Improvements in exercise performance with high-intensity interval training coincide with an increase in skeletal muscle mitochondrial content and function

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J Appl Physiol 115: 785–793, 2013. First published June 20, 2013; doi:10.1152/japplphysiol.00445.2013.—Six sessions of high-intensity interval training (HIT) have repeatedly demonstrated its proficiency as a resourceful training modality to improve exercise performance. The mechanisms explaining such improvements are unclear. Accordingly, the aim of this study was to perform a comprehensive evaluation of physiologically relevant adaptations occurring after six sessions of HIT to determine the mechanisms explaining improvements in exercise performance. Sixteen untrained (43 ± 6 ml·kg⁻¹·min⁻¹) subjects completed six sessions of repeated (8–12) 60 s intervals of high-intensity cycling (100% peak power output elicited during incremental maximal exercise test) intermixed with 75 s of recovery cycling at a low intensity (30 W) over a 2-wk period. Potential training-induced alterations in skeletal muscle respiratory capacity, mitochondrial content, skeletal muscle oxygenation, cardiac capacity, blood volumes, and peripheral fatigue resistance were all assessed prior to and again following training. Maximal measures of oxygen uptake (V̇O₂peak; ~8%; P = 0.026) and cycling time to complete a set amount of work (~5%; P = 0.008) improved. Skeletal muscle respiratory capacities increased, most likely as a result of an expansion of skeletal muscle mitochondria (~20%, P = 0.026), as assessed by cytochrome c oxidase activity. Skeletal muscle deoxygenation also increased while maximal cardiac output, total hemoglobin, plasma volume, total blood volume, and relative measures of peripheral fatigue resistance were all unaltered with training. These results suggest that increases in mitochondrial content following six HIT sessions may facilitate improvements in respiratory capacity and oxygen extraction, and ultimately are responsible for the improvements in maximal whole body exercise capacity and endurance performance in previously untrained individuals.

interval training; sprint training; HIT; oxygen extraction; mitochondria

HIGH-INTENSITY INTERVAL TRAINING (HIT) has repeatedly demonstrated its proficiency as a resourceful training modality to improve exercise performance. Over the course of six HIT training sessions, typically over 2 wk time, marked improvements in exercise performance have been observed in normal healthy individuals (3, 9, 10, 41, 63), diseased populations (39, 69), and even trained athletes (11, 24, 37, 38, 62). A comprehensive mechanistic explanation for these rapid improvements in exercise performance is however lacking.

One single session of HIT sufficiently stimulates transcriptional activation of mitochondrial biogenesis (4, 20, 40, 49), even apparent in highly trained athletes (53), and remains evident following the 2 wk of HIT (25, 41). This transcriptional stimulus translates into phenotypic changes in mitochondrial protein expression and activity, including proteins involved in fat oxidation, tricarboxylic acid (TCA) cycle and/or the electron transport chain (9, 10, 25, 39–41, 49, 63). These static measures of biochemical expression within the muscle indirectly suggest enhanced oxidative potential, however the direct assessment of respiratory capacity, substrate control, and fatigue resistance in the skeletal muscle is lacking. Measurable alterations in mitochondrial protein expression (64) do not necessarily translate into functional changes (26), and alternatively functional alterations in mitochondria can occur independently from a measured change in protein expression (32). Thus interpreting observable changes in isolated protein expression as an adaptation of complex and integrative subcellular systems may be inaccurate. Regardless, these adaptations in the skeletal muscle are primarily used to explain the improvements in exercise performance (18).

Mass-specific respiratory capacity of the skeletal muscle is the single most predictive physiological variable of endurance performance (31). Therefore, a mitochondria-centric mechanistic explanation for the improvements in endurance performance following six sessions of HIT (10, 38, 39, 41, 62, 63) is most likely appropriate. However, six sessions of HIT have also repeatedly demonstrated a proficiency for improving measures of maximal exercise performance such as maximal oxygen uptake (V̇O₂peak) and peak power output via graded exercise tests (3, 38, 39, 63, 69), although a measurable improvement does not always occur (10). Peak power output obtained during whole body exercise is largely controlled by a limitation in oxygen delivery to the locomotor muscles (2) and accordingly is best predicted by the capacity for oxygen transport as well as the ability for the working muscle to extract oxygen (31, 47, 55). Enhanced oxidative capacity of the skeletal muscle alone would not be expected to explain these improvements in measures of maximal exercise performance as the respiratory capacity of the skeletal muscle exceeds maximal oxygen delivery to the working muscles (5). Rather these improvements in exercise would most likely be attributed to an

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increase in oxygen delivery to and/or greater oxygen extraction in the lower limbs (12, 57, 59, 60).

