High-intensity aerobic interval training increases fat and carbohydrate metabolic capacities in human skeletal muscle

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Abstract: High-intensity aerobic interval training (HIIT) is a compromise between time-consuming moderate-intensity training and sprint-interval training requiring all-out efforts. However, there are few data regarding the ability of HIIT to increase the capacities of fat and carbohydrate oxidation in skeletal muscle. Using untrained recreationally active individuals, we investigated skeletal muscle and whole-body metabolic adaptations that occurred following 6 weeks of HIIT (~1 h of 10 × 4 min intervals at ~90% of peak oxygen consumption ($VO_{2\ peak}$), separated by 2 min rest, 3 d·week^{-1}). A $VO_{2\ peak}$ test, a test to exhaustion (TE) at 90% of pre-training $VO_{2\ peak}$, and a 1 h cycle at 60% of pre-training $VO_{2\ peak}$ were performed pre- and post-HIIT. Muscle biopsies were sampled during the TE at rest, after 5 min, and at exhaustion. Training power output increased by 21%, and $VO_{2\ peak}$ increased by 9% following HIIT. Muscle adaptations at rest included the following: (*i*) increased cytochrome *c* oxidase IV content (18%) and maximal activities of the mitochondrial enzymes citrate synthase (26%), β-hydroxyacyl-CoA dehydrogenase (29%), aspartate-amino transferase (26%), and pyruvate dehydrogenase (PDH; 21%); (*ii*) increased FAT/CD36, FABPpm, GLUT 4, and MCT 1 and 4 transport proteins (14%–30%); and (*iii*) increased glycogen content (59%). Major adaptations during exercise included the following: (*i*) reduced glycogenolysis, lactate accumulation, and substrate phosphorylation (0–5 min of TE); (*ii*) unchanged PDH activation (carbohydrate oxidation; 0–5 min of TE); (*iii*) ~2-fold greater time during the TE; and (*iv*) increased fat oxidation at 60% of pre-training $VO_{2\ peak}$. This study demonstrated that 18 h of repeated high-intensity exercise sessions over 6 weeks (3 d·week^{-1}) is a powerful method to increase whole-body and skeletal muscle capacities to oxidize fat and carbohydrate in previously untrained individuals.

Key words: mitochondrial oxidative capacity, fat metabolism, carbohydrate metabolism, skeletal muscle, exercise training.

Résumé : L'entraînement aérobie par intervalles de haute intensité (HIIT) constitue un compromis entre l'entraînement d'intensité modérée et l'entraînement par intervalles au sprint qui demande des efforts maximaux. Il y a cependant peu d'études concernant l'efficacité de l'HIIT pour améliorer l'oxydation des sucres et des graisses dans le muscle squelettique. Nous analysons les adaptations métaboliques du muscle et de l'organisme chez des sujets sédentaires qui se sont entraînés durant 6 semaines selon les modalités suivantes : 10 efforts d'une durée de 4 min à une intensité sollicitant ~90 % du consommation d'oxygene de pointe intercalés de périodes de repos d'une durée de 2 min pour une durée totale de 60 min à raison de 3 jours par semaine. Avant et après ce programme d'entraînement, les sujets participent à une épreuve d'évaluation du consommation d'oxygene de pointe, à un effort jusqu'à l'épuisement (TE) à une intensité correspondant à 90 % du consommation d'oxygene de pointe déterminé au début du programme d'entraînement et à une séance d'exercice sur un vélo réalisée à une intensité équivalent à 60 % du consommation d'oxygene de pointe déterminé au début du programme d'entraînement. On prélève des biopsies musculaires avant le TE, 5 min après le début du TE et au moment de l'épuisement. Au cours du programme d'entraînement, la puissance à l'effort augmente de 21 % et le consommation d'oxygene de pointe, de 9 %. Au repos, les adaptations du muscle sont : (*i*) augmentation de 18 % du contenu en cytochrome *c* oxydase IV et des activités maximales des enzymes mitochondriales (citrate synthase, 26 %, β-hydroxyacyl-CoA déshydrogénase, 29 %, aspartate aminotransférase, 26 % et pyruvate déshydrogénase (PDH), 21 %), (*ii*) augmentation de 14 à 30 % de FAT/CD36, de FABPpm, du GLUT 4 et des transporteurs protéiques MCT 1 et 4 et (*iii*) augmentation de 59 % du contenu de glycogène. Au cours de l'effort, les principales adaptations sont : (*i*) diminution de la glycogénolyse, de l'accumulation de lactate et de la phosphorylation de substrats durant les 5 premières minutes de TE, (*ii*) pas de modification de l'activation de la PDH (oxydation des sucres) durant les 5 premières minutes de TE, (*iii*) amélioration de près de 2 fois du temps de performance jusqu'à l'épuisement et (*iv*) augmentation de l'oxydation des

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graisses au cours de l'effort réalisé à une intensité correspondant à 60 % du consommation d'oxygène de pointe déterminé avant le début du programme d'entraînement. D'après ces observations, un programme d'entraînement par intervalles de forte intensité d'une durée de 18 h sur une période de 6 semaines à raison de 3 jours par semaine constitue une méthode très rentable pour améliorer les capacités organiques et musculaires d'oxydation des sucres et des graisses chez des individus préalablement sédentaires.

Mots-clés : capacité mitochondriale d'oxydation, métabolisme des graisses, métabolisme des sucres, muscle squelettique, entraînement physique.

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Introduction

Intense skeletal muscle contractions challenge the energy-producing metabolic pathways to match ATP production to demand. Moderate-intensity exercise training (MIT) (60%–75% peak oxygen consumption ($VO_{2\,peak}$), 1–2 h·d^{-1} for 2–12 weeks) increases the skeletal muscle mitochondrial volume and maximal capacity for carbohydrate and fat oxidation, and permits the maintenance of exercise at higher absolute power outputs (Holloszy 1967; Gollnick et al. 1973; Holloszy and Coyle 1984; Leblanc et al. 2004a). The greater mitochondrial volume also improves the sensitivity of mitochondria to increases in free ADP (ADP_f) at the onset of exercise, accelerating oxidative metabolism and phosphorylation and reducing the need for substrate phosphorylation from phosphocreatine degradation and glycogen use with lactate formation (Henriksson 1977; Hurley et al. 1986; Kiens et al. 1993; Green et al. 1995; Phillips et al. 1996; Kiens 1997; Leblanc et al. 2004a).

There has been renewed interest in alternative forms of exercise training that are performed at higher intensities but for shorter periods of time per week in an effort to find a more time-efficient stimulus to induce skeletal muscle and whole-body metabolic adaptations. One example is sprint interval training (SIT), in which maximal efforts (>150% of $VO_{2\,peak}$) are performed for brief periods of time (30 s), and repeated up to 6 times·d^{-1} (with 4 min rests between sprints), 3 d·week^{-1}, for 2–6 weeks (Gibala et al. 2006; Burgomaster et al. 2008). While SIT has been shown to increase the capacity for carbohydrate oxidation (greater content of pyruvate dehydrogenase (PDH), glycogen, and the glucose and lactate transport proteins GLUT 4, and MCT 1 and 4, respectively), its ability to increase the capacity for fat oxidation has been controversial. For instance, no increases in the fatty acid transport proteins FAT/CD36 or FABPpm were observed after 6 weeks of SIT (Burgomaster et al. 2007). Furthermore, increases in the maximal activity of β-hydroxyacyl CoA dehydrogenase (β-HAD), which is an indicator of the maximal capicity for β-oxidation, were observed in one study (Burgomaster et al. 2008), but no change was observed in another (MacDougall et al. 1998).

