Palmitate transport and fatty acid transporters in red and white muscles

A. Bonen, J. J. F. P. Luiken, S. Liu, D. J. Dyck, B. Kiens, S. Kristiansen, L. P. Turcotte, G. J. van der Vusse, and J. F. C. Glatz

1Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1; 2The Copenhagen Muscle Research Centre, August Krogh Institute, University of Copenhagen, DK-2100 Copenhagen, Denmark; 3Department of Exercise Sciences, University of Southern California, Los Angeles, California 90089; and 4Department of Physiology, Cardiovascular Research Institute Maastricht, Maastricht University, 6200 MD Maastricht, The Netherlands

Circulating substrates provide important fuels for cell metabolism. Glucose and lactate are known to cross the cell membrane via a facilitated transport system for which a number of transport proteins have been identified (7, 10, 14, 15). Because of the hydrophobic nature of long-chain fatty acids (LCFAs), it has generally been assumed that they can rapidly traverse the lipid bilayer of the cell membrane. This has been demonstrated using purified protein-free phospholipid bilayers, suggesting that a specific transport system is unnecessary (24). However, it has been argued that spontaneous dissociation of LCFAs from their albumin carrier is insufficient to account for uptake observed in various tissues, as well as the fact that, at physiological pH, LCFAs bound to albumin exist in an ionized form, which could hinder their unassisted diffusion across a charged membrane (6, 35, 45). Recently, evidence has begun to accumulate showing that LCFAs may, in part, enter the cell via a carrier-mediated process in a variety of physiologically important cells, including cardiac myocytes (26, 37), adipocytes (2, 3), and hepatocytes (36, 39). Unfortunately, in none of these studies has it been possible to divorce LCFA uptake from LCFA metabolism.

Skeletal muscle is dependent on the oxidation of LCFAs to sustain its ATP production. Indeed, LCFAs are the major substrate for this tissue, both at rest and during moderate exercise (18, 33). In resting muscles we have shown that 90% of the lipid metabolism is provided by exogenous fatty acids, and only 10% is derived from the endogenous triacylglycerols (13). With the onset of contraction, the LCFA uptake by muscle is greatly increased, either in vivo (17) or in vitro (12). Although it is believed that the increased LCFA uptake into skeletal muscles is dependent on the increased delivery of LCFAs (18), it has been reported that LCFA uptake by perfused skeletal muscle is saturable (41), possibly involving a carrier-mediated process. However, no direct evidence of an LCFA transport system in this tissue has been demonstrated.

Skeletal muscles are metabolically heterogeneous, with some muscles being much more dependent on oxidative metabolism than other types of muscles (4, 13, 30). We have shown that oxidative muscles exhibit much greater lactate transport (27) and insulin-stimulated glucose transport capacities (30) than glycolytic muscles. Facilitated transport of these substrates into muscle is highly correlated with the available number of transporter proteins for lactate (monocarboxylate transporter 1) (27, 28) and glucose (GLUT-4) (22, 30). Because red muscles exhibit a greater capacity for LCFA metabolism than white muscles (13), it is also possible that the LCFA transport rate in oxidative (red) muscles will be greater than in glycolytic (white) muscles. Therefore, we performed studies 1) to investigate the kinetics of palmitate transport into skeletal muscle vesicles, to determine whether the LCFA transport capacity is greater in red muscles than in white muscles, and 2) if so, whether putative LCFA transport-
ers are more abundant in red than in white muscles. We have developed a procedure to measure palmitate transport in giant sarcolemmal vesicles, derived from red and white rat skeletal muscles. Such giant vesicles have previously been used to examine the transport systems for glucose (31) and lactate (27). An advantage of these vesicles is that they are are oriented right side out and are devoid of mitochondria (31). Therefore, we were able to examine LCFA transport in the absence of LCFA metabolism.

