Acute Regulation of Fatty Acid Uptake Involves the Cellular Redistribution of Fatty Acid Translocase*

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We used muscle contraction, which increases fatty acid oxidation, as a model to determine whether fatty acid transport is acutely regulated by fatty acid translocase (FAT/CD36). Palmitate uptake by giant vesicles, obtained from skeletal muscle, was increased by muscle contraction. Kinetic studies indicated that muscle contraction increased \( V_{\text{max}} \) but \( K_{m} \) remained unaltered. Sulfo-N-succinimidyl oleate, a specific inhibitor of FAT/CD36, fully blocked the contraction-induced increase in palmitate uptake. In giant vesicles from contracting muscles, plasma membrane FAT/CD36 was also increased in parallel with the increase in long chain fatty acid uptake. Further studies showed that like GLUT-4, FAT/CD36 is located in both the plasma membrane and intracellularly (endosomally). With muscle contraction, FAT/CD36 at the surface of the muscle was increased, while concomitantly, FAT/CD36 in the intracellular pool was reduced. Similar responses were observed for GLUT-4. We conclude that fatty acid uptake is subject to short term regulation by muscle contraction and involves the translocation of FAT/CD36 from intracellular stores to the sarcolemma, analogous to the regulation of glucose uptake by GLUT-4.

Long chain fatty acids (LCFAs)† are an important substrate in many tissues for a diversity of cellular processes such as membrane synthesis, protein modification, regulation of transcription, and intracellular signaling (1–5). In addition, LCFAs represent an important source of energy for tissues such as skeletal muscle and the heart (for review, see Refs. 6 and 7).

LCFAs can enter cells via both passive diffusion and a protein-mediated mechanism (for review, see Ref. 8). In the past few years a number of putative LCFA transporter proteins have also been identified. Fatty acid translocase, the rat homolog of human glycoprotein IV or CD36 (FAT/CD36) (9), and fatty acid transport protein (FATP) (5) are integral membrane proteins, whereas the plasma membrane-bound fatty acid-binding protein (FABPpm) (10) is a peripheral membrane protein. Each of these proteins can increase LCFA uptake when expressed in various cell lines (5, 10, 11). FABPpm and FAT/CD36 are expressed ubiquitously among many tissues (for review, see Ref. 12), with the notable exception that FAT/CD36 is not expressed in liver (9).

The physiologic function and regulation of these putative fatty acid transporters remain largely unknown. A recent study has shown that FATP mRNA and mitochondrial aspartate aminotransferase mRNA (identical to FABPpm; see Ref. 10), but not FAT mRNA, increase in parallel with increases in fatty acid uptake in adipocytes (9–13) and in Zucker rats with genetic obesity (fa/fa) or non-insulin-dependent diabetes mellitus (ZDF) (13). But no changes in fatty acid uptake were observed in hepatocytes in these animals (13). We have shown recently that chronic muscle contraction (7 days) results in the overexpression of FAT/CD36 along with a concomitant increase in LCFA uptake into giant sarcolemmal vesicles (14). In addition, in transgenic mice that overexpress FAT/CD36, LCFA oxidation is increased (15). These studies (13–15) indicate that fatty acid uptake is a physiologically regulatable process involving tissue-specific and LCFA transporter-specific responses.

Whether LCFA uptake can also be regulated acutely (i.e. within minutes) by LCFA transport proteins is not known. However, this can be examined in skeletal muscle. LCFA are a key oxidizable substrate for skeletal muscle (see Ref. 7), and because of its mass (40% of body weight) and highly variable metabolic rate, skeletal muscle is a principal site for the removal of LCFA from the circulation. Moreover, LCFA uptake and metabolism can be increased rapidly in contracting muscle (16, 17). This is unlikely to be caused by an increased rate of diffusion because the LCFA gradient across the plasma membrane is very high, even in resting muscles (18). It would seem therefore that a rapid increase in LCFA uptake may be mediated, in an unknown manner, by one or more of the LCFA transporters.

