.38 min. Changes in pulmonary VO_2 throughout the 5 km time-trial are illustrated in Figure 1. The time spent over 80% of VO_{2peak} averaged 5.1 ± 2.5 min.

Mitochondrial respiration:

The rates of O_2 consumption for state 2, state 3_I, 3_{II}, 3_{I+II}, and $uncoupled_{CIV}$ respiration are summarized in Figure 2. State 3_{II} and State 3_{I+II} respiration rates were decreased immediately after the 5 km cycling time-trial ($P < 0.05$). In contrast, State 2 ($P = 0.7$), State 3_I ($P = 0.27$), and $uncoupled_{CIV}$ respiration ($P = 0.89$) were not significantly different between baseline and immediately after the exercise. As illustrated in Figure 3, the leak control ratio was not significantly changed ($P = 0.90$), whereas the ratio between State 3CI +CII and uncoupledCIV was diminished immediately after the exercise ($P < 0.05$).

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CS and SDH activity:

Although the CS activity increased by ~41% from baseline to end-exercise (Baseline: 17.2 ± 12.1 a.u., End-exercise: 24.3 ± 8.8 a.u.), likely due to between subject variability, this did not reach statistical significance (*P*=0.18). Also, SDH activity was not significantly different between baseline and immediately after the exercise (Baseline: 1.15 ± 0.6 a.u., End-exercise 0.97 ± 0.7 a.u., *P* =0.62).

Discussion

This study sought to determine the impact of high-intensity, aerobic, whole body cycling exercise on respiratory flux through the ETC of locomotor muscle mitochondria. In agreement with our hypothesis, state 3_{CI+CII} respiration was significantly diminished immediately after completion of a 5 km cycling time-trial. This decreased muscle respiratory capacity was predominantly the consequence of attenuated state 3_{CII} respiration as state 3_{CI} respiration, CS activity, state 2 respiration, and $uncoupled_{CIV}$ respiration were not significantly affected by the exercise. Collectively, these findings reveal that the metabolic challenge imposed by acute high-intensity cycling exercise, likely transiently, compromises skeletal muscle state 3_{CII} and 3_{CI+CII} respiration and therefore oxidative phosphorylation capacity. This attenuated mitochondrial respiratory function may amplify the exercise-induced development of fatigue and could also play an important role in initiating exercise-induced mitochondrial adaptations.

Acute high-intensity exercise and skeletal muscle oxidative phosphorylation capacity

The two key and novel findings of this study are that the completion of a 5 km cycling time-trial, eliciting a sustained metabolic demand close to VO_{2peak} (Figure 1), resulted in 1) a lower maximal respiratory rate, as assessed *in vitro* with substrates for convergent electron flow through complex I and II of the ETC (Figure 2), and 2) a decrease in the phosphorylation system control ratio (Figure 3). Thus, the oxidative phosphorylation capacity of the skeletal muscle, a major determinant of endurance exercise performance (31), was acutely compromised by a bout of high-intensity, whole body exercise. This lower state 3_{CI+CII} respiration appeared to be predominantly mediated by attenuated complex-II specific respiration (50%, Figure 2), and to some extent by an ~30% (non-significant) decline in complex-I specific respiration.

Consistent with the animal literature (11, 12), the present study suggests that acute high-intensity aerobic whole body exercise can, likely transiently, inhibit mitochondrial respiratory capacity. Somewhat in contrast to the current findings, it has previously been reported in humans that skeletal muscle state 3_{CI} respiration is unaffected or even increased immediately after prolonged cycling exercise at a moderate intensity (60–75% of VO_{2max}) (13–16) or after intermittent supramaximal cycling exercise (130% of VO_{2max} for an exercise duration of ~2.5 min) (17). Additionally, Rasmussen et al. (2001) also reported that, in isolated mitochondria from the *vastus lateralis*, state 3_{CI} and state 3_{CII}, assessed separately, were not significantly altered after five bouts of 1-min cycling exercise to exhaustion (32). The reason for the discrepancy between the current findings and previous studies may be attributable to several factors including the preparation used (isolated

