A short period of high-intensity interval training improves skeletal muscle mitochondrial function and pulmonary oxygen uptake kinetics

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Christensen PM, Jacobs RA, Bonne T, Flück D, Bangsbo J, Lundby C. A short period of high-intensity interval training improves skeletal muscle mitochondrial function and pulmonary oxygen uptake kinetics. J Appl Physiol 120: 1319–1327, 2016. First published February 4, 2016; doi:10.1152/japplphysiol.00115.2015.—The aim of the present study was to examine whether improvements in pulmonary oxygen uptake (Vo$_2$) kinetics following a short period of high-intensity training (HIT) would be associated with improved skeletal muscle mitochondrial function. Ten untrained male volunteers (age 26 ± 2 yr; mean ± SD) performed six HIT sessions (8-12 × 60 s at incremental test peak power; 271 ± 52 W) over a 2-wk period. Before and after the HIT period, Vo$_2$ kinetics was modeled during moderate-intensity cycling (110 ± 19 W). Mitochondrial function was assessed with high-resolution respirometry (HRR), and maximal activities of oxidative enzymes citrate synthase (CS) and cytochrome c oxidase (COX) were accordingly determined. In response to HIT, Vo$_2$ kinetics became faster ($\tau$: 20.4 ± 4.4 vs. 28.9 ± 6.1 s; $P < 0.01$) and fatty acid oxidation (ETF$_{p}$) and leak respiration (L$_{Leak}$) both became elevated ($P < 0.05$). Activity of CS and COX did not increase in response to training. Both before and after the HIT period, fast Vo$_2$ kinetics (low $\tau$ values) was associated with large values for ETF$_{p}$, electron transport system capacity (ETS), and electron flow specific to complex II (CI$$_{II}$p) ($P < 0.05$). Collectively, these findings support that selected measures of mitochondrial function obtained with HRR are important for fast Vo$_2$ kinetics and better markers than maximal oxidative enzyme activity in describing the speed of the Vo$_2$ response during moderate-intensity exercise.

high-intensity training; oxygen uptake; enzyme activity; OXPHOS; cycling economy

NEW & NOTEWORTHY

The mechanism(s) facilitating pulmonary Vo$_2$ kinetics following a training intervention remains incompletely understood. Here we provide evidence that exercise training-induced improvement in skeletal muscle mitochondrial function coincides with concurrent improvements in pulmonary Vo$_2$ kinetics.

PULMONARY OXYGEN UPTAKE (Vo$_2$) increases at the onset of constant-load moderate-intensity exercise and reaches a steady state within a few minutes (31, 56). Following the initial cardiodynamic phase (the delay time for deoxygenated blood from the exercising muscles to reach the pulmonary circulation), the increase in pulmonary Vo$_2$ follows a monoexponen-

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mated flux through the Krebs cycle, leading the authors to suggest that this enzyme likely is a poor predictor of Krebs cycle capacity (4). Therefore total mitochondrial content or maximal activities of oxidative enzymes may lack the sensitivity necessary to serve as precise markers of alterations in metabolic inertia and, hence, changes in VO2 kinetics following training interventions, at least if the enzyme activity is abundant in relation to system capacity. In contrast, high-resolution respirometry (HRR) allows for the integrative study of respiratory capacities in intact mitochondria from permeabilized muscle fibers subjected to a specific titration protocol of various substrates in combination with high abundance of oxygen in the respiratory chamber (41). Thus HRR can differentiate between simple changes in enzymatic expression vs. an alteration in the efficiency as well as functional capacity of mitochondria. In support, skeletal muscle oxidative capacity (OXPHOS capacity) and electron system capacity (ETS), obtained via HRR, correlate with time-trial performance in trained cyclists, whereas CS activity does not (23). Other studies performed on trained cyclists also indicate that CS activity is not a key determinant of performance (10, 54).

Hence, HRR may reflect endurance capacity of the muscle better than any single measure or collection of isolated enzyme activities, yet it is unclear if this is also the case when studying pulmonary VO2 kinetics, which provides a close reflection of mitochondrial function. In support, skeletal muscle oxidative capacity (OXPHOS capacity) and electron system capacity (ETS), obtained via HRR, correlate with time-trial performance in trained cyclists, whereas CS activity does not (23). Other studies performed on trained cyclists also indicate that CS activity is not a key determinant of performance (10, 54).

