Exercise training increases sarcolemmal and mitochondrial fatty acid transport proteins in human skeletal muscle

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The oxidation of long-chain fatty acids (FA) in skeletal muscle is now recognized to be a highly regulated process. Regulation occurs at a number of sites, including FA transport across the muscle membrane (sarcolemma) and the mitochondrial membranes (reviewed in Ref. 29). Transport in both cellular localization; fat oxidation

Fatty acid transport is highly regulated in skeletal muscle and involves several sites of regulation, including the transport of fatty acids across both the plasma and mitochondrial membranes. Transport across these membranes is recognized to be primarily protein mediated, limited by the abundance of fatty acid transport proteins on the respective membranes. In recent years, evidence has shown that fatty acid transport proteins move in response to acute and chronic perturbations; however, in human skeletal muscle the localization of fatty acid transport proteins in response to training has not been examined. Therefore, we determined whether high-intensity interval training (HIIT) increased total skeletal muscle FA transport protein contents. Ten untrained females (22 ± 1 yr, 65 ± 2 kg; VO2peak: 2.8 ± 0.1 l/min) completed 6 wk of HIIT, and biopsies from the vastus lateralis muscle were taken before training, and following 2 and 6 wk of HIIT. Training significantly increased maximal oxygen uptake at 2 and 6 wk (3.1 ± 0.1, 3.3 ± 0.1 l/min). Training for 6 wk increased FAT/CD36 (23%) and mitochondrial FA oxidation rates (51%) without alterations in sarcolemmal content. Whole muscle plasma membrane fatty acid binding protein (FABPpm) also increased (48%) after 6 wk of training, but in contrast to FAT/CD36, sarcolemmal FABPpm increased (23%), whereas mitochondrial FABPpm was unaltered. The changes on sarcolemmal and mitochondrial membrane proteins occurred rapidly, since differences (∼2 wk) were not observed between 2 and 6 wk. This is the first study to demonstrate that exercise training increases fatty acid transport protein content in whole muscle (FAT/CD36 and FABPpm) and sarcolemmal (FABPpm) and mitochondrial (FAT/CD36) membranes in human skeletal muscle of females. These results suggest that increases in skeletal muscle fatty acid oxidation following training are related in part to changes in fatty acid transport protein content and localization.

Fatty acid translocase; plasma membrane fatty acid-binding protein; cellular localization; fat oxidation

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likely the result of whole muscle protein or mRNA content measurements. These determinations do not reflect the “bioactive” pools of transport proteins located on the plasma and mitochondrial membranes. Therefore, the purpose of the current study was to determine the effects of 2 and 6 wk of high-intensity interval training on the content and location (sarcolemmal and mitochondrial membranes) of FAT/CD36 and FABPpm in human skeletal muscle. Mitochondrial and cytoplasmic enzymes involved in FA metabolism were also examined, as were whole body respiratory measurements throughout a peak oxygen consumption (VO₂peak) test and 60-min steady-state (~65% VO₂peak) cycling trial. We hypothesized that 1) training would increase muscle sarcolemmal content of FABPpm and FAT/CD36, 2) training would increase FAT/CD36, FABPpm, and CPT I activity in proportion to increases in mitochondrial content, and 3) these adaptations would be progressive throughout training (6 wk > 2 wk).

METHODS

Subjects

Ten healthy females (22 ± 1 yr, 65 ± 2 kg; VO₂peak: 2.82 ± 0.14 l/min) volunteered to participate in the study. Subjects were untrained but engaged in light recreational physical activity ~2 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 and oral consent. This study was approved by the Research Ethics Boards at McMaster University and the University of Guelph.

Experimental Trials

VO₂peak test. Subjects performed an incremental cycling (Lode Excalibur, Lode, Netherlands) test to exhaustion to determine VO₂peak. Respiratory gases were collected and analyzed using a metabolic cart (AEI, Moxus II Metabolic System, Pittsburgh, PA). Subjects repeated the VO₂peak test following 2 and 6 wk of high-intensity interval training (HIIT).