Another approach is high-intensity interval training (HIIT), which typically employs repeated exercise intervals at high submaximal intensities (80%–95% $VO_{2\,peak}$) and a frequency of 2–3 d·week^{-1}. Supplementing MIT with HIIT over 2 weeks in healthy untrained individuals reduced glucose utilization (Mendenhall et al. 1994; McConell et al. 2005) and increased fat oxidation (McConell et al. 2005) during 2 h of cycling at 60% of maximal oxygen consumption. Preliminary research in our laboratory demonstrated

that 2 weeks of HIIT (10 × 4 min exercise bouts at 90% of $VO_{2\,peak}$, with 2 min rest periods, 1 h·d^{-1}, 3 d·week^{-1}) in women increased $VO_{2\,peak}$, the maximal activities of 2 mitochondrial enzymes, and whole-body fat oxidation with reduced skeletal muscle glycogenolysis and phosphocreatine (PCr) utilization during 60 min of cycling at 60% of pre-training $VO_{2\,peak}$ (Talanian et al. 2007). A second study reported that 6 weeks of HIIT also increased mitochondrial oxidative capacity and prolonged time to exhaustion during exercise at 90% of pre-training $VO_{2\,peak}$ (Perry et al. 2007). A limitation to previous work done in our laboratory is that a metabolic explanation for the improved endurance performance at 90% of pre-training $VO_{2\,peak}$ is not apparent. Furthermore, it is not apparent whether long-term HIIT increases the capacity to oxidize carbohydrate in addition to fat. In the current study, we attempted to thoroughly investigate the muscle metabolic responses to the high metabolic challenge of cycling at 90% of pre-training $VO_{2\,peak}$ following 6 weeks of HIIT, and to relate them to increases in a variety of improved metabolic capacities.

Therefore, the purpose of this study was to comprehensively investigate the ability of 6 weeks of HIIT (18 h of repeated work–rest intervals at 90% of pre-training $VO_{2\,peak}$) to increase the capacity for skeletal muscle and whole-body carbohydrate and fat oxidation in recreationally active but untrained subjects. The metabolic and performance responses to exercise challenges at 90% of pre-training $VO_{2\,peak}$ were examined pre- and post-training. We hypothesized that HIIT would (*i*) increase $VO_{2\,peak}$ and cycle time to exhaustion at 90% of pre-training $VO_{2\,peak}$; (*ii*) increase the maximal activities or protein content of 5 key mitochondrial enzymes — specifically, mitochondrial aspartate aminotransferase (m-AspAt) (cytoplasm to mitochondrial electron shuttling), PDH (carbohydrate oxidation), β-HAD, citrate synthase (tricarboxylic acid (TCA) cycle), and cytochrome oxidase IV (electron transport chain) — reflecting increases in the capacity for fat and carbohydrate oxidation; (*iii*) increase the content of skeletal muscle fatty acid, glucose, and lactate transport proteins; and (*iv*) decrease skeletal muscle glycogenolysis and substrate phosphorylation from glycolysis and phosphocreatine ("anaerobic" energy production) while cycling at 90% of pre-training $VO_{2\,peak}$.

Methods

Subjects

Eight subjects (3 females, 5 males) volunteered to participate in this study. Their age, height, and mass (means ± standard error of the mean (SEM)) were 24 ± 1 year, 179 ±

Fig. 1. Study design for 6 weeks of high-intensity interval training (HIIT). $VO_{2\,peak}$, peak oxygen consumption.

$VO_{2\,peak}$ test	Trial to exhaustion @ 90% $VO_{2\,peak}$	60 min @ 60% $VO_{2\,peak}$	← 6 weeks HIIT →	$VO_{2\,peak}$ test	Trial to exhaustion @ 90% $VO_{2\,peak}$	60 min @ 60% $VO_{2\,peak}$
Day 1	**Day 3**	**Day 5**		**7 wk + 2 d**	**7 wk + 4 d**	**7 wk + 6 d**

4 cm, and 72.7 ± 4.0 kg, respectively. Prior to the study, subjects were not involved in structured training programs, but did participate in some form of aerobic activity (cycling, jogging) for ~1 $h \cdot d^{-1}$, 2–3 $d \cdot week^{-1}$. Activity logs verified that this level of activity was maintained in addition to the training sessions throughout the 6 week study, and that no additional exercise was performed during the pre- and post-training phases of the study. Subjects were medication free and were advised to refrain from taking supplements other than multivitamins. The experimental protocol and associated risks were explained orally and in writing to all subjects before written consent was obtained. The study was approved by the Research Ethics Boards of the University of Guelph, in Guelph, Ont., and McMaster University, in Hamilton, Ont., and conformed to the Declaration of Helsinki.

Study design

Each subject completed 6 weeks of cycle ergometer HIIT (see HIIT protocol). Before and after training, subjects underwent a $VO_{2\,peak}$ test, a cycling test to exhaustion (TE) at 90% of $VO_{2\,peak}$, and a 1 h cycle test at 60% of pre-training $VO_{2\,peak}$ on separate days (Fig. 1). The tests were separated by 48 h, and subjects refrained from alcohol and caffeine consumption and physical activity between experimental tests. Post-training, the $VO_{2\,peak}$ test, the TE, and the 1 h cycle test were repeated 2, 4, and 6 d after the final training session, respectively. A familiarization TE was performed approximately 1 week before the pre-training $VO_{2\,peak}$ test. Subjects were asked to consume the same diet 48 h prior to each pre- and post-training test, with the last meal being eaten 2 h before each test. This was verified by analysis of subjects' dietary records during each experimental testing week. All female subjects were eumenorrheic and underwent all experimental trials during the early to mid-follicular phase of their menstrual cycle, which occurred approximately every 4 weeks.

Cycling tests

$VO_{2\,peak}$

Subjects completed a continuous incremental cycling TE on an electromagnetically braked cycle ergometer (Lode Instrument, Groningen, the Netherlands) to determine pulmonary $VO_{2\,peak}$, using a metabolic measurement system (Vmax Series 229, SensorMedics Corporation, Yorba Linda, Calif.). Subjects familiarized themselves with a cycling test to volitional exhaustion at 90% of $VO_{2\,peak}$ and a steady-state cycling test at 60% of $VO_{2\,peak}$ at the start of the study to verify the power outputs.

Cycling TE at 90% of pre-training $VO_{2\,peak}$

Subjects arrived at the laboratory and rested on a bed

while a catheter was inserted into an antecubital vein. Three incisions were made over the vastus lateralis muscle (separated by 1.5 cm, proximally to distally) of 1 leg under local anesthesia (2% lidocaine, no epinephrine) for muscle biopsy sampling. With the subject on the bed, resting blood and muscle samples were taken. Muscle samples were immediately frozen in liquid nitrogen and stored until analysis. Subjects then cycled to volitional exhaustion at 90% of pre-training $VO_{2\,peak}$ (TE), with muscle biopsies and venous blood samples taken at 5 min and at exhaustion. All TE trials were conducted under the same conditions and without any temporal feedback to the participants. Exhaustion was taken to be the time when pedaling cadence fell below 40 $r \cdot min^{-1}$, and was immediately recorded when this occurred.