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mitochondria vs. permeabilized muscle fibers), the titration protocol (the separate assessment of the mitochondrial complexes vs. convergent electron flow through complex I and II), and/or the exercise protocol employed. Indeed, mitochondrial isolation may result in a biased selection of intact organelles (33), which might have precluded the observation of a detrimental effect of exercise on mitochondrial respiratory function. Also, the attenuated state 3_{CI+CII} respiration was, to a large extent, the consequence of compromised respiration though complex II, such that our findings are consistent with previous studies indicating that state 3_{CI} respiration is unaffected by exercise. With regards to the exercise protocol, by design, it is likely that the metabolic challenge in the current study differed from prolonged moderate exercise, which requires substantial energy supply from Beta-oxidation, employed in previous investigations (13–16, 34, 35). The current subjects, during the 5 km time-trial, demonstrated a sustained high intensity aerobic effort culminating near VO_{2max} (Figure 1), which requires that mitochondrial oxidative phosphorylation is fueled by carbohydrate substrates and operates at or near maximal capacity (36) for several consecutive minutes. This type of effort might also represent a greater metabolic challenge for the mitochondria than short duration intermittent supramaximal cycling exercise (17, 32), during which anaerobic metabolism (glycolysis and phosphocreatine) contributes significantly to ATP production.

High-intensity exercise-induced attenuation in oxidative phosphorylation: Putative mechanisms

There are multiple putative mechanisms that may explain the attenuated oxidative phosphorylation documented in this study. Among them, diminished substrate availability due to the impaired activity of some of the key enzymes of the tricarboxylic acid (TCA) cycle is likely to have played a role. Indeed, whereas oxidative phosphorylation capacity decreases as a consequence of high-intensity whole body exercise by nearly 40%, mitochondrial permeability to protons (state 2 respiration, Figure 2), leak control ratio (Figure 3), and cytochrome-c oxidase (uncoupled$_{CIV}$ respiration, Figure 2) all appear to be well preserved. These findings imply that, despite saturating substrate concentrations in the buffer solution (glutamate, malate, and succinate), factors upstream of the respiratory complexes, such as the impaired activity of some enzymes of the TCA cycle might have been responsible for the decrease in state 3_{CI+CII} respiration by limiting the amount of FADH provided to complex II. This interpretation is further supported by the lower phosphorylation system control ratio immediately after the exercise (Figure 3), which indicates a lower phosphorylative constraint relative to ETC capacity, assessed at the level of complex IV, considering that the ETC is, ultimately, limited by its terminal enzyme, cytochrome c oxidase, in human skeletal muscle (29, 30). In addition, although respirormetry measurements indicated attenuated state 3_{CII} respiration post exercise, SDH activity was not significantly different between baseline and immediately after the exercise. Therefore, in the conditions generated in the current study, the observed attenuation in oxidative phosphorylation capacity may be a consequence of decreased substrate availability, although a lower ATP synthase activity is also a possible explanation. Somewhat conflicting with the hypothesis of a deficit in substrate availability, CS activity, an enzyme of the TCA cycle, was unchanged, or even tended to increase ($P = 0.18$) following exercise. However, previous studies documented divergent effects of exercise on several enzymes of

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the TCA cycle (7, 32). For instance, after five bouts of 1-min cycling exercise to exhaustion some enzymes have been reported to be unchanged (e.g. cytochrome oxidase, citrate synthase), decreased (pyruvate dehydrogenase, α -ketoglutarate dehydrogenase, glutamate dehydrogenase), or even increased (α-Glycerophosphate dehydrogenase, NADH oxidase) (32). Therefore, CS activity could have been maintained in the current scenario, while other enzymes of the TCA such as α-ketoglutarate dehydrogenase or aconitase may well have been decreased and in turn attenuated the production of reducing equivalents.