Accordingly, the aim of the present study was to perform a comprehensive evaluation of the physiological adaptations, ranging from cardiovascular to skeletal muscle properties, following six sessions of HIT in untrained young adults and determine the mechanisms explaining rapid improvements in exercise performance. We hypothesize that improvements in respiratory capacity of the skeletal muscle will parallel any marked increases of endurance capacity (31), while a greater maximal cardiac output and/or improved oxygen extraction of the locomotor muscles will correspond to improvements in VO$_2$peak and peak power output (12, 57, 60).

MATERIALS AND METHODS

Ethical approval. These experimental protocols involving human subjects were approved by the Ethical committee for the ETH Zürich (EK 2011-N-24), in accordance with the declaration of Helsinki. Prior to the start of the experiments, informed oral and written consents were obtained from all participants.

Subjects. Sixteen untrained (VO$_2$peak, 43 ± 6 ml·kg$^{-1}$·min$^{-1}$) adult (aged 27 ± 3 yr, height 181 ± 6 cm) male subjects participated in this study (data presented as means ± SD). No subject regularly engaged in either 1) a structured exercise training program, or 2) high-intensity exercise. Aerobic activity prior to commencement of the study was limited to less than 3 h of light aerobic activity per week. All subjects were medication free throughout the duration of the study. All subjects were scanned for body composition in a dual-energy X-ray absorptiometer (Lunar IDXA, GE Healthcare, Madison, WI) prior to exercise training and again following completion of training (Table 1) with time of the day (morning) when scanned standardized for all subjects.

Experimental design. The experimental design consisted of familiarization period, baseline testing, a 2-wk exercise training intervention, and posttraining measurements.

Familiarization, baseline, and post-training exercise testing. Subjects initially performed an incremental cycling test to volitional fatigue on an electronically braked cycle ergometer (Monark E839, Varberg, Sweden) to determine VO$_2$peak and peak power output using an online gas collection system (Innocor, Innovision, Odense, Denmark) where O$_2$ and CO$_2$ concentration in the expired gas was continuously measured and monitored as breath-by-breath values. The gas analyzers and the flowmeter of the applied spirometer were calibrated prior to each test. Subjects began exercise at three consecutive 5-min workloads, increasing by 50-W increments, beginning with 50 W and finishing with 150 W. From that point the workload was increased by 30 W every 90 s thereafter until volitional fatigue.

Mean VO$_2$peak was determined as the highest value averaged over 30 s for each subject. The subjects then were allowed a 1-h rest before beginning a time trial. Following the rest each subject performed a time trial in which they completed a set workload as quickly as possible. The criteria of the time trial was completing a set absolute workload that was relative to each individual subject’s maximal exercise performance at baseline, determined as 25% more work than was completed during the baseline incremental cycling test. This workload determination was selected for the time trial exercise tests because it 1) controlled for differences in baseline aerobic capacity across subjects, and 2) normalized the time of completion of all baseline tests at ~20 min (1,306 ± 210 s). This allowed for a more standardized test of endurance across a semiheterogeneous group of subjects. All time trials were performed on an electronically braked ergometer to become familiar with these performance tests. Each subject could increase or decrease resistance manually and/or via their cadence as desired to complete the predetermined workload. Subjects were instructed to complete the tests as quickly as possible and were provided no temporal or physiological feedback. The only feedback provided was their real-time workload, cadence, and remaining workload until completion. Exercise duration and power output were recorded upon completion of each test.

Training. The specific HIT protocol used in the present study was derived from previous work (41). Briefly, following baseline testing all subjects began the training protocol, which consisted of six sessions over 2 wk (training every 2–3 days). Each training session consisted of repeated 60 s efforts of high-intensity cycling at a workload that corresponded to the peak power achieved during the incremental cycling test (249 ± 52 W). These high-intensity intervals were intermixed with 75 s of cycling at a low intensity (30 W) for recovery. Subjects completed 8 high-intensity intervals during the first two training sessions, 10 intervals during the third and fourth sessions, and 12 intervals on the final two sessions. A 3-min warm-up at 30 W was performed each day prior to training. The time commitment during each training session ranged from 21 min for the first two sessions, 25 min for the 3rd and 4th sessions, and 30 min for the final two sessions. The total time spent training over the 2-wk span, including warm-up and recovery, was under 3 h. All subjects completed all training sessions.