Cycling for 1 h at 60% of pre-training $VO_{2\,peak}$

Subjects arrived at the laboratory and rested on a bed while a catheter was inserted into an antecubital vein. A resting blood sample was taken and a saline drip was used to maintain a patent line. Subjects moved to the ergometer and began cycling for 1 h at 60% of pre-training $VO_{2\,peak}$. Respiratory samples were collected between 15–20, 35–40, and 55–60 min of exercise to measure VO_2 and VCO_2, and to calculate the respiratory exchange ratio (RER). This information was used to calculate whole-body fat and carbohydrate oxidation, using the nonprotein RER (Ferrannini 1988) and the equations of Peronnet et al. (1991). Venous blood samples were taken at 20, 40, and 60 min. Subjects were permitted to drink water ad libitum in the pre-training trial, and the same volume was ingested in the post-training trial.

HIIT protocol

Subjects conducted their first training session 1 week after the TE. Subjects trained on a cycle ergometer (Monark 894 E, Vansbro, Sweden) at a power output that elicited ~90% of $VO_{2\,peak}$, 3 $d \cdot week^{-1}$ for 6 weeks. Subjects completed 10 exercise intervals, lasting 4 min and separated by 2 min of rest during each training session. During the first and second training sessions, the power output was adjusted to the highest intensity that each subject could tolerate for a complete set of 10 intervals. Each subject's heart rate (HR) reached a steady state during the final 2 min of intervals 6–10, averaging 180 ± 2.4 $beats \cdot min^{-1}$ (~95% of HR_{max}). This HR was maintained during the remainder of the 6 weeks of training by increasing the power output during the exercise intervals and sessions. During the rest periods, subjects remained on the bike without pedaling and were allowed to consume water or sports drink ad libitum. Subjects continued with their light levels of additional aerobic exercise during the entire study (verified by activity logs). All training sessions were supervised by one of the investigators.

Muscle and blood analyses

Muscle enzyme activities

Two small pieces of frozen wet muscle (10–15 mg) were removed from each biopsy under liquid nitrogen for the determination of PDHa and PDH total (PDHt) activities (37 °C), as described elsewhere (Putman et al. 1993). A third muscle piece (6–10 mg) was removed from the pre- and post-training resting biopsies under liquid nitrogen for the spectrophotometric determination of mitochondrial citrate synthase, β-HAD, and m-AspAt maximal activities (37 °C), as described elsewhere (Srere 1969; Bergmeyer 1974).

An aliquot of each muscle enzyme homogenate (PDHa, PDHt, citrate synthase, β-HAD, m-AspAt) was then extracted with 0.5 mol·L^{-1} perchloric acid (HClO$_4$), containing 1 mmol·L^{-1} EDTA, and neutralized with 2.2 mol·L^{-1} KHCO$_3$. The supernatant from the extracts was used for the enzymatic spectrophotometric determination of total creatine (Bergmeyer 1974). Enzyme measurements were normalized to the highest total creatine content measured from all biopsies from each subject. There was no change in the average uncorrected total creatine content in the pre- and post-training samples, as reported elsewhere (Perry et al. 2007).

Muscle metabolites and glycogen

A portion of muscle from each biopsy was freeze dried, dissected free of visible blood and connective tissue, and powdered for metabolite and glycogen analyses. An aliquot of freeze-dried muscle (10–12 mg) was extracted with 0.5 mol·L^{-1} perchloric acid (HClO$_4$), containing 1 mmol·L^{-1} EDTA, and neutralized with 2.2 mol·L^{-1} KHCO$_3$. The supernatant was used to determine creatine, PCr, ATP, lactate, and hexose monophosphate contents, with enzymatic spectrophotometric assays (Bergmeyer 1974; Harris et al. 1974). Acetyl-CoA and acetylcarnitine were measured with radiometric techniques (Cederblad et al. 1990), and pyruvate was analyzed fluorometrically (Passoneau and Lowry 1993). Muscle glycogen content was determined from 2 aliquots of freeze-dried muscle (each 2–3 mg) from all biopsies, as described elsewhere (Bergmeyer 1974). Muscle metabolites were corrected for total creatine, as described above.

Western Blot analyses

An aliquot of wet muscle (30 mg) was homogenized with a Teflon pestle and glass mortar in an ice-cold buffer, containing 30 mmol·L^{-1} HEPES, 40 mmol·L^{-1} NaCl, 2 mmol·L^{-1} EGTA, 210 mmol·L^{-1} sucrose, 20 mmol·L^{-1} EDTA, and phenylmethylsulfonyl fluoride (61.4 mg·mL^{-1} dimethyl sulfoxide) for Western Blotting analyses. The homogenate was then mixed with 1.17 mol·L^{-1} KCl and 58.3 mmol·L^{-1} tetrasodium pyrophosphate, left on ice for 15 min, and centrifuged at 171 500g for 75 min at 4 °C in a Beckman Ti 50.1 rotor to collect total membranes. The pellet was homogenized, using the same Teflon pestle, with an ice-cold buffer, containing 10 mmol·L^{-1} Tris base and 1 mmol·L^{-1} EDTA. SDS (16%) was then added to the homogenate. A portion of the extract was used for the spectrophotometric determination of protein content (BCA protein assay). Muscle lysates were solubilized in Laemmeli's buffer and boiled for 5 min, resolved by SDS–PAGE on 8%–12% polyacrylamide gels,

and transferred to a PVDF membrane. Membranes were blocked with 10% nonfat milk powder or 7.5% bovine serum albumin, and immunoblotted overnight with antibodies specific for MCT 1 and 4 (gift from Dr. A.P. Halestrap, Bristol University, Bristol, U.K.), MO-25 used to detect fatty acid translocase, the homologue of human CD36 (FAT/CD36; monoclonal antibody; gift from Dr. N.N. Tandon, Otsuka Maryland Medicinal Laboratories, Rockville, Md.), plasma membrane fatty acid binding protein (FABPpm/mAsAT; polyclonal antibody; gift from Dr. J. Calles-Escandon, Wake Forest University School of Medicine, Winston-Salem, N.C.), fatty acid transport protein 4 (FATP 4; Santa Cruz Biotechnology, Santa Cruz, Calif.), GLUT 4 (Chemicon, Temecula, Calif.), hormone sensitive lipase (ProSci, Poway, Calif.), COX-IV (Molecular Probes, Ore.), and glycogen phosphorylase b (PHOS b, Biogenesis, Poole, U.K.). We used glycogen PHOS b as a marker of total phosphorylase content, because previous research has reported that training does not change the ratio of PHOS a and b (Chesley et al. 1996). Membranes were incubated for 1 h at room temperature with the corresponding secondary antibody, and the immunoreactive proteins were detected with enhanced chemiluminescence and quantified by densitometry. Ponceau staining of total protein per lane was used to verify consistent loading and transferring between lanes on each gel. A human muscle crude-membrane standard was loaded on each gel to correct for differences in blotting efficiency between gels.