It is well known that glucose uptake is increased when GLUT-4 is translocated to the plasma membrane. Whether a similar mechanism can promote LCFA uptake is not known. However, it is possible that FAT/CD36 can be translocated because this protein was present in the plasma membrane as well as in high and low density microsomal fractions in Ob17PY fibroblasts that overexpressed FAT/CD36 (11). Therefore, we hypothesized that the rapid, contraction-induced increase in LCFA uptake by intact muscle (17) may have been mediated by the translocation of FAT/CD36, analogous to the contraction-stimulated translocation of GLUT-4 to the surface of this tissue (19, 20). Therefore, we have examined the acute (1–50 min) contraction-induced uptake of palmitate by giant sarcolemmal vesicles, a preparation that diverts LCFA uptake from its metabolism and is suitable for the study of LCFA...
transport across the sarcolemma (20, 21). In addition, we have used a muscle fractionation procedure to detect surface and intracellular pools of FAT/CD36. These studies have shown, for the first time, that LCFA uptake in muscle tissue is subject to short term regulation by muscle contraction and involves the translocation of FAT/CD36 from intracellular stores to the sarcolemma, analogous to the regulation of glucose uptake by GLUT-4.

MATERIALS AND METHODS

Female Harlan Sprague-Dawley rats were used in all experiments (200–250 g). Animals were housed in a controlled environment on a 12-h light/12-h dark cycle and fed Purina rat chow ad libitum. All procedures were approved by the animal care committee at the University of Waterloo. Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (6 mg/100 g of body mass) prior to all experimental procedures.

Design of Palmitate Uptake Studies by Giant Sarcolemmal Vesicles—To examine the transport of fatty acids across the plasma membranes, giant sarcolemmal vesicles were obtained from resting and contracting rat hind limb skeletal muscles. While the animal was under anesthesia a small incision was made in one hind limb, and two uninsulated electrodes were placed on the exposed sciatic nerve. The contralateral muscles in the same animal served as resting controls in all experiments. Muscle contraction consisted of stimulating the sciatic nerve in one hind limb (10–20 V; 100 Hz, 100-ms trains, 20 tetani/min for 30 min (unless otherwise noted)). Immediately after the contraction (unless otherwise noted) the contracting muscles and the contralateral resting muscles were removed for the preparation of giant sarcolemmal vesicles (see below).

Several different experiments were performed. In one series of experiments we examined palmitate uptake into giant sarcolemmal vesicles at selected times during muscle contraction (min 1, 5, and 30) as well as 20 and 45 min after the muscle contraction had ceased. In other experiments we examined the effects of different rates of muscle contraction (20, 40 tetani/min) on palmitate uptake by giant sarcolemmal vesicles. We also examined the effects of muscle contraction (20 tetani/min) on the kinetics of palmitate uptake into giant sarcolemmal vesicles. Finally, we examined whether the contraction-induced increases in vesicular palmitate uptake were altered by sulfo-N-succinimidyl oleate (SSO), a nonpermeable sulfo succinimidyl derivative of LCFA which binds covalently to FAT/CD36 (11, 23) (parity = ~90%, gift from Dr. N. A. Abumrad, SUNY Stony Brook, Stony Brook, NY).