METHODS

Animals. Sprague-Dawley rats (male) weighing ~300 g were used in these studies. They were kept on a reverse 12:12-h light-dark cycle and were fed rat chow ad libitum. Water was freely accessible. All procedures were approved by the Committee on Animal Care at the University of Waterloo. Giant sarcolemmal vesicles. Vesicles were prepared as we (27) and others (23, 31) have described previously. Briefly, rat hindlimb muscles from both legs were divided into pools of red muscles (vastus intermedius, red vastus lateralis, soleus, red gastrocnemius, red tibialis anterior) and white muscles (plantaris, white vastus lateralis, white gastrocnemius, white tibialis anterior) on the basis of the fiber composition of these muscles as we have determined it in previous studies (27, 30). The muscle samples were cut into thin layers (~1–3 mm thick) and incubated for 1 h at 34°C in 140 mM KCl-10 mM MOPS (pH 7.4), collagenase (150 U/ml), and aprotinin (0.01 g/ml). The muscle was then washed with KCl/MOPS and supernatant was collected. Percoll (final concentration 16%) and aprotinin were added to the supernatant. This supernatant 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. The samples were spun at 60 g for 45 min at room temperature. After centrifugation, the vesicles were harvested from the interface of the two upper solutions. The vesicles were diluted in KCl-MOPS and centrifuged at 800 g for 30 min. Vesicles were immediately used for transport experiments. Some of the vesicles were placed in a blood cell counting chamber and examined with a phase contrast microscope. Vesicles from a pool of mixed fibers were photographed and sized from the photomicrographs. Vesicles were also prepared and stored at −80°C for protein and marker enzyme analysis.

Fatty acid transport. Palmitate uptake was measured by addition of unlabeled palmitate (Sigma) and radiolabeled [1H]palmitate (0.3 µCi, Amersham) and [14C]mannitol (0.06 µCi, Amersham) in a 0.1% BSA-KCl-MOPS solution to 40 µl of vesicles (~80 µg protein). The reaction was carried out at room temperature for 10 s, unless otherwise specified. Palmitate uptake was terminated by adding 1.4 ml ice-cold KCl-MOPS, 2.5 mM HgCl₂, and 0.1% BSA. The sample was quickly centrifuged at maximal speed in a microfuge for 1 min. The supernatant was discarded, and radioactivity was determined in the tip of the tube. Nonspecific uptake was measured by adding the stop solution to the membrane before the addition of the isotopes. To calculate palmitate transport, the contribution of palmitate diffusion was subtracted from the palmitate uptake as we have done previously for determining lactate transport (29) and as has been done by Abumrad et al. (3) for determining LCFA transport. In experiments in which the inhibition of palmitate uptake was examined, the inhibitors anti-plasma membrane-bound fatty acid binding protein (FABPpm, 8.1 µg/µl), phloretin (200 µM), and sulfo-N-succinimidyl oleate (SSO; 50 µM) were added 30 min before the transport studies were conducted. Vesicles were also exposed to trypsin (0.5%) for 30 min, after which the trypsin was washed from the vesicles before palmitate transport was determined. Competition for palmitate uptake was examined by adding excess oleate and octanoate during the transport measurements. Specificity of fatty acid uptake was also examined by comparing the uptake of palmitate and oleate into the vesicles.

Western blots. The putative LCFA transporters fatty acid translocase (FAT/CD36) and FABPpm, were measured in giant vesicles as well as in muscle homogenates, which we (27, 28, 29) have used for the measurement of other transport proteins. The monoclonal antibody to CD36 (Cedarlane Laboratories, Hornby, ON, Canada) was used to detect FAT/CD36. In other studies CD36 has been shown to be the human analog to rat FAT (1). Antibodies against FABPpm were those used in previous studies (41). Plasma membranes (80 µg) and prestained molecular weight markers (Bio-Rad) were separated on 12% SDS-polyacrylamide gels (150 V, 90 min). Membranes were incubated on a shaker overnight (16 h) in buffer A [20 mM Tris-base, 137 mM NaCl, 0.1 M HCl (adjusted to pH 7.5), 0.1% (vol/vol) Tween 20, and 10% (wt/vol) nonfat dried milk] at room temperature. Vesicle membranes and muscle homogenates were then incubated with FAT/CD36 in buffer A for 2 h, followed by three washes in buffer B (i.e., buffer A without dried milk; a 15-min wash and two 5-min washes) followed by incubation for 1 h with donkey antirabbit immunoglobulin G horseradish peroxidase-conjugated secondary antibody (1:3,000; Amersham, NA 934) in buffer B. Membranes were washed as before with buffer B, and then FAT/CD36 was detected using an enhanced chemiluminescence detection method by exposing the membranes to film (Hyperfilm-ECL; Amersham, Oakville, ON, Canada) at room temperature according to the instructions of the manufacturer. Film was developed and fixed in GBX fixer/replenisher (Kodak). FAT/CD36 protein band densities were obtained by scanning the blots on a densitometer connected to a Macintosh LC computer with appropriate software.