Accordingly, the aims of the present study were to 1) examine whether improvements in mitochondrial function assessed by HRR in addition to maximal oxidative enzyme activities would be found together with faster pulmonary VO2 kinetics following a short period of high-intensity training in previously untrained individuals; and 2) evaluate if fast VO2 kinetics is associated with measures of mitochondrial function. We hypothesized that training would lead to an increased skeletal muscle respiratory capacity (OXPHOS and ETS) allowing for a faster VO2 response when substrate for mitochondrial respiration (ADP, inorganic phosphate, and oxygen) is present. Moreover, we hypothesized that oxidative enzyme activity would increase and that measures of mitochondrial function obtained via HRR would reveal a greater association with VO2 kinetics than the maximal activity of oxidative enzymes.

METHODS

Subjects. Ten untrained male volunteers (age 26 ± 2 yr, body mass 77 ± 9 kg; mean ± SD) participated in the present study. This group of subjects was a subset of a larger group (n = 17) that participated in a study that investigated the mechanisms explaining the rapid improvements in exercise performance in untrained subjects following high-intensity training (26). The study procedures were approved by the Ethical committee for the ETH Zürich (EK 2011-N-24), in accordance with the Declaration of Helsinki, and all subjects gave their oral and written informed consent to participate in the study after receiving oral and written information describing the study procedures.

Study overview. Subjects performed testing for VO2 kinetics and had a muscle biopsy taken before and after a ~2-wk period with high-intensity training (HIT). A stationary cycle ergometer (Monark 839E, Varberg, Sweden) was used for testing of VO2 kinetics. The ergometer was set in power mode (meaning that power was fixed irrespective of cadence). Change in load on the flywheel was controlled automatically by an external computer connected to the cycle ergometer that registered cadence every second. The HIT sessions were also performed on a cycle ergometer with a fixed power mode (Cycle Ops 420 Pro Indoor, Saris Cycling Group).

Training intervention. During the 2-wk training period, subjects performed a total of six training sessions separated by 1–2 days of recovery as described previously (26). Training sessions encompassed repeated 60-s intervals at a fixed intensity corresponding to peak power during incremental testing before HIT (271 ± 52 W; protocol consisting of 3 steps at 50, 100, and 150 W for 5 min each followed by 30 W increments every 90 s). Subjects were given verbal support during the test, and the test terminated when subjects stopped or when cadence dropped below 60 rpm. In the training sessions each interval was separated by 75 s of recovery at 30 W. Eight intervals were performed in the first two training sessions, 10 intervals in the third and fourth sessions, and 12 intervals in the last two sessions.

VO2 kinetics. Pulmonary VO2 was assessed breath by breath prior to and again following HIT training using the Innocor system (Innovision, Odense, Denmark), which has a reported average “within day” variation during moderate-intensity cycling at 130 W (−2,000 ml/min) of 20 ml/min (95% confidence interval: −20–60 ml/min) and “day to day” variation of 10 ml/min (~50–60 ml/min) (14). Before each test, the equipment was calibrated using a 3-liter syringe for ventilation and O2 and CO2 concentration by using a calibration gas mixture. While wearing a nose-clip, subjects breathed through a mouthpiece during the exercise tests. Subjects performed three constant-load intervals at moderate intensity (110 ± 19 W; 40% incremental test peak power) lasting 6 min and separated by 20 min of recovery. All intervals were preceded by 2 min baseline cycling at 20 W. Subjects were free to choose the cadence they cycled during their recovery. All intervals were performed on a cycle ergometer with a fixed power mode (Protocol HIT sessions 19 W; 40% incremental test peak power) lasting 6 min and separated by 20 min of recovery. All intervals were preceded by 2 min baseline cycling at 20 W. Subjects were free to choose the cadence they cycled during their first interval (64 ± 7 rpm), which was then maintained (~5 rpm) throughout the remaining intervals. For the determination of VO2 kinetics, errant breaths, defined as any value lying more than 4 SDs away from the local mean (e.g., caused by swallowing and coughing), were removed. Subsequently, the VO2 responses for the three transitions were linearly interpolated to give 1-s values and then averaged. The initial cardiodynamic component was ignored by eliminating the first 20 s of data after the onset of exercise. The data were fitted using a monoeponential model:

\[ VO2(t) = VO2_{baseline} + A \left[ 1 - e^{-\left( t - Td \right)^a} \right] \]

with VO2(t) being oxygen uptake to a given time (s), VO2 baseline was calculated as average from 30 to 90 s of the 120-s baseline cycling at 20 W. A, Td, and \( \tau \) are the amplitude, time delay, and time constant, respectively, for the primary response. Three intervals were chosen, as this number of repetitions is known to reduce the 95% confidence interval surrounding the calculation of \( \tau \) relative to using only a single transition (3, 34).