Skeletal muscle sampling. Skeletal muscle biopsies were obtained under standardized conditions from the vastus lateralis muscle at baseline and again after the 2-wk, six-session regimen of HIT. Samples were collected under local anesthesia (1% lidocaine) of the skin and superficial muscle fascia, using the Bergström technique with a needle modified for suction. The biopsy was immediately dissected free of fat and connective tissue and divided into sections for measurements of mitochondrial respiration. All biopsies were taken ~48 h following the last bout of exercise.

Skeletal muscle preparation. The biopsy was sectioned into parts to measure mitochondrial respiration as previously described (26–28, 30, 32, 50). Respiration measurements were performed in mitochondrial respiration medium 06 (MR06; MR05 + catalase 280 IU/ml). Measurements of O$_2$ consumption were performed at 37°C using the high-resolution oxygraph-2k (Oroboros, Innsbruck, Austria). Standardized instrumental and chemical calibrations were performed as described previously (32). Oxygen flux was resolved by software allowing nonlinear changes in the negative time derivative of the oxygen concentration signal (DatLab, Oroboros, Innsbruck, Austria). All respirometric analyses were done in duplicate and were carried out in a hyperoxygenated environment to prevent any potential oxygen diffusion limitation with oxygen concentration within the chamber ranging between 250 and 420 nmol/ml.

Respiratory titration protocol. The titration protocol was specific to the examination of individual aspects of respiratory control through a sequence of coupling and substrate states induced via separate titrations. This titration protocol was modified from previous protocols where they are described in detail (29, 32). All titrations were added.
in series as presented. Leak respiration in absence of adenylates ($I_{leak}$) was induced with the addition of malate (2 mM) and octanoyl carnitine (0.2 mM). Maximal electron flow through electron transferring-flavoprotein (ETF) and fatty acid oxidative capacity ($P_{ETF}$) was determined following the addition of ADP (5 mM). Submaximal state 3 respiratory capacity specific to CI ($P_{CI}$) was induced following the additions of pyruvate (5 mM) and glutamate (10 mM). Maximal state 3 respiration, oxidative phosphorylation capacity (P), was then induced with the addition of succinate (10 mM). As an internal control for compromised integrity of the mitochondrial preparation, 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 (86.5 ± 15.6 to 86.6 ± 15.5 pmol O$_2$·min$^{-1}$·mg wet wt$^{-1}$, P = 0.611) or following 2 wk of HIT exercise training (99.8 ± 23.1 to 100.3 ± 23.2 pmol O$_2$·min$^{-1}$·mg wet wt$^{-1}$, P = 0.259). Oligomycin was added inhibiting ATP synthase to achieve oligomycin-induced leak respiration ($L_{OXY}$). Phosphorylative restraint of electron transport was assessed by uncoupling ATP synthase (complex V) from the electron transport system with the titration of the protonophore, carbonyl cyanide-p-(trifluoromethoxy)phenylhydrazoone (FCCP, steps of 0.5 μM) reaching electron transport system (ETS) capacity. Rotenone (0.5 μM) and antimycin A (2.5 μM) were added, in sequence, to terminate respiration by inhibiting CI and complex III (cytochrome bc1 complex), respectively. With CI inhibited, electron flow specific to CI to CII ($P_{CII}$) can be measured. Prior uncoupling with FCCP has no effect on $P_{CII}$ (32), as individual electron input to CII does not saturate the Q-cycle. Inhibition of respiration with antimycin A then allows for the determination and correction of residual oxygen consumption, indicative of non-mitochondrial oxygen consumption in the chamber. Finally, ascorbate (2 mM) and TMPD (0.5 mM) were simultaneously titrated into the chambers to assess cytochrome c oxidase (COX), complex IV, activity. TMPD and ascorbate are redox substrates that donate electrons directly to COX and activity was measured by picomoles O$_2$ per minute per mg wet weight. COX activity has been shown to strongly correlate with mitochondrial volume density and total cristae area (both measured via transmission electron microscopy) in addition to respiratory capacity (36).

