Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during exercise in women

Jason L. Talanian,[1] Stuart D. R. Galloway,[2] George J. F. Heigenhauser,[3] Arend Bonen,[1] and Lawrence L. Spriet[1]

[1]*Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada,* [2]*Department of Sport Studies, University of Stirling, Stirling, Scotland; and* [3]*Department of Medicine, McMaster University, Hamilton, Ontario, Canada*

Talanian JL, Galloway SD, Heigenhauser GJ, Bonen A, Spriet LL. Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during exercise in women. *J Appl Physiol* 102: 1439–1447, 2007. First published December 14, 2006; doi:10.1152/japplphysiol.01098.2006.—Our aim was to examine the effects of seven high-intensity aerobic interval training (HIIT) sessions over 2 wk on skeletal muscle fuel content, mitochondrial enzyme activities, fatty acid transport proteins, peak O_2 consumption ($\dot{V}O_{2\,peak}$), and whole body metabolic, hormonal, and cardiovascular responses to exercise. Eight women (22.1 ± 0.2 yr old, 65.0 ± 2.2 kg body wt, 2.36 ± 0.24 l/min $\dot{V}O_{2\,peak}$) performed a $\dot{V}O_{2\,peak}$ test and a 60-min cycling trial at ∼60% $\dot{V}O_{2\,peak}$ before and after training. Each session consisted of ten 4-min bouts at ∼90% $\dot{V}O_{2\,peak}$ with 2 min of rest between intervals. Training increased $\dot{V}O_{2\,peak}$ by 13%. After HIIT, plasma epinephrine and heart rate were lower during the final 30 min of the 60-min cycling trial at ∼60% pretraining $\dot{V}O_{2\,peak}$. Exercise whole body fat oxidation increased by 36% (from 15.0 ± 2.4 to 20.4 ± 2.5 g) after HIIT. Resting muscle glycogen and triacylglycerol contents were unaffected by HIIT, but net glycogen use was reduced during the posttraining 60-min cycling trial. HIIT significantly increased muscle mitochondrial β-hydroxyacyl-CoA dehydrogenase (15.44 ± 1.57 and 20.35 ± 1.40 mmol·min^{-1}·kg wet mass^{-1} before and after training, respectively) and citrate synthase (24.45 ± 1.89 and 29.31 ± 1.64 mmol·min^{-1}·kg wet mass^{-1} before and after training, respectively) maximal activities by 32% and 20%, while cytoplasmic hormone-sensitive lipase protein content was not significantly increased. Total muscle plasma membrane fatty acid-binding protein content increased significantly (25%), whereas fatty acid translocase/CD36 content was unaffected after HIIT. In summary, seven sessions of HIIT over 2 wk induced marked increases in whole body and skeletal muscle capacity for fatty acid oxidation during exercise in moderately active women.

fatty acid metabolism; mitochondrial enzymes; aerobic capacity; fatty acid transport

ENDURANCE EXERCISE TRAINING results in an improved capacity for whole body fat oxidation that is associated with increased mitochondrial volume as assessed by increases in citrate synthase and β-hydroxyacyl-CoA dehydrogenase (β-HAD) activities (19, 30, 37, 52). These, along with other, adaptations not only improve the potential for muscle to utilize lipids as a substrate for energy, but they are also associated with improved insulin sensitivity (20) and health. Improvement of skeletal muscle fatty acid oxidation is of considerable importance for individuals attempting to increase fat oxidation during exercise and also for athletes attempting to spare carbohydrate during competition.

It has commonly been observed that 6–12 wk of exercise training at a moderate intensity [MIT, 60–75% peak O_2 consumption ($\dot{V}O_{2\,peak}$)] can improve aerobic capacity and maximal mitochondrial enzyme activities (19, 28, 29, 37). In addition, sprint interval training (SIT) at very high power outputs (150–300% $\dot{V}O_{2\,peak}$ power) for 6–7 wk produces similar results (41, 48, 55). Recent evidence has also shown that daily sessions of MIT (2 h/day) for only 6–10 days can improve aerobic capacity and mitochondrial enzyme activities (10, 52), although not all short-term MIT protocols have reported similar increases (46, 47). Even as few as six SIT sessions in 2 wk have been shown to increase citrate synthase activity but without an increase in $\dot{V}O_{2\,peak}$ (5). The MIT and SIT short-duration (2-wk) protocols produce substantial training effects and health benefits in a short period of time. However, MIT for 2 h/day is time consuming and difficult to complete, and SIT is performed at an all-out maximal intensity that is very challenging and may be too intense for individuals beginning a training program to sustain.

Two weeks of high-intensity aerobic interval training (HIIT), performed at an exercise intensity (80–95% $\dot{V}O_{2\,peak}$) between that required for moderate training regimens and that required for sprint training regimens, may offer benefits similar to those offered by MIT and SIT. HIIT over a longer period of time (4–6 wk) has been reported to increase high-intensity exercise performance, muscle buffering capacity, whole body exercise fat oxidation rates, and aerobic capacity (15, 39, 63). However, no studies have examined whether aerobic capacity and skeletal muscle metabolic adaptations are improved in only 2 wk of HIIT.

Our aim was to investigate the effect of seven HIIT sessions over a 2-wk period on skeletal muscle metabolism during a 60-min steady-state cycling trial in recreationally active women. We measured aerobic capacity, exercise whole body fat oxidation, and muscle glycogen and triacylglycerol (TG) contents, maximal mitochondrial enzyme activities, and fatty acid transport proteins before and after training. In addition, we also evaluated the effects of training on circulatory substrates and on respiratory responses during HIIT throughout training *sessions 2* and *7*.

