TWO WEEKS OF HIGH-INTENSITY AEROBIC INTERVAL TRAINING INCREASES THE CAPACITY FOR FAT OXIDATION DURING EXERCISE IN WOMEN

Jason L. Talanian¹, Stuart D.R. Galloway², George J. F. Heigenhauser³, Arend Bonen¹ & Lawrence L. Spriet¹

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

Running Title: Fat metabolism during high intensity interval training

Corresponding author: Jason L. Talanian

Department of Human Health and Nutritional Sciences
University of Guelph
Guelph, Ontario
Canada, N1G 2W1
Tel: 1-519-824-4120 x53907
Fax: 1-519-763-5902
Email: jtalania@uoguelph.ca
ABSTRACT

Our aim was to examine the effects of seven high intensity aerobic interval training (HIIT) sessions over two weeks on skeletal muscle fuel content, mitochondrial enzyme activities, fatty acid transport proteins, VO2peak, and whole body metabolic, hormonal and cardiovascular responses to exercise. Eight females participated in the study (22.1 ± 0.2 yrs, 65.0 ± 2.2 kg, VO2peak: 2.36 ± 0.24 l·min⁻¹). Subjects performed a VO2peak test and a 60-min cycling trial at ~60% VO2peak prior to and following training. Each session consisted of ten, 4-min bouts at ~90% VO2peak with 2-min rest between intervals. Training increased VO2peak by 13%.

Following HIIT, plasma epinephrine and heart rate were lower during the final 30-min of the 60-min cycling trial at ~60% pre-training VO2peak. Exercise whole body fat oxidation (PRE: 15.0 ± 2.4, POST: 20.4 ± 2.5 g) increased 36% following HIIT. Resting muscle glycogen and triacylglycerol contents were unaffected by HIIT, but net glycogen use was reduced during the post-training 60-min cycling trial. HIIT significantly increased muscle mitochondrial β-HAD (PRE: 15.44 ± 1.57, POST: 20.35 ± 1.40 mmol·min⁻¹·kg wm⁻¹) and citrate synthase (PRE: 24.45 ± 1.89, POST: 29.31 ± 1.64 mmol·min⁻¹·kg wm⁻¹) maximal activities by 32% and 20%, while cytoplasmic HSL protein content was not significantly increased. In addition, total muscle FABPpm content increased significantly (25%), while FAT/CD36 content was unaffected following training. In summary, seven sessions of HIIT over two weeks induced marked increases in whole body and skeletal muscle capacity for fatty acid oxidation during exercise in moderately active women.

**Key words:** fatty acid metabolism, mitochondrial enzymes, aerobic capacity, fatty acid transport
INTRODUCTION

Endurance exercise training results in an improved capacity for whole body fat oxidation that is associated with increased mitochondrial volume as assessed via increases in citrate synthase and β-hydroxy-acyl-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 are also associated with improved insulin sensitivity (20) and health. Improving 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 weeks of exercise training at a moderate intensity (MIT, 60-75% VO₂peak) 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% VO₂peak power) for 6-7 weeks produces similar results (41, 48, 55). Recent evidence has also shown that daily sessions of MIT (two hours/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 little as six SIT sessions in two weeks has also been shown to increase citrate synthase activity but without an increase in VO₂peak (5). Both the MIT and SIT short duration (2 wk) protocols produce substantial training effects and health benefits in a brief period of time. However, MIT for two hours a 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 to sustain for people beginning a training program.

Two weeks of high intensity aerobic interval training (HIIT), performed at an exercise intensity (80-95% VO₂peak) between moderate and sprint training paradigms, may offer similar
benefits to MIT and SIT. Training studies utilizing HIIT over a longer period of time (4-6 wk) have reported increases in 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 as little as two weeks of HIIT.