Cycling trial at 65% pretraining VO₂peak. Subjects performed a 60-min cycling trial at ~65% pretraining VO₂peak prior to (PRE) and following 2 and 6 wk of HIIT. Subjects arrived at the laboratory at the same time in the morning 3–4 h postprandial for all trials. A Teflon catheter was inserted into an antecubital vein for blood sampling, and the catheter was kept patent with 0.9% saline. A resting blood sample was obtained, and subjects then cycled for 60 min at ~65% pretraining VO₂peak at a constant cadence (80–92 rpm) on the Lode ergometer. Respiratory gases were collected between 13 and 17, 28 and 32, 43 and 47, and 56 and 60 min of exercise for the measurements of oxygen consumption (VO₂), carbon dioxide production (VCO₂) and the calculation of the respiratory exchange ratio (RER), and blood samples were obtained at 15, 30, 45, and 60 min of exercise. Whole body fat and carbohydrate oxidation were calculated using the nonprotein RER table with the following equations: carbohydrate oxidation = 4.585 (VCO₂) – 3.226 (VO₂) and fat oxidation = 1.695 (VO₂) – 1.701 (VCO₂) (47).

Muscle biopsies. Approximately 48 h following the PRE, 2-wk, and 6-wk 60-min cycling trials, subjects arrived at the laboratory in the morning, 3–4 h postprandial, for a resting biopsy from the vastus lateralis muscle. Two incisions were made in the skin, and deep fascia under local anesthesia (2% xylocaine without epinephrine) and two muscle biopsies were obtained while the subject rested on a bed. One portion of the first biopsy was used to isolate mitochondria, and a second portion was homogenized and the extract used to measure the expression of selected proteins as well as maximal mitochondrial enzyme activities. The second biopsy was used to isolate giant sarcolemmal vesicles.

HIIT

Approximately 48 h following the pretraining muscle biopsies, subjects began training 3 days/wk, completing 18 training sessions in 6 wk. 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 was recorded throughout training and was held constant (~187–189 beats/min) at ~90% of the subjects’ maximal heart rate by increasing the power output as training progressed. Required adjustments in training power output were made at the beginning of each session. To maintain the high-intensity training stimulus, about six high-intensity intervals were completed 30 min following the 2- and 6-wk VO₂peak test and 60-min cycling trials. Throughout the 6 wk of training, subjects maintained the light recreational activities they were engaged in prior to training.

Analyses

Blood measurements. Venous blood (~5 ml) was collected in sodium-heparin tubes. A portion (200 μl) was added to 800 μl of 0.6 M perchloric acid and centrifuged, and the supernatant was analyzed for blood glucose and lactate using fluorometric techniques, as described previously (48). A second portion (1.5 ml) was centrifuged, and the plasma was analyzed for free fatty acids (FFA) using an enzymatic colorimetric technique as described by the manufacturer (Wako NEFA C test kit; Wako Chemicals, Richmond, VA).

Isolation of mitochondria from skeletal muscle. Differential centrifugation was used to obtain intact mitochondria containing both intermyofibrillar (IMF) and subsarcolemmal (SS) fractions. The isolation procedure has been described previously (12, 27). Briefly, muscle (150 ± 15 mg) was homogenized and centrifuged at 800 g for 10 min to separate the IMF and SS fractions. The IMF mitochondrial fraction was treated with protease (Subtilisin A; Sigma, St. Louis, MO) for exactly 5 min to digest the myofibrils. Further centrifugation was used to remove the myofibrils, and the supernatant containing the IMF fraction was recombined with the SS fraction. The combined samples were centrifuged twice at 10,000 g for 10 min. The pellet was resuspended in 1 μl buffer/mg tissue. A portion of the isolated mitochondria was used to measure CPT I (see next section) and citrate synthase (CS) activities. CS activity was determined in isolated mitochondria and in aliquots of homogenized whole muscle. Total muscle CS activity was assayed in a portion of muscle (~6–8 mg) that was homogenized in 100 vol/wt. Mitochondrial recovery and quality were calculated as follows:

\[
\text{Recovery} = \frac{(CS_{TS} - CS_{EM}/CS_{MM})}{100} \\
\text{Quality} = \frac{(CS_{TS} - CS_{EM}/CS_{TS})}{100} 
\]

TS is total mitochondrial suspension (1:20 dilution), EM is extra-mitochondrial suspension (1:20 dilution) in intact mitochondria, and MH is muscle homogenate. Results provided a measure of mitochondrial viability, and values were compared with total CS activity to provide a measurement of the mitochondria recovered during the isolation procedure. Recovery was used to calculate CPT I activity per gram wet muscle mass.

