A Novel Function for Fatty Acid Translocase (FAT)/CD36

INVolvement in long chain fatty acid transfer into the mitochondria*

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Fatty acid translocase (FAT)/CD36 is a long chain fatty acid transporter present at the plasma membrane, as well as in intracellular pools of skeletal muscle. In this study, we assessed the unexpected presence of FAT/CD36 in both subsarcolemmal and intermyofibril fractions of highly purified mitochondria. Functional assessments demonstrated that the mitochondria could bind ^14C-labeled palmitate, but could only oxidize it in the presence of carnitine. However, the addition of sulfo-N-succinimidyl oleate, a known inhibitor of FAT/CD36, resulted in an 87 and 85% reduction of palmitate oxidation in subsarcolemmal and intermyofibril fractions, respectively. Further studies revealed that maximal carnitine palmitoyltransferase I (CPTI) activity in vitro was inhibited by succinimidyl oleate (42 and 48% reduction). Interestingly, CPTI immunoprecipitated with FAT/CD36, indicating a physical pairing. Tissue differences in mitochondrial FAT/CD36 protein follow the same pattern as the capacity for fatty acid oxidation (heart > red muscle > white muscle). Additionally, chronic stimulation of hindlimb muscles (7 days) increased FAT/CD36 expression and also resulted in a concomitant increase in mitochondrial FAT/CD36 content (46 and 47% increase). Interestingly, with acute electrical stimulation of hindlimb muscles (30 min), FAT/CD36 expression was not altered, but there was an increase in the mitochondrial content of FAT/CD36 compared with the non-stimulated control limb (35 and 37% increase). Together, these data suggest a role for FAT/CD36 in mitochondrial long chain fatty acid uptake and demonstrate system flexibility to match FAT/CD36 mitochondrial content with an increased capacity for fatty acid oxidation, possibly involving translocation of FAT/CD36 to the mitochondria.

Circulating long chain fatty acids (LCFA)¹ are an important source of energy for tissues such as skeletal muscle and the heart. After entering the myocyte, LCFA are esterified or are transported into the mitochondria for oxidation and subsequent energy production. Traditionally, only the carnitine-palmitoyl transferase (CPT) system is thought to be involved in the movement of LCFA across the mitochondrial membranes, as CPT catalyzes the trans-esterification of fatty acyl CoA to acylcarnitine. The acylcarnitine can then be translocated to the inner mitochondrial membrane by carnitine:acylcarnitine translocase, and finally acylcarnitine is regenerated to acyl CoA by the latent CPTII in the mitochondrial matrix (1). However, recent studies (2–4) have demonstrated that the regulation of CPT cannot fully explain changes in mitochondrial fatty acid uptake, particularly when the muscle energy demand is increased. Malonyl CoA, thought to be the principal regulator of CPT (5, 6), is not responsible for increases in fatty acid oxidation during low to moderate aerobic exercise (2–4), or decreases in fatty acid oxidation during higher intensity exercise (3). Furthermore, other potential regulators have been demonstrated to have a minimal effect upon CPT activity as well (7, 8). Hence, other fatty acid-binding proteins may be involved in facilitating the movement of LCFA into the mitochondria.

Fatty acid translocase (FAT/CD36) has been implicated in multiple biological processes and is known to bind a remarkably diverse collection of ligands, including thrombospordin-I, retinal photoreceptor outer segments, anionic phospholipids, apoptotic cells, and LCFA (9). Additionally, FAT/CD36 exists in an intracellular pool in both skeletal and cardiac muscle and is translocated to the plasma membrane to assist with LCFA uptake (10, 11). Thus FAT/CD36 could possibly play a role in long chain fatty acid transport in mitochondria, but it is not known if FAT/CD36 is present at the mitochondrial membrane. However, protein expression of FAT/CD36 correlates with oxidative potential of muscle tissue, being highly expressed in heart and muscles rich in oxidative fibers, whereas lower expression occurs in muscles that are predominantly glycolytic (12). A powerful tool in the functional assessment of FAT/CD36 has been sulfo-N-succinimidyld esters of long chain fatty acids. Sulfo-N-succinimidyld oleate (SSO) and sulfo-N-succinimidyld oleate palmitate (SSP) specifically bind to the 88-kDa protein FAT/CD36 and arrest the LCFA transport into adipocytes (13), heart (14), and skeletal muscle (11, 15, 16). Neither SSO nor SSP affects cellular handling of

¹ The abbreviations used are: LCFA, long chain fatty acids; CPT, carnitine-palmitoyl transferase; FAT, fatty acid translocase; SSO, sulfo-N-succinimidyld oleate; SSP, sulfo-N-succinimidyld palmitate; SS, subsarcolemmal; IMF, intermyofibrillar.
LCFAs, with the relative contribution of oxidation and esterification remaining constant, whereas glucose and octanoate uptake are also unaffected (14, 17). Hence, SSO and SSP are excellent substrates with which to examine the functional role of FAT/CD36 in lipid metabolism.