Our aim was to investigate the effect of seven HIIT sessions over a two week 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 prior to and following training. In addition, we also evaluated the effects of training on circulatory substrates and on respiratory responses during HIIT throughout the second and seventh training session.
METHODS

Eight healthy recreationally active females (22 ± 1 years, 65.0 ± 2.2 kg; VO2peak: 2.36 ± 0.24 l·min⁻¹) volunteered to participate in the study. On average, subjects engaged in recreational physical activity 2-3 days a week. 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. Prior to the study subjects reported to the laboratory on two occasions. On the first visit, subjects performed an incremental cycling (Lode Excalibur, Quinton Instrument, Netherlands) test to exhaustion to determine VO2peak. Respiratory gases were collected and analyzed using a metabolic cart (Sensormedic, Vmax 229, Yorba Linda, CA). The second visit was to verify appropriate power outputs for the experimental trials. Subjects cycled for 15 min at 60% VO2peak to establish the power output for the 60 min trial. They then performed 4-6 bouts of cycling at 90% VO2peak, with each bout lasting 4 min and separated by 2 min of rest to establish power outputs for the HIIT sessions. Following two weeks (7 sessions) of HIIT, subjects repeated the incremental cycling test to exhaustion to establish the post-training VO2peak.

Cycle trials at ~60% VO2peak. Subjects performed a 60 min cycling trial at a moderate intensity (~60% VO2peak) before and three days following seven sessions of HIIT. Subjects arrived at the laboratory 3-4 hours post-prandial. They abstained from strenuous exercise and recorded their diet in the 24 hours prior to the trial. Three to four hours prior to the 60 min ride, subjects received a meal that was provided for them. Prior to the post-training 60 min ride subjects
replicated the same diet they ingested before the pre-training ride. A Teflon catheter was inserted into an antecubital vein for blood sampling and the catheter was kept patent by flushing with 0.9% saline. One leg was prepared for percutaneous 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 prior to 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% VO2peak at a constant cadence (78-85 rpm) on the Lode ergometer. Respiratory gases were collected between 13-17, 28-32, 43-47 and 55-59 min of exercise for the measurements of VO2 and VCO2 and the calculation of the respiratory exchange ratio (RER). These parameters were used to calculate whole body fat and carbohydrate oxidation using the non-protein RER table (16) and according to the following equations: carbohydrate oxidation = 4.585 (VCO2) – 3.226 (VO2) and fat oxidation = 1.695 (VO2) – 1.701 (VCO2) (45).

Venous blood samples were obtained at 15, 30, 45 and 60 min of exercise. Immediately following exercise, two muscle biopsies were taken with the subject sitting on the cycle ergometer. The same procedure was repeated following HIIT with muscle biopsies taken from the other leg.

High intensity interval training (HIIT). Two days following 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% VO2peak separated by 2 min of rest. Heart rate (HR) was recorded throughout training and was held constant at ~90% of HRmax by increasing 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 the seventh training session subjects cycled at the same power output as the second training session to make training related comparisons. During training sessions 2 and 7, respiratory gases and venous blood samples (Teflon catheter) were collected prior to and immediately following bouts 1, 3, 5, and 10. Throughout the two weeks of training, subjects maintained their recreational activities they were engaged in prior to training.

Analyses.

Blood measurements. Venous blood was collected in sodium-heparin tubes. A portion (1.5 ml) was added to 30 µl of EGTA and reduced glutathione, centrifuged (10,000 x g for 3 min) and the supernatant was analyzed for epinephrine using an enzymatic immunoassay (Labor Diagnostika Nord, Nordhorn, Germany). A second portion (200 µl) was added to 1 ml of 0.6M perchloric acid, centrifuged and the supernatant was analyzed for blood glucose, lactate and glycerol using fluorometric techniques (1). A third portion (1.5 ml) was centrifuged and the plasma was analyzed for free fatty acids (FFA) using an enzymatic colorimetric technique (Wako 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 KH₂PO₄ and BSA, freeze-thawed three times and the maximal activities of citrate synthase and β-HAD were determined on a spectrophotometer (at 37°C) using methods formerly described (53). The muscle homogenate was analyzed for total creatine (Cr) and enzyme measurements were normalized to the highest total pre/post Cr measured among each subject.