High-intensity cycling exercise greatly increases free radical levels (37) and, therefore, reactive O_2 species in the muscle bed, and, in particular, hydrogen peroxide (H_2O_2) (38), may be the link connecting exercise to the acute changes in mitochondrial respiratory function observed in the current study. Indeed, there is growing evidence that high-intensity exercise or *in vitro* administration of physiological concentrations of H_2O_2 can inhibit certain redox-sensitive enzymes of the Krebs cycle (α-ketoglutarate, aconitase, and SDH) (7, 32, 39, 40). For instance, during exercise, ROS mediated inhibition of aconitase activity has been suggested to result in citrate accumulation to protect the mitochondria from oxidative damage while inhibiting mitochondrial respiration through the attenuation of reducing equivalents generation (36). According to this scenario, the fall in substrate availability for oxidative phosphorylation would diminish mitochondrial membrane potential, ADP-stimulated mitochondrial respiration (41, 42), and in turn trigger the dynamic remodelling of the mitochondrial network, promoting the morphological changes documented after chronic exercise interventions. Consistent with this scenario, energetic stress has been suggested to activate AMP-activated protein kinase (AMPK), which stimulates mitochondrial biogenesis via peroxisome proliferator-activated receptor-gamma coactivator upregulation, and simultaneously triggers the destruction of existing defective mitochondria through mitophagy (43), resulting in improved mitochondrial quantity and quality. However, in conditions with excessive ROS production, the inhibition of aconitase may abolish citrate accumulation and upregulate the endogenous antioxidant system to restore the redox balance rather than stimulating mitochondrial biogenesis (36).

High-intensity exercise is commonly associated with marked metabolic disturbances that can also influence mitochondrial function. Specifically, during high-intensity exercise, inorganic phosphate, lactate, and protons accumulate in the exercising muscle (8) and have the potential to inhibit mitochondrial respiration (44). However, experimental evidence in permeabilized skeletal muscle fibers or isolated mitochondria suggests that the effects of these metabolic by-products are limited to certain non-physiological conditions (non-phosphorylating mitochondria) (45), or only appear to influence the sensitivity of respiration to some substrates without affecting maximal ADP-stimulated respiration (46). It is therefore unclear to what extent the large metabolic disturbance occurring during a 5 km cycling time-trial (8) would contribute to the impaired oxidative phosphorylation capacity observed here.

Muscle contraction generates heat which has also the potential to modulate mitochondrial respiration. For instance, heat stress prior to muscle sampling decreased state 2 and state 3_{CI} respiration without affecting muscle respiratory capacity (state 3_{CI+CII}) in *Notolabrus celidotus* (47). Also, increasing the temperature of isolated mitochondria or permeabilized

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skeletal muscle fibers, *in vitro,* augmented state 2 respiration to a larger extent than state 3_{CI+CII}, resulting in a diminished respiratory control ratio and suggesting structural damage to the mitochondria (47–49). These previous findings diverge from the decreased muscle respiratory capacity and state 3_{CII} observed in the current study (Figure 2). In addition, in this study, state 2 respiration was not significantly altered after the exercise, and only one sample (not included in the analysis) demonstrated an accelerated respiration rate with cytochrome c, implying that mitochondrial membrane integrity was preserved in these experimental conditions. Together, these findings suggest that it is unlikely that an exercise-induced increase in muscle temperature could explain the detrimental effect of high-intensity aerobic exercise on mitochondrial function.

A transient mitochondrial defect?

It should be noted, that using the current experimental design, it cannot be determined if the apparent exercise-induced attenuation in muscle respiratory capacity was transient or permanent. However, based upon the animal literature (12), it is reasonable to assume that muscle respiratory capacity was restored within several hours. Also, it is well-established that, across several days, multiple high-intensity exercise sessions, ultimately, improve muscle respiratory capacity (6, 50). Interestingly, whereas the time course for the restoration of respiratory capacity post-exercise in humans is still unknown, the molecular processes involved in mitochondrial functional recovery/improvement following exercise have been the focus of many studies. Specifically, after a single exercise session, a rapid (<4h) upregulation of transcriptional factors such as peroxisome proliferator-activated receptor gamma coactivator 1- α and β (PGC-1α and PGC-1β), and peroxisome proliferator-activated receptors β/δ (PPARβ/δ) occurs transiently. Thereafter (<24h) this is followed by an upregulation of PGC-1α and PPARα protein expression, suggesting a rapid activation of the pathways responsible for mitochondrial biogenesis (51). It is, however, important to note that this effect is thought to be cumulative, such that successive sessions are required to increase muscle mitochondrial content (50, 51). Concurrently, exercise stimulates the degradation of damaged cellular components from the mitochondria through autophagy. Some proteins involved in this process (light chain 3 I and II, and the autophagy adaptor protein p62) have been documented to be modulated within 2 hours following an exercise bout (52). This exercise-induced autophagy is intensity dependent (53), and seems to be targeting oxidatively damaged proteins (52). Besides mitochondrial biogenesis and autophagy, exercise can also rapidly (~10min) increase mitochondrial membrane interactions (28). However, changes in the abundance of key regulatory proteins involved in mitochondrial dynamic remodeling, again, seems to require multiple exercise sessions (51). Future studies investigating, in parallel, the time course of the restoration of mitochondrial function and these molecular mechanisms (biogenesis, mitochondrial dynamics, and autophagy) could provide valuable information which would improve our understanding of the adaptations in muscle in response to acute and chronic exercise.