Muscle sampling. Muscle biopsies were obtained under standardized conditions from vastus lateralis muscle at baseline and again after the 2-wk regimen of HIT. Samples were collected under local anesthesia (1% lidocaine) of the skin and superficial muscle fascia, using the Bergstrom technique with a needle modified for suction. The biopsy was immediately dissected free of fat and connective tissue and divided into sections for high-resolution respirometry and protein quantification with the latter sections being frozen immediately in liquid nitrogen and stored at −80°C until analysis. All biopsies were obtained 48 h following the last bout of exercise.

Respirometric analysis. All procedures regarding skeletal muscle preparation and HRR have been previously described in detail (26). Briefly, fat and connective tissue were first removed from the biopsies. Then the muscle tissue was added to the respiration chamber using a mitochondrial respiration medium 06 [MiR06; MiR05 + catalase 280 IU/ml at 37°C (Oroboros, Innsbruck, Austria)], with standardized instrumental and chemical calibrations performed prior to all measurements. Respirometric analyses were done in duplicate, and all titrations were added in series as presented (Fig. 1). To avoid

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a diffusional limitation, the oxygen concentration in the chamber was kept between 250 and 420 nmol/ml. Leak respiration in absence of adenylates (LOMY) was induced with the addition of malate (2 mM) and octanoyl carnitine (0.2 mM). The LN state represents the resting oxygen consumption of an unaltered and intact electron transport system free of adenylates. Maximal electron flow through electron transferring flavoprotein (ETF) representing fatty acid oxidative capacity (ETFp) was determined following the addition of ADP (5 mM). In the ETFp state, the ETF-linked transfer of electrons requires the metabolism of acetyl-CoA, and hence, the addition of malate, in order to facilitate convergent electron flow into the Q junction from both complex I (Cl) and ETF, allowing beta-oxidation to proceed. The contribution of electron flow through Cl is far below capacity, and so, here, the rate-limiting metabolic branch is electron transport through ETF, such that malate + octanoyl carnitine + ADP-stimulated respiration is representative of, rather than specific to, electron capacity through ETF (24). Submaximal state 3 respiratory capacity (P) specific to complex I (CIIP) was induced following the additions of pyruvate (5 mM) and glutamate (10 mM). Maximal state 3 respiration, oxidative phosphorylation capacity, was then induced with the addition of succinate (10 mM). This maximal state 3 respiratory state represents the cellular respiration that is resultant to saturating concentrations of ADP and substrate supply for both complex I and complex II (Cl+CIIP; OXPHOS capacity). Convergent electron input to complex I and II provides higher respiratory values compared with the isolated respiration of either complex I (pyruvate/glutamate + malate or glutamate + malate) or complex II (succinate + rotenone) (12, 45). Consequently, CI+CIIP presents more physiological relevance to the study of mitochondrial function (6) and is necessary to establish confirmation of a complete and intact electron transport system. CI+CIIP demonstrates a naturally intact electron transport system’s capacity to catalyze a sequential set of redox reactions that are partially coupled to the production of ATP via ATP synthase. Compared with a correspondent leak state with an equivalent substrate supply, CI+CIIP maintains a lower electrochemical gradient across the inner mitochondrial membrane. That gradient is dictated by the degree of coupling to the phosphorylation system (12, 41). The mitochondrial outer membrane was assessed with the addition of cytochrome c (10 μM). There was no evidence of any compromised mitochondrial membrane integrity across samples measured at baseline with the titration of exogenous cytochrome c (87.4 ± 18.7 to 87.2 ± 18.7 pmol O2·min⁻¹·mg wet wt⁻¹; P = 0.56) or following 2 wk of HIT (100.4 ± 23.6 to 100.82 ± 23.8 pmol O2·min⁻¹·mg wet wt⁻¹; P = 0.19). Oligomycin was added, inhibiting ATP synthase to achieve oligomycin-induced leak respiration (LOMY). The LOMY state is the leak state corresponding to CI+CIIP and is comparable with classic state 4 respiration (8). In LOMY, the chemiosmotic gradient is at maximum, resultant to the combination of maximal substrate supply and inhibition of ATP synthase. Oxygen flux is at a minimum and is representative of proton leak, slip, cation cycling, and overall dyscoupling (6, 12, 45). Phosphorylative restraint of electron transport was assessed by uncoupling ATP synthase (complex V) from the electron transport system with the titration of the proton ionophore, carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP; steps of 0.5 μM) reaching electron transport system (ETS) capacity. The inner mitochondrial membrane potential is completely collapsed, with an open transmembrane proton circuit, in the ETS respiratory state. The uninhibited flow of electrons through the respiratory system can, therefore, indirectly serve as an indication of maximal mitochondrial membrane potential. Rotenone (0.5 μM) and antimycin A (2.5 μM) were added, in sequence, to terminate respiration by inhibiting complex I and complex III (cytochrome bc1 complex), respectively. With complex I inhibited, electron flow specific to complex II (CIIP) can be measured. Prior uncoupling with FCCP has no effect on oxygen flux during CIIP (24), as individual electron input to succinate dehydrogenase does not saturate the Q cycle. Finally, ascorbate (2 mM) and N,N,N’,N’-tetramethyl-p-phenylenediamine (TMPD) (0.5 mM) were simultaneously titrated into the chambers to assess cytochrome c oxidase (COX), complex IV, activity.