Previous studies examining FAT/CD36 have demonstrated that, similar to GLUT4, there is an intracellular/endosomal pool of FAT/CD36 that can be mobilized to the plasma membrane upon stimulation by muscle contraction (11) or insulin (15). It appeared that the quantity of FAT/CD36 translocated to the plasma membrane scaled with energetic demands during muscle contraction (11). These studies have demonstrated the important role that FAT/CD36 plays in lipid transport at the plasma membrane, although the full extent of its cellular contribution remains unknown. Nevertheless, given the multifunctionality of this protein, its demonstrated intracellular presence, and its known involvement in LCFA transport in skeletal muscle, it is conceivable that FAT/CD36 may be involved in the transfer of LCFAEs into the mitochondria.

Translocation of proteins to the mitochondria has recently been documented. Mitochondrial proteins synthesized in the cytosol often follow a general insertion mechanism that has been studied in detail (18, 19). Briefly, translocation of outer membrane proteins recognizes the majority of precursor proteins destined for internalization into the mitochondria (18, 19), which are translocated across the mitochondrial membranes through a general insertion pore. However, it has been demonstrated recently that the voltage-dependent anion channel (also called porin) can be targeted directly to the outer membrane without being internalized (20). This alternate pathway could allow for a more rapid insertion of proteins into the mitochondrial outer membrane. Finally, protein translocation to and from the mitochondria during the process of apoptosis also demonstrates the membrane fluidity of both the inner and outer mitochondrial membranes (21). Thus, translocation of FAT/CD36 from other cellular locations to the mitochondria is perhaps feasible and could facilitate in up-regulating mitochondrial uptake of LCFAEs in response to increased oxidative demand in muscle tissue.

It is important to note that there are two distinct populations of mitochondria in skeletal muscle, the subsarcolemmal (SS) and the intermyofibrillar (IMF) mitochondria. A recent study demonstrated no differences in CPTI maximal activity or its expression remaining constant, whereas glucose and octanoate uptake are also unaffected (14, 17). Hence, SSO and SSP are excellent substrates with which to examine the functional role of FAT/CD36 in lipid metabolism.

Mitochondrial Isolation
To obtain an isolated mitochondrial fraction, differential centrifugation was used (22), with some minor modifications. Briefly, minced muscles were diluted 10-fold in Buffer 1 (100 mM KCl, 50 mM Tris-HCl, 5 mM MgSO4, 5 mM EDTA, pH 7.4) and homogenized using a polytron at the precise low setting of 3 (Kinematica, Switzerland) for 2 × 15 s. The SS mitochondria were isolated from the myofibrils by centrifugation at 800 × g for 10 min. The SS mitochondria were pelleted from the supernatant at 9000 × g (10 min). The pellet was washed twice in Buffer 1, and resuspended in 0.1 mM ATP, spun at 9000 × g (for 10 min), and resuspended in a final volume of 100 μl. The myofibrillar pellet containing the IMF mitochondria was rehomogenized using the polytron and spun again at 800 × g for 10 min. The supernatant was discarded, and the pellet was diluted 10-fold in Buffer 2 and treated with protease (P5380, 0.025 ml/g tissue, Sigma) for exactly 5 min. Addition of 15 ml of ice cold Buffer 2 arrested the protease, and the samples were centrifuged at 5000 × g for 5 min. The pellet was resuspended in a 10-fold dilution of Buffer 2 and spun at 800 × g (10 min). The supernatant was spun at 9000 × g for 10 min, and the pellet was washed twice in Buffer 2, re-spun at 9000 × g, and resuspended in a final volume of 150 μl. These separations yield viable SS and IMF mitochondria that can be used for functional in vitro studies. For Western blotting, samples were purified further by using a Percoll gradient. Samples were spun at 20,000 × g for 2 h, and the mitochondrial layer was removed. The Percoll was removed from the sample by further spinning at 21,000 × g for 5 h. At this point, the mitochondria were no longer viable, but were useful for Western blotting.