Skeletal muscle oxygenation. Absolute changes in concentrations (μmol/l) of deoxygenated hemoglobin (Hb) and myoglobin (Mb) ($\Delta$HHbMb), in addition to the skeletal muscle oxygenation index ($\Delta$mStO$_2$), the ratio of oxygenated to total tissue Hb and Mb, was obtained from the vastus lateralis muscle via spatially resolved near-infrared spectroscopy (NIRS; NIRO-200NX, Hamamatsu, Japan) with respect to an initial value arbitrarily set equal to zero. NIRS provides measurements conducted on separate days within these testing sessions. The coefficient of variation for $\Delta$mStO$_2$, assessed from duplicate measurements at baseline and expressed as the percent typical error (i.e., SD of difference scores/$\sqrt{2}$), was 2.5%.

Peripheral fatigue resistance. The magnitude of peripheral quadriceps fatigue was quantified via supramaximal magnetic stimulation of the femoral nerve, with the exercise-induced reduction in potentiated quadriceps twitch force ($Q_{tw,pot}$) assessed prior to exercise and again 30 s after termination of exercise following both the graded exercise and time trial tests.

Briefly, subjects sat semirecumbent on a chair so that their knee joint angle was between 85 and 95 degrees. A Magstim Rapid (220) stimulator (Magstim, Whitland, UK), with a figure-of-eight coil, was applied to stimulate the femoral nerve. The evoked quadriceps twitch force was measured using a strain gauge that was fixed to a rigid pole of the chair on one end with the other end connected to a noncompliant strap placed just superior to the malleolus of each subject's dominant leg. Electrodes were placed in a bipolar configuration over the center of the vastus lateralis muscle to record magnetically evoked compound action potentials. Active electrodes were placed over the motor point of the muscle, quadriceps, and the reference electrode placed in an electrically neutral site, elbow.

For evaluation of locomotor muscle strength each subject was asked to perform six 5-s maximal voluntary contractions (MVC).
Following the 3rd, 4th, and 5th MVC the femoral nerve was stimulated 3 s into the contraction to determine the superimposed twitch (TWS). Also, 3 s after each MVC, three stimulations were made, each separated by 3 s, to determine the twitch force ($Q_{\text{tw,pot}}$). The TWS force was related to the $Q_{\text{tw,pot}}$ recorded 3 s after the MVC and termed percent voluntary muscle activation: %VA = [1 – ($TWS/Q_{\text{tw,pot}}$)] × 100 (45).

Following both the maximal incremental exercise test and time trial, subjects were promptly assisted back into the chair and repositioned as they were previously. The procedure described above was repeated 30 s after the cessation of exercise. This protocol was used to assess central (neuromuscular) vs. peripheral (skeletal muscle) fatigue following each respective exercise test. To determine whether nerve stimulation was supramaximal, a MVC was followed by three single twitchs at 90, 95 and 100% of maximal stimulator power output in a nonfatigued and fatigued status.

Data analysis. For all statistical evaluations, a $P$ value of <0.05 was considered significant. Two different statistical models were used for analysis (SPSS Statistics 17.0, SPSS, Chicago, IL). Paired samples $t$-tests were used to test the null hypothesis stating no difference between all baseline measures and those obtained following 2 wk of HIT exercise training. The sequential respiratory capacities of $P$ before and following the titration of exogenous cytochrome $c$ to test mitochondrial membrane integrity were analyzed by a one-way ANOVA on repeated measurements. Mass-specific respiration was normalized to COX activity [(respiratory state/COX) × 100] and mitochondrial-specific respiratory capacities are presented as a percent of COX.

RESULTS

Exercise capacity and body composition. Following six sessions of HIT over 2 wk absolute and relative measures of $\dot{V}O_2^{\text{peak}}$ increased by 7.9% ($P = 0.031$) and 8.2% ($P = 0.026$), respectively, while peak power output increased by 7% ($P = 0.002$, Fig. 1A). Endurance capacity also improved, as the subjects were able to complete the same absolute workload ~1 min faster (4.4% decrease in time, $P = 0.008$) with a 5.1% improvement in average power output ($P = 0.013$; Fig. 1B).

Although the absolute mean power output for the time trials improved, the relative mean power output as a percent of maximal power output was the same at baseline as it was after HIT (with mean ± SD of 62.1 ± 8.9 and 60.6 ± 6.7, respectively, $P = 0.367$). Alterations in total body mass were negligible with training; however, total body fat percentage decreased ($P = 0.048$) with training (Table 1).