Physiological and practical implications for muscle performance:

Despite the large magnitude of the decrease in state 3CI+CII (~40%) immediately after high-intensity exercise, it is unlikely that the fall in mitochondrial respiratory capacity observed herein limited exercise performance during a 5 km cycling time-trial. As illustrated

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in Figure 1, subjects maintained their VO_{2peak} and even increased power output (8) during the last kilometer of the time-trial, suggesting that the acute decrease in mitochondrial capacity was not a limiting factor for VO_{2max} and exercise performance. This interpretation is also supported by several pieces of evidence collected in untrained individuals suggesting that muscle mitochondrial respiratory capacity is in excess of peripheral O_2 delivery during cycling (36). For instance, in agreement with the present results, it was recently reported that prolonged low-intensity skiing in the arctic for 6 weeks decreased muscle mitochondrial respiratory capacity by ~20% whereas peak leg and pulmonary VO_2 remained unaltered (54). Therefore, taken together, these findings suggest that it is unlikely that exercise performance during the time-trial was limited by a shortage in ATP generated by the mitochondria.

It also remains to determine whether the transient mitochondrial defect, recognized herein, may have indirectly contributed to the exercise-induced development of fatigue by exagerating the accumulation of metabolites (e.g. inorganic phosphates, ADP, or protons), increasing the firing of type III/IV afferent fibers (8), which are involved in the regulation of peripheral fatigue, or by potentiating other stress signals originating from the myocyte. In this regard, albeit using a very different experimental paradigm, it has recently been suggested that mitochondria have the potential to perturb whole-body physiological responses to stress and endocrine signals between organs via mechanisms that remain to be elucidated (55). Although still speculative at this point, this potential role of the mitochondria in the development of exercise-induced fatigue, and as a mediator of the stress-response to exercise, is of interest and warrants further investigation.

Finally, the concept of “mitohormesis” postulates that stress induces an adaptive response and triggers defence mechanisms that prevent mitochondrial damage during a subsequent similar stress (56). With this concept in mind, the impairment in mitochondrial respiratory function observed immediately after high-intensity cycling exercise, could trigger the dynamic remodelling of the mitochondrial network and promote the morphological changes reported after chronic exercise interventions. Therefore, while additional studies are warranted, mitochondrial hormesis may be one mechanism by which high-intensity exercise appears to be a suitable exercise training method when specifically targeting peripheral muscle adaptations.

Experimental considerations

It is noteworthy that in this study the inhibition of respiration with combined antimycin A and oligomycin was used to correct for residual O_2 consumption, indicative of non-mitochondrial O_2 consumption (i.e. from chemical and instrument-related respiration in the chamber), which resulted in apparently lower respiration rates compared to previously published values. Without such a correction, the respiration rates for complex I+II substrates at baseline are within the range of values previously reported by our group for healthy adults using a similar protocol and the same instrument (57–59). We also conducted some additional experiments in permeabilized skeletal muscle fibers to compare the respiratory rates obtained with the Hansatech system and a widely used apparatus (O2k-core, Oroboros, Austria) to determine whether the lower rates consistently observed by our group could be

attributed to the instrumentation employed. The muscle fibers for both experiments were obtained from the same sample of tissue (gastrocnemius muscle) and prepared similarly for the test by the same investigator. Other than initial [O2](O2k:~300 μM.ml^{-1}; Hansatech: 180 μM.ml^{-1}) the experimental conditions (T:37°C) and substrates concentrations were the same. We found that the respiratory rate (state 3 CI+II) was 1.7 fold higher with the O2k-core (64 pM.mg-1.s-1) compared to the Hansatech (37 pM.mg^{-1} .s^{-1}). This result strongly suggests that different instrumentation rather than muscle preparation contributed to the lower rates consistently reported by our group. Furthermore, using a similar preparation, we have previously reported a close correlation between the PCr recovery time constant measured by ^{31}P-MRS and state 3_{CI+II} (59), a significant correlation between uncoupled$_{CIV}$ respiration and CS activity (58), and an excess mitochondrial capacity *in vitro* compared to whole-body VO_2peak (60). We are therefore very confident that the respiratory rates reported in this study suitably reflect muscle respiratory capacity in these, *in vitro*, experimental conditions.