Muscle calculations

Muscle ADP$_f$ and free AMP (AMP$_f$) contents were calculated by assuming equilibrium of the creatine kinase and adenylate kinase reactions (Dudley et al. 1987). Specifically, ADP$_f$ was calculated using the measured ATP, creatine, and PCr values, an estimated H$^+$ concentration, and the creatine kinase constant of 1.66×10^9 (Saltin 1990). AMP$_f$ was calculated from the estimated ADP$_f$ and measured ATP content, using the adenylate kinase equilibrium constant of 1.05. Free inorganic phosphate (Pi$_f$) was calculated by adding the estimated resting free phosphate of 10.8 mmol·kg^{-1} dry weight (Dudley et al. 1987) to the difference in PCr content (Δ [PCr]) minus the accumulation of glucose 6-phosphate between rest and selected exercise time points. Substrate level phosphorylation was calculated between rest and 5 min, using the following equation:

$$\text{ATP provision rate} = 1.5(\Delta[\text{lactate}]) + \Delta[\text{PCr}]$$

where Δ is the difference between the rest and 5-min values and brackets indicate concentration (Spriet et al. 1987). No attempts were made to account for lactate efflux during the TE. Glycogenolysis and the rate of lactate accumulation were calculated by taking the difference in glycogen and lactate contents, respectively, between rest and 5 min of the TE, and dividing these values by 5 min.

Blood measurements

Venous whole blood was collected in heparinized tubes, and 200 μL was immediately deproteinized with 1 mL of 0.6% (w/v) perchloric acid. The supernatant was stored at –20 °C and analyzed for lactate, glucose, and glycerol (Bergmeyer 1974). A second portion of blood was immediately centrifuged, and 500 μL of the plasma supernatant

was stored at –20 °C and analyzed for free fatty acids with a colorimetric assay (Wako NEFA C kit, Wako Chemicals, Richmond, Va.).

Statistics

Results are expressed as means ± SEM. The level of significance was established at $p < 0.05$ for all statistics. A 2-way analysis of variance (ANOVA) with repeated measures was used to test for differences in the following: (*i*) training-power outputs and heart rates across the 6 weeks of training, (*ii*) all measurements made during the 1 h cycle trial, and (*iii*) all measurements made on the resting and 5 min biopsies during the TE. When a significant *F* ratio was obtained, post hoc analyses were completed using a Student–Newman–Keuls test. All muscle and blood measurements at the exhaustion time point during the TE were compared using a paired *t* test, because of the varied times at which subjects reached exhaustion. A paired *t* test was also used to assess the effects of training on all Western Blot analyses, maximal enzymatic activities, the rates of glycogenolysis, lactate accumulation, substrate phosphorylation, PCr utilization between rest and 5 min of the TE, and whole-body substrate oxidation during the exercise trials.

Results

Training

The mean power output during the training sessions increased by 21% between the first and sixth weeks (209 ± 47 to 252 ± 51 W). Body mass was not different before and after training (pre-training, 72.7 ± 4.0 kg; post-training, 72.5 ± 3.9 kg). $VO_{2\ peak}$ increased by 9% following training (3.29 ± 0.24 to 3.58 ± 0.26 L·min^{-1}; $p < 0.05$).

Muscle adaptations to HIIT at rest

High-energy phosphates

The resting concentrations of PCr, ATP, ADP_f, and AMP_f were unchanged following training (Table 1).

TCA cycle and electron transport chain enzyme activities

The maximal activity of citrate synthase increased by 26% and the protein content of COX-IV increased by 18% following training (Fig. 2; $p < 0.05$).

Fat metabolism

HIIT increased β-HAD maximal activity by 29% (Fig. 2; $p < 0.05$), FAT/CD36 total protein content by 16% ($p < 0.05$), and FABPpm total protein content by 30% ($p < 0.01$). FATP4 and hormone sensitive lipase protein content did not change (Fig. 3).

Carbohydrate metabolism

m-AspAt and PDHt maximal activities increased by 26% and 21%, respectively (Fig. 4; $p < 0.05$), and the resting glycogen content increased by 59% following training (Table 2; $p < 0.001$). HIIT significantly increased GLUT 4 by 21% (Fig. 5; $p < 0.01$), MCT 1 protein content by 14%, and MCT 4 protein content by 16% (Fig. 5; $p < 0.01$). There was no change in the content of PHOS *b* (Fig. 5).

Performance and metabolic responses to cycling at 90% of pre-training $VO_{2\ peak}$

Exercise performance

Time to exhaustion at 90% of pre-training $VO_{2\ peak}$ improved by 111% following HIIT (7.1 ± 0.2 to 15.0 ± 1.1 min; $p < 0.001$). The post-training power output represented ~83% of the new $VO_{2\ peak}$.

Muscle metabolic responses to cycling: initial 5 min

Compared with the initial 5 min in the pre-training TE, the post-training TE demonstrated a reduction in the amount of PCr utilized, no change in the content of ATP, a blunting of the increase in ADP_f and AMP_f content, and a 30% increase in the ATP/ADP_f ratio at 5 min of the TE (Table 1; $p < 0.05$). There was a corresponding reduction in the increase in creatine and P_i (Table 1; $p < 0.05$). Muscle glycogenolysis decreased by 32% (Fig. 6A; $p < 0.05$), and lactate accumulation was lower at 5 min of the TE following training (Fig. 6B; $p < 0.01$). Substrate phosphorylation was also reduced by 20% (pre-training, 230.8 ± 7.2; post-training, 185.0 ± 14.7 mmol ATP·kg dry wt^{-1}; $p < 0.01$). Estimated muscle pH was higher following training at 5 min (Table 2; $p < 0.001$). PDH activity was not different at 5 min (Fig. 6C), but the relative PDH activation was reduced from 91% to 77% at post-training (PDHt increased by 23%). There were no changes in any other muscle metabolites measured (Table 2).

Exhaustion

Following training, the contents of ATP and acetyl-CoA were higher and acetylcarnitine was lower at exhaustion (Table 1; $p < 0.05$). Muscle lactate was lower, whereas pH (Table 2; $p < 0.01$) and PDH activity were higher at exhaustion following training (Fig. 6C; $p < 0.05$). There were no differences in the content of PCr or creatine, but there was a larger increase in P_i (Table 1; $p < 0.05$). There were no changes in any other muscle metabolites at exhaustion (Tables 1 and 2).

Venous blood metabolites

The concentration of blood lactate was lower at 5 min and higher at exhaustion during the TE following training (Table 3; $p < 0.05$). There were no changes in any other measured blood metabolites.

Metabolic responses to 60 min of cycling at 60% of pre-training $VO_{2\ peak}$

The power output for the post-training 60 min cycling ride was 55% of the new $VO_{2\ peak}$, and the mean RER for the 60 min cycling ride was lower following training (Table 4; $p < 0.05$). The mean rate of carbohydrate oxidation (Table 4; $p < 0.05$) and the total carbohydrate oxidized for the entire 60 min ride was 16% lower (pre-training, 121.0 ± 14.8 g; post-training, 104.2 ± 12.4 g; $p < 0.05$) and the mean rate of fat oxidation (Table 4; $p < 0.05$) and total fat oxidized was 12% higher (pre-training, 15.0 ± 1.5 g; post-training, 17.1 ± 1.3 g; $p < 0.05$) following HIIT. There was a reduction in venous blood lactate concentration at each time point measured during the 60 min cycle following training, but venous plasma free fatty acid and whole blood glucose levels were not altered (Table 5).