Muscle metabolites. A portion of the resting and first post exercise 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 HClO₄/l mM EDTA and
neutralized with 2.2 M KHCO₃. 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, neutralized with 0.1 M HCl/0.2 M citric acid/0.2 M Na₂PO₄ and amyloglucosidase was added to breakdown 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 IMTG content (1). The total Cr content of freeze dried muscle samples was similar pre- and post-training and therefore 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 post-exercise 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 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 (3000 rpm for 15 min) and the supernatant was used to determine fatty acid translocase (FAT/CD36), plasma membrane fatty acid binding protein (FABPₚₘ) 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), a FABPₚₘ/mAspAT polyclonal antibody was used was to determine FABPₚₘ content (6) and a polyclonal antibody for HSL (ProSci, Poway, CA) was used to determine total HSL content.
Statistics. All data are presented as means ± SE. FABP<sub>pm</sub>, FAT/CD36, and HSL were analyzed using paired t-tests. All other data were analyzed by two way repeated measures ANOVA (time x trial) 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 a level of p < 0.05.
RESULTS

*High Intensity Interval Training.*

VO$_{2}\text{peak}$ increased from $2.36 \pm 0.24 \text{ l-min}^{-1}$ ($36.3 \pm 3.7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) prior to training to $2.66 \pm 0.21 \text{ l-min}^{-1}$ ($40.9 \pm 3.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) ($13\%$) following seven HIIT sessions consisting of ten 4 min bouts at $\sim90\%$ VO$_{2}\text{peak}$ with 2 min rest between bouts. Initial training power outputs (163-227 W) were increased by an average of $19.0 \pm 0.6$ W from the first to the sixth training session to maintain a constant HR during the exercise sessions. For comparison, training power outputs during session 7 were reduced to match the same absolute power outputs as session 2. The average absolute VO$_2$ during training sessions 2 and 7 were not different (Table 1). During training session 2, VO$_2$ reached 86% of pre-training VO$_{2}\text{peak}$ in bout 1 and 95% of pre-training VO$_{2}\text{peak}$ in bout 10, while the same power outputs represented 77% and 84% of the post-training VO$_{2}\text{peak}$ in bouts 1 and 10 of training session 7 (Table 1). The peak HR attained during session 2 ranged from $171 \pm 2 \text{ beats} \cdot \text{min}^{-1}$ during bout 1 to $181 \pm 1 \text{ beats} \cdot \text{min}^{-1}$ 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 following bout 10 (Fig. 2). There was a significantly blunted epinephrine response in session 7 following bouts 3 and 10. Whole blood lactate concentrations in session 2 increased following bouts 1 and 3 and reached a plateau during the remaining bouts (Table 1). There was no difference in the lactate response to 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 both sessions 2 and 7, but were significantly lower prior to and following bout 10 in session 7 (Table 1).
Cycling at ~60% VO2peak Pre- and Post-Training.

Subjects cycled for 60 min at 101.3 ± 4.2 W prior to and following HIIT. This power output represented 63.9 ± 2.6 % of the pre-training VO2peak and 55.2 ± 2.2 % of the post-training VO2peak (Table 2). The RER was significantly lower following 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 prior to training was 15.0 ± 2.4 g and increased 36% following HIIT to 20.4 ± 2.5 g. There was a reciprocal decrease in whole body carbohydrate oxidation at 30, 45 and 60 min following training (Fig. 3). Total carbohydrate oxidation prior to training was 80.7 ± 2.2 g and decreased 23% following HIIT to 62.1 ± 1.4 g.

HR was significantly lower following 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 post-training (Fig. 4). Plasma lactate was significantly increased above rest at all exercise time points in both trials but the increase was blunted following 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 rest following 60 min of exercise in the pre-training trial (Table 3). Post-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 post- vs. pre-training at 30 and 60 min of exercise (Table 3). Blood glucose was unchanged by exercise in both trials, but was higher at rest, 45 and 60 min of exercise following training (Table 3).

Muscle Analysis
Maximal β-HAD activity (PRE: 15.44 ± 1.57, POST: 20.35 ± 1.40 mmol-min⁻¹·kg wm⁻¹) increased by 32% and maximal citrate synthase activity (PRE: 24.45 ± 1.89, POST: 29.31 ± 1.64 mmol-min⁻¹·kg wm⁻¹) increased by 20% following HIIT (Fig. 5). There was a non-significant increase in muscle HSL protein content (~13%) following training (Fig. 6). Total muscle FABPₚₘ content increased by 25% following 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% following 60 min of exercise post-training (Table 4). IMTG content decreased 12% and 17% following 60 min of cycling pre- and post-training respectively, but there was no difference between the trials (Table 4).