METHODS

Eight healthy recreationally active women (22 ± 1 yr old, 65.0 ± 2.2 kg body wt, 2.36 ± 0.24 l/min $\dot{V}O_{2\,peak}$) volunteered to participate

Address for reprint requests and other correspondence: J. L. Talanian, Dept. of Human Health and Nutritional Sciences, Univ. of Guelph, Guelph, ON, Canada N1G 2W1 (e-mail: jtalania@uoguelph.ca).

in the study. On average, subjects engaged in recreational physical activity 2–3 days/wk. Most subjects did not limit their exercise to one type, but common activities included weight lifting, soccer, cycling, swimming, and walking. Subjects were fully informed of the purpose of the study and of potential risks before giving written consent. This study was approved by the Ethics Committees at McMaster University and the University of Guelph.

Preliminary Testing

Before the study, subjects reported to the laboratory on two occasions. On the first visit, subjects performed an incremental cycling (Lode Excalibur, Quinton Instrument, Grogingen The Netherlands) test to exhaustion for determination of $\dot{V}O_{2\,peak}$. Respiratory gases were collected and analyzed using a metabolic cart (Vmax 229, Sensormedic, Yorba Linda, CA). On the second visit, appropriate power outputs for the experimental trials were verified. Subjects cycled for 15 min at 60% $\dot{V}O_{2\,peak}$ to establish the power output for the 60-min trial. They then performed four to six bouts of cycling at 90% $\dot{V}O_{2\,peak}$, with each bout lasting 4 min and separated by 2 min of rest, to establish power outputs for the HIIT sessions. After 2 wk (7 sessions) of HIIT, subjects repeated the incremental cycling test to exhaustion to establish the posttraining $\dot{V}O_{2\,peak}$.

Cycle Trials at ~60% $\dot{V}O_{2\,peak}$

Subjects performed a 60-min cycling trial at a moderate intensity (~60% $\dot{V}O_{2\,peak}$) before and 3 days after seven sessions of HIIT. Subjects arrived at the laboratory 3–4 h after a meal. They abstained from strenuous exercise and recorded their diet in the 24 h before the trial. At 3–4 h before the 60-min ride, subjects ingested a meal that was provided for them. Before the posttraining 60-min ride, subjects replicated the same diet they ingested before the pretraining ride. A Teflon catheter, which was flushed with 0.9% saline to maintain patency, was inserted into an antecubital vein for blood sampling. One leg was prepared for percutaneuos needle biopsy sampling of the vastus lateralis muscle. Three incisions were made in the skin and deep fascia under local anesthesia (2% xylocaine without epinephrine) for three separate biopsies. Immediately before exercise, venous blood (5 ml) and one muscle biopsy were obtained while the subject rested on a bed. All muscle samples were immediately frozen in liquid nitrogen for subsequent analysis. Subjects then cycled for 60 min at ~60% $\dot{V}O_{2\,peak}$ at a constant cadence (78–85 rpm) on the Lode ergometer. Respiratory gases were collected at 13–17, 28–32, 43–47, and 55–59 min of exercise for measurements of O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) and calculation of respiratory exchange ratio (RER). These parameters were used to calculate whole body fat and carbohydrate oxidation with use of the nonprotein RER table (16) and according to the following equations: carbohydrate oxidation = 4.585 ($\dot{V}CO_2$) − 3.226 ($\dot{V}O_2$), and fat oxidation = 1.695 ($\dot{V}O_2$) − 1.701 ($\dot{V}CO_2$) (45). Venous blood samples were obtained at 15, 30, 45, and 60 min of exercise. Immediately after exercise, two muscle biopsies were taken with the subject sitting on the cycle ergometer. The same procedure was repeated after HIIT, with muscle biopsies taken from the other leg.

HIIT

At 2 days after the initial 60-min trial, subjects began training every other day, completing seven HIIT sessions in 13 days (Fig. 1). All training sessions were supervised. Each session consisted of ten 4-min cycling bouts at 90% $\dot{V}O_{2\,peak}$ separated by 2 min of rest. Heart rate (HR) was recorded throughout training and was held constant at ~90% of maximal by increases in the power output as training progressed. Required adjustments in training power output were made at the beginning of sessions, and all subjects experienced three power output increases during the initial six training sessions. During training *session 7*, subjects cycled at the same power output as during training *session 2* to enable training-related comparisons. During training *sessions 2* and *7*, respiratory gases and venous blood samples (Teflon catheter) were collected before and immediately after *bouts 1, 3, 5,* and *10*. Throughout the 2 wk of training, subjects continued the recreational activities in which they were engaged before training.

Analyses

Blood measurements. Venous blood was collected in heparin sodium tubes. A portion (1.5 ml) was added to 30 μl of EGTA and reduced glutathione and centrifuged (10,000 *g* for 3 min), and the supernatant was analyzed for epinephrine by an enzymatic immunoassay (Labor Diagnostika Nord, Nordhorn, Germany). A second portion (200 μl) was added to 1 ml of 0.6 M perchloric acid and centrifuged, and the supernatant was analyzed for blood glucose, lactate, and glycerol by fluorometric techniques (1). A third portion (1.5 ml) was centrifuged, and the plasma was analyzed for free fatty acids (FFA) by an enzymatic colorimetric technique (NEFA C test kit, Wako Chemicals, Richmond, VA).

Muscle enzyme activities. Resting frozen wet muscle samples (~6–10 mg) were homogenized in 0.1 M KH_2PO_4 and BSA and subjected to three freeze-thaw cycles, and the maximal activities of citrate synthase and β-HAD were determined on a spectrophotometer (at 37°C) using previously described methods (53). The muscle homogenate was analyzed for total creatine (Cr), and enzyme measurements were normalized to the highest total Cr measured before and after HIIT among each subject.