Giant Sarcolemmal Vesicle Preparation—Vesicles from resting and contracting muscles were prepared as we have described recently (21, 22). Briefly, rat hind limb muscles were cut into thin layers (~1–3 mm thick) and incubated for 1 h at 34 °C in 140 mM KCl and 10 mM MOPS (pH 7.4), 150 units/ml collagenase, and 0.01 g/ml aprotinin. The muscle was homogenized in 1 ml of KCl/MOPS at a ratio of 1 g muscle/3 ml of buffer A by five strokes using a tightly fitting Potter-Elvehjem glass homogenizer and subjected to a continuous Percoll gradient as described below. The LiBr-treated vesicles were preincubated with 1.4 ml of ice-cold KCl/MOPS containing 2.5 mM NaHCO3 (pH 7.0), 0.25 mM sucrose, 5 mM NaN3, and 100 μM phenylmethanesulfonyl fluoride (buffer A). The mixed vesicles were homogenized (1 g/15-ml dilution) using a Polytron (Brinkmann Instruments) at a low setting of 5 for 5 × 1 s. The resulting homogenate was centrifuged at 1,300 × g for 60 min. The supernatant was saved, and the low speed pelleted vesicles were resuspended in buffer A (2 g/15 ml), homogenized, and centrifuged again. Both supernatant fractions were pooled. The low-speed pellet was resuspended in 0.5 ml of LiBr, 50 mM Tris (pH 8.5), and 100 μM phenylmethanesulfonyl fluoride (buffer B) at a ratio of 1 g of tissue/25 ml and stirred for 4 h. The pooled, 1,300 × g supernatant fractions were centrifuged at 9,000 × g for 10 min. The 9,000 × g pellet was saved, and the supernatant was spun at 190,000 × g for 1 h. The 190,000 × g pellet was resuspended in 800 μl of buffer A by five strokes using a tightly fitting Potter-Elvehjem glass homogenizer and subjected to a continuous Percoll gradient as described below. The LiBr-treated vesicles were preincubated with 1.4 ml of ice-cold KCl/MOPS containing 2.5 mM NaHCO3 (pH 7.0), 0.25 mM sucrose, 5 mM NaN3, and 100 μM phenylmethanesulfonyl fluoride (buffer A). The mixed vesicles were homogenized (1 g/15 ml), centrifuged, and centrifuged again. Both supernatant fractions were pooled. The low-speed pellet was resuspended in 0.5 ml of LiBr, 50 mM Tris (pH 8.5), and 100 μM phenylmethanesulfonyl fluoride (buffer B) at a ratio of 1 g of tissue/25 ml and stirred for 4 h. The pooled, 1,300 × g supernatant fractions were centrifuged at 9,000 × g for 10 min. The 9,000 × g pellet was saved, and the supernatant was spun at 190,000 × g for 1 h.

RESULTS

Palmitate Uptake by Giant Sarcolemmal Vesicles

Palmitate Uptake during and after Muscle Contraction—Uptake of palmitate by giant vesicles prepared from contracting muscles was increased (Fig. 1A, p < 0.05). An increase was already apparent by 1 min (~20%), and after 5 min the transport rate was increased significantly (~29%) from that observed in control muscles (p < 0.05, Fig. 1A). By the end of the 30-min contraction period the palmitate uptake had increased even further (~75%) (p < 0.05). After muscle contraction was terminated the rate of palmitate uptake was reduced in a time-dependent manner (Fig. 1A) (p < 0.05). 50 min after the contraction had ended, palmitate uptake had returned to levels

2 A. Bonen, unpublished data.
Methods. Noncontracting muscles have been grouped at
Giant sarcolemmal vesicles were prepared at the end of contraction, and palmitate uptake was determined as described under "Materials and
min at 20 and 40 tetani/min. The contralateral muscles from the same animal served as noncontracting controls at each intensity of stimulation.

Observations of SSO-treated vesicles obtained from resting and contracting
muscles (Fig. 3). Thus, SSO was able to block completely the
increase in the contraction-induced vesicular palmitate uptake (Fig. 3).

Palmitate Uptake and FAT/CD36 Protein in Giant Sarcolemmal Vesicles

To compare the changes in palmitate palmitate uptake with the changes in FAT/CD36, each of these parameters was determined in giant vesicles obtained from muscles at rest, at the end of 30-min contraction, and after recovery from muscle contraction (Fig. 4). After 30 min of contraction there was a marked increase in palmitate uptake (+65%, p < 0.05) and in FAT/CD36 (+40%, p < 0.05). At the end of the recovery period palmitate uptake and FAT/CD36 had both returned to levels observed in vesicles obtained from resting muscles (Fig. 4, p > 0.05). During these experiments there were no changes in the membrane-associated MCT1 (data not shown).

