Training-induced elevation in $FABP_{PM}$ is associated with increased palmitate use in contracting muscle

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Turcotte, Lorraine P., Jason R. Swenberger, Michelle Z. Tucker, and Alice J. Yee. Training-induced elevation in $FABP_{PM}$ is associated with increased palmitate use in contracting muscle. *J. Appl. Physiol.* 87(1): 285–293, 1999.—To evaluate the effects of endurance training in rats on fatty acid metabolism, we measured the uptake and oxidation of palmitate in isolated rat hindquarters as well as the content of fatty acid-binding proteins in the plasma membranes ($FABP_{PM}$) of red and white muscles from 16 trained (T) and 18 untrained (UT) rats. Hindquarters were perfused with 6 mM glucose, 1,800 µM palmitate, and [1-^{14}C]palmitate at rest and during electrical stimulation (ES) for 25 min. $FABP_{PM}$ content was 43–226% higher in red than in white muscles and was increased by 55% in red muscles after training. A positive correlation was found to exist between succinate dehydrogenase activity and $FABP_{PM}$ content in muscle. Palmitate uptake increased by 64–73% from rest to ES in both T and UT and was 48–57% higher in T than UT both at rest (39.8 ± 3.5 vs. 26.9 ± 4.4 $nmol \cdot min^{-1} \cdot g^{-1}$, T and UT, respectively) and during ES (69.0 ± 6.1 vs. 43.9 ± 4.4 $nmol \cdot min^{-1} \cdot g^{-1}$, T and UT, respectively). While the rats were resting, palmitate oxidation was not affected by training; palmitate oxidation during ES was higher in T than UT rats (14.8 ± 1.3 vs. 9.3 ± 1.9 $nmol \cdot min^{-1} \cdot g^{-1}$, T and UT, respectively). In conclusion, endurance training increases *1*) plasma free fatty acid (FFA) uptake in resting and contracting perfused muscle, *2*) plasma FFA oxidation in contracting perfused muscle, and *3*) $FABP_{PM}$ content in red muscles. These results suggest that an increased number of these putative plasma membrane fatty acid transporters may be available in the trained muscle and may be implicated in the regulation of plasma FFA metabolism in skeletal muscle.

electrical stimulation; fatty acid metabolism; fatty acid transport; fatty acid uptake; hindquarter perfusion; skeletal muscle; endurance training; muscle triglycerides; fatty acid-binding proteins in plasma membranes

CIRCULATING ALBUMIN-BOUND fatty acids are a major oxidizable fuel source for skeletal muscle metabolism. Endurance training modifies substrate utilization patterns by increasing the contribution of lipids in oxidative metabolism (14). Although controversy exists regarding the source of this training-induced increase in lipid utilization, evidence suggests that part of the increase may be due to an increased utilization of plasma free fatty acids (FFA) by skeletal muscle (18, 39). Although evidence collected during whole body exercise in humans is equivocal (20, 27), substrate-exchange data collected across exercising muscles in humans suggest that plasma FFA utilization may be increased by endurance training (18, 39). To eliminate training-induced differences in the endocrine response to the exercise stimulus, we chose to study the effects of endurance training on the uptake and oxidation of plasma FFA in the perfused rat hindquarter preparation.

Until recently, permeation of FFA across the plasma membranes (PMs) of cells was believed to be achieved by a simple process of passive diffusion because of the lipid nature of the fatty acid molecule (23). However, accumulated evidence suggests that FFA permeation in skeletal muscle may partly involve a carrier-mediated process (34). The uptake rate of FFA into isolated cell systems and in perfused skeletal muscle has been shown to be a saturable function of the unbound fatty acid concentration and to be reduced by prior protease treatment of cells (2, 29, 32, 37). Putative fatty acid transporter proteins have been isolated from the PMs of several cell types and have been partially characterized (1, 25, 29, 32). The fatty acid-binding proteins in the PM ($FABP_{PM}$) isolated from both hepatic and cardiac tissues have been shown to have a molecular mass of ~40–43 kDa and a high affinity for long-chain FFA and to exist in both rat and human skeletal muscles (19, 29, 32, 40). The content of $FABP_{PM}$ has been shown to increase after short-term fasting in rats (40) and after endurance training in humans (19). Consistent with its putative role as a fatty acid transporter, antibodies raised against this class of membrane proteins inhibit both FFA binding to the PMs and transport of FFA into giant vesicles of skeletal muscle in a dose-dependent fashion (4, 34). It is not known whether the training-induced increase in muscle lipid utilization in rats is associated with an increase in muscle $FABP_{PM}$ content.

Thus the purpose of this study was to evaluate the effects of endurance training on $FABP_{PM}$ content in red and white muscles and on the rates of uptake and oxidation of plasma palmitate in perfused rat hindquarters during rest and electrical stimulation.

MATERIALS AND METHODS

Animal preparation and training protocol. Female Wistar rats were housed in pairs and maintained on a 12:12-h light-dark cycle. They received regular rat chow and water ad libitum, and they were randomly assigned to either an untrained or endurance-trained group. Trained animals (n = 16) were run on a motorized rodent treadmill 5 days/wk for 8 wk. Running time and speed, initially set at 15 min and 20 m/min, respectively, were gradually increased until total running time equaled 120 min/day at 30 m/min up a 10%

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grade (35). The untrained animals ($n = 18$) were acclimatized to treadmill running 2 days/wk for 10 min/day at 13 m/min. After 8 wk of training, the body weights were not significantly different between trained and untrained rats (275.4 ± 7.6 vs. 265.7 ± 9.9 g, respectively).

Hindquarter perfusion. Seven trained and eight untrained rats were anesthetized with ketamine/rompun (80 mg and 12 mg/kg body weight, respectively) and were prepared surgically for hindquarter perfusion as previously described (24, 38). To avoid the acute effects of exercise, the trained rats were studied 48 h after the last training bout (9, 16, 17). Before the perfusion catheters were inserted, heparin (150 IU) was administered into the inferior vena cava. The rats were killed with an intracardial injection of ketamine/rompun immediately before the catheters were inserted, and the preparation was placed in a perfusion apparatus, essentially as described by Ruderman et al. (24).