Northern blotting. Procedures for Northern blotting have been described in detail elsewhere (44). Briefly, muscles were homogenized in a K₂HPO₄ buffer (pH = 8.1) as previously described (5). Muscle homogenates were subsequently analyzed for maximal β-hydroxybutyrate dehydrogenase (β-HAD) and citrate synthase (CS) activities as previously described (13). Potassium-stimulated p-nitrophenylphosphatase activity (K⁺-
pNPPase) was assayed in muscle homogenates and giant sarcolemmal vesicles as described by Ploug et al. (31). The content of heart type cytoplasmic FABP (FABPc) in muscles was determined by a sandwich-type ELISA as previously described (46). Protein concentrations were determined by the biocinchoninic acid assay with BSA as a standard.

RESULTS

Descriptive characteristics of red and white muscles. Red [e.g., soleus (SOL) and red gastrocnemius (RG)] and white muscles [e.g., flexor digitorum brevis (FDB) and white gastrocnemius (WG)] differ considerably in their capacities for oxidative and LCFA metabolism. We characterized red and white muscles on the basis of their enzyme activities and FABPc content. Red muscles exhibited a greater maximal CS activity than white muscles (Fig. 1). With respect to the potential for lipid metabolism, red muscles 1) contained a greater sink for palmitate that is taken up, because the FABPc content was much higher in red than in white muscles (Fig. 1), and 2) also demonstrated a greater capacity for β-oxidation, because the β-HAD activities were more than twofold greater in red muscles than in white muscles (Fig. 1).

Giant sarcolemmal vesicle characteristics. To determine carrier-mediated fatty acid transport, it is important to divorce transport from its metabolism. For this purpose giant sarcolemmal vesicles obtained from rat skeletal muscles were used. The giant vesicles were spherical in appearance and averaged 9.8 ± 0.2 μm in diameter. Total vesicular protein consisted of ~90% nonmembrane-bound protein (Ref. 31 and Bonen, unpublished data). The vesicles provide an enriched fraction of muscle plasma membranes, as evidenced by the 27-fold enrichment in K⁺pNPPase in plasma membranes (8.2 ± 1.3 μmol·mg protein⁻¹·h⁻¹) compared with muscle homogenates (0.3 ± 0.2 μmol·mg protein⁻¹·h⁻¹). In other studies using the identical vesicle preparation, the characteristics of the vesicles were very similar (31) and were not contaminated by the sarcoplasmic reticulum or T tubule membranes (31).

Palmitate transport by giant sarcolemmal vesicles. In preliminary work, palmitate uptake at the highest concentration used in our studies was linear for up to 25 s in vesicles from red and white muscles. A linear increase in palmitate uptake also occurred with increasing quantities of protein (i.e., vesicle quantity; data not shown). In the present set of experiments, palmitate uptake was determined over a 10-s period with the use of 80 µg of total vesicular protein.

Palmitate uptake was determined over a range of fatty acid-to-BSA ratios designed to yield varying amounts of unbound palmitate. For these purposes we used the calculations of Richieri et al. (32). The uptake of

Fig. 1. Comparison of citrate synthase activity (A), β-hydroxybutyrate dehydrogenase (β-HAD) activity (B), and cytoplasmic fatty acid binding protein (FABP) concentrations (C) in selected white and red skeletal muscles. White muscles: white gastrocnemius (WG) and flexor digitorum brevis (FDB); red muscles: soleus (SOL) and red gastrocnemius (RG). Values are means ± SE for 5 experiments of each type. *P < 0.05, SOL or RG vs. white muscles (FDB, WG). **P < 0.05, SOL vs. RG.
palmitate into vesicles from pools of red and white rat hindlimb muscles occurred at a rapid rate, and when corrected for the diffusible component, palmitate uptake was saturable in both the red and white vesicles (Fig. 2), suggesting that palmitate was being transported into these vesicles. Maximal transport \( (V_{\text{max}}) \) into the red vesicles was 1.8 times greater than in the white vesicles (Fig. 2). The Michaelis-Menten constant \( (K_m) \) for palmitate was \(-6 \) nM in both red and white vesicles. This corresponds closely to the \( K_m \) (6 nM) that can be calculated from the palmitate uptake in perfused rat hindlimb muscles (41).

