

Jason L. Talanian, Graham P. Holloway, Laelie A. Snook, George J. F. Heigenhauser, Arend Bonen and Lawrence L. Spriet
Am J Physiol Endocrinol Metab 299:180-188, 2010. First published May 18, 2010;
doi:10.1152/ajpendo.00073.2010

You might find this additional information useful...

This article cites 59 articles, 46 of which you can access free at:

<http://ajpendo.physiology.org/cgi/content/full/299/2/E180#BIBL>

Updated information and services including high-resolution figures, can be found at:

<http://ajpendo.physiology.org/cgi/content/full/299/2/E180>

Additional material and information about *AJP - Endocrinology and Metabolism* can be found at:

<http://www.the-aps.org/publications/ajpendo>

This information is current as of October 19, 2010 .

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

Jason L. Talanian,¹ Graham P. Holloway,¹ Laelie A. Snook,¹ George J. F. Heigenhauser,² Arend Bonen,¹ and Lawrence L. Spriet¹

¹Department of Human Health and Nutritional Sciences, University of Guelph, Guelph; and ²Department of Medicine, McMaster University, Hamilton, Ontario, Canada

Submitted 1 February 2010; accepted in final form 11 May 2010

Talanian JL, Holloway GP, Snook LA, Heigenhauser GJ, Bonen A, Spriet LL. Exercise training increases sarcolemmal and mitochondrial fatty acid transport proteins in human skeletal muscle. *Am J Physiol Endocrinol Metab* 299: E180–E188, 2010. First published May 18, 2010; doi:10.1152/ajpendo.00073.2010.—Fatty acid oxidation 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, sarcolemmal, and mitochondrial membrane fatty acid transport protein contents. Ten untrained females (22 ± 1 yr, 65 ± 2 kg; $\dot{V}O_{2\text{peak}}$: 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 at the whole muscle (10%) and mitochondrial levels (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 membranes 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

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 locations has been shown to involve a number of proteins (28, 46). FA translocase (FAT/CD36) has been implicated in both sarcolemmal and mitochondrial FA membrane transport and

may represent an important protein in the context of training-induced adaptations in FA oxidation.

Plasma membrane FA transport involves a number of proteins, including a family of FA transport proteins (FATP1–6) (19, 24, 53), plasma membrane FA-binding protein (FABPpm) (33, 55, 58), and FAT/CD36 (1). These transport proteins appear to have different efficacies in influencing sarcolemmal FA transport, with FAT/CD36 displaying the greatest positive effect (46). In addition, a number of studies utilizing acute muscle contraction (8), chronic low-frequency muscle stimulation (37), and denervation (37) models have suggested that FAT/CD36 translocates between an intracellular depot and the plasma membrane, thereby regulating FA import into muscle cells (reviewed in Ref. 7). Collectively, these data have implicated FAT/CD36 as a primary regulator of skeletal muscle FA sarcolemmal transport. It has been speculated that FAT/CD36 and FABPpm coordinate, in an unknown manner, the regulation of FA sarcolemmal transport (40), and therefore, they need to be studied in concert when the muscle is challenged to increase FA transport.

Historically, the transport of FA at the mitochondria has been attributed exclusively to the carnitine palmitoyltransferase (CPT) complex (18) and specifically to the rate-limiting activity of the enzyme CPT I (reviewed in Ref. 42). However, several observations have suggested additional regulation, since mitochondrial FA oxidation can be altered independently of CPT I activity (2, 27, 38). FAT/CD36 represents one potential additional level of regulation, since gain-of-function [overexpression of FAT/CD36 in L6E9 myotubes (56)] and loss-of-function [FAT/CD36-null animals (30)] experiments resulted in increased and decreased palmitate oxidation rates, respectively, in isolated mitochondria. In addition, apparent translocation of FAT/CD36 to mitochondrial membranes during acute muscle contraction coincides with increased FA oxidation rates (12, 28, 30). Collectively, mitochondrial FAT/CD36 content and CPT I activity have been positively correlated with mitochondrial FA oxidation rates (6) and immunoprecipitate in both human (54) and rodent (12) skeletal muscle. Although there is still debate regarding the exact role of mitochondrial FAT/CD36 (36), there is substantial evidence from multiple laboratories to suggest that FAT/CD36 exists on mitochondrial membranes (6, 12, 16, 36, 54). Therefore, mitochondrial FAT/CD36 may represent a unique protein capable of synchronizing plasma membrane FA delivery and mitochondrial utilization (in an unknown manner) (26), and in this respect it may coordinate the well-characterized, training-induced adaptation of increased FA oxidation.