Muscle metabolites. A portion of the resting and first postexercise muscle biopsy were freeze-dried, powdered, and dissected free of visible connective tissue, fat, and blood. One aliquot of freeze-dried powdered muscle (~10 mg) was extracted in 0.5 M $HClO_4$-1 mM EDTA and neutralized with 2.2 M $KHCO_3$. The supernatant was used to measure Cr, phosphocreatine (PCr), ATP, and lactate. A second aliquot (2–4 mg) was extracted in 0.1 M NaOH and neutralized with 0.1 M HCl-0.2 M citric acid-0.2 M Na_2PO_4, and amyloglucosidase was added to break down glycogen to glucose, which was measured spectrophotometrically (1). The Folch extraction was used on a third freeze-dried aliquot (6–9 mg) to separate TG from the muscle (17). The TG were degraded, and the resultant glycerol was extracted for the determination of intramuscular TG (IMTG) content (1). Because the total Cr content of freeze-dried muscle samples was similar before and after training, all freeze-dried measurements were normalized to the highest total Cr measured among all six biopsies from each subject.

Western blots. Frozen wet muscle samples (50–70 mg) from the second postexercise biopsy were initially homogenized in a buffer containing 210 mM sucrose, 2 mM EGTA, 40 mM NaCl, 30 mM HEPES, 20 mM EDTA, PMSF, and DMSO. A second buffer containing 1.17 M KCl and 58.3 M tetra-sodium pyrophosphate was

Fig. 1. High-intensity interval training (HIIT) study design. $\dot{V}O_{2\,peak}$, peak O_2 consumption; S1–S7, training *sessions 1–7*.
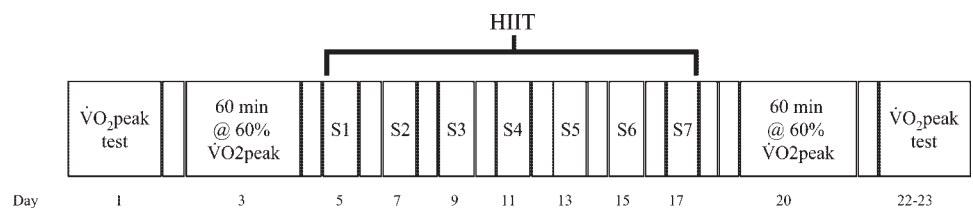
added, samples were centrifuged (50,000 rpm for 75 min), and the supernatant was discarded. Samples were then homogenized in a third buffer (10 mM Tris base-1 mM EDTA), 16% SDS was added, samples were centrifuged (3,000 rpm for 15 min), and the supernatant was used to determine fatty acid translocase (FAT)/CD36, plasma membrane fatty acid-binding protein ($FABP_{pm}$), and hormone-sensitive lipase (HSL) total content through a Western blot technique. Briefly, samples were separated on an 8% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. A monoclonal antibody (MO25) was used to specifically detect FAT/CD36 content (42), an $FABP_{pm}$/mAspAT polyclonal antibody was used was to determine $FABP_{pm}$ content (6), and a polyclonal antibody for HSL (ProSci, Poway, CA) was used to determine total HSL content.

Statistics. Values are means ± SE. $FABP_{pm}$, FAT/CD36, and HSL were analyzed using paired *t*-tests. All other data were analyzed by two-way (time × trial) repeated-measures ANOVA to determine significant differences during the 60-min trials and between training *sessions 2* and *7*. Specific differences were identified using a Student-Newman-Keuls post hoc test. Statistical significance was accepted at $P < 0.05$.

RESULTS

HIIT

$\dot{V}O_{2 peak}$ increased 13% [from 2.36 ± 0.24 l/min (36.3 ± 3.7 ml·kg^{-1}·min^{-1}) before training to 2.66 ± 0.21 l/min (40.9 ± 3.2 ml·kg^{-1}·min^{-1})] after seven HIIT sessions consisting of ten 4-min bouts at ~90% $\dot{V}O_{2 peak}$ with 2 min of rest between bouts. Initial training power outputs (163–227 W) were increased by an average of 19.0 ± 0.6 W from training *session 1* to training *session 6* to maintain a constant HR during the exercise sessions. For comparison, training power outputs during *session 7* were reduced to match the absolute power outputs during *session 2*. The average absolute $\dot{V}O_2$ during training *sessions 2* and *7* were not different (Table 1). During training *session 2*, $\dot{V}O_2$ reached 86% of
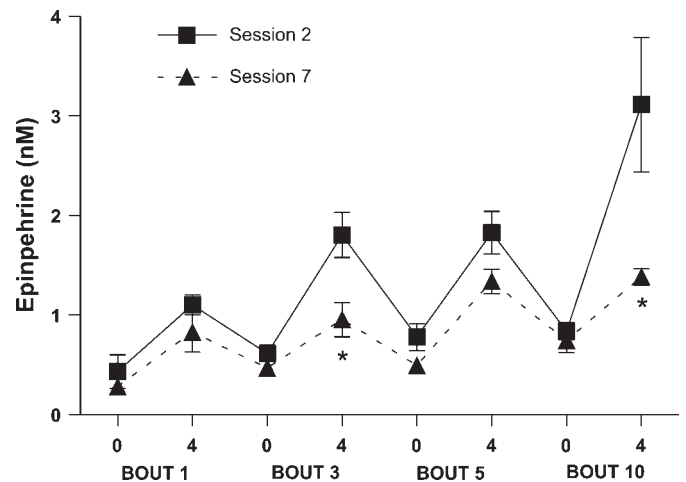

Fig. 2. Venous plasma epinephrine concentrations during HIIT *sessions 2* and *7*. Values are means ± SE ($n = 8$). *Significantly lower than the same time point during *session 2* ($P < 0.05$).

pretraining $\dot{V}O_{2 peak}$ in *bout 1* and 95% of pretraining $\dot{V}O_{2 peak}$ in *bout 10*, while the same power outputs represented 77% and 84% of the posttraining $\dot{V}O_{2 peak}$ in *bouts 1* and *10* of training *session 7* (Table 1). The peak HR attained during *session 2* ranged from 171 ± 2 beats/min during *bout 1* to 181 ± 1 beats/min during *bout 10* and was significantly lower throughout training *session 7* (Table 1).