It was important to establish that there was an appropriate intravesicular sink for palmitate. Without this sink palmitate uptake would be severely limited, and the plateau in palmitate uptake could then be due to its limited solubility in an aqueous medium. For this reason we determined the FABPc content in vesicles derived from red and white muscle. FABPc content was 7.6-fold greater in vesicles from the red muscles than in those from white muscles (Fig. 3). In both types of vesicles, however, FABPc is calculated to be present in excess, even at maximal rates of palmitate uptake, because only 3.2 and 22% of the intravesicular FABPc in red and white vesicles, respectively, would be occupied with palmitate.

To establish that palmitate was indeed transported into the vesicles, additional experiments were performed. Palmitate uptake was inhibited by anti-FABPpm, SSO, and trypsin and was reduced when transport was examined at 0°C (Fig. 4). Anti-FABPpm also inhibits fatty acid uptake in hepatocytes (39) and cardiac myocytes (38). The reduction by phloretin (\(-50\%) is similar to the phloretin-induced inhibition of fatty acid uptake in adipocytes (3). Similarly, the inhibition of palmitate uptake by 50 \( \mu \)M SSO (\(-43\%\)), a nonpermeable sulfo-N-succinimidyl LCFA derivative that specifically inhibits LCFA transport in adipocytes (19), was similar to the inhibition of LCFA uptake (\(-65\%) by 200 \( \mu \)M SSO in adipocytes (19). It was also observed that palmitate uptake was lowered in the presence of excess (100 \( \mu \)M) oleate but not in the presence of octanoate (100 \( \mu \)M) or glucose (1 mM) (Fig. 4). When the osmolarity was increased from 300 to 600 mosM, glucose transport was reduced (data not shown) as has been reported previously for glucose (25), and palmitate transport was increased by \( 35 \pm 5\% \) \( (n = 3 \) experiments). Thus both substrates enter an osmotically reactive space rather than binding to the plasma membrane. Differences between glucose and palmitate responses to the increase in extravascular osmolarity presumably reflect a reduction in the intravesicular sink for glucose (i.e., cell water) and an increase in the intravesicular sink for palmitate, namely an increase in FABPc concentrations. The increased FABPc concentration would reduce the intravesicular diffusion distance for palmitate once it had been taken across the sarcolemmal membrane.

To further examine the fate of the palmitate taken up by the vesicles, we lysed the vesicles immediately at the end of the transport period. From these lysed vesicles we were able to recover 100% of the \(^3\)H label in the cytosol compartment (Table 1). It was also found that the intravesicular radiolabel was present in the vesicles as unesterified \[^3\]Hpalmitate (100%) (Table 1). These data clearly indicate that palmitate sequestration by these vesicles is due to the traversal of palmitate across the sarcolemmal membrane rather than to a physical partitioning in the membranes.

Fatty acid transporters. Because palmitate was being transported into red and white vesicles at different maximal rates, it was important to determine whether putative LCFA transporters were present in greater abundance in red muscles compared with white muscles. Both FAT mRNA and FATP mRNA were present in red and white muscles (Fig. 5). The abundance of these transcripts was five- to sixfold greater in red (SOL) than in white (FDB) muscle \( (P < 0.05) \). The FAT/CD36 protein content in the plasma membranes from red muscles was 1.4-fold greater than in plasma membranes from white muscles (Fig. 6), whereas FABPpm was 1.2-fold greater in red vesicles than in white vesicles (Fig. 6).

**DISCUSSION**

This study is the first to demonstrate 1) that palmitate is transported across the sarcolemmal membrane...
of skeletal muscles and 2) that transport into giant vesicles from red muscles is greater than that into vesicles obtained from white muscles. In addition, we showed 3) that the transcripts of two putative LCFA transporters (FAT and FATP) are present in greater quantities in a red muscle (SOL) than in a white muscle (FDB), and 4) we found a greater content of the

Table 1. Palmitate uptake and recovery from giant sarcolemmal vesicles

<table>
<thead>
<tr>
<th>Palmitate Uptake</th>
<th>Palmitate Recovery</th>
<th>Palmitate Recovery by Thin-Layer Chromatography</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pellet</td>
<td>Cytosol</td>
</tr>
<tr>
<td>Pellet Cytosol</td>
<td>2.1 ± 0.1</td>
<td>2.0 ± 0.2</td>
</tr>
</tbody>
</table>

After uptake period (10 s), palmitate recovery was determined by lysing vesicle membranes and separating membranes (pellet) and cytosolic fractions of the same vesicles. Palmitate recovery in cytosolic fraction from lysed vesicles was also determined using thin-layer chromatography. Values are means ± SE of separate experiments expressed in pmol/mg protein. FA, fatty acid; PL, phospholipids; MG, monoacylglycerol; DG, diacylglycerol; TG, triacylglycerol; ND, not detectable.

