Endurance training increases FFA oxidation and reduces triacylglycerol utilization in contracting rat soleus

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FREE FATTY ACIDS (FFA) REPRESENT an important source of energy during low- and moderate-intensity exercise (4, 11, 16, 32, 33). Endurance training results in an increase in the activities of enzymes of β-oxidation, the tricarboxylic acid cycle, and the electron transport system (18, 25) and a shift toward greater total lipid utilization during submaximal exercise (21). This shift is indicated by reduced rates of glycogen depletion (5, 15, 23) and increased utilization of plasma FFA (25, 41) during exercise after endurance training. An increased use of muscle triacylglycerol (TG) after training (20) has also been demonstrated, but this is controversial. Therefore, the effect of endurance training on the relative contribution from exogenous FFA and intramuscular lipids during contraction is unknown.

Preferential use of FFA derived from intramuscular TG hydrolysis may be advantageous, because this would avoid the constraint of transporting FFA from the vascular compartment to the cytoplasmic space, as well as to offer a FFA supply in close vicinity to the mitochondrion, circumventing a long diffusion distance (43). However, the relative contribution of exogenous and intramuscular sources of FFA toward increased lipid oxidation in the trained state is uncertain. Various studies have demonstrated an increased reliance on intramuscular lipid during exercise in trained humans (20, 21, 27), whereas others have not (9, 25). These discrepancies may arise from limitations in the various methods that have been used to assess lipid metabolism in human muscle. For example, studies utilizing stable isotopes (32, 33) assess simultaneous FFA and TG metabolism by measuring expired 13CO2 and respiratory exchange ratio, without determining actual changes in TG content and assuming that FFA taken up by muscle are not esterified during exercise. We have recently shown that this assumption is incorrect (13). Studies measuring net changes in muscle TG have not assessed the specific effects of contraction on synthesis and degradation of the intramuscular TG pool. Finally, in trained individuals, the endocrine and substrate milieu is altered; thus observations of training-induced changes in lipid metabolism have been made under different conditions from those in untrained humans. Therefore, it is necessary to examine FFA and TG metabolism in trained and untrained muscle under controlled conditions. Furthermore, it is difficult to determine the relative contribution of exogenous and intramuscular lipids in exercising humans, due to the inability to directly quantify the turnover of various intramuscular lipids, as well as the variability in exogenous substrate and hormone concentrations, both of which can be affected by training. Collectively, these problems may account for the discrepancy regarding the contribution of exogenous FFA and intramuscular TG to lipid metabolism during exercise in the trained state. The use of an isolated muscle preparation, although removed from the normal circulatory and neural influences, permits direct and simultaneous determination of lipid synthesis and degradation, the direct quantification of oxidation from both exogenous systems.

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and intramuscular lipids, as well as the ability to control exogenous variables such as substrate and hormonal concentrations.

In recent years, it has also been shown that FFA enter the cell via a protein-mediated mechanism (2). Such a mechanism has also been identified in skeletal muscle (8). Three putative fatty acid transporters have been identified (FAT/CD36, FABPpm, FATP), although their precise function remains unknown. With seven days of chronic electrical stimulation, protein-mediated FFA uptake is increased (7). FFA uptake is also increased after endurance training in perfused rat skeletal muscle (40), as well as in humans (39). Because this increase in FFA uptake is associated with a concurrent increase in the expression of the putative fatty acid transporters FAT/CD36 (7) and FABPpm (40), it is possible that a training-induced increase in FFA utilization is attributable to a concurrent increase in fatty acid transporters. However, no study has investigated the expression of all three putative transporters in response to intense aerobic training in conjunction with detailed measurements of FFA oxidation.

Our purpose in the present study was to determine the effect of endurance training on 1) the expression of putative FFA transporters and fatty acid transport in giant sarcolemmal vesicles and 2) the contribution of intramuscular and exogenous lipid sources for energy provision during rest and contraction. To this purpose, we used the recently characterized pulse-chase technique, which enables us to examine simultaneously the synthesis and degradation of intramuscular lipid pools as well as to compare the oxidation of exogenously provided palmitate and the intramuscular lipid depots under a variety of conditions (13, 14, 30). We have previously demonstrated that increasing the oxidative capacity of muscle using chronic stimulation results in significant increases in FFA oxidation and TG esterification during rest (8). However, the effect of endurance training on these aspects of lipid metabolism at rest and during contraction remains to be clarified.