CS activity was assayed in muscle lysates using a commercially available CS assay kit (CS0720, Sigma-Aldrich) according to the manufacturer guidelines with minor adjustments. All activities were normalized to milligram protein.

Statistics. Changes in VO₂ kinetics, respiratory states, and aerobic enzymes following the training period were examined with a Wilcoxon signed rank test given our small sample size as a conservative approach to lower the chance of type I error. Pearson’s correlation coefficient (r) was calculated between variables before and after HIT and also between changes in the speed of the VO₂ response and changes in HRR variables and enzyme activity. To evaluate if a significant association was present between variables, a one-tailed test was used throughout the study with the a priori hypothesis that fast VO₂ kinetics would be associated with high values for the various variables describing mitochondrial function.

RESULTS

Pulmonary VO₂ kinetics. The primary VO₂ response was faster than before HIT (r: 20.4 ± 4.4 vs. 28.9 ± 6.1 s; P = 0.004) whereas end-exercise VO₂ (4–6 min: 2,261 ± 311 vs. 2,304 ± 304 ml/min; P = 0.16) was not altered (Figs. 2 and 3 and Table 1).

High-resolution respirometry of skeletal muscle tissue. Higher values relative to before HIT were observed for LN (10 ± 3 vs. 8 ± 1 pmol O2·min⁻¹·mg wet wt⁻¹; P = 0.049)

![Fig. 1. Changes in oxygen concentration (left; high starting values) and oxygen flux (right; low starting values) during high-resolution respirometry used for evaluation of mitochondrial function in a representative subject. See METHODS section for details. LN, leak respiration in absence of adenylates; ETFp, capacity for fatty acid oxidation; CI+CIIP, maximal state 3 respiration oxidative phosphorylation capacity; LOMY, oligomycin-induced leak respiration; ETS, electron transport system capacity; CIIP, electron flow specific to complex II in the mitochondria.](http://jap.physiology.org/ by 10.220.33.6 on October 14, 2017)
response to HIT was observed for CIIP (68 ± 19 pmol O$_2\cdot$min$^{-1}$; $P = 0.02$) (Fig. 4B). No significant increase in response to HIT was observed for CI (68 ± 14 vs. 60 ± 13 pmol O$_2\cdot$min$^{-1}$·mg wet wt$^{-1}$; $P = 0.13$) (Fig. 4C), CI+CIIP (100 ± 24 vs. 87 ± 19 pmol O$_2\cdot$min$^{-1}$·mg wet wt$^{-1}$; $P = 0.13$) (Fig. 4D), L$_{OMY}$ (38 ± 8 vs. 34 ± 6 pmol O$_2\cdot$min$^{-1}$·mg wet wt$^{-1}$; $P = 0.38$) (Fig. 4E), ETS (118 ± 30 vs. 99 ± 14 pmol O$_2\cdot$min$^{-1}$·mg wet wt$^{-1}$; $P = 0.06$) (Fig. 4F), and CIIP (74 ± 17 vs. 66 ± 11 pmol O$_2\cdot$min$^{-1}$·mg wet wt$^{-1}$; $P = 0.19$) (Fig. 4G).

Skeletal muscle oxidative enzyme activity. COX (196 ± 51 vs. 160 ± 35 pmol O$_2\cdot$min$^{-1}$·mg wet wt$^{-1}$; $P = 0.10$) (Fig. 4H) and CS (120 ± 35 vs. 128 ± 19 μmol·min$^{-1}$·mg protein$^{-1}$; $P = 0.65$; $n = 9$) (Fig. 4I) were not increased with HIT.