Table 1. Skeletal muscle pH, high-energy phosphates, and creatine contents during a cycling trial to exhaustion at 90% of pre-training peak oxygen consumption before and after 6 weeks of high-intensity interval training.

Parameter	Pre-training			Post-training		
	Rest	5 min	Exh	Rest	5 min	Exh
Cr, mmol·kg dry wt^{-1}	49.8±2.1	114.1±3^b	105.6±3.5	49.3±2.7	101.7±4.9^{a,b}	103.2±4.8
PCr, mmol·kg dry wt^{-1}	76.1±3.6	13.6±1.5^b	20.2±3.1	76.5±4.0	24.2±4.4^{a,b}	22.7±3.8
ATP, mmol·kg dry wt^{-1}	23.6±1.0	20.5±0.8^b	20.6±0.7	22.8±0.8	22.6±0.9^a	22.3±0.5^a
ADP$_f$, μmol·kg dry wt^{-1}	83.9±4.1	366±42.5^b	231.9±35.7	78.8±5.0	265.4±34.1^{a,b}	284.6±45.8
ATP/ADP$_f$	287.2±21.5	61.4±7.8^b	103.6±15.4	298.9±24.1	103.9±22.2^{a,b}	97.8±18.9
AMP$_f$, μmol·kg drywt^{-1}	0.29±0.03	6.79±1.45^b	2.91±0.89	0.27±0.04	3.39±0.69^{a,b}	4.23±1.49
Pi$_f$, mmol·kg dry wt^{-1}	10.8	80.9±3.8	7.8±1.0	10.8	68.2±6.4^a	13.1±2.0^a
pH	6.95±0.00	6.52±0.02^b	6.49±0.02	6.95±0.01	6.60±0.03^{a,b}	6.59±0.03^a

Note: Values are means ± standard error of the mean (SEM), n = 8. Cr, creatine; wt, weight; PCr, phosphocreatine; ADP$_f$, free ADP; AMP$_f$, free AMP; Pi$_f$, free inorganic phosphate. Exh, exhaustion (pre-training, 7.1 ± 0.2 min; post-training, 15.0 ± 1.1 min).

^{a}Significantly different from the same pre-training time point ($p < 0.05$).
^{b}Significantly different from rest in the same trial ($p < 0.05$).

Table 2. Skeletal muscle glycogen and metabolite data during a cycling trial to exhaustion at 90% of pre-training peak oxygen consumption before and after 6 weeks of high-intensity interval training.

Parameter	Pre-training			Post-training		
	Rest	5 min	Exh	Rest	5 min	Exh
Glycogen, mmol·kg dry wt^{-1}	521.8±47.0	351.5±41.4^b	ND	829.8±29.7^a	713.4±25.6^{a,b}	606.5±25.7
Glucose, mmol·kg dry wt^{-1}	1.2±0.1	8.3±0.7	12±1.4	1.9±0.3	9.9±1.3	12.6±1.5
G-6-P, mmol·kg dry wt^{-1}	0.4±0.1	8.0±0.9	7.7±0.8	0.5±0.1	5.5±1.1	6.4±1.5
F-6-P, mmol·kg dry wt^{-1}	0.04±0.01	0.90±0.13	0.79±0.15	0.11±0.02	0.66±0.18	0.80±0.29
G-3-P, mmol·kg dry wt^{-1}	0.61±0.17	7.29±0.96	8.60±0.45	1.85±0.57	8.20±1.45	7.72±0.94
Lactate, mmol·kg dry wt^{-1}	6.5±1.2	118.8±6.1^b	125.4±6.4	8.5±1.4	98±7.8^{a,b}	100.4±7.4^a
Pyruvate, mmol·kg dry wt^{-1}	0.22±0.02	0.41±0.06	0.47±0.05	0.29±0.05	0.61±0.07	0.46±0.03
Acetyl-CoA, mmol·kg drywt^{-1}	2.9±0.6	25.6±2.1	24.5±1.8	2.9±0.3	24.9±3.2	28.8±3.3^a
Acetylcarnitine, mmol·kg dry wt^{-1}	2.5±0.5	13.1±0.9	14.7±0.8	2.5±0.7	12.0±0.7	13.0±0.6^a

Note: Values are means ± standard error of the mean (SEM), n = 8. Exh, exhaustion; wt, weight; ND, no data; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; G-3-P, glycerol-3-phosphate. Values for the 5 min exercise time points were significantly greater than rest for each metabolite (main effect of time, $p < 0.05$).

^{a}Significantly different from the same pre-training time point ($p < 0.05$).
^{b}Significantly different from rest in the same trial ($p < 0.05$).

Fig. 2. Maximal activities of β-hydroxyacyl CoA dehydrogenase (β-HAD), citrate synthase (CS), and protein content of cytochrome c oxidase subunit IV (COX IV) in skeletal muscle before and after 6 weeks of high-intensity interval training. Values are means ± standard error of the mean (SEM) for 8 subjects. w.w., wet weight. *, $p < 0.05$, post-training (Post) > pre-training (Pre).
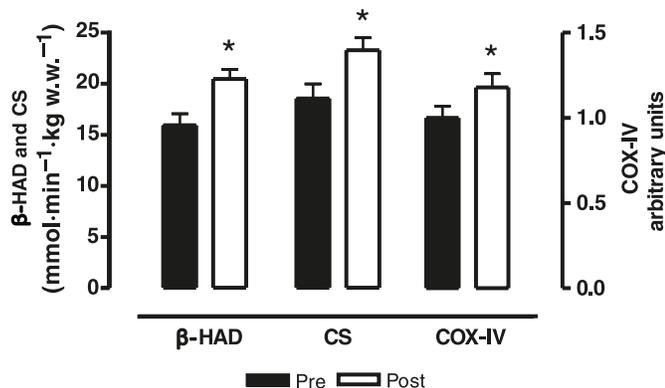