Conclusion

The present study revealed that, largely as a consequence of attenuated complex II-specific respiration, skeletal muscle oxidative phosphorylation capacity is diminished immediately following high-intensity aerobic whole body exercise in healthy active adults. This transient mitochondrial defect induced by high-intensity cycling exercise might amplify the exercise-induced development of fatigue and play an important role in initiating exercise-induced mitochondrial adaptations.

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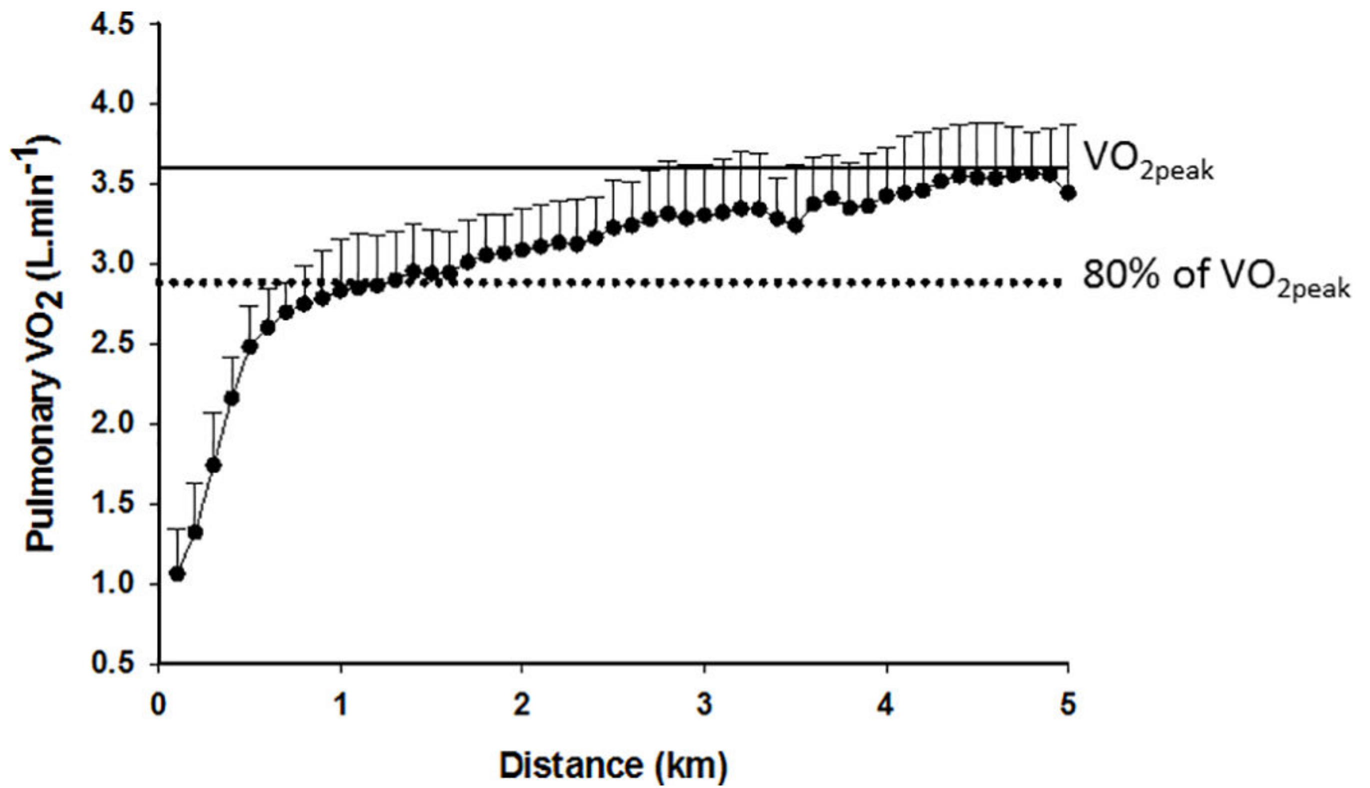

Figure 1. Pulmonary oxygen consumption (VO_2) during the 5 km cycling time-trial.