Correlations. Both before and after HIT, fast VO$_2$ kinetics (low values of $\tau$) was associated with large values for ETF$^p$ (P < 0.05) (Fig. 4B), ETS (P < 0.05) (Fig. 4F), and CIIP (P < 0.05) (Fig. 4G). Changes following HIT in $\tau$ were associated with changes in CIIP (P < 0.05) (Fig. 5C) but not ETF$^p$ (Fig. 5A) and ETS (Fig. 5B). In addition, VO$_2$ kinetics was associated with large values for $L_0$ before HIT (P < 0.01) (Fig. 4A) and large values for L$_{OMY}$ (P < 0.05) (Fig. 4E) and CS (P < 0.05) (Fig. 4I) after HIT. COX (Fig. 4H) was a poor predictor of fast VO$_2$ kinetics, and no significant associations were

![Image](https://via.placeholder.com/150)

Fig. 2. Top: pulmonary oxygen uptake (VO$_2$) shown in 5-s intervals for graphical clarity with exponential fits of the response during 6-min of cycling at moderate intensity (110 ± 19 W) before (open circles; A) and after (filled circles; B) a short period of high-intensity training in 10 untrained subjects. Bottom: modeled VO$_2$ response shown before (dashed line) and after training (full line) in absolute values (C) and expressed relative to end-exercise VO$_2$ (D).

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Fig. 3. Mean and individual values for the primary pulmonary VO$_2$ response ($\tau$; the time to attain 63% of the amplitude) (A) and steady-state energy expenditure from 4 to 6 min of exercise (B) during 6-min of cycling at moderate intensity (110 ± 19 W) in 10 untrained subjects before (pre; open bars) and after (post; filled bars) a short period of high-intensity training. **Value post significantly different from pre (P < 0.01).

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Table 1. Changes in pulmonary oxygen uptake (VO$_2$) kinetics during cycling exercise for 6 min at moderate intensity (110 ± 19 W) in 10 untrained subjects before (pre) and after (post) a short period of high-intensity training:

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline, ml/min</td>
<td>909 ± 121</td>
<td>923 ± 100</td>
</tr>
<tr>
<td>Td, s</td>
<td>11.4 ± 4.0</td>
<td>17.5 ± 3.3*</td>
</tr>
<tr>
<td>$\tau$, s</td>
<td>28.9 ± 6.1</td>
<td>20.4 ± 4.4*</td>
</tr>
<tr>
<td>A, ml/min</td>
<td>1393 ± 256</td>
<td>1337 ± 286</td>
</tr>
<tr>
<td>End-exercise VO$_2$ (4–6 min), ml/min</td>
<td>2304 ± 304</td>
<td>2261 ± 311</td>
</tr>
<tr>
<td>Delta VO$_2$ (4–6 min), ml/min</td>
<td>21 ± 49</td>
<td>−20 ± 42</td>
</tr>
</tbody>
</table>

Td, time delay, $\tau$, the time to attain 63% of the amplitude of the response, A, amplitude. *Value post significantly different from pre (P < 0.01).
present either with CI (Fig. 4C) or CI + CIIP (Fig. 4D). The functional gain was only associated with LOMY pre-HIT ($P < 0.05$).

**DISCUSSION**

The primary and novel finding in the present study was that dynamic measures of mitochondrial function in the form of high electron transport system capacity (ETS), fatty acid oxidation (ETFP), and respiratory capacity specific to complex II (CIIP) were associated with the speed of the primary VO$_2$ response during moderate-intensity cycling, both before and after a 2-wk high-intensity training intervention in previously untrained subjects. Moreover, ETF$_P$ increased by training, and ETS strongly tended to increase together with the attainment of faster VO$_2$ kinetics.

The faster VO$_2$ kinetics during moderate exercise was observed after just six high-intensity training sessions with a total training volume of ~60 min for the entire training period. This is in line with previous studies in which a speeding of the VO$_2$ response has been observed after as little as two to six sessions with either high-intensity low-volume training (1, 38) or low-intensity high-volume training (38, 42). In the present study the faster VO$_2$ kinetics was attained together with improved oxidative muscle capacity in the form of increased leak respiration in the absence of adenylates (LN) (Fig. 4A) and fatty acid oxidative capacity (ETFP) (Fig. 4B). In turn, the majority of the remaining variables assessed with HRR tended to increase ($P < 0.05, <0.15$), demonstrating that the intense albeit brief training period provided a potent stimulus for the variables measured with HRR. Moreover, the observation of associations both before and after HIT between fast VO$_2$ kinetics and selected HRR measures (Fig. 4, B, F, and G) does suggest that these associations were not incidental, also in light of the faster VO$_2$ response attained after HIT together with increases in