Venous plasma epinephrine concentrations increased during each cycling bout during training *session 2* and reached the highest level after *bout 10* (Fig. 2). There was a significantly blunted epinephrine response in *session 7* after *bouts 3* and *10*. Whole blood lactate concentrations in *session 2* increased after *bouts 1* and *3* and reached a plateau during the remaining bouts (Table 1). There was no difference in the lactate response to

Table 1. *Respiratory, heart rate, and venous blood measurements during HIIT sessions 2 and 7*

	Bout 1		*Bout 3*		*Bout 5*		*Bout 10*	
	0 min	4 min	0 min	4 min	0 min	4 min	0 min	4 min
$\dot{V}O_2$, l/min								
Session 2		2.03±0.17		2.19±0.16		2.19±0.17		2.24±0.15
Session 7		2.07±0.17		2.17±0.19		2.21±0.26		2.25±0.20
$\dot{V}O_{2 peak}$								
Session 2, % pre training $\dot{V}O_{2 peak}$		86.1±2.5		92.8±2.5†		92.9±2.5†		94.8±2.3†
Session 7, % post training $\dot{V}O_{2 peak}$		77.7±2.3*		81.5±2.6*†		83.0±3.5*†		84.4±2.6*†
Heart rate, beats/min								
Session 2	72±2	171±2	125±4†	180±2†	133±3†	181±2†	132±3†	181±1†
Session 7	69±4	167±2	125±3†	174±2*†	129±3†	177±2*†	126±4†	177±2*†
Lactate, mM								
Session 2	0.6±0.1	2.1±0.3	3.1±0.2†	3.4±0.2†	3.5±0.1†	3.6±0.2†	3.2±0.2†	3.3±0.2†
Session 7	0.6±0.1	2.0±0.2	2.8±0.1†	3.0±0.2†	2.9±0.1†	3.3±0.2†	3.3±0.2†	3.5±0.2†
Glucose, mM								
Session 2	4.84±0.12	4.58±0.25	4.90±0.23	4.53±0.21	5.16±0.32	4.94±0.17	5.34±0.19	4.51±0.18
Session 7	4.91±0.14	4.68±0.10	4.46±0.19	4.11±0.17	4.80±0.20	4.58±50.21	5.34±0.28	4.77±0.24
FFA, mM								
Session 2	0.44±0.10	0.27±0.04	0.34±0.06	0.26±0.03	0.43±0.11	0.28±0.04	0.59±0.14†	0.38±0.11
Session 7	0.32±0.06	0.27±0.04	0.28±0.03	0.22±0.03	0.31±0.03	0.27±0.03	0.42±0.04	0.27±0.03
Glycerol, μM								
Session 2	35.4±6.3	41.6±7.6	59.2±8.0†	67.0±7.5†	87.2±11.9†	84.2±10.4†	109.4±12.0†	127.7±13.5†
Session 7	45.8±4.6	55.9±3.8	64.0±4.6†	65.2±6.0†	74.7±4.6†	79.2±5.6†	88.6±3.9*†	97.6±5.9*†

Values are means ± SE, ($n = 8$). HIIT, high-intensity interval training; $\dot{V}O_2$, O_2 consumption; FFA, free fatty acids. *Significantly lower ($P < 0.05$) than the same time point during *session 2*. †Significantly higher ($P < 0.05$) than the same time point during *bout 1*.

cycling during *session 7*. Whole blood glucose and plasma FFA concentrations were unchanged throughout the training bouts in both training sessions (Table 1). Whole blood glycerol concentrations increased during the training bouts in *sessions 2* and *7* but were significantly lower before and after *bout 10* in *session 7* (Table 1).

Cycling at ~60% $\dot{V}_{O_2\,peak}$ Before and After Training

Subjects cycled for 60 min at 101.3 ± 4.2 W before and after HIIT. This power output represented 63.9 ± 2.6% of the pretraining and 55.2 ± 2.2% of the posttraining $\dot{V}_{O_2\,peak}$ (Table 2). The RER was significantly lower after HIIT (Table 2), and the estimated whole body fat oxidation was significantly higher at 30, 45, and 60 min of cycling (Fig. 2). Total fat oxidation during the 60-min trial before training was 15.0 ± 2.4 g and increased by 36% after HIIT to 20.4 ± 2.5 g. There was a reciprocal decrease in whole body carbohydrate oxidation at 30, 45, and 60 min after training (Fig. 3). Total carbohydrate oxidation before training was 80.7 ± 2.2 g and decreased by 23% after HIIT to 62.1 ± 1.4 g.

HR was significantly lower after training at 45 and 60 min of cycling (Fig. 4). Plasma epinephrine concentrations increased during exercise in both trials but were blunted at 30 and 60 min of exercise after training (Fig. 4). Plasma lactate was significantly increased above rest at all exercise time points in both trials, but the increase was blunted after HIIT at 15, 30, and 45 min of exercise (Fig. 4).

Plasma FFA decreased from rest at 15 min and then increased over time and was significantly higher than the level at rest after 60 min of exercise in the pretraining trial (Table 3). After training, plasma FFA was not altered from rest for 45 min but increased significantly above rest at 60 min. Whole blood glycerol was elevated above rest at all exercise time points in both trials and was significantly higher following training at 30 and 60 min of exercise (Table 3). Blood glucose was unchanged by exercise in both trials but was higher at rest and at 45 and 60 min of exercise after training (Table 3).

Muscle Analysis

Maximal β-HAD activity (15.44 ± 1.57 and 20.35 ± 1.40 mmol·min^{-1}·kg wet mass^{-1} before and after training, respectively) increased by 32% and maximal citrate synthase activity (24.45 ± 1.89 and 29.31 ± 1.64 mmol·min^{-1}·kg wet mass^{-1} before and after training, respectively) increased 20% after HIIT (Fig. 5). There was a nonsignificant increase in muscle

HSL protein content (~13%) after training (Fig. 6). Total muscle $FABP_{pm}$ content increased 25% after training, while muscle FAT/CD36 protein content was unchanged (Fig. 6).