Subcellular Distribution of GLUT-1, GLUT-4, Transferrin, and FAT/CD36

Fractions of the muscle samples obtained by continuous Per-
coll density gradients were characterized immunologically and enzymatically (Table I and Fig. 5). GLUT-1, MCT1, and 5'-nucleotidase were most prominently present in the surface muscle fractions and not detected in the intracellular fractions (Table I). The transferrin receptor has been used as a marker of the light microsomal/endosomal compartment (33, 34). In the present studies, the transferrin receptor was present both in the intracellular fraction and at the surface of the membrane (Table I). GLUT-4 was present in greater quantities in the intracellular fraction and at the surface of the membrane (Fig. 5). The transferrin receptor was present both in the intracellular fraction and at the surface of the membrane (Table I). GLUT-4 was present in greater quantities in the intracellular fraction and at the surface of the muscle (Fig. 5). As expected, insulin reduced the intracellular GLUT-4 and increased the surface GLUT-4 (Fig. 5). The fractions obtained from resting muscle showed a large pool of FAT/CD36 at the

FIG. 1. Palmitate uptake by giant sarcolemmal vesicles at selected time points during muscle contraction (panel A) and at selected
rates of muscle contraction (panel B) (mean ± S.E.). Panel A, muscles were electrically stimulated via the sciatic nerve to contract for 1, 5, and 30 min. Thereafter, muscles recovered from contraction for 20 and 45 min. The contralateral muscles from the same animal served noncontracting controls at each time point. Giant sarcolemmal vesicles were prepared at each time point, and palmitate uptake was determined as described under "Materials and Methods." Noncontracting muscles have been grouped at

Palmitate Kinetics during Muscle Contraction—To examine the effects of muscle contraction on the kinetics of palmitate uptake, rat hind limb muscles remained at rest or were stimulated to contract at either 20 or 40 tetani/min. The increase in palmitate uptake increased in direct proportion to
the rate of muscle stimulation (Fig. 1B). At 20 and 40 tetani/
min the palmitate uptake rates were increased 28% (p < 0.05)
and +56% (p < 0.05), respectively, compared with the uptake
rates in resting control muscles (Fig. 1B).

Palmitate Kinetics during Muscle Contraction—To examine the effects of muscle contraction on the kinetics of palmitate uptake, rat hind limb muscles remained at rest or were stimulated to contract at either 20 or 40 tetani/min. Palmitate uptake (Vmax), by vesicles prepared from contracting muscles, was increased ~40% (p < 0.05). The Km (~ 15 nM) was not altered (Fig. 2).

Effects of Transporter Inhibitor on Contraction-induced Palmitate Uptake

To determine if the contraction-induced increase in palmitate uptake was protein-mediated we compared palmitate uptake at rest and at the end of the 30-min contraction, either in the absence or presence of SSO, which binds covalently (23, 31, 32) and specifically only to FAT/CD36 (22). For these purposes vesicles were obtained from resting and contracting muscles. The vesicles from each treatment were then subdivided into
two pools, those that were pretreated with SSO and those that were not. In the absence of SSO there was a contraction-
induced increase in palmitate uptake (+47%, p < 0.05) (Fig. 3). In vesicles from resting muscles, SSO reduced vesicular palmitate uptake by 58% (p < 0.05). Palmitate uptake rates did not differ (p > 0.05) in SSO-treated vesicles obtained from resting and contracting

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surface of the muscle as well as an intracellular pool of FAT/CD36 (Fig. 5).