The initial perfusate (400 ml) consisted of Krebs-Henseleit solution, 1- to 2-day-old washed bovine erythrocytes (hematocrit, 30%), 5% bovine serum albumin (Cohn fraction V; Sigma Chemical, St. Louis, MO), 6 mmol/l glucose, 1,800 µmol/l albumin-bound palmitate, and 5 µCi of albumin-bound [1-^{14}C]palmitate (ICN Pharmaceuticals, Costa Mesa, CA). No insulin was added. This concentration of palmitate was chosen because it has been shown that, at concentrations >1,500 µmol/l, the maximal velocity of palmitate uptake and oxidation are attained in the perfused hindquarter preparation (37, 38). The perfusate (37°C) was continuously gassed with a mixture of 95% O_2-5% CO_2, which yielded arterial pH values of 7.3–7.4 and arterial P_{CO_2} and P_{O_2} values that were typically 38–43 and 250–350 Torr, respectively, in both the trained and untrained rats. Mean perfusion pressures were 94 ± 7 and 131 ± 10 mmHg during unilateral hindquarter perfusion at rest and during electrical stimulation, respectively, in both the trained and untrained rats.

The first 25 ml of perfusate that passed through the hindquarter were discarded; then the perfusate was recirculated at a flow of 12.5 ml/min (0.60 and 0.64 $ml \cdot min^{-1} \cdot g^{-1}$ perfused muscle in trained and untrained rats, respectively). After an equilibration period of 20 min, the left superficial fast-twitch white (predominantly type IIb) and the deep fast-twitch red (predominantly type IIa) sections of the gastrocnemius muscles, as well as the slow-twitch red soleus muscle (predominantly type I), were taken out and freeze-clamped with aluminum clamps precooled in liquid N_2. The left iliac vessels were then tied off, and a clamp was fixed tightly around the proximal part of the leg to prevent bleeding. The right leg was then perfused at rest for 20 min at a perfusate flow of 9 ml/min (0.43 and 0.46 $ml \cdot min^{-1} \cdot g^{-1}$ perfused muscle in trained and untrained rats, respectively). Resting arterial and venous perfusate samples were taken at 10, 15, and 20 min. After perfusion at rest, the right leg was quickly immobilized at the tibiopatellar ligament, and a hook electrode was placed around the sciatic nerve and connected to a Grass S48 stimulator (Astro-Med, West Warwick, RI). Perfusate flow was increased to 15 ml/min (0.72 and 0.77 $ml \cdot min^{-1} \cdot g^{-1}$ perfused muscle in trained and untrained rats, respectively). The resting length of the gastrocnemius-soleus-plantaris muscle group was adjusted to obtain maximal active tension on stimulation. Isometric contractions were induced by electrical stimulation of the sciatic nerve with supramaximal (10–20 V) trains of 100 ms and 100 Hz, with an impulse duration of 1 ms, delivered every 3 s. During muscle stimulation, the tension developed by the gastrocnemius-soleus-plantaris muscle group was measured with a force displacement transducer, model FT03 (Astro-Med) and recorded on a linear recorder. The decrease in tension development over the stimulation period was used as an indicator of performance. Arterial and venous perfusate samples were taken after 5, 10, 15, 20, and 25 min of electrical stimulation. Arterial and venous samples for the analysis of [^{14}C]FFA and $^{14}CO_2$ radioactivities were taken after 10, 15, and 20 min of perfusion at rest and after 15, 20, and 25 min of perfusion during electrical stimulation. Arterial and venous perfusate samples for determinations of P_{CO_2}, P_{O_2}, pH, and hemoglobin were taken after 15–20 min of equilibration at rest and during electrical stimulation. At the end of the 25-min stimulation period, muscle samples from the right leg of the animal were taken and treated as described above. The exact muscle mass perfused was determined by infusion of a colored solution of methyl blue into the arterial catheter and weighing the colored muscle mass at the end of the perfusions.

$FABP_{PM}$ content. Ten trained and 10 untrained rats were anesthetized with ketamine/rompun and then decapitated. The white and red portions of the gastrocnemius muscles of both legs were immediately removed and trimmed of fat and connective tissues. PM fractions were prepared fresh, as previously described (3, 40), by combining the muscle tissues from two rats. Briefly, the muscles were minced thoroughly with scissors, diluted fourfold [in Tris-15% sucrose buffer with 0.1 mmol/l phenylmethylsulfonyl fluoride, 10 mmol/l EGTA, and 10 mg/ml trypsin inhibitor made fresh daily (pH 7.5)], and homogenized by one 10-s burst with a Polytron homogenizer on level 6. The homogenates were filtered through a multifilter system, and the filtered homogenates were centrifuged at 100,000 g for 1 h. The pellets were resuspended in Tris-15% sucrose buffer, and a small aliquot of the resulting suspension, termed the crude membrane (CM) fractions, was retained for analysis. The remaining suspension of CM fractions was layered onto continuous sucrose gradients (35–70%) that were centrifuged at 120,000 g for 2 h by using a Beckman SW28 swing-out rotor. The PM layers were harvested, washed in Tris buffer, and centrifuged at 100,000 g for 1 h. The pellets were reconstituted in a small volume of Tris buffer and frozen in liquid N_2 for analysis. To assess the purity of the PM preparations, the protein content and the activity of the PM marker enzyme 5′-nucleotidase (33), were measured and compared between the CM and PM fractions. Protein content was measured with the commercially available Bio-Rad (Richmond, CA) microassay procedure. The PM and CM fractions were analyzed for $FABP_{PM}$ content by Western blotting and for succinate dehydrogenase (SDH) activity, respectively. SDH activity was determined by the ferricyanide method as modified by Veeger et al. (41) and is expressed as the amount of succinate oxidized per minute per milligram of protein.

Blood and muscle sample analysis. Arterial and venous perfusate samples were analyzed for glucose, lactate, and FFA concentrations as well as for [^{14}C]FFA and $^{14}CO_2$ radioactivities. Samples for glucose and lactate were immediately deproteinized by using 6% perchloric acid (wt/vol). After centrifugation at 4,500 g for 5 min, the pellet was discarded, and the supernatant was analyzed for glucose spectrophotometrically by using the enzymatic glucose oxidase method (Sigma Chemical) and for lactate by using the YSI 1500 lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH), respectively. Samples for FFA were put in 200 µmol/l EGTA (pH 7) and centrifuged; the supernatant was frozen until analyzed spectrophotometrically by using the WAKO NEFA-C test (Biochemical Diagnostics, Edgewood, NY). Because the FFA concentration was low in the absence of added palmitate (<80 µmol/l) and because palmitate was the only fatty acid added, measured FFA concentrations were taken to equal palmitate concentrations.