Resting muscle glycogen content was unaffected by training, but net muscle glycogen utilization was decreased by 12% after 60 min of exercise after training (Table 4). IMTG content decreased 12% and 17% after 60 min of cycling before and after training, respectively, but there was no difference between the trials (Table 4).

Resting muscle PCr was similar in both trials, but PCr was higher 60 min after training, such that net PCr degradation was significantly decreased by 40% after HIIT. Muscle ATP was unchanged by exercise after both trials, but ATP contents were lower after than before training at 60 min (Table 4). Muscle free ADP at 60 min was lower after the posttraining trial (Table 4). Muscle lactate contents increased from rest after 60 min of exercise to the same extent in both trials (Table 4).

DISCUSSION

This study examined the effects of 2 wk of HIIT at ~90% $\dot{V}_{O_2\,peak}$ on whole body and muscle metabolic responses to exercise at ~60% pretraining $\dot{V}_{O_2\,peak}$ in recreationally active females. This is the first study using this short-duration HIIT protocol to measure whole body responses and metabolic adaptations in skeletal muscle. Training resulted in increased $\dot{V}_{O_2\,peak}$, whole body fat oxidation during exercise, and maximal mitochondrial enzyme activities (citrate synthase and β-HAD) after only seven HIIT sessions in 2 wk. Training also increased the skeletal muscle content of $FABP_{pm}$, which may have contributed to the increases in whole body fat oxidation.

Training-Induced Increases in $\dot{V}_{O_2\,peak}$ and Muscle Mitochondrial Enzymes

Classic responses to the traditional long-duration (>24 h of accumulated training) submaximal training protocols are an improved aerobic capacity (9, 19, 28), increased whole body fat oxidation, and increased skeletal muscle mitochondrial enzyme activities (28, 29, 37). It has also been shown that as few as six to seven 2-h sessions at ~60–70% $\dot{V}_{O_2\,peak}$ increase aerobic capacity, whole body fat oxidation, and mitochondrial enzyme activities (10, 52). Although a lack of a control group training for a similar duration at a lower cycling intensity limits our interpretations of our results, we are confident that previous literature on short-term endurance training reveals the significance of our short-term HIIT protocol.

Table 2. *Effects of HIIT on $\dot{V}_{O_2}$ and RER during cycling at ~60% pretraining $\dot{V}_{O_2\,peak}$*

	Cycling at 60% $\dot{V}_{O_2\,peak}$			
	15 min	30 min	45 min	60 min
$\dot{V}_{O_2}$, l/min				
Pre	1.47±0.06	1.50±0.06	1.51±0.05	1.51±0.05
Post	1.46±0.06	1.45±0.06	1.44±0.05	1.45±0.06
$\dot{V}_{O_2\,peak}$				
Pre, % pretraining $\dot{V}_{O_2\,peak}$	62.1±2.6	64.1±2.9	64.6±2.3	64.8±3.1
Post, % posttraining $\dot{V}_{O_2\,peak}$	55.5±2.3*	55.4±2.6*	54.9±2.2*	55.2±2.9*
RER				
Pre	0.92±0.02	0.91±0.02	0.88±0.01*	0.88±0.02
Post	0.89±0.02	0.85±0.02*†	0.84±0.02*†	0.84±0.02*†

Values are means ± SE, (*n* = 8). RER, respiratory exchange ratio; Pre, pretraining; Post, posttraining. *Significantly different ($P < 0.05$) from the same time point during pretraining trial. †Significantly different ($P < 0.05$) from 15 min of the same trial.
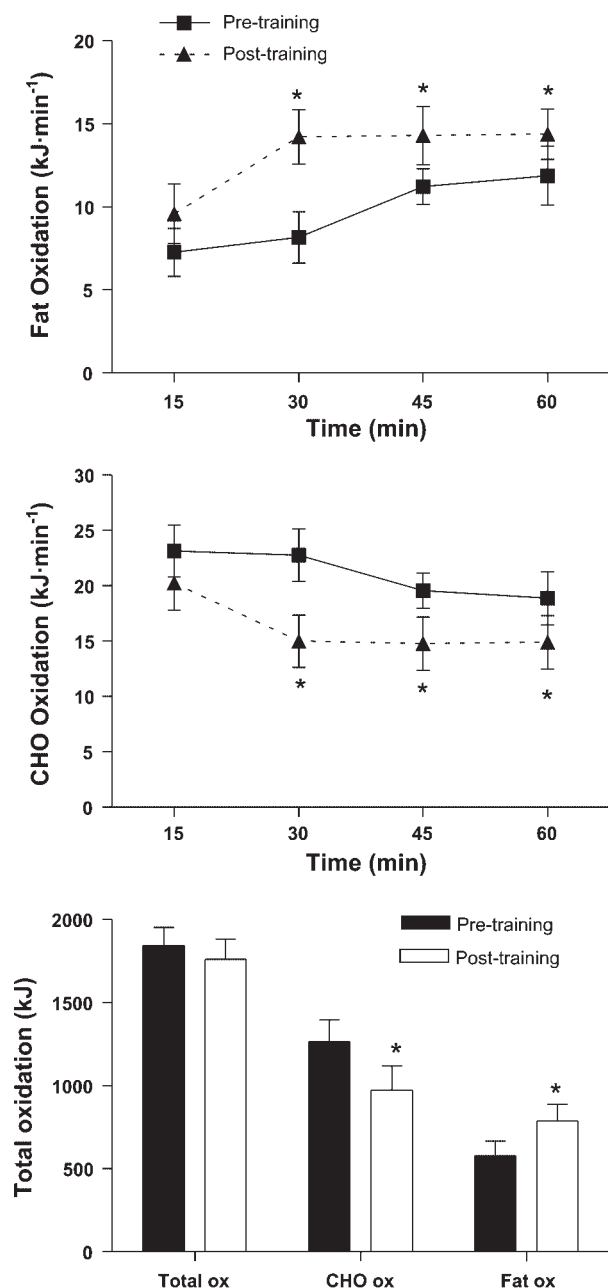
Fig. 3. Effects of HIIT on whole body fat and carbohydrate (CHO) oxidation (ox) measurements during 60 min of cycling at ~60% pretraining $\dot{V}O_{2\ peak}$. Values are means ± SE (*n* = 8). *Significantly different from the same time point before training (*P* < 0.05).