Effects of Muscle Contraction on the Redistribution of FAT/CD36

Our primary aim was to examine the effects of muscle contraction on the subcellular redistribution of FAT/CD36. As a positive control for these studies we also examined the effects of electrically induced muscle contraction on GLUT-1, MCT1, transferrin receptor (Table I), and GLUT-4 (Fig. 5). The GLUT-1, MCT1, and transferrin receptor distributions were not altered by muscle contraction (Table I), whereas GLUT-4 was reduced in the intracellular pool \((p < 0.05; \text{Fig. } 5)\) and was increased in muscle surface fractions \((p < 0.05; \text{Fig. } 5)\). These results for GLUT-1 and GLUT-4 are consistent with previous reports (20, 29, 30).

With 30 min of electrically induced muscle contraction the intracellular FAT/CD36 was reduced, and the surface FAT/CD36 was increased \((p < 0.05; \text{Fig. } 5)\). This paralleled the responses of GLUT-4 (Fig. 5).

DISCUSSION

There are a number of novel observations in the present studies. First, by using a muscle contraction model we have shown, for the first time, that fatty acid uptake can be acutely regulated. Second, FAT/CD36, one of the fatty acid transport proteins, is present both at the plasma membrane and in an intracellular pool in skeletal muscle. Third, with muscle contraction FAT/CD36 is translocated from an intracellular pool to the surface of the muscle. Finally, SSO, which binds covalently to FAT/CD36 (23), inhibited the contraction-induced increase in LCFA uptake. Thus, our studies have shown that the uptake of fatty acids across the plasma membrane is regulated acutely by muscle contraction, involving the translocation of FAT/CD36 from an intracellular pool to the surface of the muscle.

One of the strengths of our work is that we have used the giant sarcolemmal vesicle preparation to examine LCFA transport. It is well known that the determination of protein-mediated uptake of substrates across the plasma membrane requires a system in which transport and metabolism are necessarily divorced. To date, determinations of protein-mediated LCFA uptake in tissues such as adipocytes (13, 35), hepato-
Heart are an ideal system for the study of protein-mediated events at the plasma membrane or enzymatic steps in LCFA metabolism. The giant sarcolemmal vesicle preparation used in this study avoids entirely the problem of metabolism (diabetes (40), obesity (13), fasting (41)) or by chronically increased muscle activity (14, 42). Until the present studies there had been no evidence to indicate that LCFA uptake is regulated acutely by a fatty acid transport protein, although based on work in perfused, contracting muscle it was speculated that this might be the case (43).

We have shown previously that in muscle and heart, vesicular palmitate uptake is protein-mediated because it can be inhibited by trypsin and phloretin. Moreover, oleate competes with palmitate uptake (22). There are a number of candidate LCFA transporters (FAT/CD36, FATP1, FABPpm) that are implicated in LCFA uptake in a number of tissues (8, 12). Among these, FAT/CD36 appears to be a key LCFA transport protein in skeletal muscle. When FAT/CD36 expression was increased in chronically stimulated muscles (24 h/day, 7 days), palmitate uptake by giant sarcolemmal vesicles was increased (14). In transgenic mice that overexpress FAT/CD36, palmitate oxidation is increased, but only when muscles are contracting (15). A null mutation in FAT/CD36 reduced adipocyte LCFA uptake and increased circulating levels of fatty acids (44), indicating that LCFA uptake by peripheral tissues was impaired. Collectively, these studies have begun to show that FAT/CD36 is a key LCFA transport protein in adipocytes and skeletal muscle.

The inhibition of the contraction-induced increase in LCFA uptake by giant vesicles is fully recovered as palmitate from inside the vesicles; and (d) none of the palmitate taken up is esterified, oxidized, or associated with the plasma membrane. Thus, the giant vesicle preparation used in the present study is a well characterized, appropriate system for examining protein-mediated LCFA uptake across the plasma membrane.