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To determine plasma palmitate radioactivity, duplicate 100-µl aliquots of the perfusate plasma were mixed with liquid scintillation fluid (BudgetSolve; Research Product International, Mount Prospect, IL) and counted in a Tri-carb liquid scintillation counter (model 4000 CA; United Technologies Packard, Downers Grove, IL). To ascertain that the radioactivity in the plasma was due solely to FFA, lipid extraction and separation were performed on a subsample ($n = 3$ each for the trained and untrained groups) of perfusate samples, as previously described (36, 39). The recovered palmitate fraction contained 97% of the total radioactivity on the plate, and that amount corresponded to >90% of the total radioactivity present in the plasma samples. Thus total plasma radioactivity was used to calculate the specific activity of palmitate in the perfusate. The liberation and collection of $^{14}CO_2$ from the blood were performed within 4–5 min of anaerobic collection (2 ml) as previously described (36, 39). In this system, arterial perfusate plasma palmitate concentration and specific activity, as well as the arteriovenous difference in radioactivity, varied by <5% during the last 20 min of perfusion at rest and during the last 15 min of perfusion during electrically induced muscle contractions. Thus steady-state conditions were achieved. Perfusate samples for the determination of P_{CO_2}, P_{O_2}, pH, and hemoglobin were collected anaerobically, placed on ice, and measured within 5 min of collection with an ABL-5 acid-base laboratory (Radiometer America, Westlake, OH) and spectrophotometrically (Sigma Chemical), respectively.

Muscle triglyceride concentration was determined as glycerol residues after extraction and separation of the muscle samples, as previously described (10, 30). Briefly, lipids were extracted from powdered muscle samples by centrifugation at 1,000 *g* in 2:1 chloroform-methanol solution and 4 mmol/l magnesium chloride. The organic extract was evaporated and reconstituted in chloroform, and silicic acid was added for the removal of phospholipids by centrifugation. The resulting supernatant was evaporated, saponified in ethanolic potassium hydroxide for 30 min at 70°C, and centrifuged with 0.15 mol/l magnesium sulfate. The final supernatant was analyzed spectrophotometrically for glycerol by the enzymatic glycerol kinase method (Sigma Chemical). To measure the incorporation of [^{14}C]palmitate into muscle triglycerides, lipids from the extracted organic layer were separated by liquid chromatography as previously described (39).

For Western-blot analysis, solubilized PM proteins (30 µg) were separated by SDS-PAGE on a 12% resolving gel and transferred electrophoretically to a polyvinylidene difluoride membrane (40). The membrane was blocked in 1% bovine serum albumin in Tween-Tris-buffered saline (TTBS: 500 mmol/l NaCl, 20 mmol/l Tris, 0.05% Tween-20, pH 7.5) for 2 h at 23°C, rinsed twice with TTBS, and incubated overnight at 4°C with a polyclonal antibody to the rat hepatic $FABP_{PM}$ (1:200). (The antibody was kindly donated by Dr. Dario Sorrentino, Mount Sinai Medical Center, New York, NY). After further washing with TTBS, the membrane was incubated with goat-anti-rabbit ^{125}I-labeled IgG for 2 h at 23°C. The membrane was then washed and dried, and the density of the bands was quantified by using a phosphorImager (Molecular Dynamics, Sunnyvale, CA). To analyze for differences in $FABP_{PM}$ protein content among groups, we used a rat liver CM preparation as standard and expressed density as relative units. To determine whether there was a fiber type difference in $FABP_{PM}$ protein content, we measured and compared band density from PM fractions of red and white skeletal muscles isolated from each rat. To determine whether there were training-induced changes in $FABP_{PM}$ protein content, we measured and compared band density from PM fractions of either red or white skeletal muscles isolated from trained and untrained rats. In all cases, multiple gels were analyzed.

Calculations and statistics. Fractional uptake was calculated as the difference in radioactivity between the arterial and venous perfusate samples divided by the radioactivity in the arterial sample (38). Palmitate delivery was calculated by multiplying perfusate plasma flow by the arterial perfusate plasma palmitate concentration. Palmitate uptake was calculated by multiplying plasma delivery by the fractional uptake (38). Percent palmitate oxidation was calculated by dividing the total amount of radioactivity recovered as $^{14}CO_2$ by the total amount of radioactivity that was taken up by the muscles (38). Total palmitate oxidation was calculated by multiplying palmitate uptake by percent oxidation. Both percent and total palmitate oxidation were corrected for label fixation by using acetate correction factors of 1.9 and 1.3 for rest and electrical stimulation, respectively. These correction factors were estimated from hindquarter perfusions with [1-^{14}C]acetate ($n = 4$) and were found to be similar to the correction factors estimated by others (26). Palmitate accumulation into muscle triglycerides was calculated as the radioactivity accumulated in the triglyceride fraction and was expressed per milligram of wet muscle weight. Uptake and release of substrates and uptake of O_2 across the hindquarter were calculated by multiplying perfusate flow by the arteriovenous difference in concentration and were expressed per gram of perfused muscle, which was measured to be 7.3 ± 0.5 and 7.4 ± 0.4% of body weight for unilateral hindquarter perfusion in trained and untrained rats, respectively.

During both rest and electrical stimulation periods, the arterial and venous specific activities for palmitate did not vary over time. The arterial and venous specific activities for palmitate were not significantly different between the trained and untrained groups and averaged 0.88 ± 0.07 and 0.82 ± 0.07 µCi/mmol at rest and 0.86 ± 0.06 and 0.82 ± 0.06 µCi/mmol during electrical stimulation, respectively ($P > 0.05$). Because the calculated rates of palmitate uptake and oxidation did not change significantly during the last 15 min of perfusion at rest and electrical stimulation, the averages of the values were used to make comparisons between groups.

Statistical evaluation of the data was done by using analysis of variance with Newman-Keul's test for post hoc multiple comparisons when appropriate. Pearson product-moment correlations were computed for the assessment of a correlation between $FABP_{PM}$ protein content and SDH activity. In all instances, an α of 0.05 was used to determine significance.