citrate synthase and β-HAD activity, and whole body fat oxidation. Even though subjects trained for only 2 wk in the present study and the short-duration sprint studies, the total training time was ~4.7 h in our study vs. 15–18 min in the sprint studies. This finding supports the idea that, with interval training, an increase in $\dot{V}O_{2\ peak}$ requires a specific amount of exercise.

With our HIIT protocol, as well as other protocols in which subjects trained for 2 h/day at ~60–70% $\dot{V}O_{2\ peak}$ (52), similar
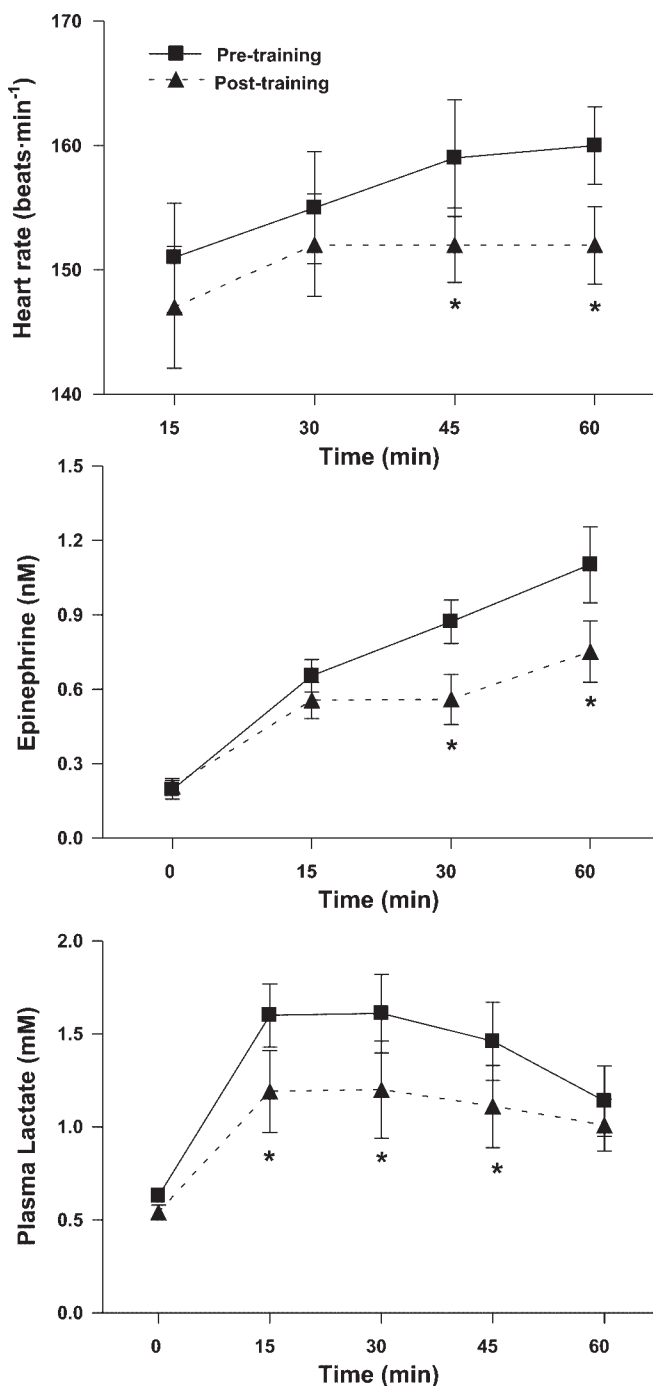
Fig. 4. Effects of HIIT on heart rate and venous plasma epinephrine and whole blood lactate concentrations during 60 min of cycling at ~60% pretraining $\dot{V}O_{2\ peak}$. Values are means ± SE (*n* = 8). *Significantly lower than the same time point before training (*P* < 0.05).

Long-duration (6–7 wk) intermittent sprint protocols have also produced significant improvements in $\dot{V}O_{2\ peak}$ and mitochondrial enzyme activity (25, 41, 48, 55). Moreover, there has also been recent interest in the adaptive responses of as few as six sprint training sessions over 2 wk (~15–18 min of training) (4, 5, 18). These studies reported significant increases in exercise performance and skeletal muscle citrate synthase activity and cytochrome *c* oxidase protein content without increases in $\dot{V}O_{2\ peak}$ or β-HAD activity. The uniqueness of the present study is that a training intensity (~90% $\dot{V}O_{2\ peak}$) that is intermediate between classic submaximal and sprint training paradigms resulted in increases in $\dot{V}O_{2\ peak}$, skeletal muscle

Table 3. *Effects of HIIT on venous blood measurements during cycling at ~60% pretraining $\dot{V}_{O_2\,peak}$*