Previous studies examining the responses of FATP contents to aerobic training have yielded equivocal results (11, 59, 61),

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

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 ($\dot{V}O_{2\text{peak}}$) test and 60-min steady-state ($\sim 65\% \dot{V}O_{2\text{peak}}$) 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; $\dot{V}O_{2\text{peak}}$: 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

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

Cycling trial at 65% pretraining $\dot{V}O_{2\text{peak}}$. Subjects performed a 60-min cycling trial at $\sim 65\%$ pretraining $\dot{V}O_{2\text{peak}}$ 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 $\sim 65\%$ pretraining $\dot{V}O_{2\text{peak}}$ 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 ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) 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 = $4.585 (\dot{V}CO_2) - 3.226 (\dot{V}O_2)$ and fat oxidation = $1.695 (\dot{V}O_2) - 1.701 (\dot{V}CO_2)$ (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 anaesthesia (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 $90\% \dot{V}O_{2\text{peak}}$ separated by 2 min of rest. Heart rate was recorded throughout training and was held constant (~ 187 – 189 beats/min) at $\sim 90\%$ 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 $\dot{V}O_{2\text{peak}}$ 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 μ l) was added to 800 μ l 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 \pm 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 μ l 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:

$$\text{Recovery} = (\text{CS}_{\text{TS}} - \text{CS}_{\text{EM}} / \text{CS}_{\text{MH}}) \times 100$$

$$\text{Quality} = (\text{CS}_{\text{TS}} - \text{CS}_{\text{EM}} / \text{CS}_{\text{TS}}) \times 100$$

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 μ l of mitochondrial suspension to 80 μ l of a standard reaction medium containing L-[^3H]carnitine (GE Healthcare, Amersham Biosciences) with either 75 or 300 μ M palmitoyl CoA. The reaction was stopped after 6 min with the addition of ice-cold 1 M HCl. Palmitoyl-

[³H]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 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 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 $\dot{V}O_{2peak}$ 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). $\dot{V}O_{2peak}$ 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*	154 ± 5	168 ± 5	183 ± 4	189 ± 6	197 ± 6	205 ± 6
Heart rate, beats/min	187 ± 1	188 ± 2	188 ± 2	189 ± 2	189 ± 2	188 ± 3

Values are means ± SE; $n = 10$. *All power outputs were significantly different ($P < 0.05$) from each other.

Cycling at ~65% of Pretraining $\dot{V}O_{2peak}$ 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. $\dot{V}O_2$ 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, blood 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 ~65% PRE $\dot{V}O_{2peak}$

	Min			
	15	30	45	60
$\dot{V}O_2$, l/min				
PRE	1.88 ± 0.07	1.88 ± 0.07	1.97 ± 0.09	1.97 ± 0.09
2 Wk	1.92 ± 0.09	1.91 ± 0.08	1.95 ± 0.09	2.01 ± 0.10
6 Wk	1.85 ± 0.08	1.90 ± 0.08	1.94 ± 0.09	1.94 ± 0.08
% $\dot{V}O_{2peak}$				
PRE (%PRE)				
$\dot{V}O_{2peak}$	67 ± 3	67 ± 3	70 ± 3*	70 ± 3*
2 Wk (%2 wk)				
$\dot{V}O_{2peak}$	63 ± 3	63 ± 3	64 ± 4	66 ± 4
6 Wk (%6 wk)				
$\dot{V}O_{2peak}$	57 ± 3†	58 ± 3	60 ± 3†	60 ± 2†
RER				
PRE	0.95 ± 0.02	0.93 ± 0.03*	0.91 ± 0.01*	0.90 ± 0.01*
2 Wk	0.90 ± 0.01	0.88 ± 0.01*	0.86 ± 0.01*	0.85 ± 0.01*
6 Wk	0.90 ± 0.01	0.87 ± 0.01*	0.85 ± 0.01*	0.84 ± 0.01*
Heart rate, beats/min				
PRE	166 ± 3	171 ± 3*	175 ± 3*	177 ± 3*
2 Wk	159 ± 3	167 ± 3*	168 ± 4*	169 ± 4*
6 Wk	157 ± 3	159 ± 3†	163 ± 3*†	164 ± 3*†

Values are means ± SE; n = 10. $\dot{V}O_2$, oxygen consumption; RER, respiratory exchange ratio; $\dot{V}O_{2peak}$, 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.

creased 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.

Sarcolemmal FATP

In the present study, contrary to our hypothesis, basal resting sarcolemmal FAT/CD36 content was not altered with training. However, it seems likely that, when performing exercise in the trained state, the distribution of FAT/CD36 to the sarcolemma during exercise is increased when FA oxidation increases severalfold above resting rates. Acute electrical muscle stimulation (30 min) has been shown to significantly increase FAT/CD36 on the sarcolemma and rates of palmitate uptake (8). However, following less than 1 h of recovery, FAT/CD36 content on the plasma membrane returned to preexercise levels