The remaining mitochondria were further purified for Western blot analysis using consecutive Percoll (GE Healthcare, Aurora, OH) gradients (20,000 g for 1 h and 20,000 g for 5 h). CPT I activity. The radioisotope assay used for the determination of CPT I activity has been described previously (2, 43). Briefly, the assay was conducted at 37°C and initiated by the addition of 10 μl of mitochondrial suspension to 80 μl of a standard reaction medium containing l-[3H]carnitine (GE Healthcare, Amersham Biosciences) with either 75 or 300 μM palmitoyl CoA. The reaction was stopped after 6 min with the addition of ice-cold 1 M HCl. Palmitoyl-
[14]H]carnitine was extracted in water-saturated butanol in a process involving three washes with distilled water and subsequent centrifugation steps to separate the butanol phase, in which the radioactivity was counted.

Muscle enzyme activities. Fresh muscle (~6–10 mg of tissue) was homogenized in 0.1 M KH2PO4 and BSA and freeze-thawed two times, and the maximal activities of CS (57), β-hydroxyacyl-CoA dehydrogenase (β-HAD) (4), and mitochondrial-aspartate aminotransferase (mAspAT) (4, 31) were determined on a spectrophotometer (at 37°C) using formerly described methods.

Preparation of giant sarcolemmal vesicles. Giant vesicles from muscle samples (184 ± 9 mg) were generated as described previously (8, 9, 27). Briefly, the tissue was cut into 1- to 3-mm-thick layers and incubated for 1.5 h at 34°C in 140 mM KCl/10 mM MOPS (pH 7.4), aprotinin (30 µg/ml), and collagenase (type VII, 150 U/ml) in a shaking water bath. At the end of the incubation, the supernatant fraction was collected and the remaining tissue was washed with KCl/ MOPS and 10 mM EDTA, which resulted in a second supernatant fraction. Both supernatant fractions were pooled; Percoll (GE Healthcare) and aprotinin were added to final concentrations of 3.5% (vol/vol) and 10 µg/ml, respectively. The resulting suspension was placed at the bottom of a density gradient consisting of a 3-mL middle layer of 4% Nycodenz (wt/vol) and a 1 mL KCl/MOPS upper layer. This sample was centrifuged at 60,000 g for 45 min at room temperature (25°C). Subsequently, the vesicles were harvested from the interface of the upper and middle layer, diluted in KCl/MOPS, and recentrifuged at 12,000 g for 5 min. The pellet was resuspended in KCl/ MOPS. The pellet was then frozen for subsequent Western blot analysis.

Western blot analysis. Wet muscle (20–30 mg) was initially homogenized in a buffer containing 210 mM sucrose, 2 mM EGTA, 40 mM NaCl, 30 mM HEPES, 20 mM EDTA, and PMSF dissolved with DMSO. A second buffer containing 1.17 M KCl and 58.3 M tetrasodium 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, and samples were centrifuged (3,000 rpm for 15 min). The muscle supernatant, the isolated sarcolemma, and mitochondria were used to determine hormone-sensitive lipase (HSL), FAT/CD36, FABPpm, GLUT4, and cytochrome c oxidase complex IV (COX-IV) content by Western blot analyses. Briefly, samples were separated on a 10% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. A monoclonal antibody (MO25) was used to detect FAT/CD36 content (46), and a FABPpm/mAspAT polyclonal antibody was used to determine FABPpm content (46), whereas commercially available probes were used to measure total HSL (ProSci, Poway, CA), COX-IV (Invitrogen, Burlington, ON, Canada) content, and sarcolemmal GLUT4 content (Chemicon International, Temecula, CA).

Statistics. All data are presented as means ± SE. A one-way repeated-measures ANOVA (trial) was used to determine significant differences between muscle biopsy measurements and VO2peak tests. A two-way repeated-measures ANOVA (trial × time) was used to determine significant differences during the 60-min cycling trials. Specific differences were identified using Fisher’s least significant difference post hoc analysis. Statistical significance was accepted at P < 0.05.