	Cycling at 60% $\dot{V}_{O_2\,peak}$				
	0 min	15 min	30 min	45 min	60 min
FFA, mM					
Pre	0.60±0.14	0.39±0.06†	0.49±0.10	0.65±0.14	0.87±0.16†
Post	0.52±0.11	0.42±0.06	0.48±0.06	0.56±0.14	0.72±0.16†
Glycerol, μM					
Pre	60.0±5.6	67.2±5.1†	89.6±5.1†	118.9±7.1†	140.5±10.5†
Post	54.4±3.8	79.2±3.8†	110.0±12.6*†	131.3±12.8†	166.4±13.6*†
Glucose, mM					
Pre	4.4±0.2	4.8±0.3	4.7±0.3	4.5±0.3	4.4±0.3
Post	5.2±0.2*	4.8±0.3	5.1±0.4	5.2±0.3*	5.2±0.4*

Values are means ± SE, ($n = 8$). *Significantly different ($P < 0.05$) from the same time point during pretraining trial. †Significantly different ($P < 0.05$) from 0 min of the same trial.

increases in β-HAD activity were observed after only seven training sessions. In contrast, with other protocols in which subjects trained for 2 h/day for 5–7 days (46, 47) and six sprint (5) training sessions, no significant increases were observed in β-HAD. The data from our HIIT protocol suggest that a combination of high training intensities, the duration of each bout (4 min), and several rest-to-exercise transitions provides a powerful stimulus for increasing the enzyme contents of many of the metabolic pathways in the mitochondria in a short period of time. It is not clear why the 90% $\dot{V}_{O_2\,peak}$ intermittent training protocol increases citrate synthase and β-HAD activity, but HIIT offers a mechanism to quickly increase muscle mitochondrial capacity, as well as whole body fat oxidation and $\dot{V}_{O_2\,peak}$, in untrained individuals.

Training-Induced Metabolic Responses to 60 Minutes of Cycling at ~60% Pretraining $\dot{V}_{O_2\,peak}$

Whole body fat oxidation. In the present study, seven intermittent HIIT sessions at ~90% $\dot{V}_{O_2\,peak}$ increased posttraining whole body fat oxidation during 60 min of cycling at ~60% pretraining $\dot{V}_{O_2\,peak}$. This is a classic response typically observed with longer-duration endurance-training studies (27, 34, 49), but the present adaptations in whole body fat oxidation occurred with only 2 wk of training. Previously, incorporation of interval training into cyclists' exercise regimen yielded similar results. Well-trained cyclists replaced a portion (~15%) of their normal training with 6 wk of HIIT bouts at ~80% $\dot{V}_{O_2\,peak}$, resulting in an enhanced whole body fat

oxidation during exercise (62). Therefore, HIIT offers a short-duration stimulus for elite endurance athletes to increase fat oxidation during exercise above an already high endurance training-induced level of fatty acid oxidation.

Reduced glycogenolysis. Muscle glycogenolysis was reduced by 12% during 60 min of cycling after training. Muscle glycogen phosphorylase, a key regulatory enzyme in glycogenolysis, is activated by epinephrine via the cAMP second messenger system and the release of calcium during contractions. The activity of phosphorylase in the active "a" form is also stimulated via the contraction-induced accumulation of allosteric regulators, free ADP and AMP. The blunted plasma epinephrine response and reduced accumulations of free ADP and AMP in the present study are classic training-induced alterations in traditional moderate-intensity endurance protocols (21, 36, 46). These changes were consistent with the decreased glycogen use during the 60-min cycling trial in the present study. Once again, the uniqueness of the present work is that the classic training-induced shifts in fuel use during exercise were present after as few as seven HIIT sessions over 2 wk.

In contrast to most training studies where resting muscle glycogen content increased after training (5, 10, 22, 48), resting glycogen content was unchanged in the present study. It appears that the present training stimulus (glycogen degradation each training day) and number of training days did not appear to be sufficient to increase resting muscle glycogen.
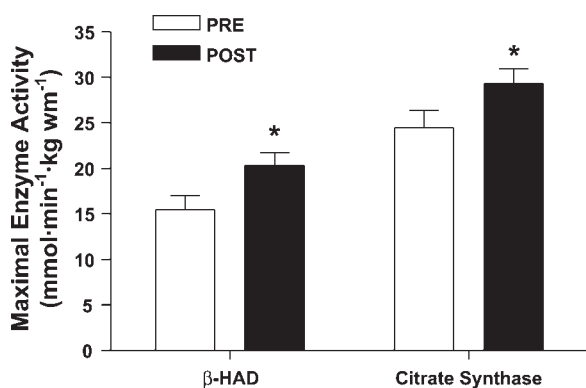

Fig. 5. Maximal mitochondrial enzyme activities before (Pre) and after (Post) HIIT. Values are means ± SE ($n = 8$). β-HAD, β-hydroxyacyl-CoA dehydrogenase; wm, wet mass. *Significantly higher than Pre ($P < 0.05$).
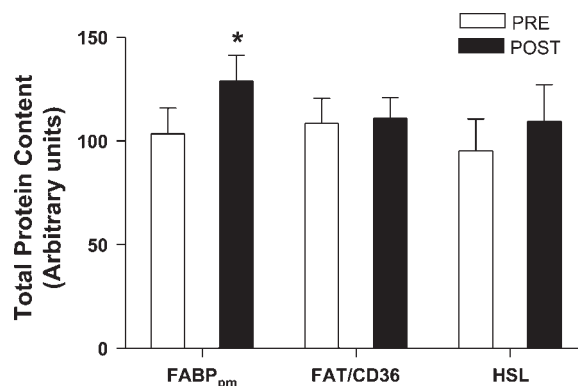

Fig. 6. Plasma membrane fatty acid-binding protein (FABP$_{pm}$), fatty acid translocase (FAT)/CD36, and hormone-sensitive lipase (HSL) protein content before and after HIIT. Values are means ± SE ($n = 8$). *Significantly higher than Pre ($P < 0.05$).