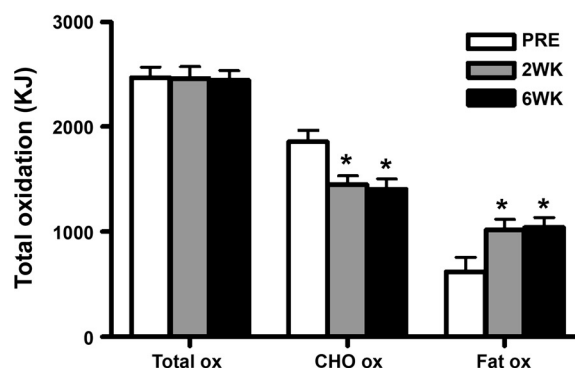
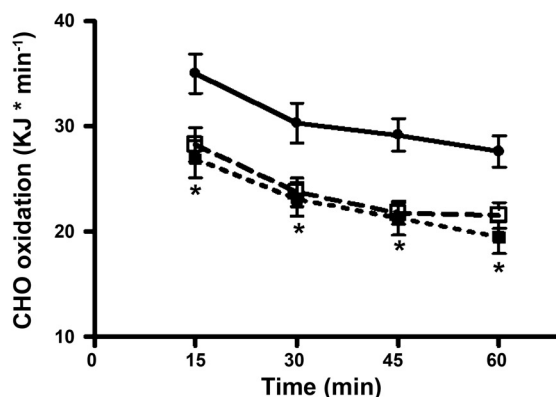
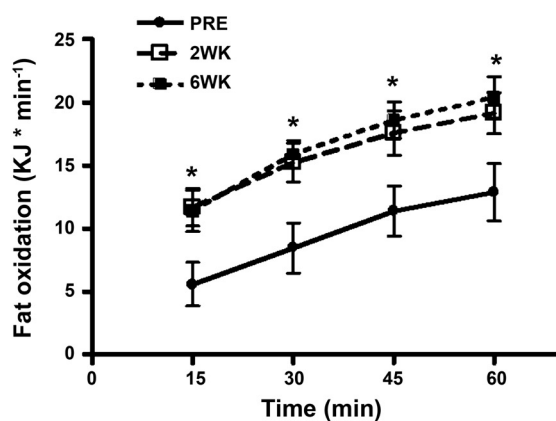


Fig. 1. Effect of high-intensity interval training (HIIT) on whole body fat and carbohydrate (CHO) oxidation (ox) measurements during 60 min of cycling at ~65% pretraining (PRE) peak oxygen consumption. Values are means ± SE (n = 10); 2 and 6 wk, following 2 and 6 wk of HIIT, respectively. *Significantly different from the same time point prior to training ($P < 0.05$).

Table 3. Effect of high-intensity interval training on venous blood measurements during 60 min of cycling at ~65% PRE $\dot{V}O_{2peak}$

	Min				
	0	15	30	45	60
Lactate, mM					
PRE	1.0 ± 0.2	3.1 ± 0.7*	2.8 ± 0.6*	2.8 ± 0.5*	2.7 ± 0.6*
2 Wk	0.9 ± 0.2	1.8 ± 0.3*	1.8 ± 0.2*	1.6 ± 0.2*	1.4 ± 0.3
6 Wk	0.9 ± 0.1	1.5 ± 0.3*	1.2 ± 0.1	1.1 ± 0.2	1.0 ± 0.1
FFA, mM					
PRE	0.27 ± 0.07	0.35 ± 0.09	0.40 ± 0.09*	0.59 ± 0.09*	0.66 ± 0.08*
2 Wk	0.21 ± 0.04	0.22 ± 0.07	0.35 ± 0.08*	0.54 ± 0.13*	0.68 ± 0.09*
6 Wk	0.27 ± 0.06	0.24 ± 0.08	0.45 ± 0.08*	0.69 ± 0.09*	0.77 ± 0.11*
Glucose, mM					
PRE	4.4 ± 0.2	4.4 ± 0.2	4.6 ± 0.2	4.8 ± 0.2	4.6 ± 0.2
2 Wk	4.7 ± 0.2	3.9 ± 0.2*	4.1 ± 0.2	4.5 ± 0.2	4.1 ± 0.2
6 Wk	4.8 ± 0.2	4.1 ± 0.2*	4.6 ± 0.3	4.2 ± 0.2	4.3 ± 0.2

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.

(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 FABPm content was increased with training. Overexpression of FABPm 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 FABPm 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, FABPm has been shown to acutely translocate to the plasma membrane during muscle contraction (34).

Although studies examining the rate of FABPm internalization (endocytosis) have not been conducted, the current data suggest that FABPm 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 FABPm work in a concerted fashion, as has been proposed (40), this may suggest that FABPm 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 FABPm 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 FATP 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 FABPm, translocate to the sarcolemmal membrane 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.