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

This study examined the effects of two weeks of high-intensity interval training (HIIT) at ~90% VO₂peak on whole body and muscle metabolic responses to exercise at ~60% pre-training VO₂peak in recreationally active females. This is the first study using this short duration HIIT protocol to measure both whole body responses and metabolic adaptations in skeletal muscle. Training resulted in increased VO₂peak, whole body fat oxidation during exercise and maximal mitochondrial enzyme activities (citrate synthase, β-HAD) following only seven HIIT sessions in two weeks. Training also increased the skeletal muscle content of the fatty acid transport protein FABPₘₘ, which may have contributed to the observed increases in whole body fat oxidation.

*Training Induced Increases in VO₂peak and Muscle Mitochondrial Enzymes*

Classic responses to the traditional long duration (> 24 hr accumulated training) submaximal training protocols are an improved aerobic capacity (9, 19, 28), increased whole body fat oxidation, and increases in skeletal muscle mitochondrial enzyme activities (28, 29, 37). It has also been shown that as little as 6-7 two hour sessions at ~60-70% VO₂peak increases aerobic capacity, whole body fat oxidation and mitochondrial enzyme activities (10, 52). While 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.

Long duration (6-7 wk) intermittent sprint protocols have also produced significant improvements in VO₂peak and mitochondrial enzyme activity (25, 41, 48, 55). Moreover, there has also been recent interest into the adaptive responses of as little as six sprint training sessions over two weeks (~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 VO$_2$peak or $\beta$-HAD activity. The uniqueness of the present study is that a training intensity (~90% VO$_2$peak) that is intermediate between classic submaximal and sprint training paradigms resulted in increases in VO$_2$peak, skeletal muscle citrate synthase and $\beta$-HAD activity, and whole body fat oxidation. Even though in both the present study and the short duration sprint studies subjects trained for only two weeks, the total training time was ~4.7 hr in our study vs. 15-18 min of training in the sprint studies. This argues that with interval training, there is a specific amount of exercise that is required for VO$_2$peak to increase.

Our HIIT protocol as well as others training for two hours a day at ~60-70% VO$_2$peak (52) observed similar increases in $\beta$-HAD activity following only seven training sessions. In contrast, other 2 hour/day protocols lasting 5-7 days (46, 47) and six sprint (5) training sessions did not observe significant increases in $\beta$-HAD. The data from our high intensity intermittent training protocol suggests 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% VO$_2$peak intermittent training protocol increases both citrate synthase and $\beta$-HAD activity, but HIIT offers a mechanism to quickly increase muscle mitochondrial capacity as well as whole body fat oxidation and VO$_2$peak in untrained individuals.

*Training Induced Metabolic Responses to 60 min of Cycling at ~60% Pre-Training VO$_2$peak Whole Body Fat Oxidation.* In the present study, seven intermittent HIIT sessions at ~ 90% VO$_2$peak increased post-training whole body fat oxidation during 60 min of cycling at ~60% of pre-training VO$_2$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 two weeks of training. Previously, incorporation of interval training into cyclists’ exercise regime yielded similar results. Well-trained cyclists replaced a portion (~15%) of their normal training with six weeks of HIIT bouts at ~80% of VO2peak resulting in an enhanced whole body fat oxidation during exercise (62). Therefore, HIIT offers a short duration stimuli 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 post-training. Muscle glycogen phosphorylase, a key regulatory enzyme in glycogenolysis, is activated by epinephrine via the cyclic AMP 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 that occurred 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 following as little as seven HIIT sessions over two weeks.

Unlike most training studies where resting muscle glycogen content increased following 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.

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 triglycerides (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 being derived 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 intramuscular TG 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 (PRE: 5.4 ± 3.5 vs. POST: 7.3 ± 3.7 mmol·kg DM⁻¹), 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 following HIIT. HSL is believed to be 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 if adaptations increase further or plateau following longer HIIT training protocols.