Table 4. *Effects of HIIT on skeletal muscle measurements during cycling at ~60% pretraining* $\dot{V}_{O_2\,peak}$

| | Pretraining | | Posttraining | | | |
	0 min	60 min	0 min	60 min	ΔPre	ΔPost
Glycogen, mmol/kg dry mass	468.6±25.0	136.5±17.4†	474.6±25.8	182.4±15.5*†	332.1±19.1	292.2±22.3*
IMTG, mmol/kg dry mass	46.4±2.6	41.0±3.0	43.1±3.3	35.8±3.4	5.4±3.5	7.3±3.7
PCr, mmol/kg dry mass	76.9±3.3	53.5±4.3†	77.2±3.2	63.1±3.3†	23.4±5.8	14.1±3.3*
ATP, mmol/kg dry mass	24.1±1.2	24.2±1.6	22.4±0.8	21.5±0.8*	−0.1±1.1	0.9±0.3
Free ADP, µmol/kg dry mass	101.8±10.5	198.0±41.5†	88.1±2.3	120.1±9.7*†	96.1±39.9	32.1±9.7*
Free AMP, µmol/kg dry mass	0.46±0.12	1.87±0.81†	0.33±0.01	0.66±0.08†	1.41±0.8	0.33±0.09*
Lactate, mmol/kg dry mass	3.9±0.4	11.1±0.9†	3.7±0.5	9.9±0.8†	7.2±1.0	6.2±1.6

Values are means ± SE, ($n = 8$). PCr, phosphocreatine; IMTG, intramuscular triacylglycerol; Δ, change. *Significantly different ($P < 0.05$) from the same time point during pretraining trial. †Significantly different ($P < 0.05$) from 0 min of the same trial.

Skeletal Muscle Fat Metabolism

Increases in skeletal muscle fat oxidation likely result from a number of adaptations, including an increase in mitochondrial volume (30) and alterations at several regulatory steps; adipose tissue lipolysis of TG to fatty acids (60), transport of fatty acids into the cell, intramuscular lipolysis of TG to fatty acids, and, ultimately, fatty acid transport into the mitochondria (2, 3). Exercise training results in a greater contribution of energy from fatty acids that is stored in peripheral adipose tissue and IMTG stores (34, 58, 59). It has also been shown that exercise-trained individuals use more IMTG as an energy source than untrained individuals (34, 50). However, in the present study, training did not result in a significant increase (35%) in IMTG utilization (5.4 ± 3.5 and 7.3 ± 3.7 mmol/kg dry mass before and after training, respectively), but 60 min of exercise may have been too short to detect a training effect.

In the present study, we did not see a significant increase in HSL protein content after HIIT. HSL is believed to be a key regulatory enzyme in lipolysis of IMTG stores (33, 61). However, it may be that our training protocol was not long enough to stimulate significant adaptations in skeletal muscle HSL content, and further studies are warranted to assess whether adaptations increase further or plateau after longer HIIT protocols.

A third regulatory step that may limit skeletal muscle fat oxidation is the transport of fatty acids across the plasma and mitochondrial membranes. Although previously viewed as a completely passive process (24), evidence suggests that long-chain fatty acid (LCFA) membrane transport is a highly regulated process involving several transporters (3, 40). We measured two transport proteins of interest, FABP$_{pm}$ and FAT/CD36. Training resulted in a significant increase in total FABP$_{pm}$ content but no change in FAT/CD36 content. FABP$_{pm}$ has been identified as a plasma membrane LCFA transport protein, and inhibition of this transporter decreases LCFA uptake (56). Three weeks of long-duration (15 training sessions lasting 1–2 h per session) knee extension exercise resulted in an increased whole muscle FABP$_{pm}$ content (38), but the present study is the first to demonstrate an increase in FABP$_{pm}$ content using HIIT over only seven training sessions.

The absence of an increase in FAT/CD36 content does not necessarily imply no increase in the transport potential of LCFA through FAT/CD36. Research suggests that FAT/CD36 is located at the plasma membrane (3), within the intracellular fraction, and on the mitochondrial membrane (2, 32), with FAT/CD36 content on the mitochondria following a trend similar to that of oxidative capacity within tissue types (heart > red muscle > white muscle) in rodents (7). Therefore, it remains possible that there was a shift in the fractional concentrations of FAT/CD36 on the mitochondria and plasma membrane that could increase LCFA uptake (2, 7, 32).

Female Exercise-Training Studies

Similar to men, aerobic and mitochondrial enzyme capacities in well-trained women are enhanced compared with those in less-trained women (12, 13). As well, traditional endurance training studies using mixed male and female populations have shown that training increases these markers of fitness, as well as whole body fat oxidation (11, 31, 44, 51). However, the present study is the first to use an interval-training protocol at ~90% $\dot{V}_{O_2\,peak}$ and exclusively female subjects to observe increases in mitochondrial enzyme activities, $\dot{V}_{O_2\,peak}$, and whole body fat oxidation. The results vary: two studies showed a slight difference between women and men in proportions of carbohydrate and fat sources utilized for fuel (43, 57), and another showed no gender differences (8). As well, some studies showed that substrate utilization varies during different phases of the menstrual cycle (23, 64), and others showed no difference in substrate utilization between menstrual cycle phases (26, 35, 54). In this study, our whole body fat oxidation rates after HIIT were very convincing, with all eight subjects using a higher absolute rate and a greater percentage for energy than before training.

Future studies are necessary to compare genders after HIIT.

In summary, seven sessions of HIIT over a 2-wk period offer a short-duration stimulus to improve whole body fat oxidation and the capacity for skeletal muscle to oxidize fat. HIIT is a realistic type of exercise that can be performed by elite athletes as well as untrained individuals. Our protocol, along with other HIIT and SIT protocols, reveals "the potency of exercise intensity for stimulating adaptations in skeletal muscle that improve performance and have implications for improving health" (14). The short duration of our training provides a tool that can be incorporated into existing training protocols to maximize training adaptations in a short period of time or can be used by untrained individuals to improve initial fitness with training for only 3 h/wk for 2 wk.

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