Table 4. Maximal mitochondrial enzyme activities and protein contents

	PRE	2 Wk	6 Wk
Whole muscle			
CPT I, $\text{mmol} \cdot \text{min}^{-1} \cdot \text{kg} \text{wm}^{-1}$	211.6 ± 21.9	252.1 ± 27.4*	309.6 ± 26.3*
β -HAD, $\text{mmol} \cdot \text{min}^{-1} \cdot \text{kg} \text{wm}^{-1}$	8.5 ± 1.3	10.6 ± 1.0*	11.8 ± 1.1*
CS, $\text{mmol} \cdot \text{min}^{-1} \cdot \text{kg} \text{wm}^{-1}$	18.4 ± 3.0	24.2 ± 3.4*	28.5 ± 2.9*
mAspAT, $\text{mmol} \cdot \text{min}^{-1} \cdot \text{kg} \text{wm}^{-1}$	86.4 ± 8.8	107.9 ± 13.4*	120.8 ± 10.4*
COX-IV (relative to control)	1.00 ± 0.00	1.29 ± 0.10*	1.57 ± 0.20*
Isolated mitochondria			
CPT I, $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg} \text{mp}^{-1}$	11.9 ± 1.9	8.6 ± 0.9	11.1 ± 1.6
CS, $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg} \text{mp}^{-1}$	38.9 ± 5.2	40.1 ± 1.4	41.5 ± 2.0

Values are means ± SE; $n = 10$. CPT I, carnitine palmitoyltransferase I; β -HAD, β -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 ($P < 0.05$).

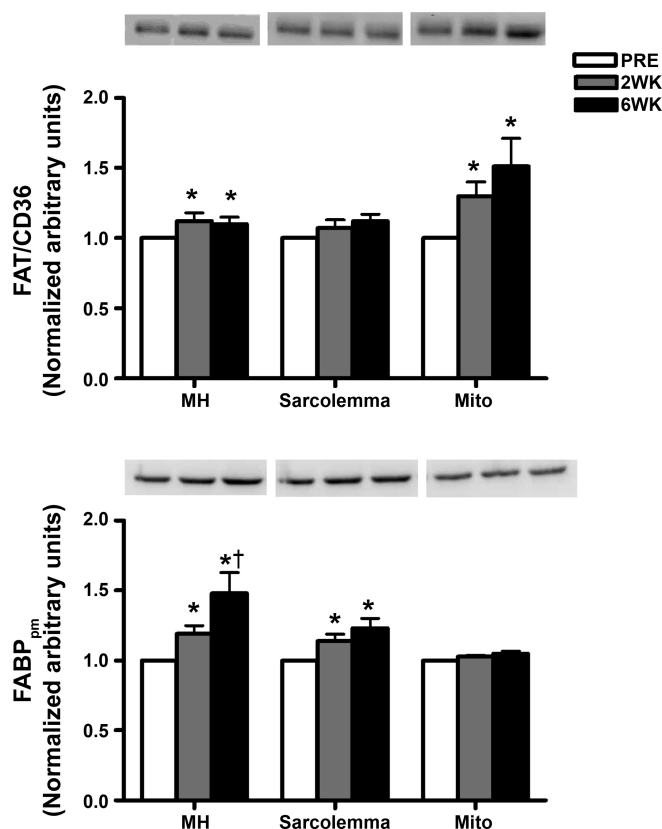


Fig. 2. Effect of HIIT on fatty acid transport protein (FATP) content. Values are means \pm SE ($n = 10$). MH, muscle homogenate; mito, mitochondria; FAT/CD36, fatty acid translocase; FABP^{pm}, plasma membrane fatty acid-binding protein. *Significantly higher than PRE ($P < 0.05$); †significantly higher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

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 mitochondria. Despite these limitations, it is known that FAT/CD36 resides on the outer mitochondrial membrane (16), and acute overexpression of FAT/CD36 in L6E9 myotubes increases mitochondrial FA oxidation (56). Models utilizing FAT/CD36 ablation approaches have yielded equivocal results (30, 36), which may reflect the belief that mitochondrial FAT/CD36 is more important in regulating changes in mitochondrial oxidation as opposed to basal FA oxidation rates (30).

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 mitochondria 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 ex-

pressed 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.

Contrary to our hypothesis, training did not alter the content of FABP^{pm} per mitochondria. However, these results may not reflect limitations in FA transport into the organelle, since it is presently believed that FABP^{pm} does not contribute to mitochondrial FA oxidation. In mitochondria, it has been shown that FABP^{pm} is identical in structure to mAspAT (5, 10, 33), which contributes to the shuttle of reducing equivalents across the mitochondrial membranes. This interpretation is strengthened by the observation that acute overexpression of FABP^{pm} in rodent muscle increased mitochondrial FABP^{pm} 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 FABP^{pm} (a.k.a. mAspAT) per milligram of mitochondrial protein.

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.