![Fig. 4. Associations between the speed of the primary pulmonary VO$_2$ response ($\tau$; the time to attain 63% of the amplitude) cycling at moderate intensity (110 ± 19 W) and variables describing mitochondrial function in 10 untrained subjects before (pre; open circles and bars) and after (post; filled circles and bars) a short period of high-intensity training. A: LN, leak respiration without adenylates. B: ETF$_P$, capacity for fatty acid oxidation. C: CIIP, respiration capacity specific to complex I in the mitochondria. D: CI + CIIP, maximal state 3 respiration OXPHOS capacity. E: LOMY, oligomycin-induced leak respiration. F: ETS, electron transport system capacity. G: CIIP, electron flow specific to complex II in the mitochondria. H: COX, cytochrome c oxidase activity in complex IV. I: CS, citrate synthase activity ($n = 9$). Asterisk post significantly different from pre ($P < 0.05$).](http://jap.physiology.org/10.1152/japplphysiol.00115.2015)
ETF_p (Fig. 4B) and the strong tendency for an increase in ETS (Fig. 4F). Future studies remain to be done to see if similar findings are observed, also with the use of higher exercise intensities as well as different subject groups (elite athletes, patient groups). In addition, an even larger sample size to increase the statistical power is also considered of importance. However, on the basis of the present findings, HRR appears to be a promising tool in elucidating the various components of primary V\textsubscript{O}_2 response. Collectively, the present findings suggest that a reduced metabolic inertia was implicated in the faster V\textsubscript{O}_2 kinetics following the training period. Nonetheless, it cannot be ruled out that the faster V\textsubscript{O}_2 response following training was at least partly due to higher bulk blood flow (32, 52) and/or improved local distribution of blood flow to the contracting fibers (38) during the initial phase of exercise, although limited evidence supports the idea that increases in oxygen delivery speed V\textsubscript{O}_2 kinetics at moderate intensity (22, 37, 40, 57). Nevertheless, factors other than HRR-derived measures could be important for fast V\textsubscript{O}_2 kinetics at moderate intensity, which is supported by the observation that single HRR measures in the present study “only” explained ~30–50% of the variation in the speed of the V\textsubscript{O}_2 kinetics. A large proportion of slow-twitch fibers could be considered of importance for fast V\textsubscript{O}_2 kinetics since this fiber type appears to contain more aerobic enzymes than fast-twitch fibers (12). This is supported by findings from a cross-sectional study in which a large proportion of slow-twitch fibers was associated with fast V\textsubscript{O}_2 kinetics during heavy- but not moderate-intensity exercise (43). In line herewith, it is worth emphasizing that it likely is the quality (training) of the mitochondria rather than fiber type per se that is of physiological functional importance, since highly trained subjects have been found to have similar aerobic enzyme activity in pools of slow- and fast-twitch muscles (27). Irrespective of “mitochondrial training status,” creatine phosphate content in the various fiber types may also influence V\textsubscript{O}_2 kinetics. Accordingly, slow-twitch fibers have less creatine phosphate than fast-twitch fibers (17), and recently it has been observed that an increase in the creatine phosphate content through dietary interventions leads to slower V\textsubscript{O}_2 kinetics during intense exercise (11). Moreover, middle-distance runners (800-1,500 m) had slower V\textsubscript{O}_2 kinetics during moderate-intensity running than long-distance runners (5,000–10,000 m) (29), which may relate in part to an expected higher fast-twitch composition in the former group (46) and hence a higher creatine phosphate pool. Change in muscle fiber composition was not assessed in the present study, so the potential influence from this cannot be evaluated. Uncoupling protein 3 (UCP3) may influence metabolism during rest and exercise (50). Therefore faster V\textsubscript{O}_2 kinetics in theory could arise from higher UCP3 levels (higher V\textsubscript{O}_2 for the same workload in both the transient phase and during steady state), but that seems unlikely since UCP3 has been found to be lowered in the endurance-trained relative to the untrained state (49).