RESULTS
Training power output initially averaged 154 ± 5 W (129–188 W) and increased every week throughout training, reaching 205 ± 6 W (180–240 W) during the final week. Heart rate averaged between 187 and 189 beats/min throughout the 6 wk of training (Table 1). VO2peak increased significantly, by 11 (2.8 ± 0.1 to 3.1 ± 0.1 l/min) and 18% (3.3 ± 0.1 l/min) following 2 and 6 wk, respectively.

| Table 1. Average power output and heart rate throughout 6 wk of high-intensity interval training |
|-------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                               | Week 1            | Week 2            | Week 3            | Week 4            | Week 5            | Week 6            |
| Power output, W*              | 194 ± 5           | 168 ± 5           | 183 ± 4           | 189 ± 6           | 197 ± 6           | 205 ± 6           |
| Heart rate, beats/min         | 187 ± 1           | 188 ± 2           | 182 ± 2           | 189 ± 2           | 189 ± 2           | 182 ± 3           |
| Values are means ± SE; n = 10. *All power outputs were significantly different (P < 0.05) from each other. |

Cycling at ~65% of Pretraining Vo2peak Before and After Training
Subjects cycled for 60 min at 123 ± 6 W prior to training (PRE) and following 2 and 6 wk of training. VO2 was similar between the three cycling trials, but training resulted in a progressive decrease in heart rate (Table 2).

Whole Body Fuel Utilization
Compared with PRE, RER was significantly lower at all time points following both 2 and 6 wk (Table 2). As a result, calculated rates of whole body (Fig. 1) and total fat oxidation (PRE, 15.2 ± 3.4 g; 2 wk, 25.0 ± 2.5 g; 6 wk, 25.6 ± 2.4 g) were higher throughout cycling following training. Therefore, there was an ~20% decrease in whole body carbohydrate oxidation following training (PRE, 109.7 ± 6.6 g; 2 wk, 89.5 ± 5.0 g; 6 wk, 87.0 ± 6.0 g).

Blood Measurements
Blood lactate concentrations increased during the PRE exercise trial, but this trend was not observed following 2 and 6 wk of training. As a result, compared with PRE, lactate concentrations were lower at all exercise time points following 2 and 6 wk (Table 3). Plasma FFA concentrations increased above resting values at 30, 45, and 60 min of exercise during all three trials, and there was no effect of training on plasma FFA (Table 3).

Muscle Analyses
Mitochondrial enzyme activities and protein contents. Whole muscle maximal activities of CPT I, β-HAD, CS, and mAspAT as well as COX-IV content were significantly increased by ~30% following 2 wk of training (Table 4). Four additional weeks of training resulted in further increases of ~20% in these parameters (Table 4).

CPT I activity measured in the presence of palmitoyl-CoA concentrations that elicit ~50% maximal activity (75 µM) also significantly increased following training (PRE, 103.0 ± 10.0 mmol·min⁻¹·kg wet muscle⁻¹; 2 wk, 138.3 ± 15.8 mmol·min⁻¹·kg wet muscle⁻¹; 6 wk, 165.1 ± 15.1 mmol·min⁻¹·kg wet muscle⁻¹). In contrast, when CPT I and CS were determined in isolated mitochondria and expressed per milligram of mitochondrial protein, there were no significant differences between PRE, 2 wk, and 6 wk (Table 4).

Long-chain FA transport proteins. Whole muscle FAT/ CD36 content increased following 2 (+13%) and 6 wk (+10%) of training (Fig. 2). Under resting conditions, the sarcolemmal FAT/CD36 content did not increase following training. In contrast, mitochondrial FAT/CD36 content in-
Table 2. Effect of high-intensity interval training on $\dot{V}_O_2$, RER, and heart rate during 60 min of cycling at $\sim$65% PRE $\dot{V}_O_2$peak