The dashed line indicates the oxygen consumption corresponding to 80% of the group VO_{2peak}, while the solid upper line represents the group VO_{2peak}. Values are presented as mean ± SD.

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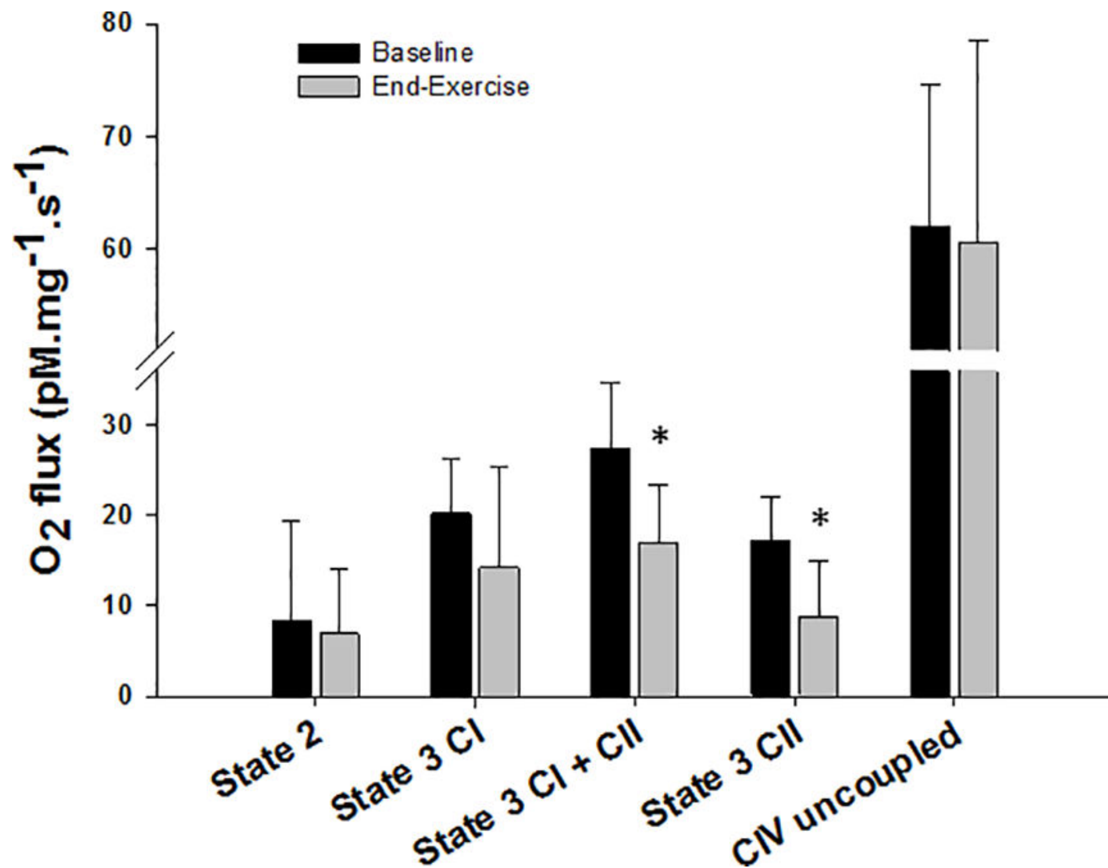

Figure 2. Skeletal muscle respiratory fluxes (oxygen ([O_2] consumption rates per mg of tissue) at resting baseline and immediately following a 5 km time-trial.

The following substrates were used: State 2: malate + glutamate, State 3 CI: malate + glutamate + ADP, State 3 CI+II: malate + glutamate + succinate + ADP, State 3 CII: malate + glutamate + succinate + ADP + rotenone (complex I inhibitor), and uncoupled$_{CIV}$ respiration, corresponding to ETC capacity: malate + glutamate + succinate + ADP + rotenone + antimycin A (complex III inhibitor) + oligomycin (complex V inhibitor) + Ascorbate + TMPD. Values are presented as mean ± SD after correction for non-mitochondrial O_2 consumption. *, $P < 0.05$; significantly different from baseline.