Skeletal muscle respiratory capacity. All respiratory states measured improved in response to the six HIT sessions except for submaximal state 3 respiration specific to mitochondrial complex II ($P_{\text{CI}}$; Fig. 2A). Mitochondrial content as assessed by COX activity (Fig. 2B), an established biomarker of mitochondrial volume density in human skeletal muscle (36), also increased with training ($P = 0.026$). All measured increases in respiratory capacity were silenced when normalizing mass-specific respirometric values to measures of mitochondrial content (Fig. 2B), suggesting that the improvements in the oxidative capacity of the skeletal muscle were due to an expansion of the mitochondrial network as opposed to qualitative alterations in function.

Tissue oxygenation. NIRS assessed parameters of skeletal muscle oxygenation changed with HIT training. Measures of $\Delta mStO_2$ and $\Delta HHbMb$ decreased ($P = 0.037$) and increased ($P = 0.001$), respectively, at peak power output (Table 2). Average values of $\Delta mStO_2$ obtained during the time trial also decreased ($P = 0.022$) while $\Delta HHbMb$ showed a tendency ($P = 0.062$) to increase. These alterations in skeletal muscle oxygenation observed during each matched relative intensity (100% and 60%, respectively) are not surprising as peak power output and average power output produced during the time trial both increased with training (Fig. 1). Alterations in skeletal muscle oxygenation at the same absolute intensities, however, were also observed to change as $\Delta mStO_2$ at 150, 180, 210, and 240 W diminished (Fig. 3A) and $\Delta HHbMb$ increased at 100, 150, 180, 210, 240, and 270 W (Fig. 3B).

Cardiovascular parameters. Maximal cardiac output failed to improve after two wk of HIT ($A < 0.001\%$, $P = 0.965$; Fig. 4). Similarly, $Hb_{\text{mass}}$, [Hb], Hct, RCV, PV, and TBV were all unaltered after six HIT sessions (Table 1).

Peripheral fatigue resistance. The change in $Q_{\text{tw,pot}}$ and MVC prior to and following exercise, regardless of exercise test, was no different when comparing baseline measures to those obtained following HIT (Fig. 5, A and B).

DISCUSSION

In the present study we performed an evaluation of physiological adaptation to six sessions of HIT in untrained young adults in attempt to determine the mechanisms explaining improvements in exercise performance with this abbreviated training modality. Improvements in $\dot{V}O_2^{\text{peak}}$, peak power output, and time trial performance were observed over the 2-wk span (Fig. 1). These improvements occurred concurrently with an increase in skeletal muscle respiratory capacity (Fig. 2) and...
greater leg deoxygenation (Fig. 3). Moreover, the improvements in exercise performance occurred independent from any alterations in maximal cardiac capacity (Fig. 4) or blood characteristics, specifically oxygen-carrying capacity and total blood volume (Table 1). Together these data empirically corroborate previous findings and postulates stating that improvements in exercise performance after six sessions of HIT over the span of 2 wk are primarily attributed to enhanced oxidative potential in the skeletal muscle.

Discernible improvements in exercise capacity have been observed after only six HIT training sessions in nonhealthy populations (39, 69), normal healthy individuals (3, 9, 10, 41, 63), and even trained athletes (11, 24, 37, 38, 62). We also observed marked improvements in $\dot{V}O_2$peak, peak power output, and time trial completion (Fig. 1).

Here the results indicate that a greater skeletal muscle oxidative capacity contributes to the improvements in endurance performance observed (Fig. 1B). Our measured improvements in endurance capacity are in line with previous reports of improvement (10, 38, 39, 63, 69). Congruent with this premise is the fact that mass-specific respiratory capacity is the strongest determining factor of endurance performance in highly trained athletes (31) as well as in race horses (65). It is, however, not clear exactly how a greater oxidative capacity in human skeletal muscle may improve $\dot{V}O_2$peak and peak power output as was observed in this (Fig. 1A) as well as previous studies (3, 38, 39, 63, 69).