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<td>15</td>
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<tr>
<td>$\dot{V}_O_2$, l/min</td>
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<tr>
<td>PRE</td>
<td>1.88 ± 0.07 1.88 ± 0.07 1.97 ± 0.09 1.97 ± 0.09</td>
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<td>2 Wk</td>
<td>1.92 ± 0.09 1.91 ± 0.08 1.95 ± 0.09 2.01 ± 0.10</td>
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<td>6 Wk</td>
<td>1.85 ± 0.08 1.90 ± 0.08 1.94 ± 0.09 1.94 ± 0.08</td>
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<td>% $\dot{V}_O_2$peak PRE (%PRE $\dot{V}_O_2$peak)</td>
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<tr>
<td>PRE</td>
<td>67 ± 3 67 ± 3 70 ± 3* 70 ± 3*</td>
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<tr>
<td>2 Wk (%2 wk $\dot{V}_O_2$peak)</td>
<td>63 ± 3 63 ± 3 64 ± 4 66 ± 4</td>
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<td>6 Wk (%6 wk $\dot{V}_O_2$peak)</td>
<td>57 ± 3‡ 58 ± 3 60 ± 3† 60 ± 2†</td>
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<td>RER</td>
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<tr>
<td>PRE</td>
<td>0.95 ± 0.02 0.93 ± 0.03* 0.91 ± 0.01* 0.90 ± 0.01*</td>
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<tr>
<td>2 Wk</td>
<td>0.90 ± 0.01 0.88 ± 0.01* 0.86 ± 0.01* 0.85 ± 0.01*</td>
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<td>6 Wk</td>
<td>0.90 ± 0.01 0.87 ± 0.01* 0.85 ± 0.01* 0.84 ± 0.01*</td>
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<tr>
<td>Heart rate, beats/min</td>
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<tr>
<td>PRE</td>
<td>166 ± 3 171 ± 3* 175 ± 3* 177 ± 3*</td>
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<tr>
<td>2 Wk</td>
<td>159 ± 3 167 ± 3* 168 ± 4* 169 ± 4*</td>
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<tr>
<td>6 Wk</td>
<td>157 ± 3 159 ± 3† 163 ± 3† 164 ± 3*†</td>
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Values are means ± SE; n = 10. $\dot{V}_O_2$, oxygen consumption; RER, respiratory exchange ratio; $\dot{V}_O_2$peak, peak oxygen consumption; PRE, pretraining.

*Significantly different (P < 0.05) from 15 min of the same trial and from the same time point during the PRE trial. †Significantly different from the same time point during the 2-wk trial.

Increased by 30% following 2 wk and 51% following 6 wk (Fig. 2). Whole muscle FABPpm content increased significantly after 2 (+19%) and 6 wk (+48%) of training (Fig. 2). The sarcolemmal FABPpm content increased significantly after 2 (+14%) and 6 wk (+23%); however, mitochondrial FABPpm content was not increased following 2 or 6 wk of training.

HSL and GLUT4 content. Whole muscle HSL content increased with training (n = 9; PRE, 1.00 ± 0.0 normalized arbitrary units; 2 wk, 1.12 ± 0.05 normalized arbitrary units; 6 wk, 1.28 ± 0.14 normalized arbitrary units). Both total muscle and sarcolemmal GLUT4 contents increased significantly following 2 wk (~30%) and increased further following 6 wk (~50%) of training (Fig. 3). No GLUT4 was detected in the mitochondrial fraction.

**DISCUSSION**

This is the first study to measure fatty acid transport protein contents at the whole muscle, sarcolemmal, and mitochondrial membrane fractions in human skeletal muscle before and after training. We observed that following training, 1) total muscle FABPpm and FAT/CD36 contents increased, 2) at rest, FABPpm but not FAT/CD36 increased on the sarcolemma, and 3) FAT/CD36 but not FABPpm content increased on mitochondrial membranes. These changes occurred rapidly, since 4) differences in sarcolemmal and mitochondrial contents were not altered between 2 and 6 wk. Last, 5) basal sarcolemmal GLUT4 content remained elevated 48 h following the final training sessions after 2 and 6 wk of training. These results suggest that increases in skeletal muscle fatty acid oxidation following training are in part related to changes in fatty acid transport protein content and localization.

Fig. 1. Effect of high-intensity interval training (HIIT) on whole body fat and carbohydrate (CHO) oxidation (ox) measurements during 60 min of cycling at ~65% pretraining (PRE) peak oxygen consumption. Values are means ± SE (n = 10); 2 and 6 wk, following 2 and 6 wk of HIIT, respectively. *Significantly different from the same time point prior to training (P < 0.05).
(8), suggesting that translocation to the sarcolemmal is transient and likely occurs when needed for increased rates of FA oxidation. In this respect, sarcolemmal FAT/CD36 likely only contributes a small component of basal FA transport. In support of this suggestion, ablating FAT/CD36 only minimally inhibits sarcolemmal FA transport (~15%) at rest (30) but substantially inhibits (~100%) the ability of skeletal muscle to respond to situations that require an increase in FA energy provision (AICAR) (22). It is clear that studies are needed to examine the potential for translocation of FAT/CD36 to the sarcolemma in human skeletal muscle during exercise and whether an increased content participates in regulating FA oxidation.