**METHODS**

**Animals**

Female Sprague-Dawley rats were randomly placed in a control (n = 10) or 8-wk-training (n = 10) group. Animals were housed in a controlled environment on a reversed 12:12-h light-dark cycle and fed Purina rat chow ad libitum. Ethical approval was obtained from the Animal Ethics Committee at the University of Waterloo.

The training protocol used in the present study is similar to that of Duan and Winder (12). Animals were run on a treadmill at a 15% incline for 5 days/wk, beginning at 30 min/day, with the duration gradually increased to 2 h/day by the end of week 5. Rats initially began running at 21 m/min, and by week 5 this had increased to 31 m/min. For weeks 6 to 8, rats were trained for 2 h each day at 31 m/min. All animals initially weighed 93 ± 3 g, and by the end of week 8 they weighed 243 ± 3 g (trained, 242 ± 3 g; untrained, 244 ± 3 g). At the end of the training period (48 h after the last training session), animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/100 g body mass) before any experimental procedures.

**Muscle Characterizations**

Citrate synthase activity. Samples of soleus (SOL) and red gastrocnemius (RG) were excised and frozen in tongs precooled in liquid N2 and stored at −80°C until analyzed. The frozen muscle was homogenized in a K2HPO4 buffer (pH = 8.1), as previously described (6), and analyzed for maximal citrate synthase activity (26, 38). Activity was determined by initiating the reaction with oxaloacetate and linking the release of free CoASH to a colorometric agent, 5,5-dithiobis(2-nitrobenzoate) (DTNB), which was monitored at a wavelength of 412 nm.

Cytosolic fatty acid binding protein content. Cytosolic fatty acid binding protein (FABP) content was determined by a sandwich-type ELISA, as previously described (44). Briefly, polynuclear chloride plates were coated with 0.1 M carbonate buffer (pH = 9.6) containing 100 ng of anti-FABP IgG. Homogenized muscle samples and standards were added to the plates, and the FABP was allowed to bind to the immobilized IgG for 90 min. All samples were assayed in triplicate. FABP antigen was detected by a second antibody and complexed with biotin (streptavidin-biotin complex), which can be detected enzymatically.

**Fatty Acid Transporters**

RNA isolation. Total RNA was isolated from muscle tissues (RG and plantaris (PL)) by using the guanidine isothiocyanate/cesium chloride centrifugation method (10) with some modifications. The tissues were homogenized in 10 ml of 4 M guanidine isothiocyanate and layered on top of 3.3 ml of 5.7 M of cesium chloride solution. The samples were centrifuged in an SW-41 Ti rotor (Beckman Canada, Mississauga, ON, Canada), at 30,000 rpm for 23 h. The RNA pellets were recovered and purified by two precipitations in ethanol.

Northern blot analysis. Five micrograms of total RNA were used for electrophoresis on 1.2% formaldehyde agarose gels (35) and then transferred to positively charged nylon membrane (Boehringer Mannheim, Laval, QC, Canada). The Northern blots were ultraviolet-cross-linked with a GS-Gene linker (Bio-Rad, Hercules, CA). cDNA for FATP (36) was subcloned into BamH I/Xho I restriction enzyme sites of pBluescript (KS). Template DNA was linearized with BamH I restriction enzyme and digoxigenin (DIG)-labeled antisense riboprobe was generated by in vitro transcription with T3 RNA polymerase. FAT cDNA (1) was subcloned into Eco R I site of pBluescript (KS). The orientation was checked by digestion of template DNA with Acc I restriction enzyme. DIG-labeled antisense riboprobe, 1.6 kb long, was generated by digestion of the template DNA with Asa I restriction enzyme, and in vitro transcription with T3 RNA polymerase. cDNA for mitochondrial AspAT (36) was subcloned into Eco R I site of pBluescript (KS). The orientation was checked by digestion of template DNA with Hind III restriction enzyme. DIG-labeled antisense riboprobe was generated by in vitro transcription with T7 RNA polymerase after linearization of template DNA with Xho I restriction enzyme.