An increase in L\textsubscript{N} in response to training (Fig. 4A) suggests loss of coupling efficiency across the electron transport system. Change in mitochondrial efficiency in response to exercise training is far from understood. L\textsubscript{N} positively correlates with training status ranging from normal individuals to elite athletes when measured near sea level (25). Both findings suggest that improvements in respiratory capacity with training may come at the expense of some loss of mitochondrial efficiency in environments near sea level. However, the functional significance of a lowering of mitochondrial efficiency (increase in L\textsubscript{N}) is unclear in light of the unchanged steady-state V\textsubscript{O}_2 after the HIT period and the finding that highly trained cyclists do not appear to have a lower energy requirement during exercise than untrained subjects (39). Electron coupling efficiency was observed to improve while respiratory capacities decreased in individuals staying at high altitude for 4 wk (24). Throughout that study, subjects maintained their level of fitness. Collectively, these findings suggest that the regulation of mitochondrial efficiency may be dependent on the environmental oxygen pressure. To evaluate whether oxygen pressure during training or recovery impacts on changes in V\textsubscript{O}_2 kinetics through distinct response on L\textsubscript{N}, it will be of interest to conduct a training study on a group of untrained subjects—having a large improvement potential—exposed to either normoxia or hypoxia.
Whether the observed increased fatty acid oxidative capacity after HIT (Fig. 4B) was of importance for facilitating fast VO\(_2\) kinetics is not clear, owing to the fact that studies typically evaluated fatty acid turnover either from the bloodstream or from muscular stores later in exercise (>5 min) (13, 47), which is a time when the rapid increase in VO\(_2\) (0–1 min) has passed. Breakdown of intramuscular triacylglycerol during moderate-intensity exercise was not apparent after 90 min in one study (47) whereas it tended to be lowered (with ~20%) after 5 min in another study also reporting that uptake of free fatty acids from the blood amounted to 11% of the energy turnover during 60 min of exercise (13). Thus the relative contribution to the aerobic energy turnover in the first minute of exercise from fatty acid metabolism is probably low. However, a diet high in fat has recently been reported to slow VO\(_2\) kinetics (44), hereby showing that fat as a substrate likely impacts on the speed of the VO\(_2\) kinetics. Therefore it seems plausible that a high mitochondrial capacity for oxidizing fat may play a role in the speed of the VO\(_2\) response. Moreover, it may be that intramuscular triacylglycerol is a substrate that actually contributes more than previously expected to the increase in VO\(_2\) during a rest-to-moderate-exercise transition, which could be caused by the close vicinity to the muscle mitochondria.

ETS represents maximal uncoupled respiration and hence the maximal velocity that electrons can travel from complex I through IV in mitochondria, independent of efficiency, and this likely reflects maximal oxidative capacity of the muscle (24). In turn, it also appears to be a valid indicator of how rapid oxidative metabolism can be accelerated on the basis of the present findings (Fig. 4F). During exercise, respiration is considered to be coupled to ADP phosphorylation, and thus it needs to be considered whether oxidative capacity (CI + CIIP, maximal state 3 respiration) is more relevant to an in vivo situation. Nevertheless, ETS and not CI + CIIP was linked with fast VO\(_2\) kinetics, but it is worth noting that ETS and CI + CIIP were associated in the present study (\(r^2 = 0.58\) and 0.90 pre- and post-HIT, respectively). The correlation of fast VO\(_2\) kinetics with the ETS respiratory state, opposed to CI + CIIP, may reiterate implications of our data suggested by the correlation of LS. Adaptation to exercise training, especially that observed following several sessions of high-intensity low-volume exercise near sea level, may come at the expense of mitochondrial efficiency, and thus fast VO\(_2\) kinetics and ETS correlate better than measures of CI + CIIP. These interpretations require further research.

Maximal activities of COX and CS were not augmented by training, although a significant increase in COX was identified in the larger sample size that the subjects in the present study were a part of (26). In training studies of similar duration, COX activity has been observed to increase with training (7, 36), CS was not elevated by training, which has been observed by some (42) but not all training studies of similar short duration (7, 36, 53). In the present study, COX, an established biomarker of mitochondrial volume density (35), was a poor predictor of fast VO\(_2\) kinetics both before and after the intervention, suggesting that this enzyme per se is not a good marker of the metabolic inertia in untrained subjects. CS activity after the training period was associated with fast VO\(_2\) kinetics, but since this was not the case before the intervention this may reflect that the association post-HIT was incidental or measuring error in CS activity pre-HIT. During aerobic metabolism, CS is a catalyst early in the Krebs cycle whereas COX is a catalyst later in the oxidative phosphorylation. The lack of association between COX and the speed of the VO\(_2\) response does suggest that this step late in oxidative phosphorylation is not limiting, but as mentioned previously caution is warranted because of the moderate sample size (n = 10). Thus it will be of interest to see if a similar finding is present in future studies with a larger sample size. Studies performed on trained cyclists have reported that the maximal enzyme activity is an insensitive measure of endurance performance (10, 23, 54). Since HRR-derived measures but not enzyme activity improved together with faster VO\(_2\) kinetics it could be argued that HRR provides a better reflection of changes occurring in vivo than the isolated study of maximal activities of oxidative enzymes.