RESULTS

Palmitate metabolism. Perfusate palmitate concentration and delivery to the hindquarter were not significantly different between the trained and untrained groups during rest and electrical stimulation (Table 1). In both the trained and untrained groups, perfusate plasma palmitate concentration did not vary from rest to electrical stimulation. Palmitate delivery to the hindquarter increased by 55–60% from rest to electrical stimulation in both the trained and untrained groups.

The fractional uptake of palmitate did not increase significantly from rest to electrical stimulation, but it was 38–44% higher in the trained than the untrained group during rest and electrical stimulation (Table 1, Fig. 1*A*). Total palmitate uptake increased by 64–73%

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Table 1. *Effects of endurance training on palmitate metabolism, glucose uptake, and lactate release in perfused rat hindquarters during rest and electrical stimulation*

	Trained		Untrained	
	Rest	ES	Rest	ES
Oxygen uptake, $\mu mol \cdot g^{-1} \cdot h^{-1}$	24.9 ± 5.3	59.4 ± 9.8†	23.4 ± 2.4	55.8 ± 9.2†
Palmitate concentration, µmol/l	1,905.7 ± 74.3	1,875.0 ± 94.5	1,676.8 ± 89.9	1,707.1 ± 82.9
Palmitate delivery, $nmol \cdot min^{-1} \cdot g^{-1}$	570.6 ± 36.6	888.5 ± 56.1†	519.8 ± 43.7	841.4 ± 66.0†
Palmitate fractional uptake	0.070 ± 0.006*	0.076 ± 0.006*	0.051 ± 0.006	0.053 ± 0.005
Palmitate uptake, $nmol \cdot min^{-1} \cdot g^{-1}$	39.8 ± 3.5*	69.0 ± 6.1*†	26.9 ± 4.4	43.9 ± 4.4†
Oxidation, %	7.0 ± 1.6*	21.4 ± 1.9†	12.6 ± 1.4	21.1 ± 2.3†
Palmitate oxidation, $nmol \cdot min^{-1} \cdot g^{-1}$	2.8 ± 0.5	14.8 ± 1.3*†	3.4 ± 0.4	9.3 ± 1.9†
Glucose concentration, mmol/l	5.9 ± 0.1	5.7 ± 0.2	6.1 ± 0.3	5.9 ± 0.2
Glucose uptake, $\mu mol \cdot g^{-1} \cdot h^{-1}$	3.6 ± 0.9	12.1 ± 1.4†	3.6 ± 0.6	11.5 ± 2.0†
Lactate concentration, mmol/l	0.8 ± 0.1*	1.7 ± 0.2*†	1.3 ± 0.2	2.3 ± 0.1†
Lactate release, $\mu mol \cdot g^{-1} \cdot h^{-1}$	5.7 ± 0.9	18.9 ± 3.1†	4.3 ± 1.5	20.7 ± 2.7†

Values are means ± SE; $n = 6$ trained and 8 untrained rats. ES, electrical stimulation. Resting values, average of values measured after 10, 15, and 20 min of perfusion. ES values, average of values measured after 15, 20, and 25 min of perfusion during ES. Percent and total palmitate oxidation values were corrected for label fixation, as described in MATERIALS AND METHODS. * $P < 0.05$ compared with untrained group; † $P < 0.05$ compared with rest.

from rest to electrical stimulation in both groups and was 48–57% higher in the trained than in the untrained group during rest and electrical stimulation (Table 1, Fig. 1*B*). The percentage of palmitate oxidized increased by 67–205% from rest to electrical stimulation in both groups (Table 1, Fig. 2*A*). At rest, the percentage of palmitate oxidized was significantly lower in the trained group and resulted in similar total rates of palmitate oxidation among the trained and untrained groups (Table 1, Fig. 2*B*). During electrical stimulation, the percentage of palmitate oxidized was similar between the trained and untrained groups and resulted in a 60% higher total rate of palmitate oxidation in the trained group.

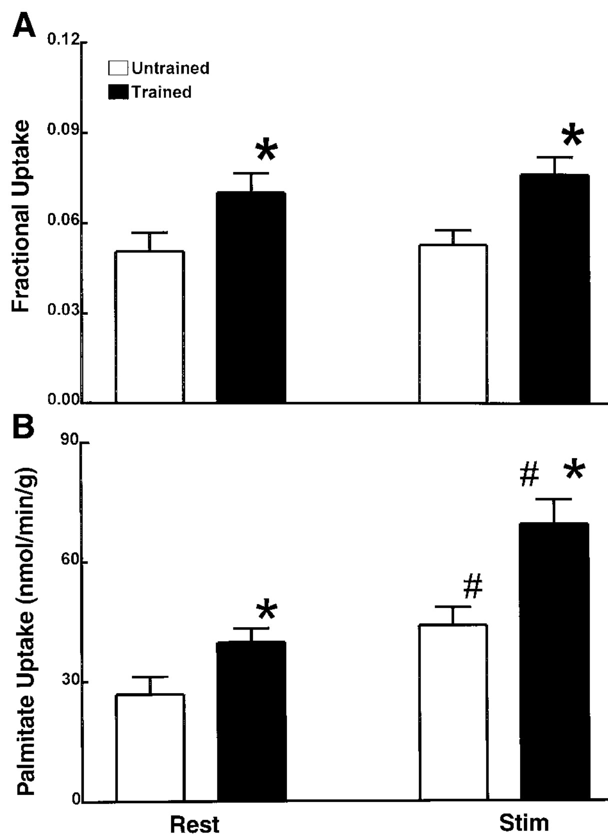

Fig. 1. Fractional (*A*) and total (*B*) uptake of palmitate in perfused rat hindquarters of trained and untrained rats during rest and electrical stimulation. Values are means ± SE for 6 trained and 8 untrained rats. Because there were no significant change in values measured after 10, 15, and 20 min of perfusion at rest as well as after 15, 20, and 25 min of perfusion during electrical stimulation, average resting and electrical stimulation values were used for each rat. *Significantly different compared with untrained group, $P < 0.05$. #Significantly different compared with rest, $P < 0.05$.

Substrate exchange across the hindquarter. Arterial perfusate glucose concentrations were not significantly different between groups during rest and electrical stimulation and did not change significantly from rest to electrical stimulation in both the trained and untrained groups (Table 1). Glucose uptake was not significantly different between the trained and untrained groups during rest and electrical stimulation. Glucose uptake increased by 216–238% from an average resting value of 3.6 ± 0.6 $\mu mol \cdot g^{-1} \cdot h^{-1}$, to reach an average of value of 11.8 ± 1.7 $\mu mol \cdot g^{-1} \cdot h^{-1}$ during the last 15 min of the stimulation period (Table 1, Fig. 3*B*).