In recent years the identification of a number of LCFA transport proteins has led to studies showing that LCFA transporters or their transcripts may be altered by chronic alterations in metabolism (diabetes (40), obesity (13), fasting (41)) or by chronically increased muscle activity (14, 42). Until the present studies there had been no evidence to indicate that LCFA uptake is regulated acutely by a fatty acid transport protein, although based on work in perfused, contracting muscle it was speculated that this might be the case (43).

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transport by SSO provides evidence that the fatty acid transport protein FAT/CD36 promoted the contraction-induced increase in vesicular fatty acid uptake. We have shown previously that SSO, a sulfoamidomethyl derivative of oleate, inhibits LCFA uptake in muscle and heart, but not in liver, which does not express FAT/CD36 (22). The inhibition of LCFA uptake by SSO varies among tissues (8). Our studies suggest that this is attributable to differences in the surface availability of FAT/CD36. In giant vesicles from the heart, which contain more FAT/CD36 than giant vesicles obtained from skeletal muscles, SSO inhibits LCFA uptake more (70% inhibition) than in skeletal muscle vesicles (50% inhibition) (22). Similarly, in the present studies, SSO reduced vesicular LCFA uptake further in contracting muscles (58% inhibition) than in resting muscles (30% inhibition) because plasma membrane concentrations of FAT/CD36 were increased in contracting muscles. In our studies (22) and others (8) SSO does not fully suppress LCFA uptake, and this implies that other mechanisms such as LCFA diffusion and/or the role of other LCFA transport proteins are also important in taking up LCFA across the plasma membrane. Nevertheless, the complete inhibition of the contraction-induced uptake of palmitate by SSO in the present studies indicates that the function of this transport protein is associated with a need to increase the uptake of LCFA when the rate of their oxidation is increased (i.e. during muscle contraction). Support for this is also derived from our studies with transgenic mice that overexpress FAT/CD36. In muscles from these animals, skeletal muscle LCFA oxidation was increased, but only when muscles were contracting, not when muscles remained at rest (15).

Several lines of evidence pointed toward a translocation mechanism for increasing LCFA uptake by FAT/CD36. The kinetic studies demonstrated an increase in $V_{\text{max}}$ in vesicles derived from stimulated muscles, suggesting that muscle contraction increased the number of transport proteins at the surface of the muscle. This was confirmed because contraction increased the amount of vesicular FAT/CD36. This increase was not attributable to de novo protein synthesis because there was no increase in total FAT/CD36 during the 30-min contraction period (data not shown). Therefore, the increase in vesicular FAT/CD36 was most likely accounted for by a translocation of FAT/CD36 from an intracellular compartment(s) to the surface.

Although the translocation of GLUT-4 is a well recognized mechanism by which glucose flux into the muscle is increased (for review, see Ref. 29), it was not known previously that there was also an intracellular, translocatable FAT/CD36 pool in muscle. Our fractionation studies established that like GLUT-4, FAT/CD36 was present both at the surface of the muscle and in an intracellular pool. Consistent with other studies (29, 30), muscle contraction translocated GLUT-4, but not GLUT-1, to the muscle surface. The important new observation is that muscle contraction also translocated FAT/CD36.

The redistribution of GLUT-4 among subcellular compartments in muscle or fat cells has been taken, for many years, as evidence that this glucose transporter is translocated from intracellular sites to the surface of the tissue, thereby stimulating glucose uptake. A similar interpretation is therefore warranted in the present studies, namely that FAT/CD36 is translocated from an intracellular compartment to the surface of the muscle, which then facilitates an increase in LCFA uptake. The changes in LCFA uptake and FAT/CD36 in contracting muscle are not simply correlate, but these are causally related. The evidence for this are the studies with SSO. SSO binds covalently to FAT/CD36 (23), and SSO inhibits LCFA transport in tissues that express FAT/CD36 (muscle and heart (22)), but not in tissues in which FAT/CD36 is not expressed (i.e. liver (22)). In the present studies SSO inhibited the contraction-induced palmitate transport. Because of this critical experiment and other studies (22, 23), the parallel changes in vesicular LCFA uptake and FAT/CD36 in the plasma membrane, during and after contraction, can be seen as being related in a causal manner. Presumably, there are also other physiologic stimuli that can also increase LCFA uptake via the translocation of FAT/CD36; these, however, remain to be identified.