FAT/CD36 and FABP<sub>pm</sub> protein in homogenates and in membranes obtained from red muscles than in those obtained from white muscles. A major advantage of the giant sarcolemmal vesicle preparation is that LCFA transport occurred in the absence of any LCFA metabolism and esterification. Thus we are now able to examine LCFA transport into vesicles obtained from a metabolically important tissue in which LCFA metabolism may be altered by physiological (exercise), pathological (diabetes), and dietary (fasting) conditions.

Palmitate uptake by red and white muscles. Skeletal muscles in the rat have a wide range of metabolic capacities. These can be roughly divided into red and white muscles, reflecting a great capacity for oxidative and glycolytic metabolism, respectively. The diversity for LCFA metabolism in red and white muscles has been shown in recent studies (13). Differences in LCFA uptake in these studies between red and white muscles could not be attributed to differences either in the delivery of palmitate or in the vascularization of these two muscles, because red and white muscles were incubated with identical palmitate concentrations (13).
Therefore, the differences in palmitate uptake, oxidation, and esterification in the intact red and white muscles reflect differences in their biochemical machinery for these processes. One of these processes is the uptake of palmitate by these muscles. The saturable nature of LCFA uptake by perfused rat muscle (41) pointed toward a potentially important role for a protein-mediated transport process. Alternatively, the saturation of LCFA uptake could also have been related to a saturation of LCFA metabolism. To establish conclusively whether cellular LCFA uptake occurs via a carrier-mediated process, a vesicle preparation was used to examine the transsarcolemmal movement of palmitate.

The giant sarcolemmal vesicle preparation in the present study offers a number of advantages over other systems. 1) Metabolism is divorced from transport in the vesicles, thereby avoiding the potential problems of esterification and oxidation that have occurred in other preparations used to date [adipocytes, cardiac myocytes, and hepatocytes (3, 8, 9)] in which LCFA transport has been investigated. 2) The giant sarcolemmal vesicles (~10 µm in diameter) are more suited for transport studies than small sarcolemmal vesicles (<1 µm diameter), because the volume of the larger vesicles avoids the problem of backflux of the substrate that can occur in small vesicles, making it difficult to obtain initial rates of transport (31). 3) Finally, the metabolic distinction, with respect to the uptake of LCFA between intact red and white muscles, is preserved when LCFA transport is examined in vesicles derived from red and white muscles.

A number of observations lead us to the conclusion that palmitate uptake by giant sarcolemmal vesicles occurs via a facilitated transport system. First, we showed that palmitate uptake was a saturable process in the giant sarcolemmal vesicles. Second, palmitate uptake was inhibited by antibodies to FABP$_{pm}$ by phloretin [an inhibitor of many transport processes, including LCFA transport (3, 37)], and by SSO, a specific inhibitor of LCFA transport in adipocytes (19). Palmitate uptake was also reduced when the temperature was lowered from 20°C to 0°C, which is largely taken to be due to a reduction in palmitate transport rather than diffusion. That palmitate uptake is protein mediated is also shown by the selectivity of fatty acid transport (i.e., palmitate uptake rate >> octanoate uptake rate) and the competition for transport between oleate and palmitate but not between octanoate and palmitate. Our experiments also demonstrate that the palmitate was not esterified, because 1) 95% of the palmitate was able to efflux from the vesicles when not impeded by phloretin (data not shown), and 2) we recovered 100% of the radio-labeled palmitate in the cytosol from lysed vesicles. Importantly, we also found that all of the radio-labeled material was palmitate, thereby confirming the complete absence of LCFA metabolism in these vesicles. Collectively, these experiments indicate very strongly that palmitate was taken up by the vesicles via a carrier-mediated transport mechanism similar to that described for adipocytes (3, 8), hepatocytes (8), and cardiac myocytes (8, 26, 37). However, unlike these latter preparations, in which LCFA metabolism confounds the determination of transport, the absence of LCFA metabolism in the giant sarcolemmal vesicles provides an excellent model for examining LCFA transport in a metabolically important tissue.