Measures of maximal whole body exercise capacity are largely controlled by a limitation in oxygen delivery to the locomotor muscles (2) with respiratory capacity of the skeletal muscle able to outstrip maximal oxygen delivery to the working muscles (5). Traditional endurance training improves maximal incremental exercise capacity in untrained individuals by means of an increased maximal cardiac output (12, 59), attributable to a greater stroke volume (16, 23, 59), in addition to greater oxygen extraction at the locomotor muscle (12, 54, 57, 59). Accordingly, we expected an increase in maximal cardiac output during graded exercise due to a training-induced expansion of total blood volume. We originally thought that 2 wk of HIT would increase total blood volume by means of plasma expansion. Plasma volume responds rather rapidly in response to high-intensity exercise training (14, 21, 22, 46), and an acute expansion of PV in untrained individuals has shown to improve maximal exercise capacity (33). Unlike these previous findings, however, we were unable to detect a change either PV, TBV (Table 1), or, consequently, maximal cardiac output (Fig. 4). However, 7 wk of sprint training does not facilitate an

| Table 2. Skeletal muscle oxygenation during exercise |
|---------------------------------|----------|----------|----------------|----------------|
|                                | Pre MAX | Post MAX | Pre TTavg     | Post TTavg     |
| ΔmStO₂, %                      | 63.4 ± 6.7 | 56.4 ± 9.3* | 66.0 ± 6.8 | 59.6 ± 12.2* |
| ΔHHbMb, μmol/l                 | 15.5 ± 19.3 | 36.5 ± 27.0*** | 33.2 ± 16.3 | 60.6 ± 39.7† |

Values are presented as absolute changes, means ± SE, from an initial value obtained prior to each respective exercise test that was arbitrarily set equal to zero. Near-infrared spectroscopy derived measures of skeletal muscle oxygenation (ΔmStO₂) and deoxygenated hemoglobin and myoglobin (ΔHHbMb) at peak power output achieved during an incremental exercise test (MAX) and averaged throughout a set-workload time trial (TT) prior to (Pre) and following (Post) 6 sessions of high-intensity training (HIT). Difference between corresponding pre and post HIT measurements: *P ≤ 0.05, ***P ≤ 0.001. †A trend toward significance of P = 0.062.
increase in PV or BV (44) and 12 wk of both low- and high-intensity training also failed to increase BV, PV, and Hbmass in women (7). Thus, any acute high-intensity training-induced expansion of PV appears to be a transient phenomenon with a failure of initial fluid shifts to remain over time (44).

We did not expect improvements in oxygen-carrying capacity to explain improvements in maximal whole body exercise capacity, and this assumption was substantiated by our findings (Table 1). The rapid training-induced expansion of PV is not a phenomenon mirrored by red blood cell volume (21, 22) or total hemoglobin mass (22), both of which take much more time to increase (59) if at all (61).

Two weeks of HIT has repeatedly demonstrated an ability to increase protein expression and mitochondrial enzyme activity, both suggestive of an enhanced oxidative capacity of the skeletal muscle. Mitochondrial protein expression and enzyme activity, including proteins involved in fat oxidation, TCA cycle and/or the electron transport chain (9, 10, 25, 39–41, 49, 63), all improve with 2 wk of HIT training. Mitochondrial enzymatic protein expression has been reported to increase after as few as three to five sessions of HIT with enzymes involved in the TCA cycle and β-oxidation increasing before mitochondrial respiratory complexes of the electron transport system (49). Hitherto, these alterations in skeletal muscle
protein expression have primarily served as the foundation of mechanistic explanations for the improvements in exercise performance with HIT (18). Measures of in vivo oxidative capacity using phosphorous magnetic resonance spectroscopy have also been reported to improve following 6 sessions of HIT, further supporting these mechanistic suppositions (17, 35). While it has previously been demonstrated that the same HIT protocol as implemented in the current study results in increased expression and activity of COX and citrate synthase (41), here we substantiate and expound upon these claims with direct evidence that HIT increases skeletal muscle respiratory capacity (Fig. 2). These improvements in skeletal muscle respiratory capacities are most likely attributable to mitochondrial reticular expansion within the skeletal muscle as there is primarily no difference in respiratory capacity between pre and post HIT measurements when normalized to mitochondrial content (Fig. 2C). Qualitative alterations in mitochondrial function that exist across individuals that differ in aerobic capacities (28) were not apparent after just six sessions of HIT. Unfortunately we are unable to verify that the improvements in exercise performance were directly facilitated by the increases in skeletal muscle oxidative capacity and/or expansion of skeletal muscle mitochondria as our experimental set-up does not allow for a proper time course analysis. Future studies could attempt to verify our findings by monitoring mitochondrial protein expression and function in parallel with measures of exercise performance throughout a regimen of HIT. Our results, however, do suggest that improvements in exercise performance, oxidative capacity of the skeletal muscle and leg oxygenation may all be attributable to an increase in skeletal muscle mitochondria.