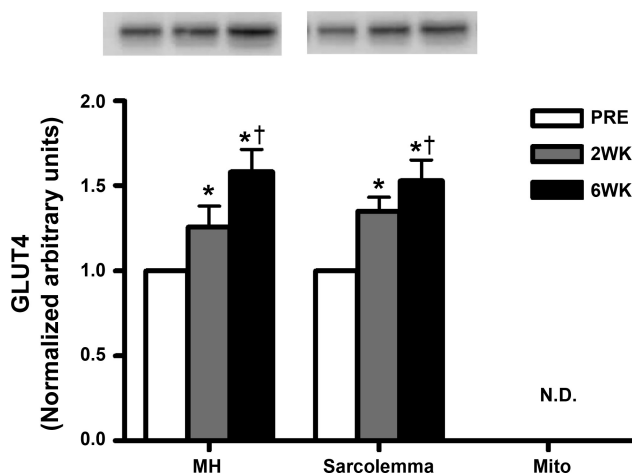
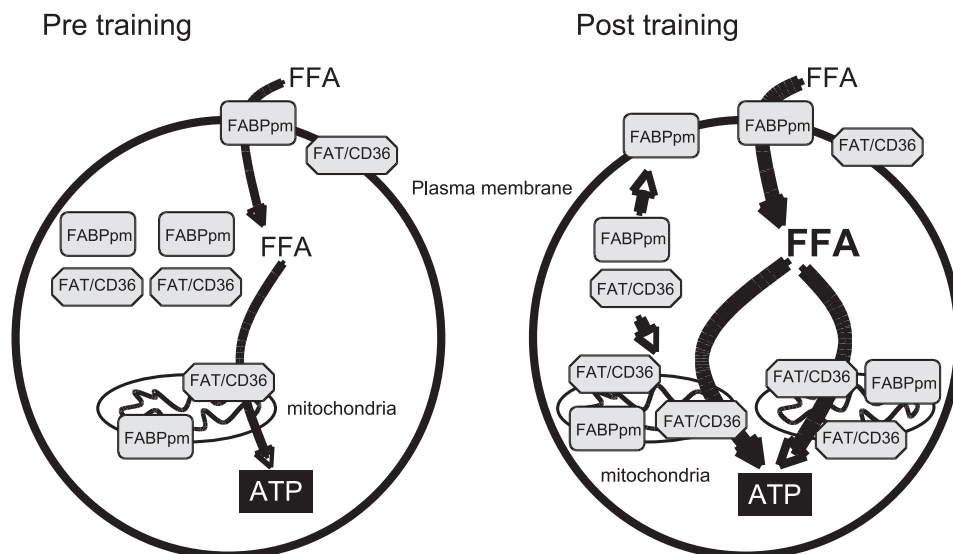


Fig. 3. Effect of HIIT on GLUT4 (glucose transport protein) protein content. Values are means \pm SE ($n = 10$). ND, not detected. *Significantly different than PRE ($P < 0.05$); †significantly higher than 2 wk.

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 FATP 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 sarcolemmal 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% $\dot{V}O_{2peak}$, which is in the range that would elicit maximal fat oxidation rates (52, 62). However, this absolute

power output represented only 64% $\dot{V}O_{2peak}$ following 2 wk and 59% $\dot{V}O_{2peak}$ 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 $\dot{V}O_{2peak}$ 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 alter 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 sarcolemma 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.

ACKNOWLEDGMENTS

Present address for J. L. Talanian: School of Education, Bacone College, Muskogee, OK 74403 (e-mail: talaniaj@bacone.edu).

GRANTS

This study was supported by grants from the Natural Sciences and Engineering Research Council of Canada (L. L. Spriet and A. Bonen) and the Canadian Institutes of Health Research (L. L. Spriet, G. J. F. Heigenhauser, and A. Bonen). A. Bonen is the Canada Research Chair in Metabolism and Health.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