The ingredients for RNA transcription included 1–2 μg of DNA template plus the NTP mix [2.5 mM rCTP, 2.5 mM rGTP, 2.5 mM rATP, 1.625 mM uridine triphosphate (UTP) (Promega) and 0.875 mM Dig-11 UTP (Boehringer Mannheim)], 20 mM dithiothreitol (Promega), 1 IU/1 μg template DNA of RNase inhibitor (Promega) and 1X RNA polymerase. DNA was added to 10 μg DNA plus the NTP mix for 2 h at 37°C. The
DNA template was then digested for 10 min at 37°C with RNase-free DNase (1 IU/µg of DNA template; Promega). After precipitation in ethanol and centrifugation at 12,000 rpm for 15 min, the probe was resuspended in 10–20 ml DIG Easy-Hyb hybridization buffer (Boehringer Mannheim) at concentrations 50–100 ng/ml. After prehybridization of the membrane for at least 4 h at 68°C, the DIG Easy-Hyb hybridization buffer was replaced with buffer containing DIG-labeled antisense RNA probe and membrane was incubated with the probe overnight at 68°C. Chemiluminescent detection was performed in accordance with the protocol supplied by the manufacturer (Boehringer Mannheim), and the membrane was exposed to Kodak BioMax film. After exposure, the film was developed in Kodak developer and fixed in Kodak fixer.

Transport Studies

Giant sarcolemmal vesicles were prepared, as previously described (22, 28, 31), from pooled oxidative muscles of the rat hindlimb (vastus intermedius, red vastus lateralis, red gastrocnemius and red tibialis anterior). Palmitate uptake into vesicles was measured by the addition of unlabeled palmitate (Sigma, St. Louis, MO) and radiolabeled [3H]palmitate (0.3 µCi, Amersham Life Science, Oakville, ON, Canada) and [14C]mannitol (0.06 µCi, Amersham) in a 0.1% BSA-MOPS solution to 40 µl of vesicles (~80 µg protein). The reaction was carried out at room temperature for 10 s and was terminated by the addition of 1.4 ml of ice-cold KCl-MOPS solution, 2.5 mM HgCl2, and 0.1% BSA. The sample was quickly centrifuged at maximal speed in a microfuge for 1 min and the supernatant was discarded, and the radioactivity was counted in the tip of the tube. Palmitate transport was calculated by subtracting the contribution of nonspecific diffusion, as has been previously done (3, 29).

Pulse-Chase Studies

Preparation of soleus muscle strips. Longitudinal strips of soleus [SOL, 60% type I; 31% type IIA (14)] were carefully stripped longitudinally without damage to the tendons, tied, and placed in a 4-ml glass incubation reservoir (Radtomi Glass Technology, Monrovia, CA). The lower tendon was secured by passing it through a two-way stopcock and closing the valve.

Pulse phase: preexperimental labeling of intramuscular lipids. Three milliliters of warmed (30°C), pregassed (5% O2-5% CO2, pH 7.4) Krebs-Henseleit buffer containing 4% fatty acid-free BSA (Boehringer Mannheim), 10 mM glucose, 100 µM porcine insulin, 1.0 mM palmitate, and 2 µCi of [1-14C]palmitate (Amersham) were immediately added to the incubation reservoir. The incubation medium was continuously gassed with 95% O2-5% CO2, and temperature was maintained at 30°C via an outer water jacket of the incubation reservoir. Muscles were “pulsed” with [1-14C]palmitate for 30 min to prelabel all endogenous lipid pools.

After the pulse incubation buffer was drained, muscles were washed for 15 min with incubation medium containing no radiolabeled palmitate. After the wash, some of the muscles were randomly removed and processed for lipid extraction to determine the amount of 14C label incorporated into the endogenous lipid pools.

Chase phase: experimental phase examining exogenous and endogenous lipid metabolism. The remaining muscles were incubated with 1.0 mM palmitate and 2 µCi [9,10-3H]palmitate (Amersham) for an additional 30 min, either at rest or while electrically stimulated to contract. Muscles were stimulated with 150 ms trains comprised of 0.1-ms impulses (20–40 V; 60 Hz) at 20 tetani/min. This had previously been determined to elicit maximal rates of TG pool turnover and rates of oxidation without any significant development of fatigue (13). Muscle force production was monitored at 10-min intervals during this period. Exogenous palmitate oxidation and esterification were monitored by the production of 3H2O and incorporation of tritium into endogenous lipids. Intramuscular lipid hydrolysis and oxidation were simultaneously monitored by measuring the decrease in prelabeled [14C]palmitate content and production of 14CO2, respectively. A 0.5-cm layer of heavy mineral oil was placed on top of the incubation medium to prevent any escape of the 14CO2 produced from the oxidation of intramuscular lipids.