In agreement with our original hypothesis, sarcolemmal FABPpm content was increased with training. Overexpression of FABPpm has previously been shown to increase sarcolemmal FA content as well as FA transport independent of changes in the other transport proteins (FAT/CD36 and FATPs) (14, 31, 46). These data strongly suggest that the increase in sarcolemmal FABPpm content following training improves the capacity of FA uptake across the sarcolemma. We did not measure maximal FA transport rates in the present study, since direct measurements of FA transport in sarcolemmal vesicles following training in humans would require ~500–1,000 mg of muscle. Similarly to FAT/CD36, FABPpm has been shown to acutely translocate to the plasma membrane during muscle contraction (34). Although studies examining the rate of FABPpm internalization (endocytosis) have not been conducted, the current data suggest that FABPpm represents a more “stable” adaptation than FAT/CD36 similar to changes in sarcolemmal GLUT4 following exercise (present study and Ref. 13). If FAT/CD36 and FABPpm work in a concerted fashion, as has been proposed (40), this may suggest that FABPpm works in a regulatory fashion to mediate larger fluctuations in sarcolemmal FAT/CD36 during subsequent exercise bouts. Regardless of the speculation on the interactive nature of these proteins, it appears that independently upregulating FABPpm on sarcolemmal membranes increases FA transport (14, 31, 46), and therefore, it is feasible to speculate in the present study that FA transport was increased following 2 and 6 wk of training.

The FAT family of FA transport proteins has also been shown to regulate FA transport with different efficacies in yeast (15), as well as mature mammalian muscle (46), and, similarly to FAT/CD36 and FABPpm, translocate to the sarcolemma in response to metabolic demands (34). This family of transport protein also represents additional regulation of FA transport that needs to be examined in the context of training-induced improvements in FA oxidation. These proteins were not studied in the present study because of 1) a lack of tissue and 2) poor quality of antibodies for human skeletal muscle. Hopefully these methodological limitations will be rectified in future studies.

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Table 3. Effect of high-intensity interval training on venous blood measurements during 60 min of cycling at ~65% \( \dot{V}O_2\text{peak} \)

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<tr>
<td>Lactate, mM</td>
<td>PRE</td>
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<tr>
<td>PRE</td>
<td>1.0 ± 0.2</td>
<td>3.1 ± 0.7*</td>
<td>2.8 ± 0.6*</td>
<td>2.8 ± 0.5*</td>
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<td>1.8 ± 0.3*</td>
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<td>6 Wk</td>
<td>0.9 ± 0.1</td>
<td>1.5 ± 0.3*</td>
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<td>FFA, mM</td>
<td>PRE</td>
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<tr>
<td>PRE</td>
<td>0.27 ± 0.07</td>
<td>0.35 ± 0.09</td>
<td>0.40 ± 0.09*</td>
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<td>2 Wk</td>
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<td>Glucose, mM</td>
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<tr>
<td>PRE</td>
<td>4.4 ± 0.2</td>
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Values are means ± SE; n = 9. FFA, free fatty acid. *Significantly different (P < 0.05) from 0 min of the same trial and from the same time point during PRE.

Table 4. Maximal mitochondrial enzyme activities and protein contents

<table>
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<th>2 Wk</th>
<th>6 Wk</th>
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<td>CPT I, mmol·min⁻¹·kg wm⁻¹</td>
<td>211.6 ± 21.9</td>
<td>252.1 ± 27.4*</td>
<td>309.6 ± 26.3*</td>
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<tr>
<td>( \beta )-HAD, mmol·min⁻¹·kg wm⁻¹</td>
<td>8.5 ± 1.3</td>
<td>10.6 ± 1.0*</td>
<td>11.8 ± 1.1*</td>
</tr>
<tr>
<td>CS, mmol·min⁻¹·kg wm⁻¹</td>
<td>18.4 ± 3.0</td>
<td>24.2 ± 3.4*</td>
<td>28.5 ± 2.9*</td>
</tr>
<tr>
<td>mAspAT, mmol·min⁻¹·kg wm⁻¹</td>
<td>86.4 ± 8.8</td>
<td>107.9 ± 13.4*</td>
<td>120.8 ± 10.4*</td>
</tr>
<tr>
<td>COX-IV (relative to control)</td>
<td>1.00 ± 0.00</td>
<td>1.29 ± 0.10*</td>
<td>1.57 ± 0.20*</td>
</tr>
<tr>
<td>Isolated mitochondria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPT I, mmol·min⁻¹·mg mp⁻¹</td>
<td>11.9 ± 1.9</td>
<td>8.6 ± 0.9</td>
<td>11.1 ± 1.6</td>
</tr>
<tr>
<td>CS, mmol·min⁻¹·mg mp⁻¹</td>
<td>38.9 ± 5.2</td>
<td>40.1 ± 1.4</td>
<td>41.5 ± 2.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10. CPT I, carnitine palmitoyltransferase I; \( \beta \)-HAD, \( \beta \)-hydroxyacyl-CoA dehydrogenase; wm, total muscle wet mass; CS, citrate synthase; mAspAT, mitochondrial-aspartate aminotransferase; COX-IV, cytochrome c oxidase complex IV; mp, mitochondrial protein. *Significantly different from PRE (P < 0.05) and from 2 wk (P < 0.05).
Mitochondrial Long-Chain FA Transport Proteins