A third regulatory step that may limit skeletal muscle fat oxidation is through the transport of fatty acids across the plasma and mitochondrial membranes. Although previously viewed as a completely passive process (24), evidence now suggests that LCFA membrane transport is a highly regulated process involving several transporters (3, 40). We measured two transport proteins of interest, FABPₚₘ and FAT/CD36. Training resulted in a significant increase in total FABPₚₘ content, but no change in FAT/CD36 content. FABPₚₘ has been identified as a plasma membrane LCFA transport proteins and inhibition of this transporter decreases LCFA uptake (56). Three weeks of long duration (15 training sessions lasting 1-2 hr 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 that there was 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 similar trend to 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, well-trained women have enhanced aerobic and mitochondrial enzyme capacities compared to women less trained (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 increasing whole body fat oxidation (11, 31, 44, 51). However, the present study is the first to use an interval training protocol at ~90% VO_{2}peak using exclusively female subjects to observe increases in mitochondrial enzyme activities, VO_{2}peak and whole body fat oxidation. There have been varying results showing that women utilize slightly different proportions of carbohydrate and fat sources for fuel than men (43, 57) and others that have observed no gender differences (8). As well, some studies have shown that substrate utilization varies during different phases of the menstrual cycle (23, 64) and others have shown no difference in substrate utilization between varying menstrual cycle phases (26, 35, 54). In this
study, our whole body fat oxidation rates following HIIT were very convincing with all eight subjects using a higher absolute rate and a greater percentage for energy than prior to training. Future studies are necessary to compare genders following HIIT.

In summary, seven sessions of HIIT training over a two week period offers 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 athlete as well as untrained individuals. Our protocol along with other HIIT and SIT protocols reveal “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 only three hours of training a week for two weeks.

Acknowledgements:
The authors thank Lindsay Crabbe and Erin Weersink for excellent technical assistance. This study was supported by operating grants from the Canadian Institutes of Health Research (L.L.S., G.J.F.H. and A.B.), a Gatorade Sports Science Institute Award (J.L.T.), the Natural Science and Engineering Research Council of Canada (L.L.S and A.B.), the Physiological Society and Carnegie Trust for the Universities of Scotland (S.D.R.G.) and the Canada Research Chair Program (A.B.). A. Bonen is the Canada Research Chair in Metabolism and Health.
REFERENCES


Table 1. Respiratory, heart rate and venous blood measurements during high intensity interval training sessions 2 and 7.

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<tr>
<td>Session 2</td>
<td>0.6 ± 0.1</td>
<td>2.1 ± 0.3</td>
<td>3.1 ± 0.2</td>
<td>†</td>
<td>3.4 ± 0.2</td>
<td>†</td>
<td>3.5 ± 0.1</td>
<td>†</td>
</tr>
<tr>
<td>Session 7</td>
<td>0.6 ± 0.1</td>
<td>2.0 ± 0.2</td>
<td>2.8 ± 0.1</td>
<td>†</td>
<td>3.0 ± 0.2</td>
<td>†</td>
<td>2.9 ± 0.1</td>
<td>†</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 2</td>
<td>4.84 ± 0.12</td>
<td>4.58 ± 0.25</td>
<td>4.90 ± 0.23</td>
<td>4.53 ± 0.21</td>
<td>5.16 ± 0.32</td>
<td>5.16 ± 0.32</td>
<td>4.94 ± 0.17</td>
<td>5.34 ± 0.19</td>
</tr>
<tr>
<td>Session 7</td>
<td>4.91 ± 0.14</td>
<td>4.68 ± 0.10</td>
<td>4.46 ± 0.19</td>
<td>4.11 ± 0.17</td>
<td>4.80 ± 0.20</td>
<td>4.58 ± 0.20</td>
<td>5.34 ± 0.28</td>
<td>4.77 ± 0.24</td>
</tr>
<tr>
<td>FFA (mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 2</td>
<td>0.44 ± 0.10</td>
<td>0.27 ± 0.04</td>
<td>0.34 ± 0.06</td>
<td>0.26 ± 0.03</td>
<td>0.43 ± 0.11</td>
<td>0.28 ± 0.04</td>
<td>0.59 ± 0.14</td>
<td>†</td>
</tr>
<tr>
<td>Session 7</td>
<td>0.32 ± 0.06</td>
<td>0.27 ± 0.04</td>
<td>0.28 ± 0.03</td>
<td>0.22 ± 0.03</td>
<td>0.31 ± 0.03</td>
<td>0.27 ± 0.03</td>
<td>0.42 ± 0.04</td>
<td>0.27 ± 0.03</td>
</tr>
<tr>
<td>Glycerol (µM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 2</td>
<td>35.4 ± 6.3</td>
<td>41.6 ± 7.6</td>
<td>59.2 ± 8.0</td>
<td>†</td>
<td>67.0 ± 7.5</td>
<td>†</td>
<td>87.2 ± 11.9</td>
<td>†</td>
</tr>
<tr>
<td>Session 7</td>
<td>45.8 ± 4.6</td>
<td>55.9 ± 3.8</td>
<td>64.0 ± 4.6</td>
<td>†</td>
<td>65.2 ± 6.0</td>
<td>†</td>
<td>74.7 ± 4.6</td>
<td>†</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 8. S2, Session 2; S7, Session 7; FFA, free fatty acids. † Significantly higher (p < 0.05) than the same time point during bout 1.
Table 2. Effects of high intensity interval training on VO₂ and respiratory exchange ratio during 60 min of cycling at ~60% pre-training VO₂ peak.