Arterial perfusate lactate concentration was significantly lower in the trained than in the untrained group during rest and electrical stimulation (Table 1). Resting arterial perfusate lactate concentration increased by 74–116% during electrical stimulation in both the trained and untrained groups. Lactate release was not significantly different between the trained and untrained groups during rest and electrical stimulation (Table 1). In both groups of rats, lactate release was highest after 5–10 min of stimulation and decreased thereafter to reach values of 18.9 ± 3.1 and 20.7 ± 2.7 $\mu mol \cdot g^{-1} \cdot h^{-1}$ in the trained and untrained groups, respectively, at the end of the stimulation period (Fig. 3*A*).

Resting O_2 uptake was not significantly different between the trained and untrained groups (Table 1). O_2 uptake increased significantly during electrical stimulation in both the trained and untrained groups. The initial amount of tension developed by the contracting muscles was not significantly different between groups

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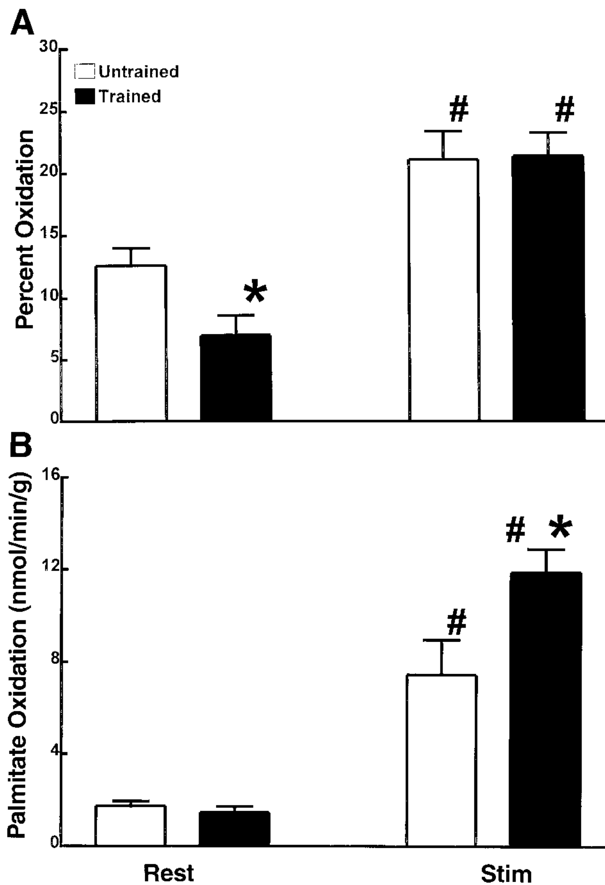

Fig. 2. Percent (*A*) and total (*B*) oxidation of palmitate in perfused rat hindquarters of trained and untrained rats during rest and electrical stimulation. Values are means ± SE for 6 trained and 8 untrained rats. Because there was no significant change in values measured after 10, 15 and 20 min of perfusion at rest, as well as after 15, 20 and 25 min of perfusion during electrical stimulation, average resting and electrical stimulation values were used for each rat. Percent and total palmitate oxidation values were corrected for label fixation as described in MATERIALS AND METHODS. * Significantly different compared with untrained group, $P < 0.05$. # Significantly different compared with rest, $P < 0.05$.

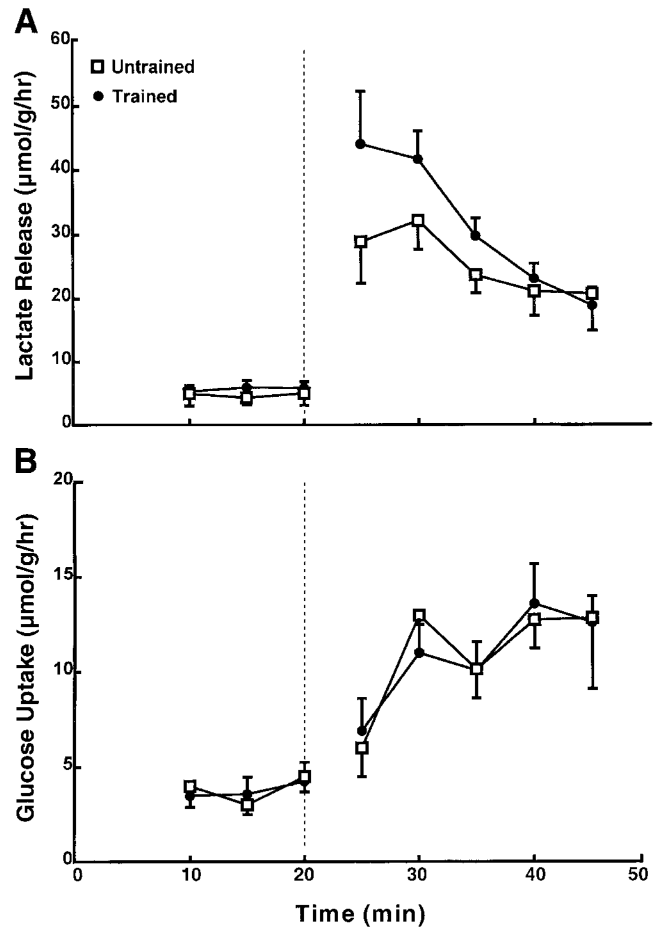

Fig. 3. Lactate release (*A*) and glucose uptake (*B*) in perfused rat hindquarters of trained and untrained rats during rest and electrical stimulation. Values are means ± SE for 6 trained and 8 untrained rats. Glucose uptake and lactate release values were higher during electrical stimulation (*minutes 25–45*) than during rest (*minutes 10–20*); $P < 0.05$.

and averaged 950 ± 59 g ($P > 0.05$). Muscle tension development decreased markedly during the first few minutes of electrical stimulation and was followed by a more gradual decrease. By the end of the stimulation period, muscle tension development had decreased by 48.5 ± 2.8 and 59.9 ± 3.7% in the trained and untrained groups, respectively ($P < 0.05$).