Measurement of Substrate Oxidation and Palmitate Incorporation into Muscle Lipids

Lipid oxidation. Palmitate oxidation and intramuscular lipid oxidation during contraction were determined as previously outlined (13, 14). Briefly, 14CO2 was determined by adding 0.5 ml of incubation buffer with 1.0 ml of 1 M H2SO4 in a sealed glass vial holding a suspended center well containing benzenthionium hydroxide. Center wells were placed in scintillation vials and counted with standard liquid scintillation techniques. Oxidation of [9,10-3H]palmitate was determined by separation of 3H2O from the labeled substrate. One milliliter of incubation buffer was mixed with 5.0 ml of 2:1 chloroform-methanol for 20 min. Two milliliters of 2 M KCl-2 M HCl were added, and the solution was mixed for an additional 20 min and then centrifuged for 5 min at 3,000 g. One milliliter of the aqueous phase was removed for liquid scintillation counting.

Radiolabeled palmitate incorporation into lipid pools. After incubation, soleus strips were removed, blotted and weighed, and ultraturaxed (Polytron homogenizer) in 1.5 ml of ice-cold 1:2 chloroform-methanol solution. Connective tissue was blotted and weighed and then subtracted from the muscle wet weight. Endogenous lipid extraction was performed as previously outlined (13, 14). Samples were spotted on silica gel plates (Silica Gel GF 250 µm, Analtech, Newark, DE) and resolved in solvent (60:40:4, heptane-isopropyl ether-acetic acid) for 45 min. Individual lipid bands were sprayed with dichlorofluorescein dye (0.02% wt/vol in ethanol) and visualized with long-wave ultraviolet light. Samples contained internal standards of phosphatidylcholine, palmitate, mono-, di-, and tripalmitin (Sigma Chemical) to insure proper lipid identification.

Calculations and Statistics

The quantity of palmitate (in nanomoles) esterified and oxidized was calculated from the specific activity of labeled palmitate in the incubation medium (i.e., radiolabeled palmitate in disintegrations per minute per total palmitate in nanomoles). Hydrolysis of intramuscular lipids at rest was calculated as the net loss of preloaded [3H]palmitate (in nanomoles per gram wet weight) from each pool. We have previously documented that, during contraction at 20 tetani/min, the loss of each nanomole of incorporated [14C]palmitate represents ~145 nmol of total FFA from the TG pool (13). This ratio was used to calculate the actual rates of net TG hydrolysis and endogenous lipid oxidation during muscle contraction.

Results were analyzed with ANOVA procedures, and a Fisher protected least significant difference post hoc test was used to test significant differences revealed by the ANOVA. Significance was accepted at P ≤ 0.05. All data are reported as means ± SE.
RESULTS

Citrate synthase activity and cytosolic FABP content. Maximal citrate synthase activity was significantly increased in SOL and RG after 8 wk of endurance training (Table 1). Cytosolic FABP (heart isoform) was unaffected by training (Table 1).

Muscle tension. Tension produced during contraction was recorded at 10-min intervals. Initial tension production was similar in SOL from trained and untrained rats (76.7 ± 9.5 g/g wet wt) and did not decline by 30 min (74.3 ± 8.9 g/g wet wt).

Palmitate esterification and oxidation. Total exogenous palmitate uptake (i.e., sum of all pools plus oxidation) at rest and during tetanic contraction is shown in Fig. 1. Training did not alter total palmitate uptake at rest. In contrast, training increased total palmitate uptake 1.6-fold during contraction (P ≤ 0.05).

Esterification of [3H]palmitate into PL, MG, and DG pools at rest was unaffected by endurance training (P ≥ 0.05, Table 2). In contrast, during contraction, the esterification of FFA into all these smaller endogenous pools was significantly increased (P ≤ 0.05) after endurance training (Table 2). Palmitate esterification into muscle TG was significantly increased both at rest (50%; P ≤ 0.05) and during electrical stimulation (69%; P ≤ 0.05) after 8 wk of training (Fig. 2A). Endurance training had no significant effect on exogenous palmitate oxidation at rest (9%; P ≤ 0.05, Table 2). In contrast, during contraction, the rate of palmitate oxidation was increased (52%; P ≤ 0.05) during contraction (Fig. 2B).

Intramuscular lipid hydrolysis and oxidation. Endurance training generally had no effect on the loss of [14C]palmitate (hydrolysis) from the smaller PL, MG, and DG pools either at rest or with contraction (Table 3). Hydrolysis of the TG pool was also unaffected by training at rest (Fig. 3A). Endurance training had no significant effect on exogenous palmitate oxidation at rest (9%; P ≤ 0.05, but palmitate oxidation was increased (52%; P ≤ 0.05) during contraction (Fig. 2B).