<table>
<thead>
<tr>
<th></th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VO₂ (l•min⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pre</em></td>
<td>1.47 ± 0.06</td>
<td>1.50 ± 0.06</td>
<td>1.51 ± 0.05</td>
<td>1.51 ± 0.05</td>
</tr>
<tr>
<td><em>Post</em></td>
<td>1.46 ± 0.06</td>
<td>1.45 ± 0.06</td>
<td>1.44 ± 0.05</td>
<td>1.45 ± 0.06</td>
</tr>
<tr>
<td><strong>% VO₂peak</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pre (% of Pre-training VO₂ peak)</em></td>
<td>62.1 ± 2.6</td>
<td>64.1 ± 2.9</td>
<td>64.6 ± 2.3</td>
<td>64.8 ± 3.1</td>
</tr>
<tr>
<td><em>Post (% of Post-training VO₂ peak)</em></td>
<td>55.5 ± 2.3*</td>
<td>55.4 ± 2.6*</td>
<td>54.9 ± 2.2*</td>
<td>55.2 ± 2.9*</td>
</tr>
<tr>
<td><strong>RER</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pre</em></td>
<td>0.92 ± 0.02</td>
<td>0.91 ± 0.02</td>
<td>0.88 ± 0.01*</td>
<td>0.88 ± 0.02</td>
</tr>
<tr>
<td><em>Post</em></td>
<td>0.89 ± 0.02</td>
<td>0.85 ± 0.02†*</td>
<td>0.84 ± 0.02†*</td>
<td>0.84 ± 0.02†*</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 8. VO₂, oxygen consumption; RER, respiratory exchange ratio; Pre, pre-training; Post, post-training.

† Significantly different (p < 0.05) from 15 min of the same trial. * Significantly different than the same time point during the pre-training trial.
Table 3. Effects of high intensity interval training on venous blood measurements during 60 min of cycling at ~60% pre-training VO\textsubscript{2} peak.