Western-blot analysis. The PM fractions isolated from the red and white skeletal muscles were enriched, as shown by an increase in the specific activity of 5′-nucleotidase relative to their respective CM fractions (Table 2). SDH activity in the PM fractions was not detectable; this indicates that contamination from mitochondrial membrane proteins was negligible. These results are consistent with those of previous reports that used similar PM isolation procedures (3, 40) and thus demonstrate the integrity of our PM preparation. Although the protein yield and 5′-nucleotidase activity were somewhat elevated in the CM fractions of the red skeletal muscle group, no significant differences were observed between groups (Table 2; $P > 0.05$). This result was probably due in part to the inherent variability associated with the isolation procedure in combination with the small number of preparations produced. However, these differences between groups became

Table 2. *Protein yield and 5′-nucleotidase activity of membrane fractions isolated from red and white skeletal muscles of trained and untrained rats*

Condition	Protein Yield, $mg \cdot g$ wet wt^{-1}		5′-Nucleotidase Activity, $nmol \cdot min^{-1} \cdot mg\ protein^{-1}$	
	Red	White	Red	White
	Crude membranes			
Trained	68.6 ± 0.8	34.7 ± 0.8	16.3 ± 2.5	11.1 ± 4.7
Untrained	70.4 ± 0.1	38.4 ± 0.6	10.3 ± 1.2	4.8 ± 1.2
	Plasma membranes			
Trained	0.10 ± 0.01	0.14 ± 0.04	74.1 ± 9.4	84.9 ± 7.6
Untrained	0.09 ± 0.03	0.07 ± 0.02	76.7 ± 8.3	74.2 ± 9.2

Values are means ± SE; $n = 5$ independent membrane preparations. No significant differences were observed between skeletal muscle groups or between trained and untrained groups; $P > 0.05$.

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negligible for the PM fractions, especially for the red gastrocnemius muscle. This is an important point, because $FABP_{PM}$ protein levels were measured in this membrane fraction. As previously shown by us and others (29, 40), Western-blot analysis by using a polyclonal antibody to the rat hepatic $FABP_{PM}$ detected a single protein band of ~42–43 kDa. Scanning densitometry of multiple gels revealed that red skeletal muscle PM contained 226 and 43% more $FABP_{PM}$ protein than white skeletal muscle PM in trained and untrained rats, respectively (Fig. 4*B*). SDH activity measured in the CM fractions was 140–155% higher in red than in white skeletal muscles (Fig. 4*A*). Compared with the untrained rats, $FABP_{PM}$ protein content in the trained rats was significantly higher by 55% in red skeletal muscle, whereas no significant difference in $FABP_{PM}$ protein content was found between groups in white skeletal muscle (Fig. 4*B*). A positive correlation ($y = 0.60x + 2.8$, $r = 0.85$, $P < 0.05$) was found to exist between SDH activity and $FABP_{PM}$ protein content in red muscle of trained and untrained rats (Fig. 5).

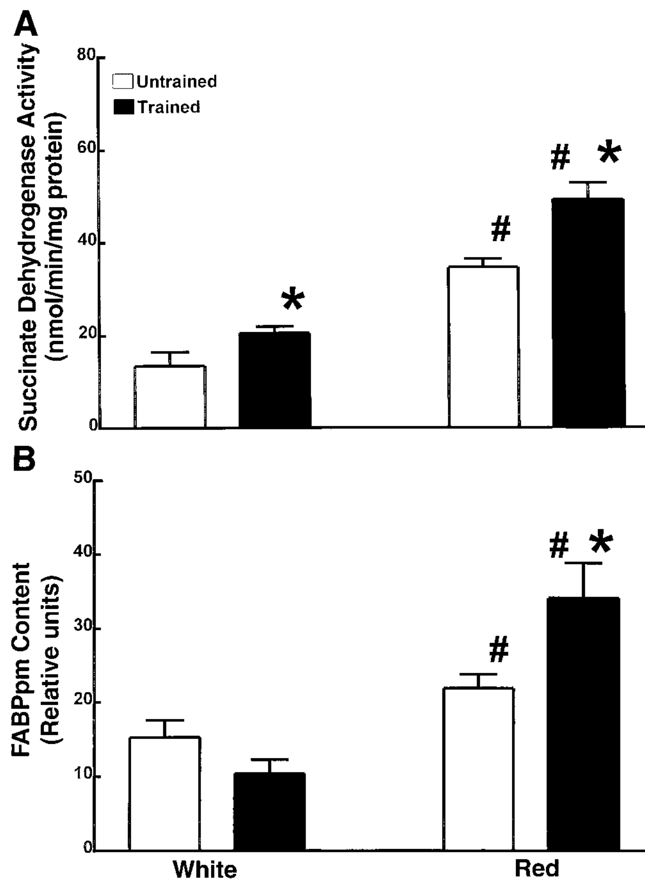

Fig. 4. Succinate dehydrogenase activity (*A*) and protein content of fatty acid-binding protein in plasma membranes ($FABP_{PM}$; *B*) in red and white muscles of trained and untrained rats. $FABP_{PM}$ protein was measured by immunoblotting plasma membrane (PM) fractions and was quantitated by scanning densitometry. $FABP_{PM}$ results are expressed as %liver standard. Each value represents mean ± SE of immunoblots of separate PM preparations from trained ($n = 5$) and untrained ($n = 5$) muscles. Succinate dehydrogenase activity was measured in corresponding crude membrane fractions. * Significantly different compared with untrained group, $P < 0.05$. # Significantly different compared with white muscle, $P < 0.05$.

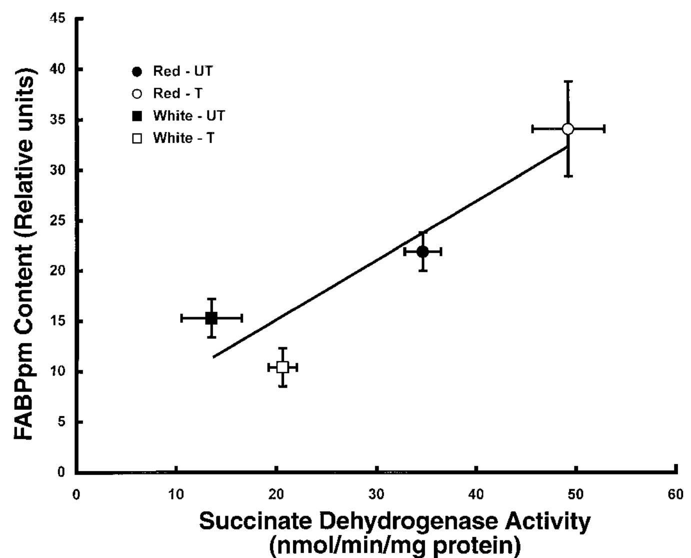

Fig. 5. Correlation between $FABP_{PM}$ content and succinate dehydrogenase activity in red muscles. Succinate dehydrogenase activity and $FABP_{PM}$ content were measured in crude membrane and PM fractions of gastrocnemius muscles from trained (T; $n = 5$) and untrained (UT; $n = 5$) rats. ●, Red muscles in untrained rats; ○, red muscles in trained rats; ■, white muscles in untrained rats; □, white muscles in trained rats. Regression equation and correlation coefficient are $y = 0.60x + 2.8$; $r = 0.85$; $P < 0.05$.