- Abumrad NA, el-Maghrabi MR, Amri EZ, Lopez E, Grimaldi PA. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. *J Biol Chem* 268: 17665–17668, 1993.
- Benton C, Holloway GP, Campbell SE, Yoshida Y, Tandon NN, Glatz JF, Luiken JJ, Spriet LL, Bonen A. Rosiglitazone increases fatty acid oxidation and fat/CD36 but not CPT1 in rat muscle subsarcolemmal and intermyofibrillar mitochondria. *J Physiol* 586: 1755–1766, 2008.
- Bergmeyer HU. *Methods of Enzymatic Analysis*. New York: Verlag Chemie Weinheim, 1974.
- Berk PD, Wada H, Horio Y, Potter BJ, Sorrentino D, Zhou SL, Isola LM, Stump D, Kiang CL, Thung S. Plasma membrane fatty acid-binding protein and mitochondrial glutamic-oxaloacetic transaminase of rat liver are related. *Proc Natl Acad Sci USA* 87: 3484–3488, 1990.
- Bezaire V, Bruce CR, Heigenhauser GJ, Tandon NN, Glatz JF, Luiken JJ, Bonen A, Spriet LL. Identification of fatty acid translocase on human skeletal muscle mitochondrial membranes: essential role in fatty acid oxidation. *Am J Physiol Endocrinol Metab* 290: E509–E515, 2006.
- Bonen A, Campbell SE, Benton CR, Chabowski A, Coort SL, Han XX, Koonen DP, Glatz JF, Luiken JJ. Regulation of fatty acid transport by fatty acid translocase/CD36. *Proc Nutr Soc* 63: 245–249, 2004.
- Bonen A, Luiken JJ, Arumugam Y, Glatz JF, Tandon NN. Acute regulation of fatty acid uptake involves the cellular redistribution of fatty acid translocase. *J Biol Chem* 275: 14501–14508, 2000.
- Bonen A, Luiken JJ, Liu S, Dyck DJ, Kiens B, Kristiansen S, Turcotte LP, Van Der Vusse GJ, Glatz JF. Palmitate transport and fatty acid transporters in red and white muscles. *Am J Physiol Endocrinol Metab* 275: E471–E478, 1998.
- Bradbury MW, Berk PD. Mitochondrial aspartate aminotransferase: direction of a single protein with two distinct functions to two subcellular sites does not require alternative splicing of the mRNA. *Biochem J* 345: 423–427, 2000.
- Burgomaster KA, Cermak NM, Phillips SM, Benton CR, Bonen A, Gibala MJ. Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval training and detraining. *Am J Physiol Regul Integr Comp Physiol* 292: R1970–R1976, 2007.
- Campbell SE, Tandon NN, Woldegiorgis G, Luiken JJ, Glatz JF, Bonen A. A novel function for fatty acid translocase (FAT)/CD36: involvement in long chain fatty acid transfer into the mitochondria. *J Biol Chem* 279: 36235–36241, 2004.
- Cartee GD, Young DA, Sleeper MD, Zierath J, Wallberg-Henriksson H, Holloszy JO. Prolonged increase in insulin-stimulated glucose transport in muscle after exercise. *Am J Physiol Endocrinol Metab* 256: E494–E499, 1989.
- Clarke DC, Miskovic D, Han XX, Calles-Escandon J, Glatz JF, Luiken JJ, Heikkila JJ, Bonen A. Overexpression of membrane-associated fatty acid binding protein (FABPpm) in vivo increases fatty acid sarcolemmal transport and metabolism. *Physiol Genomics* 17: 31–37, 2004.
- DiRusso CC, Li H, Darwis D, Watkins PA, Berger J, Black PN. Comparative biochemical studies of the murine fatty acid transport proteins (FATP) expressed in yeast. *J Biol Chem* 280: 16829–16837, 2005.
- Distler AM, Kerner J, Peterman SM, Hoppel CL. A targeted proteomic approach for the analysis of rat liver mitochondrial outer membrane proteins with extensive sequence coverage. *Anal Biochem* 356: 18–29, 2006.
- Dudley GA, Tullson PC, Terjung RL. Influence of mitochondrial content on the sensitivity of respiratory control. *J Biol Chem* 262: 9109–9114, 1987.
- Fritz IB, Yue KT. Long-chain carnitine acyltransferase and the role of acylcarnitine derivatives in the catalytic increase of fatty acid oxidation induced by carnitine. *J Lipid Res* 4: 279–288, 1963.
- Gimeno RE, Ortegon AM, Patel S, Punreddy S, Ge P, Sun Y, Lodish HF, Stahl A. Characterization of a heart-specific fatty acid transport protein. *J Biol Chem* 278: 16039–16044, 2003.