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FFA (mM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.60 ± 0.14</td>
<td>0.39 ± 0.06\textsuperscript{†}</td>
<td>0.49 ± 0.10</td>
<td>0.65 ± 0.14</td>
<td>0.87 ± 0.16\textsuperscript{†}</td>
</tr>
<tr>
<td>Post</td>
<td>0.52 ± 0.11</td>
<td>0.42 ± 0.06</td>
<td>0.48 ± 0.06</td>
<td>0.56 ± 0.14</td>
<td>0.72 ± 0.16\textsuperscript{†}</td>
</tr>
<tr>
<td><strong>Glycerol (µM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>60.0 ± 5.6</td>
<td>67.2 ± 5.1\textsuperscript{†}</td>
<td>89.6 ± 5.1\textsuperscript{†}</td>
<td>118.9 ± 7.1\textsuperscript{†}</td>
<td>140.5 ± 10.5\textsuperscript{†}</td>
</tr>
<tr>
<td>Post</td>
<td>54.4 ± 3.8</td>
<td>79.2 ± 3.8\textsuperscript{†}</td>
<td>110.0 ± 12.6\textsuperscript{†}</td>
<td>131.3 ± 12.8\textsuperscript{†}</td>
<td>166.4 ± 13.6\textsuperscript{†}</td>
</tr>
<tr>
<td><strong>Glucose (mM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>4.4 ± 0.2</td>
<td>4.8 ± 0.3</td>
<td>4.7 ± 0.3</td>
<td>4.5 ± 0.3</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>Post</td>
<td>5.2 ± 0.2\textsuperscript{*}</td>
<td>4.8 ± 0.3</td>
<td>5.1 ± 0.4</td>
<td>5.2 ± 0.3\textsuperscript{*}</td>
<td>5.2 ± 0.4\textsuperscript{*}</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 8. Pre, pre-training; Post, post-training; FFA, free fatty acid. † Significantly different (p < 0.05) from 0 min of the same trial. * Significantly different than the same time point during the Pre trial.
Table 4. Effects of high intensity interval training on skeletal muscle measurements during 60 min of cycling at ~60% pre-training VO$_2$ peak.

<table>
<thead>
<tr>
<th></th>
<th>Pre-training</th>
<th></th>
<th>Post-training</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>60 min</td>
<td>0 min</td>
<td>60 min</td>
</tr>
<tr>
<td><strong>Glycogen</strong></td>
<td>468.6 ± 25.0</td>
<td>136.5 ± 17.4†</td>
<td>474.6 ± 25.8</td>
<td>182.4 ± 15.5†</td>
</tr>
<tr>
<td><strong>IMTG</strong></td>
<td>46.4 ± 2.6</td>
<td>41.0 ± 3.0</td>
<td>43.1 ± 3.3</td>
<td>35.8 ± 3.4</td>
</tr>
<tr>
<td><strong>Phosphocreatine</strong></td>
<td>76.9 ± 3.3</td>
<td>53.5 ± 4.3†</td>
<td>77.2 ± 3.2</td>
<td>63.1 ± 3.3†</td>
</tr>
<tr>
<td><strong>ATP</strong></td>
<td>24.1 ± 1.2</td>
<td>24.2 ± 1.6</td>
<td>22.4 ± 0.8</td>
<td>21.5 ± 0.8*</td>
</tr>
<tr>
<td><strong>ADPf</strong></td>
<td>101.8 ± 10.5</td>
<td>198.0 ± 41.5†</td>
<td>88.1 ± 2.3</td>
<td>120.1 ± 9.7†*</td>
</tr>
<tr>
<td><strong>AMPf</strong></td>
<td>0.46 ± 0.12</td>
<td>1.87 ± 0.81†</td>
<td>0.33 ± 0.01</td>
<td>0.66 ± 0.08†</td>
</tr>
<tr>
<td><strong>Lactate</strong></td>
<td>3.9 ± 0.4</td>
<td>11.1 ± 0.9†</td>
<td>3.7 ± 0.5</td>
<td>9.9 ± 0.8†</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 8. IMTG, intramuscular triacylglycerol; ADPf, free adenosine diphosphate; AMPf, free adenosine monophosphate.

Data are mmol•kg dry mass$^{-1}$ except for ADP and AMP (µmol•kg dry mass$^{-1}$). † Significantly different (p < 0.05) from 0 min of the same trial.

* Significantly different than the same time point Pre-training
**FIGURE 1.** High intensity interval training study design. HIIT, High intensity interval training; HIIT; S#, training session #.

**FIGURE 2.** Venous plasma epinephrine concentrations during high intensity interval training sessions 2 and 7. Values are mean ± SE, n = 8. * Significantly lower than the same time point during session 2 (p < 0.05).