Fig. 3. Total skeletal muscle protein content of the fatty acid transporters FAT/CD36, FABPpm, and FATP 4, and total cytoplasmic hormone sensitive lipase (HSL) before and after 6 weeks of high-intensity interval training. Values are means ± standard error of the mean (SEM) for 8 subjects. *, $p < 0.05$, post-training (Post) > pre-training (Pre).
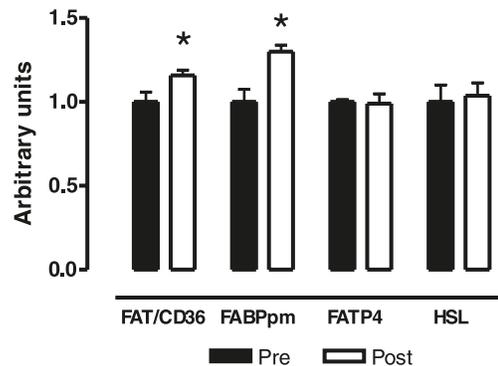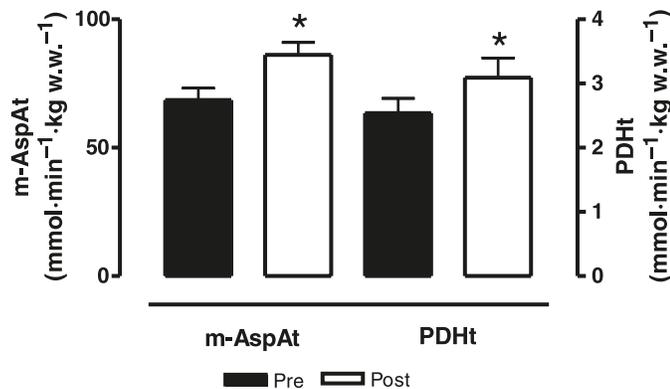
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Fig. 4. Maximal activities of mitochondrial aspartate-amino transferase (m-AspAt) and total pyruvate dehydrogenase (PDHt) in skeletal muscle before and after 6 weeks of high-intensity interval training. Values are means ± standard error of the mean (SEM) for 8 subjects. w.w., wet weight. *, $p < 0.01$, post-training (Post) > pre-training (Pre).
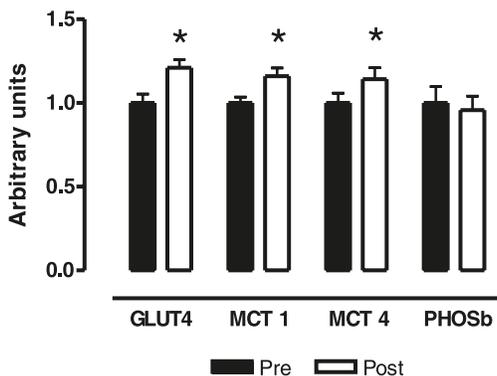

Fig. 5. Total skeletal muscle protein content of the MCT 1 and MCT 4 lactate transporters, the GLUT 4 glucose transporter, and phosphorylase (PHOS) *b* before and after 6 weeks of high-intensity interval training. Values are means ± standard error of the mean (SEM) for 8 subjects. *, $p < 0.01$, post-training (Post) > pre-training (Pre).

Fig. 6. Rates of skeletal muscle glycogenolysis (A) and lactate accumulation (B) between rest and 5 min, and rates of pyruvate dehydrogenase activation (PDHa) (C) during a cycling trial to exhaustion at 90% of pre-training $VO_{2\,peak}$ before and after 6 weeks of high-intensity interval training. Values are means ± standard error of the mean (SEM) for 8 subjects. d.w., dry weight; w.w., wet weight. *, $p < 0.01$ post-training (Post) > pre-training (Pre); †, significantly different from the previous time point in the same trial ($p < 0.05$). ‡, significantly different from the same pretaining time point ($p < 0.05$).
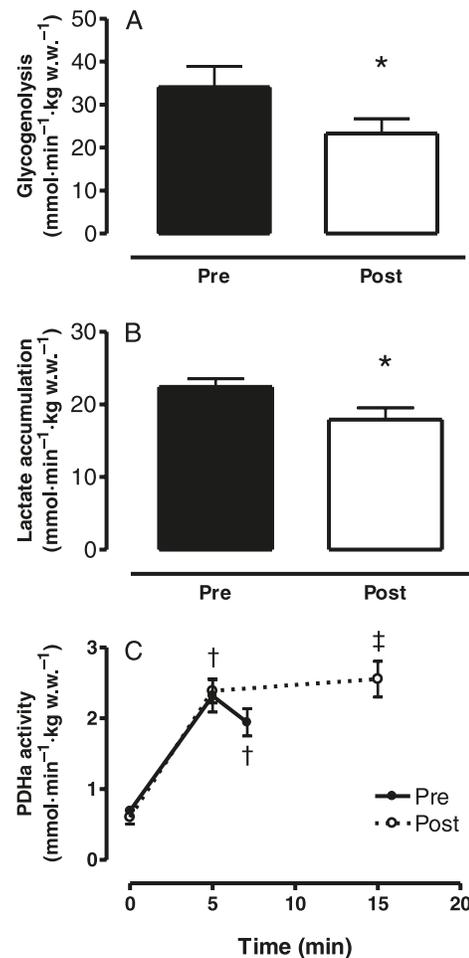

Discussion

This study demonstrated that 18 h of HIIT over a 6 week period provided a powerful training stimulus in previously untrained recreationally active individuals. This commitment is approximately two thirds of traditional MIT (>30 h over 6 weeks), although the actual contraction time of 12 h is approximately one third of MIT. Importantly, this is the first study to provide this broad characterization of improved metabolic capacities following HIIT, as well as the specific responses to challenges at both high (90% of pre-training $VO_{2\,peak}$) and moderate submaximal exercise intensities (60% of pre-training $VO_{2\,peak}$) following any form of high-intensity training. Our findings thoroughly demonstrate the potency of HIIT for improving substrate utilization at a variety of metabolic demands in humans.

Whole-body and skeletal muscle measurements were made pre- and post-training at rest, during a cycle ride to exhaustion at 90% of pre-training $VO_{2\,peak}$, and a 1 h cycle at 60% of pre-training $VO_{2\,peak}$ to corroborate previous findings of improved fat oxidation at moderate submaximal ex-

ercise intensities following HIIT (Talanian et al. 2007). The training adaptations included (*i*) increased whole-body aerobic capacity; (*ii*) increased maximal activities and protein content of 5 skeletal muscle mitochondrial enzymes; (*iii*) increased content of fatty acid transport proteins; (*iv*) increased glucose and lactate transport protein contents; (*v*) increased glycogen content; (*vi*) reduced glycogenolysis and reliance on substrate phosphorylation from glycolysis and PCr at 90% of pre-training $VO_{2\,peak}$; (*vii*) increased time to exhaustion at 90% pre-training $VO_{2\,peak}$; and (*viii*) increased fat oxidation at 60% of pre-training $VO_{2\,peak}$. Collectively, these results demonstrated that only 12 h of actual contraction time during repeated high-intensity exercise bouts (18 h total, including rest intervals) produced significant improvements in skeletal muscle metabolism and whole-body aerobic capacity and exercise performance at high intensities.

Table 3. Venous blood metabolite concentrations during a cycling trial to exhaustion at 90% of pre-training peak oxygen consumption before and after 6 weeks of high-intensity interval training.

Time	Lactate, mmol·L^{-1}		Glucose, mmol·L^{-1}		Glycerol, μmol·L^{-1}		Free fatty acids, mmol·L^{-1}	
	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training
Rest	0.6±0.1	0.6±0.1	5.0±0.4	4.2±0.3	40.0±4.9	35.2±6.2	0.19±0.02	0.19±0.04
5 min	3.9±0.5^b	2.8±0.4^{a,b}	4.7±0.5	4.4±0.5	46.9±4.7	41.7±8.6	0.16±0.02	0.27±0.08
Exhaustion	5.6±0.6	7.7±0.9^a	5.0±0.3	5.1±0.5	46.8±4.0	77.7±12.0^a	0.17±0.02	0.32±0.08^a

Note: Values are means ± standard error of the mean (SEM), $n = 8$.

^{a}Significantly different from the same time pretaining time point ($p < 0.05$).

^{b}Significantly different from rest in the same trial ($p < 0.05$).

Table 4. Whole-body metabolic responses to cycling for 60 min at 60% of peak oxygen consumption before and after 6 weeks of high-intensity interval training.