- Goodyear LJ, Hirshman MF, Napoli R, Calles J, Markuns JF, Ljungqvist O, Horton ES. Glucose ingestion causes GLUT4 translocation in human skeletal muscle. *Diabetes* 45: 1051–1056, 1996.
- Gulve EA, Spina RJ. Effect of 7–10 days of cycle ergometer exercise on skeletal muscle GLUT-4 protein content. *J Appl Physiol* 79: 1562–1566, 1995.
- Han XX, Chabowski A, Tandon NN, Calles-Escandon J, Glatz JF, Luiken JJ, Bonen A. Metabolic challenges reveal impaired fatty acid metabolism and translocation of FAT/CD36 but not FABPpm in obese Zucker rat muscle. *Am J Physiol Endocrinol Metab* 293: E566–E575, 2007.
- Hawley JA, Hargreaves M, Zierath JR. Signalling mechanisms in skeletal muscle: role in substrate selection and muscle adaptation. *Essays Biochem* 42: 1–12, 2006.
- Hirsch D, Stahl A, Lodish HF. A family of fatty acid transporters conserved from mycobacterium to man. *Proc Natl Acad Sci USA* 95: 8625–8629, 1998.
- Holloszy JO, Coyle EF. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol* 56: 831–838, 1984.
- Holloway GP. Mitochondrial function and dysfunction in exercise and insulin resistance. *Appl Physiol Nutr Metab* 34: 440–446, 2009.
- Holloway GP, Benton CR, Mullen KL, Yoshida Y, Snook LA, Han XX, Glatz JF, Luiken JJ, Lally J, Dyck DJ, Bonen A. In obese rat muscle transport of palmitate is increased and is channeled to triacylglycerol storage despite an increase in mitochondrial palmitate oxidation. *Am J Physiol Endocrinol Metab* 296: E738–E747, 2009.
- Holloway GP, Bezaire V, Heigenhauser GJ, Tandon NN, Glatz JF, Luiken JJ, Bonen A, Spriet LL. Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content and carnitine palmitoyl-transferase I activity in human skeletal muscle during aerobic exercise. *J Physiol* 571: 201–210, 2006.
- Holloway GP, Bonen A, Spriet LL. Regulation of skeletal muscle mitochondrial fatty acid metabolism in lean and obese individuals. *Am J Clin Nutr* 89: 455S–462S, 2009.
- Holloway GP, Jain SS, Bezaire V, Han XX, Glatz JF, Luiken JJ, Harper ME, Bonen A. FAT/CD36-null mice reveal that mitochondrial FAT/CD36 is required to upregulate mitochondrial fatty acid oxidation in contracting muscle. *Am J Physiol Regul Integr Comp Physiol* 297: R960–R967, 2009.
- Holloway GP, Lally J, Nickerson JG, Alkhateeb H, Snook LA, Heigenhauser GJ, Calles-Escandon J, Glatz JF, Luiken JJ, Spriet LL, Bonen A. Fatty acid binding protein facilitates sarcolemmal fatty acid transport but not mitochondrial oxidation in rat and human skeletal muscle. *J Physiol* 582: 393–405, 2007.
- Houmard JA, Hickey MS, Tyndall GL, Gavigan KE, Dohm GL. Seven days of exercise increase GLUT-4 protein content in human skeletal muscle. *J Appl Physiol* 79: 1936–1938, 1995.
- Isola LM, Zhou SL, Kiang CL, Stump DD, Bradbury MW, Berk PD. 3T3 fibroblasts transfected with a cDNA for mitochondrial aspartate aminotransferase express plasma membrane fatty acid-binding protein and saturable fatty acid uptake. *Proc Natl Acad Sci USA* 92: 9866–9870, 1995.
- Jain SS, Chabowski A, Snook LA, Schwenk RW, Glatz JF, Luiken JJ, Bonen A. Additive effects of insulin and muscle contraction on fatty acid transport and fatty acid transporters, FAT/CD36, FABPpm, FATP1, 4 and 6. *FEBS Lett* 583: 2294–2300, 2009.
- Jeppesen J, Mogensen M, Prats C, Sahlin K, Madsen K, Kiens B. FAT/CD36 is localized in sarcolemma and in vesicle-like structures in subsarcolemma regions but not in mitochondria. *J Lipid Res* 51: 1504–1512, 2010.
- King KL, Stanley WC, Rosca M, Kerner J, Hoppel CL, Febbraio M. Fatty acid oxidation in cardiac and skeletal muscle mitochondria is unaffected by deletion of CD36. *Arch Biochem Biophys* 467: 234–238, 2007.
- Koonen DP, Benton CR, Arumugam Y, Tandon NN, Calles-Escandon J, Glatz JF, Luiken JJ, Bonen A. Different mechanisms can alter fatty acid transport when muscle contractile activity is chronically altered. *Am J Physiol Endocrinol Metab* 286: E1042–E1049, 2004.

