Palmitate transport and fatty acid transporters in red and white muscles

A. Bonen,1 J. J. F. P. Luiken,2 S. Liu,3 D. J. Dyck,3 B. Kiens,2 S. Kristiansen,2 L. P. Turtotte,3 G. J. Van der Vusse,3 and J. F. C. Glatz4

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

Bonen, A., J. J. F. P. Luiken, S. Liu, D. J. Dyck, B. Kiens, S. Kristiansen, L. P. Turtotte, G. J. van der Vusse, and J. F. C. Glatz. Palmitate transport and fatty acid transporters in red and white muscles. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E471–E478, 1998.—We performed studies 1) to investigate the kinetics of palmitate transport into giant sarcolemmal vesicles, 2) to determine whether the transport capacity is greater in red muscles than in white muscles, and 3) to determine whether putative long-chain fatty acid (LCFA) transporters are more abundant in red than in white muscles. For these studies we used giant sarcolemmal vesicles, which contained cytoplasmic fatty acid binding protein (FABPc), an intravesicular fatty acid sink. Intravesicular FABPc concentrations were sufficiently high so as not to limit the uptake of palmitate under conditions of maximal palmitate uptake (i.e., 4.5-fold excess in white and 31.3-fold excess in red muscle vesicles). All of the palmitate taken up was recovered as unesterified palmitate. Palmitate uptake was reduced by phloretin (−50%), sulfo-N-succinimidyl oleate (−43%), anti-plasma membrane-bound FABP (FABP pm, −30%), trypsin (−45%), and when incubation temperature was lowered to 0°C (−70%). Palmitate uptake was also reduced by excess oleate (−65%), but not by excess octanoate or by glucose. Kinetic studies showed that maximal transport was 1.8-fold greater in red muscles than in white vesicles. The Michaelis-Menten constant in both types of vesicles was ~6 nM. Fatty acid transport protein mRNA and fatty acid translocase (FAT) mRNA were about fivefold greater in red muscles than in white muscles. FAT/CD36 and FABP pm proteins in red vesicles or in homogenates were greater than in white vesicles or homogenates (P < 0.05). These studies provide the first evidence of a protein-mediated LCFA transport system in skeletal muscle. In this tissue, palmitate transport rates are greater in red than in white muscles because more LCFA transporters are available.

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 LCFA 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 muscle 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 sarcoclemmal 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 sarcoclemmal 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 10 mM EDTA, and the 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% Nycodeenz (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 recentrifuged 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 addition of 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. Non-specific 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 determinations of fatty acid transport in giant vesicles (16). To determine LCFA transport, experiments in which the inhibition of palmitate uptake was examined, the inhibitors anti-plasma membrane-bound fatty acid binding protein (FABPpm; 8.1 µg/ml), phloretin (200 µM), and sulfon-N- succinimidyl diolate (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 (22, 27, 28) 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 (100 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 anti-rabbit 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 rapidly excised and frozen in liquid N₂. RNA was extracted from 100–150 mg of muscle by use of the method of Chomczynski and Sacchi (11). RNA integrity was determined on a 1% formaldehyde denaturing gel, separated on an agarose gel, and transferred overnight to an uncharged nylon membrane. To check that RNA (10 µg/lane) was intact and evenly loaded and to check transfer to the nylon membrane, RNA was stained with methylene blue to visualize 28S and 18S ribosomal bands (corrections for total RNA loading errors were made using the 18S signal RNA). RNA was probed with cDNAs for FAT (1) and fatty acid transport protein (FATP) (34) labeled with a randomized priming kit. Filters were prehybridized (1 h) and hybridized overnight (58°C, 6× standard sodium citrate containing 0.5 g/l each of Ficoll, polyvinylpyrrolidone, and BSA, 0.5% SDS, and 100 g/ml salmon sperm DNA). After filters were washed, they were exposed to X-ray film at ~80°C and to imaging screens for scanning and quantitation.

Determination of maximal enzyme activities and cytoplasmic FABP content. For the purposes of enzyme activity determinations, frozen muscle samples 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-nitrophenolphosphatase 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 bicinchoninic 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 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 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 $9.8 \pm 0.2$ 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 ($8.2 \pm 1.3$ µmol·mg protein⁻¹·h⁻¹) compared with muscle homogenates ($0.3 \pm 0.2$ 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
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 up-
take was saturable in both the red and white vesicles
(Fig. 2), suggesting that palmitate was being trans-
ported 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 $6 \text{nM}$ in both red and white
vesicles. This corresponds closely to the $K_m$ ($6 \text{nM}$) that
can be calculated from the palmitate uptake in per-
fused 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 be severely lim-
ited, and the plateau in palmitate uptake could then be
due to its limited solubility in an aqueous medium. For
this reason we determined the FABP$_c$ content in vesicles
derived from red and white muscle. FABP$_c$ 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, FABP$_c$ is calculated to be present in
excess, even at maximal rates of palmitate uptake,
because only 3.2 and 22% of the intravesicular FABP$_c$
in red and white vesicles, respectively, would be occu-
pied with palmitate.

To establish that palmitate was indeed transported
into the vesicles, additional experiments were per-
formed. Palmitate uptake was inhibited by anti-
FABP$_{pm}$ SSO, and trypsin and was reduced when
transport was examined at 0°C (Fig. 4). Anti-FABP$_{pm}$
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 µM 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 µM SSO in adipocytes (19). It was also
observed that palmitate uptake was lowered in the
presence of excess (100 µM) oleate but not in the
presence of octanoate (100 µM) 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 osmoti-
cally reactive space rather than binding to the plasma
membrane. Differences between glucose and palmitate
responses to the increase in extravesicular 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 FABP$_c$ concentrations. The increased FABP$_c$
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 $^3\text{H}$ label in the
cytosol compartment (Table 1). It was also found
that the intravesicular radiolabel was present in the vesicles
as unesterified $[^3\text{H}]$palmitate (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 mem-
branes from white muscles (Fig. 6), whereas FABP$_{pm}$
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

![Fig. 2. Palmitate transport in giant sarcolemmal vesicles obtained from red (A) and white (B) muscles (means ± SE; $n = 5$ separate experiments).](image-url)
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

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<td>Pellet</td>
<td>Cytosol</td>
<td>FA</td>
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<td>Palmitate</td>
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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 pm 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.

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 FABPp (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 radiolabeled palmitate in the cytosol from lysed vesicles. Importantly, we also found that all of the radiolabeled 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 FABPc (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 FABPc could limit the uptake of transport into the vesicles. However, we can calculate that the giant sarcolemmal vesicles contained more than adequate amounts of FABPc 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 FABPc is complexed with palmitate in red and white vesicles, respectively, at the maximal palmitate concentrations used in our studies. Alternatively stated, it appears that FABPc 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 FABPc 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_{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) cloned 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} (1), and FATP migrates at 63 kDa (34). Previously, rad et al. (1) and Schaffer and Lodish (34) cloned LCFA transporters FAT/CD36 and FABP_{pm} were present in red and white muscles. This greater number of LCFA transport proteins in the plasma membranes obtained from these oxidative muscles.

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. Therefore, we would expect that LCFA transport should also be greater in red than in white muscles. This was indeed observed: the V_{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.

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 divided 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.

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

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