Triglyceride concentration. Precontraction triglyceride concentrations and [^{14}C]palmitate accumulations into muscle triglycerides were found to be higher in the soleus than in the red or white gastrocnemius muscles of both trained and untrained rats (Table 3). In both trained and untrained rats, postcontraction triglyceride concentrations were not significantly different from their respective precontraction level ($P > 0.05$). Although triglyceride concentrations were lower by 26–40% in the trained than the untrained rats, this was not significant ($P = 0.14$). Compared with the precontraction level, the postcontraction [^{14}C]palmitate accumulations into muscle triglycerides were significantly higher in all three fiber types and were not affected by training. The change in [^{14}C]palmitate accumulation from pre- to postcontraction was found to be higher in both soleus and red gastrocnemius muscles than in white gastrocnemius muscle.

DISCUSSION

Our results show that endurance training increases plasma palmitate uptake during rest and electrical stimulation and increases plasma palmitate oxidation during electrical stimulation. For the same plasma palmitate delivery, the training-induced increase in plasma palmitate uptake was associated with a higher fractional uptake of palmitate. Although oxidative enzyme capacity was increased by training, the training-induced increase in palmitate uptake could have been due in part to an increase in the content of $FABP_{PM}$, a PM protein that has been implicated in the regulation of transsarcolemmal FFA transport (34). Whereas resting palmitate oxidation was not affected by training, palmitate oxidation during electrical stimulation was

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Table 3. *Intramuscular triglyceride concentration and [14C]palmitate accumulation into intramuscular triglycerides in soleus and red and white gastrocnemius muscles of trained and untrained rats*

	IMTG Concentration, μmol/g wet wt		[14C]Palmitate Accumulation, dpm/mg wet wt	
	Trained	Untrained	Trained	Untrained
Soleus				
Before	9.0 ± 1.9	14.6 ± 4.1	1.4 ± 0.6	1.9 ± 0.4
After	10.3 ± 3.1	14.4 ± 5.3	4.3 ± 0.5*	3.9 ± 0.7*
Change			2.9 ± 0.4	1.9 ± 0.6
n	5	5	5	5
Red gastrocnemius				
Before	1.4 ± 0.27†	2.6 ± 0.49†	0.83 ± 0.1†	0.77 ± 0.03†
After	2.0 ± 0.38†	1.9 ± 0.27†	3.6 ± 0.47*	3.2 ± 0.92*
Change			2.7 ± 0.5	2.4 ± 0.9
n	7	7	7	7
White gastrocnemius				
Before	1.6 ± 0.30§	2.4 ± 0.66§	0.21 ± 0.01§	0.22 ± 0.01§
After	1.2 ± 0.14§	1.5 ± 0.40§	0.97 ± 0.14*‡§	1.7 ± 0.3*‡§
Change			0.8 ± 0.1‡§	1.5 ± 0.3‡§
n	7	7	7	7

Values are means ± SE; *n*, no. of rats. IMTG, intramuscular triglyceride; dpm, disintegrations per min; before, samples taken immediately before resting period; after, samples taken immediately after 25-min stimulation period. * Before vs. after, $P < 0.05$; † red gastrocnemius vs. soleus, $P < 0.05$; ‡ white gastrocnemius vs. red gastrocnemius, $P < 0.05$; § white gastrocnemius vs. soleus, $P < 0.05$.

higher in the trained muscle. Thus, when faced with an increased energy demand, the trained muscle was able to increase its contribution of fats to total oxidative metabolism. This may have been due in part to an increased availability of long-chain fatty acyl-CoA for mitochondrial oxidation. The existence of a positive correlation between $FABP_{PM}$ content and SDH activity in muscle suggests that the ability to take up FFA parallels the capacity for fatty acid oxidation in muscles.

The present results collected in intact perfused skeletal muscle agree with substrate exchange data collected across exercising knee extension muscles in humans (18, 39) and suggest that, with an equal delivery of FFA to the working muscles, the endurance-trained muscle is better able to utilize plasma FFA than is the untrained muscle. Because perfusate plasma palmitate concentration was maintained at a constant level from rest to electrical stimulation, the increase in perfusion flow rate with electrical stimulation was accompanied by an increase in the delivery of plasma palmitate to the rat hindquarter. We have previously shown that variations in perfusate flow rates from 9 to 15 ml/min have no effect on the rates of palmitate uptake and oxidation during unilateral perfusion of resting and contracting hindquarters (36, 38), and this has been found to be true for the rate of triglyceride synthesis during hindquarter perfusion at rest (5). In the present study, the fact that the fractional uptake of palmitate was maintained from rest to electrical stimulation indicates that the contraction-induced increase in palmitate uptake was independent of the change in perfusate flow rate.

With the physiological system used in the present study, the training-induced adaptations responsible for the increased utilization of plasma FFA by the muscle could be located at any steps from the dissociation of FFA from albumin to cellular metabolism. Taking into consideration that FFA delivery to the muscle was similar between the trained and untrained muscles, factors inherent in the perfused muscle mass must be responsible for the increased plasma FFA utilization. Muscle tissue blood flow is an important factor that can affect substrate utilization by the muscle. However, in contracting perfused skeletal muscle, the impact of training on blood flow distribution to the contracting muscles was found to be minimal (21).