The sarcolemmal vesicles used in these experiments contained FABP$_c$ (5.4 µg FABP/mg protein in red vesicles and 0.7 µg FABP/mg protein in white vesicles) that was somewhat higher than has been found in intact red skeletal muscles, such as the SOL (~2 µg FABP/mg protein) and RG (0.75 µg FABP/mg protein), and in a white muscle, such as the FDB (~0.1 µg FABP/mg protein) and WG (~0.2 µg FABP/mg protein) (13). The cytosolic FABP content of the vesicles is critical, because this sink is required to bind palmitate once it has traversed the sarcolemmal membrane. Inadequate concentrations of FABP$_c$ could limit the uptake of transport into the vesicles. However, we can calculate that the giant sarcolemmal vesicles contained more than adequate amounts of FABP$_c$ to provide the necessary intravesicular sink for palmitate. In the course of our 10-s experiments to measure palmitate uptake, only 3.2 and 22% of the FABP$_c$ is complexed with palmitate in red and white vesicles, respectively, at the maximal palmitate concentrations used in our studies. Alternatively, it appears that FABP$_c$ is present in 4.5-fold and 31.3-fold excess in red and white vesicles, respectively. Thus, as in vivo, most of the intravesicular FABP$_c$ is not complexed with fatty acids (47).

Presumably, the LCFA transport system in muscle is functionally coordinated with the different metabolic capacities for lipid metabolism in red and white muscles.
We (13) have clearly established that the capacity for LCFA metabolism is greater in red than in white muscles. Therefore, we would expect that LCFA transport should also be greater in red than in white muscles. This was indeed observed: the $V_{\text{max}}$ for palmitate transport was greater in red muscle vesicles than in white muscle vesicles, whereas the $K_m$ was ~6 nM in vesicles from both types of muscles. This greater maximal transport capacity in red muscles appears to reflect the greater number of LCFA transport proteins in the plasma membranes obtained from these oxidative muscles.

Sarcolemmal fatty acid transporters. Recently, Abumrad et al. (1) and Schaffer and Lodish (34) identified distinct LCFA transporters. FAT migrates at 88 kDa (1), and FATP migrates at 63 kDa (34). Previously, Stremmel et al. (40) implicated a 40- to 43-kDa FABP$_{pm}$ with the transport of fatty acids in liver. FABP$_{pm}$ is also present in muscle (42). There is evidence that each of these proteins stimulates fatty acid transport when their respective cDNAs are transfected into various cell lines (16, 20, 21, 34, 43). In our studies, transcripts for the putative LCFA transporters FATP and FAT were present in red and white muscles, with red muscle (SOL) containing five- to sixfold more FAT mRNA and FATP mRNA than the white muscles (FDB). A similar difference between red and white rodent skeletal muscle FAT mRNA has recently been shown in other studies (44). Unfortunately, we cannot resolve the debate as to which of the putative LCFA transporters is more important in muscle. Thus, although all putative LCFA transporters are coexpressed in muscle, it is not known whether they are functionally independent or interdependent. However, studies by Berk et al. (8) showed that putative fatty acid transporters are regulated in a tissue-specific manner in diabetic animals and that the three LCFA transporters (FAT, FATP, and FABP$_{pm}$) appear to be selectively regulated in diabetic animals (8).

In summary, we have provided evidence that palmitate is transported into skeletal muscle by a mechanism involving membrane-associated fatty acid transport proteins. Palmitate transport studies were performed with giant sarcolemmal vesicles obtained from rat hindlimb skeletal muscles. In these vesicles palmitate was not metabolized and esterified, and thus transport was diverted from metabolism. Palmitate transport was greater in red than in white muscle vesicles, consistent with differences in palmitate uptake and metabolism in isolated, intact red and white muscles. Skeletal muscles contained FAT mRNA and FATP mRNA, with five- to sixfold more of these transcripts in red compared with white muscles. The putative LCFA transporters FAT/CD36 and FABP$_{pm}$ were greater in vesicles from red muscles compared with those derived from white muscles.

We thank P. M. H. W. Willemsen, Y. F. de Jong, and Y. Arumugam for expert technical assistance. We also thank Dr. N. Abumrad, SUNY (Stony Brook, NY), for providing us with the FAT cDNA and SSO and Dr. J. Schaffer, Washington University School of Medicine (St. Louis, MO), for providing us with the FATP cDNA.

This study was supported by a collaborative grant from the Canadian Natural Sciences and Engineering Research Council, the Medical Research Council of Canada, the Danish National Research Foundation, National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR-45168-01, and The Netherlands Heart Foundation.

Address for reprint requests: A. Bonen, Dept. of Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada N2L 3G1.

Received 6 October 1997; accepted in final form 22 April 1998.

REFERENCES