**FIGURE 3.** Effects of high intensity interval training on whole body fat and carbohydrate oxidation measurements during 60 min of cycling at ~60% pre-training VO₂peak. Values are means ± SE, n = 8. * Significantly different than the same time point pre training (p < 0.05).

**FIGURE 4.** Effects of high intensity interval training on heart rate, venous plasma epinephrine and whole blood lactate concentrations during 60 min of cycling at ~60% pre-training VO₂peak. Values are mean ± SE, n = 8. * Significantly lower than the same time point pre training (p < 0.05).

**FIGURE 5.** Maximal mitochondrial enzyme activities pre and post high intensity interval training. Values are mean ± SE, n = 8. β-HAD, β-hydroxy-acy-CoA dehydrogenase; wm, wet mass. * Significantly higher pre training (p < 0.05).

**FIGURE 6.** FABP<sub>pm</sub>, FAT/CD36 and HSL protein content pre and post high intensity
interval training. Values are mean ± SE, n = 8. FABP<sub>pm</sub>, plasma membrane fatty acid binding protein; FAT/CD36, fatty acid translocase, HSL, hormone sensitive lipase. * Significantly higher than pre training (p < 0.05).
Figure 1

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
<th>13</th>
<th>15</th>
<th>17</th>
<th>20</th>
<th>22-23</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VO_{2peak} test</td>
<td></td>
<td>60 min @ 60% VO_{2peak}</td>
<td>S1</td>
<td>S2</td>
<td>S3</td>
<td>S4</td>
<td>S5</td>
<td>S6</td>
<td>S7</td>
<td>VO_{2peak} test</td>
</tr>
</tbody>
</table>

HIIT
Figure 2

Epinphrine (nM)

- Session 2
- Session 7

BOUT 1  BOUT 3  BOUT 5  BOUT 10

0  1  2  3  4

0  1  2  3  4

Session 2 and Session 7 show similar trends in epinephrine release with higher peaks observed at specific bout times. The figure indicates a significant increase at BOUT 10 for Session 7 compared to Session 2.
Figure 3

![Graph showing Fat Oxidation (kJ min⁻¹) vs Time (min) for Pre-training and Post-training, with significant differences marked by asterisks.]

![Graph showing CHO Oxidation (kJ min⁻¹) vs Time (min) for Pre-training and Post-training, with significant differences marked by asterisks.]

![Bar graph showing Total oxidation (kJ) for Total ox, CHO ox, and Fat ox between Pre-training and Post-training, with significant differences marked by asterisks.]

- Fat Oxidation (kJ min⁻¹) increases over time for both Pre-training and Post-training with significant differences at certain time points.
- CHO Oxidation (kJ min⁻¹) decreases over time for both Pre-training and Post-training with significant differences at certain time points.
- Total oxidation (kJ) shows a decrease in Total ox and CHO ox and an increase in Fat ox for Post-training compared to Pre-training with significant differences at certain time points.
Figure 4

- **Heart rate (beats·min⁻¹)**
  - Y-axis: 0 to 170
  - X-axis: 0 to 60 minutes
  - Two lines: Pre-training (solid) and Post-training (dashed)
  - Error bars
  - Significant differences indicated by asterisks

- **Epinephrine (nM)**
  - Y-axis: 0.0 to 1.5
  - X-axis: 0 to 60 minutes
  - Two lines: Pre-training (solid) and Post-training (dashed)
  - Error bars
  - Significant differences indicated by asterisks

- **Plasma Lactate (mM)**
  - Y-axis: 0.0 to 2.0
  - X-axis: 0 to 60 minutes
  - Two lines: Pre-training (solid) and Post-training (dashed)
  - Error bars
  - Significant differences indicated by asterisks
Figure 5

Maximal Enzyme Activity
(mmoll·min⁻¹·kg wm⁻¹)

β-HAD

Citrate Synthase

* PRE

POST
Figure 6

The graph shows the total protein content for different proteins: FABP<sub>pm</sub>, FAT/CD36, and HSL, measured in arbitrary units. The bars indicate the protein content before (PRE) and after (POST) treatment. The PRE group shows a lower protein content compared to the POST group for all three proteins. The POST group for FABP<sub>pm</sub> shows a significant increase indicated by an asterisk (*).