CPT I catalyzes the first and committed step in FA transport into the mitochondria and is considered a rate-limiting enzyme in the FA carnitine-dependent transport of FA into the mitochondria (42). However, CPT I has been shown to immunoprecipitate with FAT/CD36 in rodent (12) and human (54) muscle mitochondria, and it has been postulated that FAT/CD36 and CPT I work together to coordinate the transport of FA into the mitochondria (6). However, very little is understood of how FAT/CD36 aids or facilitates transport of FA across the mitochondrial membranes. This interpretation is strengthened by the observation that acute overexpression of FABPpm in rodent muscle increased mitochondrial FABPpm content and mAspAT activity proportionately without altering mitochondrial FA oxidation rates (31). In the present study, whole muscle mAspAT responded in a mirror image to that of CS, β-HAD, and COX-IV, classical markers of mitochondrial content. This suggests that mAspAT increased simply in response to mitochondrial proliferation as opposed to increasing out of proportion to the mitochondrial increases. This interpretation is supported by the constant mitochondrial FABPpm (a.k.a. mAspAT) per milligram of mitochondrial protein.

Contrary to our hypothesis, training did not alter the content of FABPpm per mitochondria. However, these results may not reflect limitations in FA transport into the organelle, since it is presently believed that FABPpm does not contribute to mitochondrial FA oxidation. In mitochondria, it has been shown that FABPpm is identical in structure to mAspAT (5, 10, 33), which contributes to the shuttle of reducing equivalents across the mitochondrial membranes. In this interpretation is strengthened by the observation that acute overexpression of FABPpm in rodent muscle increased mitochondrial FABPpm content and mAspAT activity proportionately without altering mitochondrial FA oxidation rates (31). Our present results revealed that training augmented FAT/CD36 content on the mitochondria (per gram of mitochondrial protein) by 30% following 2 wk of training and 51% following 6 wk of training, suggesting that mitochondrial FA oxidation may also be increased. In contrast, although whole muscle CPT I maximal activity (per gram wet mass) increased 19 and 46% following 2 and 6 wk of training, respectively, this reflects changes in mitochondrial content, since CPT I activity expressed per milligram of mitochondrial protein was unaltered with training [similar to rodent studies (38)]. The one exception in the literature suggesting that FAT/CD36 does not exist on mitochondrial membranes is difficult to explain (35). Studies showing that FAT/CD36 immunoprecipitates with CPT I (12, 54) and a targeted proteomic approach (16) on purified mitochondrial outer membranes provide convincing evidence that FAT/CD36 is present at the mitochondrion.

Fig. 2. Effect of HIIT on fatty acid transport protein (FATP) content. Values are means ± SE (n = 10). MH, muscle homogenate; mito, mitochondria; FAT/CD36, fatty acid translocase; FABPpm, plasma membrane fatty acid-binding protein. *Significantly higher than PRE (P < 0.05); †significantly higher than 2 wk.

Recently FATP1, another sarcolemmal FA transport protein, has been implicated in regulating mitochondrial FA oxidation since overexpression of FATP1 in L6E9 myotubes increased palmitate oxidation (56). It is likely that this reflects changes in acyl-CoA synthetase activity as opposed to a direct “transport” of FA into mitochondria, since in isolated mitochondria both acyl-CoA synthetase and palmitate oxidation increased ~70% in FATP1-transfected cells (56). FATP1 was not measured in the present study because the quality of the commercially available FATP1 antibodies is presently very poor in human tissue. Therefore, we cannot comment on the potential training-induced alterations in localization of this protein at either the sarcolemmal or mitochondrial membranes, but changes at both locations remain possible.