Palmitate transport into the muscle. The mRNA content of putative long-chain fatty acid transport proteins (FAT, FATP, FABPpm), fatty acyl-CoA synthase (FACS), and the heart isoform of cytosolic FABP (H-FABPc) failed to show any significant increases after endurance training (Fig. 4). Protein content of the putative fatty acid transporter FAT/CD36 was also unaffected with training (data not shown). This is consistent with our finding that transport of palmitate into giant sarcolemmal vesicles prepared from resting oxidative hindquarter muscle was also not significantly increased after training (control, 7.4 ± 0.8 pmol·g wet wt⁻¹·10 s⁻¹; trained, 8.0 ± 0.7 pmol·g wet wt⁻¹·10 s⁻¹).

DISCUSSION

In this study, we have utilized the pulse-chase technique in an isolated muscle preparation to examine the effect of endurance training on lipid turnover and oxidation. An isolated preparation has the advantage of permitting precise control of hormone and substrate concentrations, which allows for a more direct examination of the effects of endurance training on muscle lipid metabolism. This is an important consideration when examining the effects of training, because both hormone and substrate concentrations in the plasma may become altered. This study has shown that endurance training results in a marked increase in the capacity of muscle to incorporate palmitate both at rest and during contraction. However, at rest, the increase in palmitate uptake was directed toward TG esterification, whereas the rate of oxidation remained unaltered. During contraction, palmitate esterification into TG and oxidation were both increased. Intramuscular TG hydrolysis and oxidation were not altered at rest in trained muscles, but during contraction, these were significantly lower. This suggests that training induced a sparing effect on...
intramuscular TG utilization due to the enhanced capacity to incorporate and oxidize exogenous FFA. To our knowledge, this is the first demonstration of a reduced reliance on intramuscular TG during contraction in the trained state. Furthermore, the increase in FFA incorporation into skeletal muscle after endurance training does not appear to be due to increases in abundance of fatty acid transporters or to transport capacity.

Lipid metabolism during muscle contraction. The relative contributions of endogenous lipids compared with exogenous FFA during exercise in the untrained or trained state are controversial. In a previous study using an identical stimulation paradigm, we calculated that 70% of aerobically produced ATP was derived from intramuscular lipid oxidation. This is similar to the value of 62% previously reported by Spriet et al. (37) in tetanically contracting rat hindlimb muscles. Studies in humans by Hurley et al. (20) and Martin et al. (27) have indicated that in the trained state the reliance on exogenous FFA is reduced and that the contribution from intramuscular TG is increased. Other studies have failed to demonstrate greater utilization of intramuscular TG following endurance training (9, 25). These findings are complicated by the fact that FFA delivery to the muscle may be lowered during exercise in trained individuals due to a blunted sympathoadrenal response (17, 45), reducing adipocyte lipolysis and circulating FFA. Therefore, it is possible that an increased reliance on endogenous lipids may be the result of impaired fatty acid delivery in the trained state in vivo (20, 45). We have eliminated this variable by using an isolated muscle preparation in which the FFA concentration of the incubating medium remains constant. The observed increase in palmitate oxidation in trained contracting muscle parallels findings in both humans (41) and rats (40) when circulating FFA are kept constant. Thus in such studies (the present one and (40, 41)), FFA availability is not limiting to its oxidation. We now have very good evidence indicating that altering FFA availability at the level of the plasma membrane results in a concomitant alteration in FFA uptake (8).

Intramuscular TG hydrolysis and oxidation during contraction. Our finding that endogenous lipid oxidation was decreased during contraction in the trained state was unexpected; however, this can probably be reconciled with the fact that the trained muscle has an increased capacity to incorporate and oxidize exogenous FFA, resulting in a sparing of intramuscular TG stores. A reciprocal relationship between exogenous and endogenous lipid oxidation has been demonstrated previously in both resting (14, 46) and contracting muscle (13).

Increased fatty acid uptake by trained muscles. Palmitate uptake into SOL from trained animals was increased at rest and during contraction. It has been demonstrated that there is a protein-mediated mechanism facilitating the uptake of FFA into skeletal muscle (7, 8). We have previously demonstrated that increasing the oxidative capacity of muscle through chronic stimulation results in dramatic increases in palmitate transport and expression of the fatty acid transporter FAT/CD36, as well as palmitate oxidation and esterification into TG at rest (7). During contraction, the

### Table 2. Esterification of [3H]palmitate into PL, MG, and DG at rest and during contraction before and after 8 wk of endurance training