Muscle oxidative capacity is another determining factor of the capacity to oxidize FFA (14). As previously shown by others (14), we found that SDH activity was 42–52% higher in the trained than in the untrained muscles. However, the oxidative potential of skeletal muscles certainly does not completely dictate the rate of fat utilization, because similar rates of fat utilization can be measured in individuals with different enzymatic profiles (6). It has been suggested that malonyl-CoA, a potent inhibitor of carnitine palmitoyl transferase-1 activity, is an important factor regulating substrate utilization in contracting muscle (42). In rats, muscle malonyl-CoA levels have been shown to decrease during exercise or electrical stimulation (42). However, contrary to expectations, endurance training was found to attenuate the exercise-induced decrease in malonyl-CoA level (15). These results suggest that, although malonyl-CoA appears to play a role in the regulation of oxidative metabolism under some physiological conditions in rat skeletal muscle, its primacy in regulating the training-induced increase in fat oxidation is not certain.

As an alternative explanation, the training-induced increase in fat oxidation could also be due in part to an increased availability of long-chain fatty acyl-CoA in the cytosol and to an increased fatty acid entry into the mitochondria (27). Results in exercising humans have shown that fatty acid entry into the mitochondria may be enhanced with training (27). In line with this, we measured a training-induced increase in palmitate uptake during rest and electrical stimulation, and this may have increased the availability of fatty acyl-CoA for mitochondrial oxidation. Our results of an increase in $FABP_{PM}$ content in red muscles suggest that the training-induced increase in the uptake of FFA may have been partially due to an increase in the number of FFA transporters on the PMs. Although evidence exists to show that part of the uptake of FFA across the PMs of cells may occur by passive diffusion (13), other data support the existence of a fatty acid transport system (23, 28, 31). Several proteins have been proposed as candidate long-chain fatty acid transporters (1, 25, 29, 34), but only three of those are known to be present in skeletal muscle; namely, the FATP, FAT, and $FABP_{PM}$. In line with the well-established oxidative potential of

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different muscle fibers, FATP and FAT mRNA levels and FAT and $FABP_{PM}$ protein levels have been shown to be higher in red oxidative fibers (4), and muscle $FABP_{PM}$ protein levels have been shown to vary during different physiological conditions (19, 34, 40). If the function of $FABP_{PM}$ is to contribute to the transport FFA from the plasma to the cytoplasm of cells for subsequent metabolism, then it follows that the higher $FABP_{PM}$ content in trained muscle could account for part of the increase in palmitate uptake.

As reported by others, we showed that muscle triglyceride concentrations were higher in red oxidative than in white glycolytic muscle fibers, were not affected by 25 min of electrical stimulation, and tended to be lower in the trained than in the untrained rats (11, 12, 17, 30). Although the lack of change in muscle triglyceride concentrations with electrical stimulation has been previously reported in untrained rats (12), we report for the first time that similar results were obtained in trained rats. Two points are important to consider when considering our data on muscles. First, the lack of change in muscle triglyceride concentrations with electrical stimulation does not exclude the possibility that muscle triglycerides were used as a substrate. While FFA were being hydrolyzed from the muscle triglycerides pool, triglycerides could also have been synthesized so that no net change in concentration was observed (8). Our measured rates of [^{14}C]palmitate accumulation into triglycerides would suggest that synthesis of triglycerides did occur. Second, when looking at the data on muscle triglycerides, it is important to keep in mind that, with the protocol design used in this study, differences between pre- and postcontraction data actually reflect both the resting and the electrical stimulation periods. Keeping these considerations in mind and taking into account the contributions of both plasma FFA and blood glucose, we estimated the contribution of muscle triglycerides to total oxidative metabolism to be ~30% at rest. During 20 min of rest, this would have represented the utilization of ~0.9–1.1 µmol of triglycerides for the perfused muscle mass. If similar calculations are done to account for the leftover uptake of plasma FFA, then the synthesis of triglycerides from plasma FFA would have resulted in the accumulation of ~0.2 µmol of triglycerides/g perfused muscle mass. These changes in muscle triglyceride levels would be undetectable by the assay. Whether or not muscle triglycerides were used as substrate during electrical stimulation is difficult to ascertain. Previously collected data on changes in muscle triglyceride levels with electrical stimulation have shown minimal to moderate use of this fuel during similar stimulation protocols (12, 30). At the present time, kinetic data on the rates of hydrolysis and synthesis of muscle triglycerides during muscle contractions are limited to rates of accumulation of [^{14}C]FFA into muscle triglycerides (12). Our results of an increased accumulation of [^{14}C]palmitate in the postcontraction samples agree with other data (12). Although these rates of accumulation are higher than those obtained at rest, our calculated changes in muscle triglyceride levels would also be undetectable by the assay. Thus, while it is possible that muscle triglycerides contributed to total oxidative metabolism, it is difficult to make accurate estimations of their contribution with the data at our disposal.

Rates of glucose uptake and lactate release during rest and electrical stimulation were not affected by training. These results are in agreement with other reports (7, 9, 16). Thus, in the absence of insulin, resting glucose uptake by the perfused hindquarter was not affected by training in spite of a training-induced increase in oxidative capacity and reported increases in GLUT-4 protein levels (16). Glucose transport rates in all three fiber types were also found to be unaffected by training (9). We extend these results to include a lack of training effect on glucose uptake during electrical stimulation. Although rates of lactate release were not affected by training, lactate concentrations were found to be lower in the trained rats. In whole body experiments, it has been shown that for a specific rate of lactate infusion, trained rats were able to maintain lower arterial lactate concentrations (7). This was attributed to an increased efficiency of lactate removal in the trained rats, with gluconeogenesis and oxidation contributing approximately equally to total lactate removal. With the experimental method used in the present study, oxidation and possibly incorporation into muscle glycogen would be the possible fates for the training-induced increase in lactate removal (22).

In summary, the present study has shown that endurance training increases palmitate uptake in resting and contracting perfused skeletal muscle, palmitate oxidation in contracting perfused skeletal muscle, and $FABP_{PM}$ content in red muscles. Intramuscular triglyceride utilization during muscle contractions was found to be minimal and was not modified by endurance training. The training-induced increase in palmitate uptake could have been due in part to an increased availability of fatty acid transporters at the PMs and could have contributed to the higher rates of palmitate oxidation during electrical stimulation. In line with this, $FABP_{PM}$ content was found to be related to the oxidative capacity of the muscle fibers. These results support the notions that factors inherent in the muscle are important in regulating the contribution of fats to total oxidative metabolism in skeletal muscle and that fatty acid transporter proteins located in the PMs may play an important role in the regulation of plasma FFA utilization by skeletal muscle.

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