Time	RER		Rate of fat oxidation, g·min^{-1}		Rate of carbohydrate oxidation, g·min^{-1}	
	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training
Rest	0.93±0.01	0.91±0.01	0.17±0.03	0.27±0.04	2.20±0.28	1.88±0.22
20 min	0.91±0.01	0.89±0.01	0.24±0.03	0.34±0.04	2.06±0.30	1.74±0.20
40 min	0.89±0.01	0.87±0.01	0.34±0.03	0.41±0.04	1.80±0.21	1.59±0.21
60 min	0.91±0.01	0.89±0.01^a	0.25±0.03	0.34±0.04^a	2.02±0.25	1.74±0.21^a

Note: Values are means ± standard error of the mean (SEM), $n = 7$. RER, respiratory exchange ratio.

^{a}Significantly different from pre-training ($p < 0.05$). Main effect of training for RER, such that post-training < pre-training ($p < 0.05$); main effect of time for RER and both fat and carbohydrate oxidation rates between 0 and 60 min ($p < 0.001$).

Table 5. Venous blood metabolite concentrations during 60 min of cycling at 60% of pre-training peak oxygen consumption before and after 6 weeks of high-intensity interval training.

Time	Lactate, mmol·L^{-1}		Glucose, mmol·L^{-1}		Glycerol, μmol·L^{-1}		Free fatty acids, mmol·L^{-1}	
	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training
Rest	0.5±0.1	0.4±0.1	4.5±0.3	4.4±0.3	38.6±6.3	39.0±4.7	0.52±0.10	0.42±0.11
20 min	1.4±0.2^b	0.8±0.1^{a,b}	3.8±0.3	4.5±0.3	70.5±12.2	65.9±6.6	0.32±0.09	0.32±0.09
40 min	1.2±0.2^b	0.5±0.1^{a,b}	4.3±0.4	4.4±0.3	109.2±17.2	104.1±11.7	0.48±0.11	0.44±0.08
60 min	1.1±0.2^b	0.6±0.0^{a,b}	4.2±0.3	4.5±0.4	165.1±19.5	141.8±16.1	0.71±0.12	0.58±0.11

Note: Values are means ± standard error of the mean (SEM), $n = 8$.

^{a}Significantly different from the same pre-training time point ($p < 0.05$).

^{b}Significantly different from rest in the same trial ($p < 0.01$). There was a main effect of time for glycerol and free fatty acids ($p < 0.001$).

Training-induced metabolic adaptations in skeletal muscle: implications for untrained individuals

Previous investigations with well-trained cyclists have found that incorporating HIIT into a regular training regimen is effective at increasing fat oxidation at high-power outputs, reducing substrate phosphorylation, and increasing endurance performance (Westgarth-Taylor et al. 1997; Weston et al. 1997; Stepto et al. 1999; Clark et al. 2004). The current study demonstrated that significant adaptations can also occur in untrained individuals with HIIT. Moreover, the current study extends previous findings (Mendenhall et al. 1994; McConell et al. 2005; Perry et al. 2007; Talanian et al. 2007) by showing that the metabolic responses to HIIT occur at many different levels of muscle substrate utilization during moderate and high-intensity exercise, including membrane transport, rates of substrate phosphorylation, glycogenolysis, and fat oxidation.

$VO_{2\ peak}$ and mitochondrial oxidative capacity

The current study demonstrated that only 18 h of accumulated high-intensity interval-based exercise over 6 weeks resulted in a 9% increase in $VO_{2\ peak}$ and 18%–29% increases in the maximal activities or content of several key mitochondrial enzymes (citrate synthase, COX-IV, β-HAD, m-AspAt, and PDH). Thus, HIIT increases the capacity for both fat and carbohydrate oxidation. The responses of citrate synthase and β-HAD are similar to previous findings following 2–6 weeks of HIIT (Perry et al. 2007; Talanian et al. 2007), suggesting that the increases occur rapidly during HIIT in untrained recreationally active individuals. The increases in maximal enzyme activities, representing 5 key mitochondrial metabolic pathways, clearly document the increase in skeletal muscle mitochondrial volume and oxidative potential that occurred in response to the high-intensity, low-volume exercise stimulus of HIIT. The current results

are also generally similar to those reported following 2–6 weeks of very-high-intensity intermittent training involving repeated Wingate sprints (Burgomaster et al. 2005, 2006, 2007, 2008; Gibala et al. 2006). Many of these adaptive increases are similar to those reported following classic MIT protocols as well (Holloszy and Coyle 1984; Schantz 1986; Dudley et al. 1987; Kiens et al. 1993; Leblanc et al. 2004a, 2004b). While the lack of a control group training with a different protocol limits a comparison of our results to other paradigms, we believe that previous reports of metabolic responses to training serve as useful benchmarks and reveal the significance of this HIIT protocol.

Reduced glycogenolysis and substrate phosphorylation at 90% of pre-training $VO_{2\,peak}$

Exercise at 90% of $VO_{2\,peak}$ requires a high demand for ATP in skeletal muscle (Jansson et al. 1987; Green 1997). The energy demand is initially met through the degradation of PCr, increased glycolytic activity, and a rapid response in oxidative phosphorylation. Following HIIT, less glycogenolysis and substrate phosphorylation (PCr degradation and glycolytic flux) occurred in the initial 5 min of exercise at 90% of pre-training $VO_{2\,peak}$, suggesting that oxidative metabolism was more rapidly activated. Muscle [ATP] decreased less, and the increases in P_i, AMP_f, ADP_f, and [H^+] were significantly blunted in the initial 5 min of exercise following training. This indicates that the larger mitochondrial oxidative capacity following training afforded a tighter coupling between ATP supply and demand at the onset of exercise.

Muscle glycogenolysis, and therefore flux through PHOS, was reduced by 30% during the first 5 min of the TE in HIIT-trained individuals. Exercise-induced accumulations of AMP_f and ADP_f allosterically control the more active a form of PHOS, and the lower accumulations of these metabolites following training could account for the lower flux (Chesley et al. 1996; Phillips et al. 1996; Rush and Spriet 2001). The lower post-training accumulation of P_i might have also contributed to the lower glycogenolytic flux, given that P_i is a substrate for PHOS (Chesley et al. 1996; Phillips et al. 1996; Rush and Spriet 2001). Epinephrine levels were not measured in the current study, but previous studies have demonstrated lower levels following HIIT training (Mendenhall et al. 1994; Talanian et al. 2007). Thus, lower post-HIIT epinephrine levels might have resulted in less PHOS a during exercise at 90% of pre-training $VO_{2\,peak}$.

This study reported no change in PDH activation during cycling at 90% of pre-training $VO_{2\,peak}$ following HIIT, indicating that carbohydrate oxidation was maintained during high-intensity exercise after training. It might be argued that reduced production of ATP from substrate phosphorylation following HIIT would be compensated for by a greater oxidation of carbohydrates (increased flux through PDH). However, this occurred at the onset of exercise and would not have been detected 5 min into the cycling bout when PDH activation was measured. In addition, the amount of energy that was no longer derived from substrate phosphorylation following training would be small and difficult to detect, even if PDH activation had been measured earlier. Ultimately, the similar PDH activation, despite reduced glycogenolysis and lactate production, demonstrated a tightening of

metabolic control, in which reduced PHOS activation, glycolytic flux, and pyruvate production more closely matched the rate of carbohydrate oxidation, resulting in less lactate production. Furthermore, the increase in PDHt demonstrates that the capacity for carbohydrate oxidation was also increased. This could lead to a greater potential rate of carbohydrate oxidation at higher power outputs.