38. **Koves TR, Noland RC, Bates AL, Henes ST, Muoio DM, Cortright RN.** Subsarcolemmal and intermyofibrillar mitochondria play distinct roles in regulating skeletal muscle fatty acid metabolism. *Am J Physiol Cell Physiol* 288: C1074–C1082, 2005.
39. **Kraniou GN, Cameron-Smith D, Hargreaves M.** Effect of short-term training on GLUT-4 mRNA and protein expression in human skeletal muscle. *Exp Physiol* 89: 559–563, 2004.
40. **Luiken JJ, Turcotte LP, Bonen A.** Protein-mediated palmitate uptake and expression of fatty acid transport proteins in heart giant vesicles. *J Lipid Res* 40: 1007–1016, 1999.
41. **Lund S, Holman GD, Zierath JR, Rincon J, Nolte LA, Clark AE, Schmitz O, Pedersen O, Wallberg-Henriksson H.** Effect of insulin on GLUT4 cell surface content and turnover rate in human skeletal muscle as measured by the exofacial bis-mannose photolabeling technique. *Diabetes* 46: 1965–1969, 1997.
42. **McGarry JD, Brown NF.** The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur J Biochem* 244: 1–14, 1997.
43. **McGarry JD, Mills SE, Long CS, Foster DW.** Observations on the affinity for carnitine, and malonyl-CoA sensitivity, of carnitine palmitoyltransferase I in animal and human tissues. Demonstration of the presence of malonyl-CoA in non-hepatic tissues of the rat. *Biochem J* 214: 21–28, 1983.
44. **Mikines KJ, Sonne B, Farrell PA, Tronier B, Galbo H.** Effect of physical exercise on sensitivity and responsiveness to insulin in humans. *Am J Physiol Endocrinol Metab* 254: E248–E259, 1988.
45. **Nesher R, Karl IE, Kipnis DM.** Dissociation of effects of insulin and contraction on glucose transport in rat epitrochlearis muscle. *Am J Physiol Cell Physiol* 249: C226–C232, 1985.
46. **Nickerson JG, Alkhateeb H, Benton CR, Lally J, Nickerson J, Han XX, Wilson MH, Jain SS, Snook LA, Glatz JF, Chabowski A, Luiken JJ, Bonen A.** Greater transport efficiencies of the membrane fatty acid transporters FAT/CD36 and FATP4 compared with FABPpm and FATP1 and differential effects on fatty acid esterification and oxidation in rat skeletal muscle. *J Biol Chem* 284: 16522–16530, 2009.
47. **Peronnet F, Massicotte D.** Table of nonprotein respiratory quotient: an update. *Can J Sport Sci* 16: 23–29, 1991.
48. **Perry CG, Talanian JL, Heigenhauser GJ, Spriet LL.** The effects of training in hyperoxia vs. normoxia on skeletal muscle enzyme activities and exercise performance. *J Appl Physiol* 102: 1022–1027, 2007.
49. **Perseghin G, Price TB, Petersen KF, Roden M, Cline GW, Gerow K, Rothman DL, Shulman GI.** Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. *N Engl J Med* 335: 1357–1362, 1996.
50. **Ploug T, Galbo H, Vinten J, Jorgensen M, Richter EA.** Kinetics of glucose transport in rat muscle: effects of insulin and contractions. *Am J Physiol Endocrinol Metab* 253: E12–E20, 1987.
51. **Richter EA, Jensen P, Kiens B, Kristiansen S.** Sarcolemmal glucose transport and GLUT-4 translocation during exercise are diminished by endurance training. *Am J Physiol Endocrinol Metab* 274: E89–E95, 1998.
52. **Romijn JA, Coyle EF, Sidossis LS, Rosenblatt J, Wolfe RR.** Substrate metabolism during different exercise intensities in endurance-trained women. *J Appl Physiol* 88: 1707–1714, 2000.
53. **Schaffer JE, Lodish HF.** Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. *Cell* 79: 427–436, 1994.
54. **Schenk S, Horowitz JF.** Coimmunoprecipitation of FAT/CD36 and CPT I in skeletal muscle increases proportionally with fat oxidation after endurance exercise training. *Am J Physiol Endocrinol Metab* 291: E254–E260, 2006.
55. **Schwieterman W, Sorrentino D, Potter BJ, Rand J, Kiang CL, Stump D, Berk PD.** Uptake of oleate by isolated rat adipocytes is mediated by a 40-kDa plasma membrane fatty acid binding protein closely related to that in liver and gut. *Proc Natl Acad Sci USA* 85: 359–363, 1988.
56. **Sebastián D, Guitart M, García-Martínez C, Mauvezin C, Orellana-Gavaldà JM, Serra D, Gómez-Foix AM, Hegardt FG, Asins G.** Novel role of FATP1 in mitochondrial fatty acid oxidation in skeletal muscle cells. *J Lipid Res* 50: 1789–1799, 2009.
57. **Srere PA.** Citrate synthase. In: *Methods in Enzymology*. New York: Academic, 1969.
58. **Stremmel W, Lotz G, Strohmeyer G, Berk PD.** Identification, isolation, and partial characterization of a fatty acid binding protein from rat jejunal microvillous membranes. *J Clin Invest* 75: 1068–1076, 1985.
59. **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.
60. **Thorell A, Hirshman MF, Nygren J, Jorfeldt L, Wojtaszewski JF, Dufresne SD, Horton ES, Ljungqvist O, Goodyear LJ.** Exercise and insulin cause GLUT-4 translocation in human skeletal muscle. *Am J Physiol Endocrinol Metab* 277: E733–E741, 1999.
61. **Tunstall RJ, Mehan KA, Wadley GD, Collier GR, Bonen A, Hargreaves M, Cameron-Smith D.** Exercise training increases lipid metabolism gene expression in human skeletal muscle. *Am J Physiol Endocrinol Metab* 283: E66–E72, 2002.
62. **van Loon LJ, Greenhaff PL, Constantin-Teodosiu D, Saris WH, Wagenmakers AJ.** The effects of increasing exercise intensity on muscle fuel utilisation in humans. *J Physiol* 536: 295–304, 2001.