The exact mechanism of protein-facilitated LCFA transport is not known. Besides FAT/CD36 there are several additional fatty acid transporter proteins (FABPpm (10) and FATP (5, 45)) that may also be involved in the transport process. A model involving their potential interactions has been presented (46), but experimental evidence is lacking for this model. Recently, we have shown that FATP expression in giant vesicles is correlated inversely with fatty acid uptake, whereas both FAT/CD36 and FABPpm are correlated positively with fatty acid uptake in giant vesicles obtained from heart and red and white muscles (22). We have also observed that FABPpm and FAT/CD36 may interact with each other to facilitate LCFA uptake across the sarcolemma (22). Thus, if FABPpm is present in excess at the plasma membrane, then the translocation of FAT/CD36, and its possible association with FABPpm, may augment the rate of LCFA transfer from FABPpm because of an increased number of FAT/CD36 proteins.

Our findings may also have clinical relevance. In animal models of metabolic diseases (obesity, diabetes) LCFA uptake is increased in adipocytes and cardiac myocytes (13). Although this may be associated with altered expression of LCFA transport proteins, it is also possible that the translocation of FAT/CD36 is altered. It is now well known that insulin resistance is often associated with postreceptor defects in the signaling pathway that stimulates GLUT-4 translocation to the cell’s surface (29, 47). Moreover, it will also be important to determine how alterations in glucose and LCFA transport are related because LCFA can impair insulin action (48).

In summary, we have shown that muscle contraction increases LCFA uptake into giant sarcolemmal vesicles. Kinetic studies revealed that contraction increased $V_{\text{max}}$ but not $K_{m}$ of palmitate uptake. Inhibition of the contraction-induced LCFA uptake by SSO, a specific inhibitor of FAT/CD36, indicated that this transport protein was the key component in augmenting LCFA uptake. The increase in $V_{\text{max}}$ is consistent with the increase in FAT/CD36 in the plasma membrane of giant sarcolemmal vesicles. Further studies have shown that FAT/CD36 is redistributed from an intracellular location to the surface of the muscle.

**Fig. 5.** Effects of muscle contraction on the subcellular redistribution of GLUT-4 (upper panel) and FAT/CD36 (lower panel) in continuous Percoll gradient fractions obtained from rat skeletal muscles. Muscles were electrically stimulated via the sciatic nerve to contract for 30 min at 40 tetani/min. The contralateral muscles from the same animal served as noncontracting control muscles. Percoll gradients were prepared as described under “Materials and Methods.” Western blotting was performed on the Percoll fractions as described under “Materials and Methods.” Representative blots for GLUT-4 and FAT/CD36 are shown. These blot is, however, not directly comparable to the plotted data. Western blots are based on 5 μg of protein in each lane, whereas the plotted data are based on the total protein content of each of the fractions examined. Data are the means of four independent control and contraction experiments (error bars have been omitted for the sake of clarity). Fractions 1–15 are derived from the 190,000 × g pellet (muscle surface) and fractions 16–30 from the 190,000 × g pellet derived from the LiBr extraction of a low spin pellet.
the muscle, indicating that this protein is being translocated in skeletal muscle, when the need for LCFA metabolism is increased by muscle contraction. This is similar to the contraction-induced translocation of GLUT-4 from an intracellular compartment to the surface of the muscle. Whether other physiologic stimuli can also translocate FAT/CD36 is not known. Thus, the present studies have identified an entirely new mechanism regulating fatty uptake by contracting skeletal muscle. Whether fatty acid transport represents the rate-limiting step in fatty acid metabolism remains to be determined.

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