Leg oxygenation during exercise is also a primary determinant of maximal exercise performance (31) and may explain measured improvements in maximal incremental performance. Our measures of leg oxygenation, Δ[HbHbMb and ΔmStO2], can be used as surrogate measures of oxygen extraction (6, 15). It is generally accepted that oxygen extraction improves to some degree with training (12, 48, 54, 56–58), with the greatest improvements observed in previously untrained individuals (59). However, reported alterations in tissue oxygenation following HIT are disputed. Six sessions of HIT training reportedly improved muscle fractional oxygen extraction as measured by the changes in [HHb] kinetics with NIRS (3) whereas negligible improvements in fractional oxygen extraction were observed following eight sessions of HIT (43). Our results agree with the former study (3), as we observed marked increases in leg deoxygenation during exercise after HIT (Fig. 3) suggesting greater oxygen extraction. These results are also in line with another study that reported greater muscle deoxygenation of the vastus lateralis muscle, suggesting improved oxygen extraction following HIT (52). We observed that skeletal muscle deoxygenation increases at peak power output (Table 2), at specific workloads during the graded exercise test (Fig. 3 A and B), and throughout the time trial (Table 2). The suggested mechanism explaining these increases in tissue deoxygenation relates back to the expansion of mitochondria within the skeletal muscle (Fig. 2B). Oxygen extraction in the skeletal muscle during exercise, the amount of oxygen transported per unit time between capillaries and mitochondria, is a product of the diffusive capacity of the muscle for oxygen and the difference between oxygen tension in the capillary and mitochondria (66). An expansion of the mitochondrial reticular network (Fig. 2B) creates a larger oxygen sink during exercise, reducing overall oxygen tension within a contracting myocyte and increasing the oxygen gradient between capillaries and mitochondria. Although not measured in the present study, a linear association between skeletal muscle capillarization and mitochondrial content has been established (51). Moreover, capillary-to-fiber surface area and mitochondrial volume develop concurrently with a negligible change in capillary-to-fiber surface area ratio per fiber unit mitochondrial volume (42). Taken together with our data, this would suggest that HIT-mediated mitochondrial expansion (Fig. 2B) might improve the diffusive capacity of the muscle. Collectively, the larger oxygen gradient and improved diffusive capacity would improve skeletal muscle oxygen extraction capacity during exercise (66) as was observed (Table 2 and Fig. 3).

Resistance to the development of peripheral fatigue plays an important role in exercise performance (13). We observed no difference in measures of peripheral fatigue (Fig. 5, A and B) despite decreased time to complete a set workload with a greater mean power output (Fig. 1B) and greater peak power output achieved (Fig. 1A). Together these data suggest that the development of peripheral fatigue was delayed with HIT, allowing the subjects to perform better during each respective exercise test while maintaining the same relative level of power output and peripheral fatigue. Accumulation of metabolic by-products, especially inorganic phosphate (Pi), factor in the development of peripheral fatigue (1, 68). Completing six sessions of HIT over the course of 2 wk has been shown to improve skeletal muscle buffer capacity (19) and reduce phosphocreatine recovery time, indirectly indicating an improved functional oxidative capacity in skeletal muscle following training (17, 35). Our data contribute to these previous findings by providing direct evidence of the improvement in the oxidative capacity of the skeletal muscle with HIT (Fig. 2, A and B). The enhanced oxidative capacities of the skeletal muscle most likely facilitated the delayed onset of peripheral fatigue (Fig. 5), permitting improvements in overall exercise performance (Fig. 1).

The present study could have been strengthened with the addition of a group that engaged in more typical form of endurance training (lower intensity and longer duration), matching work across groups. There is evidence to suggest that the transcriptional regulation of several genes involved in skeletal muscle mitochondrial biogenesis is similar with interval and endurance training when matching both work and time (67); however, one primary advantage of HIT is the time efficiency of training. How the physiological effects of a more typical endurance exercise program would compare to those presented in this study are unknown and require further attention.

In conclusion, we provide evidence indicating that an improved oxidative capacity of the skeletal muscle resulting from an increase in mitochondrial content may facilitate an improvement in tissue oxygenation, delayed peripheral fatigue, and explain the improvement in both maximal whole body exercise capacity as well as endurance performance following 6 sessions of HIT over a 2-wk period in previously untrained adults.

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