Collectively, these adaptations likely contributed to the 2-fold increase in endurance (TE) at 90% of pre-training $VO_{2\,peak}$ following training. HIIT was therefore very effective at (i) increasing mitochondrial oxidative capacity, (ii) increasing oxidative metabolism and reducing substrate phosphorylation and glycogen use early during intense exercise, and (iii) improving whole-body performance at high exercise intensities in previously untrained recreationally active individuals.

Increased content of muscle glycogen, GLUT4, and MCT1 and 4

Six weeks of HIIT increased muscle glycogen content by 59%. This increase is one of the largest reported following training. MIT studies have typically reported increases of 10%–40% (Gollnick et al. 1973; Henriksson 1977; Kiens et al. 1993; Chesley et al. 1996), although 1 study reported a 58% increase (Leblanc et al. 2004a). Interestingly, 2 weeks of HIIT did not increase resting glycogen content (Talanian et al. 2007). Furthermore, variable training-induced increases in glycogen content of 0%–30% have been reported following 2–7 weeks of SIT (Harmer et al. 2000; Rodas et al. 2000; Burgomaster et al. 2005, 2008; Gibala et al. 2006), with 1 study showing a ~55% increase (Burgomaster et al. 2006). The greater content of GLUT4 supports the findings of a larger PDHt and increased capacity for carbohydrate oxidation. Likewise, the greater MCT1 and 4 contents indicate an improved capacity to transport lactate and might have assisted in reducing lactate concentration during the high-intensity exercise trial. These responses in GLUT4 and the MCTs have also been observed following SIT (Burgomaster et al. 2007).

Increased fat oxidation

Six weeks of HIIT increased whole-body fat oxidation and decreased carbohydrate oxidation during cycling at 60% of pre-training $VO_{2\,peak}$, as has been reported in classic MIT studies (Henriksson 1977; Hurley et al. 1986; Kiens et al. 1993; Leblanc et al. 2004a). A similar shift in fuel utilization has also been reported following only 2 weeks (7 sessions) of HIIT in untrained females (Talanian et al. 2007). A small effect has also been observed following 6 weeks of SIT (Burgomaster et al. 2008).

The increases in FAT/CD36 and FABPpm reported in this study could have contributed to the enhanced fat oxidation by increasing the rate of free fatty acid transfer across the muscle and mitochondrial (FAT/CD36) membranes. Both transporters are found on the sarcolemma, the mitochondrial membranes, and in a cytoplasmic pool in skeletal muscle (Berk et al. 1990; Stump et al. 1993; Bonen et al. 2000; Bezaire et al. 2005; Holloway et al. 2006). Inhibition of FAT/CD36 on either the sarcolemma (Bonen et al. 1998, 2000) or mitochondrial membranes (Campbell et al. 2004; Bezaire et al. 2005; Holloway et al. 2006) results in de-

creased long-chain fatty acid (LCFA) transport or oxidation, respectively. FABPpm has been shown to regulate LCFA transport across the sarcolemma (Turcotte et al. 2000; Clarke et al. 2004; Holloway et al. 2007), but does not appear to be rate-limiting for mitochondrial LCFA transport (Holloway et al. 2007). Interestingly, FABPpm and m-AspAt are identical proteins (Berk et al. 1990; Stump et al. 1993), functioning as a fatty acid transport protein on the sarcolemma, and facilitating electron transfer between the cytoplasm and mitochondria (Lehninger et al. 1993; Holloway et al. 2007). FABPpm adapts quickly to HIIT, given that the 25% increase in whole muscle content reported after 2 weeks of HIIT (Talanian et al. 2007) is similar to the 31% increase after 6 weeks in the current study. However, 2 weeks of HIIT did not increase FAT/CD36, which is surprising considering the improved fat oxidation observed in the study by Talanian et al. (2007). Neither FAT/CD36 nor FABPpm increased following 6 weeks of SIT (Burgomaster et al. 2007). Collectively, these findings suggest that a longer duration (in minutes) of high-intensity intervals are required to increase the expression of these proteins, and a period of greater than 2 weeks is necessary for FAT/CD36 to respond. The lack of change in FATP4 content, another fatty acid transport protein shown to influence fatty acid transport across membranes (DiRusso et al. 2005; Nickerson and Bonen 2005), suggests that the improved fat oxidation in the current study was due to other mechanisms. Finally, it should be remembered that whole muscle protein measures do not reveal the location of HIIT-induced increases and (or) redistributions of transport proteins among sarcolemmal, cytoplasmic, and mitochondrial pools.

Speculations on the mechanisms of HIIT-induced increases in mitochondrial oxidative capacity

We can only speculate on the mechanisms by which HIIT stimulated mitochondrial biogenesis with such short training durations. Elevations in cytoplasmic calcium, AMP_f, and other signals occurring during muscle contraction activate several signaling pathways, and downstream transcription factors known to regulate the expression of genes encoding mitochondrial proteins in skeletal muscle (Hood 2001; Freyssenet 2007). Thus, it is likely that the intense contractions during HIIT produce higher levels of these stimuli than MIT, albeit for a shorter duration. The apparently similar increases in mitochondrial oxidative capacity following HIIT (current study; Perry et al. 2007; Talanian et al. 2007), SIT (MacDougall et al. 1998; Rodas et al. 2000; Burgomaster et al. 2005, 2006, 2007, 2008), and MIT (Holloszy and Coyle 1984; Schantz 1986; Dudley et al. 1987; Kiens et al. 1993; Leblanc et al. 2004a, 2004b) suggest that greater changes in these metabolic signals compensate for a reduced stimulus duration and volume (h·d^{-1}, h·week^{-1}). Alternatively, the real potency might simply be in the repetitive nature of this training, such that the repeated surges in these signals at the onset of each work interval could contribute to a form of pulsatile signaling. Clearly, future work is required to determine the mechanisms by which different training regimens induce similar adaptations.

Summary and conclusions

The current study demonstrates that 6 weeks of this unique HIIT protocol in previously untrained individuals increases whole body $VO_{2\ peak}$, the maximal activities or protein content of 5 skeletal muscle mitochondrial enzymes, and the content of transport proteins for fatty acids, glucose, and lactate, as well as resting glycogen. These static responses were associated with reduced glycogenolysis and reliance on substrate phosphorylation from glycolysis and PCr at 90% of pre-training $VO_{2\ peak}$, a greater time to exhaustion at 90% of pre-training $VO_{2\ peak}$, and increased fat oxidation at 60% of pre-training $VO_{2\ peak}$. Collectively, these findings indicate that HIIT increases the capacity for both fat and carbohydrate oxidation in skeletal muscle. The result is a tightening of metabolic control during both moderate and high exercise challenges, such that oxidative phosphorylation is optimized and carbohydrate use is spared. These results are impressive given that they occurred in untrained individuals who were previously recreationally active with only 18 h of repeated high-intensity exercise bouts (12 h of actual contraction time) every other day for 6 weeks.

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