Fig. 3. Effect of HIIT on GLUT4 (glucose transport protein) protein content. Values are means ± SE (n = 10). ND, not detected. *Significantly different than PRE (P < 0.05); †significantly higher than 2 wk.
Training Increases Muscle Fatty Acid Transport Proteins

Fig. 4. Summary of HIIT effects on FATP localization. HIIT resulted in an increase in total FAT/CD36 and FABPpm protein as well as an increase in the amount of FABPpm on plasma membranes and FAT/CD36 on mitochondrial membranes. FABPpm (mitochondrial-aspartate aminotransferase) increased at the whole muscle level, but not on isolated mitochondria, reflecting changes in mitochondrial number. FFA, free fatty acid; ATP, adenosine 5’-triphosphate. Note that although FAT isoforms have been found on both plasma and mitochondrial membranes, we have not depicted these because of a lack of information regarding how the localization is altered with HIIT.

Plasma Membrane GLUT4 Content

In the present study we show a progressive increase in both total and plasma membrane GLUT4 content throughout training. Several studies in human muscle have previously shown that total GLUT4 content is increased following aerobic training (11, 21, 32, 39); however, the plasma membrane compartment has remained largely unexplored. The pronounced increase in sarcosomal GLUT4 content following training in the present study is in contrast to a previous report (51); however, this likely reflects differences in cross-sectional (51) and longitudinal (present study) study designs. In both human and rodent muscle, GLUT4 content has been shown to translocate from an intracellular depot to the plasma membrane in response to a variety of physiological stimuli (20, 41, 45, 50, 60). In the present study, biopsies were taken 48 h following a training bout, and therefore, it is difficult to determine how much of the increase in plasma membrane GLUT4 content represents the residual effect of the previous training session. Regardless, our data show that training results in a progressive increase in both total and plasma membrane GLUT4, which may account for the previous reports of increased glucose transport following muscle contraction that persists for at least 48 h in humans (44, 49).

Classic Training Adaptations

Corroborating the training-induced increases in skeletal muscle FA transport protein content was an ~66% increase in whole body fat oxidation during a 60-min submaximal cycle following only 2 wk of HIIT. These results are even greater than the 36% increase in whole body fat oxidation observed in our previous 2-wk HIIT training study with women (59). It is not clear why we observed a greater adaptation in whole body fat oxidation in the present study or why we saw an improvement only following 2 wk of training. However, it may have been due to differences in the initial training status of the subjects and the relative intensity of the PRE rides. The pretraining ride in the present study was at a relative intensity of ~69% \( V_{\text{O2peak}} \), which is in the range that would elicit maximal fat oxidation rates (52, 62). However, this absolute power output represented only 64% \( V_{\text{O2peak}} \) following 2 wk and 59% \( V_{\text{O2peak}} \) following 6 wk of training. Hence, it is likely that whole body fat oxidation would have been higher if the subjects had exercised at ~69% of their new \( V_{\text{O2peak}} \) following 2 and 6 wk of training.

Intriguingly, both studies (present study and Ref. 59) revealed that performance of a high-intensity exercise program that predominantly requires carbohydrate to supply the large energy demand during training improved whole body fat oxidation when exercise was done at a moderate submaximal intensity. This may be of no surprise since calcium, which increases at all exercise intensities, and the strong shift in the energy charge of the cell during high-intensity exercise are both strong stimuli augmenting a number of signaling mechanisms that alternate both carbohydrate and fat metabolism in skeletal muscle (23). Improvements in whole body fat oxidation and glycogen sparing that have commonly been observed following exercise training can be attributed to improvements in skeletal muscle oxidative capacity (17, 25). Although HIIT relies primarily on carbohydrate for fuel, this form of training has consistently been shown to augment mitochondrial enzyme activities (48, 59). This study showed that training can augment HSL content as well as long-chain FA transport protein content and location, suggesting that training improves the capacity of skeletal muscle to utilize long-chain FA for fuel at a number of regulatory sites.

In summary, this is the first study to show that exercise training by young female subjects is a potent stimulus increasing FA transport protein contents on skeletal muscle sarcotact and mitochondria 48 h following the final training bout. Specifically, training increased mitochondrial content and FABPpm on the plasma membrane and FAT/CD36 on mitochondrial membranes, as depicted in Fig. 4. The results suggest that the subcellular locations of FABPpm and FAT/CD36 may be important determinants of FA oxidation.

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