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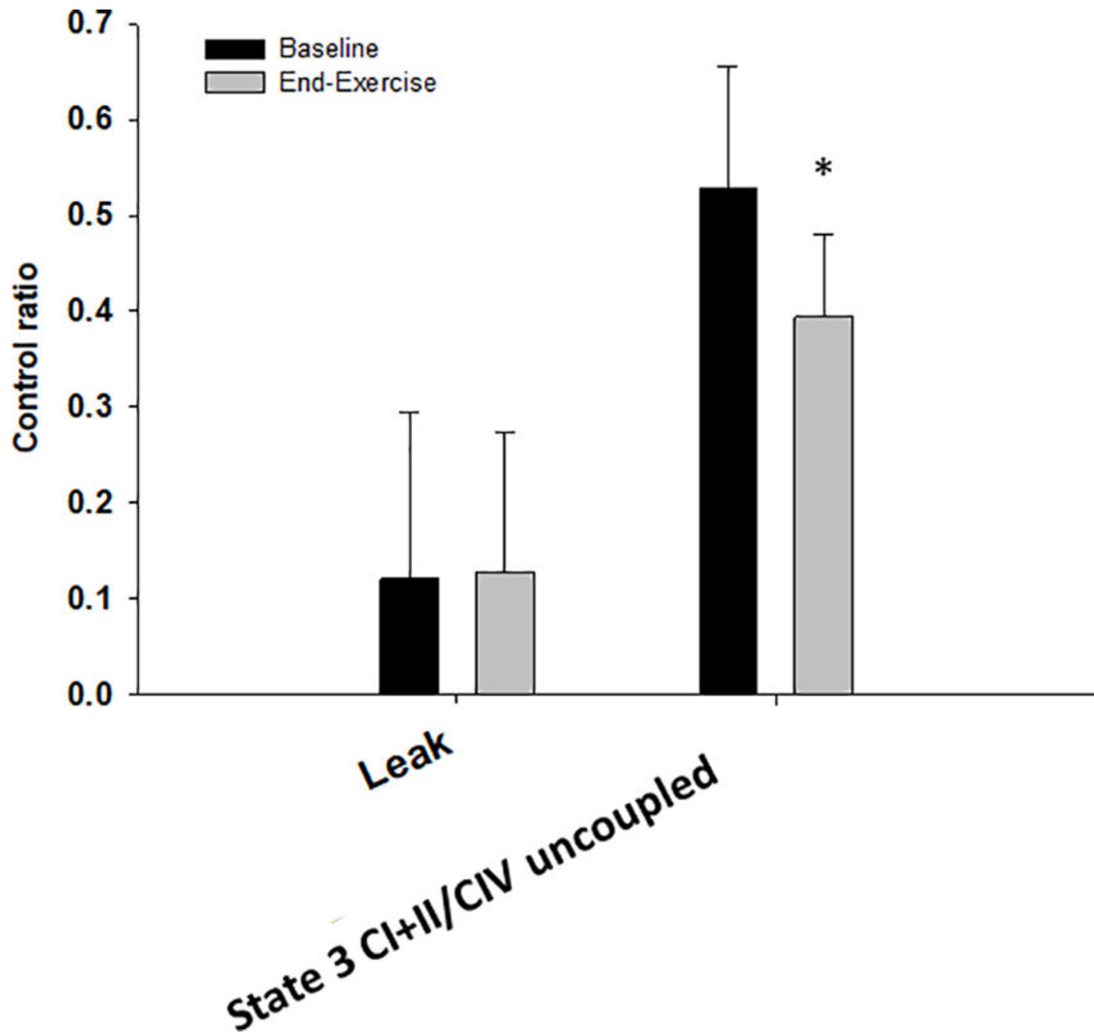

Figure 3. Respiratory control ratio for leak (state 2/uncoupled$_{CIV}$ respiration) and the ratio between state 3_{CI+CII} and uncoupled$_{CIV}$ respiration, an index of the relative excess capacity of cytochrome-c oxidase, at resting baseline and immediately following a 5 km time-trial.

Values are presented as mean ± SD. *, $P < 0.05$; significantly different from baseline.