Pulmonary VO\(_2\) during steady state was not reduced with training (Figs. 2 and 3B), but 8 of the 10 subjects had a lower VO\(_2\) relative to before HIT (range 13–155 ml/min). A cross-sectional study has found that the energy requirement for constant-load exercise is not different between world-class cyclists and untrained individuals (39), although a lowering of steady-state VO\(_2\) has been reported in some training studies (38, 58). The absolute VO\(_2\) during steady state (~2,300 ml/min for 110 W) was high in the present study compared with other studies with similar work rates (1, 14, 44). This likely relates to the VO\(_2\) equipment measuring to high values since no subjects experienced any slow component (delta VO\(_2\) from 4 to 6 min) being 21 ± 49 pre-HIT (range −53 to 107 ml/min) and −20 ± 42 post-HIT (range −98 to 51 ml/min) (Table 1). In turn, since the VO\(_2\) baseline and amplitude are used to calculate τ (time to attain 63% of the amplitude), any small measuring noise in absolute values is not expected to affect the calculation of τ and hence the associations with HRR variables in the present study.

During HRR the respiratory chambers are hyperoxygenated and do not represent a limitation for respiration of the muscle fibers (15). Likewise, oxygen delivery appears to be more than adequate in the exercising human muscle during moderate-intensity exercise (5, 16, 40); thus oxygen delivery to the contracting fibers may be comparable in HRR and moderate cycling as in the present study. Still, if training improved either bulk oxygen delivery or distribution, as suggested in other studies (32, 28, 52), the abundance of oxygen during HRR is not truly reflective of in vivo conditions, at least in the untrained state. Thus we cannot exclude that an improvement in local oxygenation can have impacted on in vivo conditions for respiration (e.g., ADP levels and reactive oxygen species) in the mitochondria during cycle exercise post-HIT, whereas conditions in vitro during HRR were similar both pre- and post-HIT. Nevertheless, the HRR data do show that a number of the respiratory steps are improved with training (Fig. 4, A and B), suggesting that the mitochondria are capable of a higher respiration rate and this is also mirrored by the faster VO\(_2\) kinetics post-HIT.

In a training study, several factors are changed. Therefore the precise contribution from a single variable (e.g., in HRR) to the measured change in the in vivo VO\(_2\) response is not easy to quantify. As such, the present study does not provide the possibility of manipulating variables as in computer modeling studies of respiration (30). Nevertheless, the findings do point toward which links in the respiratory chain assessed with HRR appear to impact the most on the speed of the VO\(_2\) response measured in vivo. Respiration during HRR is expressed in
relation to the amount of muscle. It is considered unlikely that all fibers in the quadriceps muscle are active during moderate exercise as used in the present study. In turn, the intense nature of the HIT intervention likely resulted in mitochondrial adaptations in muscle fibers in high-order motor units (51), but even with this limitation HRR appears to reflect the functional capacity of the muscle in terms of obtaining a rapid increase in \( \text{VO}_2 \) during exercise. Yet, it could be that associations between HRR variables and \( \text{VO}_2 \) kinetics would be stronger after a moderate-intensity training intervention since the confounding influence from adaptations in high-order motor units would be minimized.

In summary, 2 wk of high-intensity training in untrained subjects resulted in faster \( \text{VO}_2 \) kinetics during moderate-intensity cycling together with improved fatty acid oxidation and leak respiration in the absence of adenylates assessed with high-resolution respirometry, whereas maximal activity of CS and COX-4 and energy turnover during steady-state conditions were unchanged. In turn, electron transport system capacity, fatty acid oxidation, and electron transport specific to complex II appeared to be good predictors of fast \( \text{VO}_2 \) kinetics both before and after the training intervention.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

P.M.C., R.A.J., and C.L. conception and design of research; P.M.C., R.A.J., T.C.B., D.F., and C.L. performed experiments; P.M.C., R.A.J., and C.L. drafted manuscript; P.M.C., R.A.J., T.C.B., D.F., J.B., and C.L. edited and revised manuscript; P.M.C., R.A.J., and C.L. edited and revised manuscript.

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