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<th>Rest vs. Contraction</th>
<th>Untrained</th>
<th>Trained</th>
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<td>MG</td>
<td></td>
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<td>DG</td>
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<td>20.1 ± 0.8</td>
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Values are expressed as nanomoles per gram wet weight (means ± SE). PL, phospholipid; MG, monoacylglycerol; DG, diacylglycerol. aSignificantly different from untrained; b significantly different from rest.
Table 3. Hydrolysis of PL, MG, and DG at rest and during contraction before and after 8 wk of endurance training

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<td>Trained</td>
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<td>Rest 4.2 ± 1.25</td>
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<td>Contraction 5.9 ± 2.1</td>
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<td>0.45 ± 0.11</td>
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Values expressed as nanomoles per gram wet weight (means ± SE). b Significantly different from rest.

Fig. 3. TG hydrolysis and intramuscular lipid oxidation at rest (A) and during contraction (B; 20 tetani/min). a Significantly different from untrained condition; b significantly different from rest.

Fig. 4. Messenger RNA content of several proteins related to sarcolemmal free fatty acid (FFA) transport in control and trained muscles (A: red gastrocnemius, B: plantaris). a Significantly different from untrained condition.
during contraction in untrained SOL muscle. This is similar to the values that can be calculated from the data of Hopp and Palmer (19) in electrically stimulated flexor digitorum brevis muscles incubated in vitro. During contraction in trained SOL muscles, endogenous lipid provision decreased to 772 µmol ATP·g⁻¹·30 min⁻¹, and exogenous FFA contribution increased to 28 µmol ATP·g⁻¹·30 min⁻¹. This indicates a 29% mismatch in calculated total ATP provision from lipid oxidation in the trained and untrained muscles. The reasons for this mismatch are unclear, but it is noteworthy that a similar discrepancy (~20%) was also reported by Saddik and Lopashuk (34) in beating heart during fat-free and high-fat conditions as determined by the pulse-chase technique. The discrepancy in the present study could be due to an overestimation of the calculated endogenous lipid oxidation, which is based on the specific activity of the TG pool. TG content is determined by traditional enzymatic techniques that are highly variable. Alternatively, it is possible that there is actually no mismatch in energy provision if glycogen contribution to ATP provision was greater in the trained muscle. Unfortunately, we did not determine this in the present study.

Palmitate transport. In the present study, we were unable to demonstrate an increase in palmitate transport into giant sarcolemmal vesicles prepared from oxidative muscles of the hindlimb following training. This is in agreement with the lack of increase in mRNA abundance for three of the putative fatty acid transporters (FAT/CD36, FATP, and FABPpm). Furthermore, mRNA abundance for FACS and protein content of FABPc also did not increase in response to training. These data suggest that the training intensity in the present study was sufficient to cause an increase in the oxidative capacity of the muscle (i.e., citrate synthase) and in FFA uptake and metabolism, but not as a consequence of an enhanced capacity to transport FFA into the cell. However, previous studies have demonstrated an increase in FABPc (24) and FAT/CD36 (7) after chronic stimulation, indicating that these parameters can be increased, given a sufficiently intense demand on the muscle. A lack of effect of endurance training on FABPc content has also been previously reported (42), and one recent study found an increase in FABPpm after aerobic training in rats (40). Overall, the data from the present study and others suggest that an increase in several key proteins involved in lipid uptake and metabolism (FAT/CD36, FABPpm, FATP, FACS, and FABPc) may not be necessary to facilitate modest increases in FFA uptake and oxidation. However, further increases in lipid metabolism induced by more intense stimulation may require a parallel increase in these proteins (7).

Alternatively, it is possible that the lack of increase in mRNA abundance of the putative fat transporter and FACS in the present study is not indicative of the actual changes that occurred at the protein level. However, FABPc was also measured in the present study by the ELISA method (data not shown), and it also showed no increase after training. It should be noted that the mRNA values for these various proteins are determined on muscle homogenates and do not reflect increases in these proteins that may have occurred in specific cellular compartments. It is also possible that any increase in the putative fat transporter mRNA after training may have decreased to control levels during the 48 h between the last training session and the sampling of the tissues. Furthermore, palmitate transport determinations were made in giant sarcolemmal vesicles prepared from resting hindlimb muscle. We have previously shown that muscle contraction acutely increases the total incorporation of FFA into the cell independently of changes in FFA concentration or delivery (13), suggesting an increase in transporter activity or translocation to the plasma membrane. Therefore, the lack of increase in palmitate transport in vesicles prepared from resting muscle may not be reflective of changes which occurred during muscle contraction.

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