Palmitate Binding and Oxidation
Viable mitochondria were pre-incubated in a 20-ml vial with a rubber cap containing 1.0 ml of modified Krebs buffer (MKB; 115 mM NaCl, 2.6 mM KCl, 1.2 mM KH2PO4, 10 mM NaHCO3, 10 mM HEPES, pH 7.4) at 37 °C for 15 min (gassed with 5% CO2:95% O2 and constant shaking). For palmitate oxidation studies, the buffer was supplemented with 5.0 mM ATP, 1.0 mM NAD+, 0.5 mM Na-carnitine, 0.1 mM coenzyme A, 25 μM cytochrome c, and 0.5 mM malate. The radiolabel [14C]palmitate (0.5 μCi, 185 nM palmitate) was used in all experiments. Reactions were initiated by the addition of a 6.1 palmitate:BSA complex, proceeded at 37 °C for 60 min (unless otherwise noted), and was terminated by the addition of ice-cold 3 M perchloric acid administered by syringe through the rubber cap. For palmitate binding studies, mitochondria were washed 3× with MKB, 5 ml of scintillation fluid was added, and the radioactivity was determined. Palmitate oxidation was determined as described previously (27, 28), ensuring that both the CO2 and the acid-soluble products were measured.

In Vivo Acute and Chronic Electrical Stimulation
To ascertain whether FAT/CD36 in SS and IMF mitochondria was increased with perturbations known to increase the capacity for fatty acid oxidation, muscles were chronically (7 days) or acutely (30 min) stimulated electrically as described previously in work from our laboratory (11, 29).

Chronic Stimulation—In anesthetized rats, stainless steel electrodes were sutured to underlying muscles on either side of the peroneal nerve. These electrodes were passed subcutaneously from the thigh of the stimulated limb to the back of the neck, where they were attached to an external miniature electronic stimulator. The contralateral limb was sham-operated to act as a control. Animals were allowed 7 days to recover from surgery before stimulating pulses (12 Hz, 50-μs duration) were initiated. The peroneal nerve, which innervates the tibiae anterior and extensor digitorum longus muscles, was stimulated for 24 h/day for 7 days. Twenty-four hours after terminating stimulation, the contracting muscles and the contralateral resting muscle were removed for mitochondrial isolation.

Acute Stimulation—Under anesthesia, a small incision was made in one hind limb, and stimulating 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 V, 100 Hz, 100-ms trains, 20 tetani/min for 30 min). Immediately after stimulation, the contracting muscles and the contralateral resting muscle were removed for mitochondrial isolation.

Carnitine Palmitoyltransferase I Activity
CPT I (EC 2.3.1.21) activity measurements were carried out with the sensitive forward radioisotope assay, as described previously (30) and modified by Starritt et al. (7). Briefly, this assay measures the amount of carnitine palmitoyltransferase I activity.
of labeled palmitoyl-l-carnitine formed when both palmitoyl-CoA and radiolabeled [3H]carnitine were added to the medium surrounding the intact mitochondria. The reaction was initiated by the addition of 10 μl of mitochondrial suspension and was stopped 6 min later by the addition of 60 μl of ice-cold 1 M HCl. Palmitoyl-[3H]carnitine formed during the reaction was extracted with 400 μl of water-saturated butanol in a process involving three washes with distilled water and subsequent centrifugations (1000 × g for 5 min) to separate the butanol phase. Radioactivity was measured in 100 μl of the butanol layer and 5 ml of liquid scintillation mixture. All assays were performed in duplicate at 37 °C.

Immunoprecipitation and Western Blotting

Samples for immunoprecipitation were prepared using the Catch & Release immunoprecipitation system as described by the manufacturer (Upstate Biotechnology). Immunoprecipitated samples and purified, isolated mitochondria fractions were analyzed for total protein (BCA protein assay), and 100 μg of denatured proteins from each sample were separated by electrophoresis on a SDS-7.5% polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. The monoclonal antibody MO26 was used to detect FAT/CD36 (31) and a polyclonal 1° antibody was used to detect CPTI (32). Commercially available antibodies were used to detect cytochrome c oxidase (Santa Cruz Biotechnology), Na+/K’ATPase α1 (Upstate Biotechnology), and SERCA1 ATPase (Affinity Bioreagents Inc.). An internal control of previously extracted rat muscle was used in each gel to normalize for variation in signal observed across the membranes. Blots were quantitated using chemiluminescence and the ChemiGenius2 bio-imaging system (Syngene, Cambridge, UK).

Statistics

All data are reported as mean ± S.E. Statistical comparisons were performed using an analysis of variance, with a least squares Fisher test for post hoc comparison. Significance was accepted at p < 0.05.

RESULTS

Mitochondrial Purification

Western blotting performed on purified mitochondrial extract demonstrated the presence of FAT/CD36 (88 kDa) and the cytochrome c oxidase IV subunit (22 kDa) (Fig. 1, A and B, respectively). In contrast, the plasma membrane protein Na+/K’ATPase α-1, the sarcoplasmic reticulum protein Ca2+-ATPase and the transferrin receptor, an endosomal marker, were not detectable (Fig. 1, C–E, respectively). These results indicate that the isolation procedure successfully yielded highly purified SS and IMF mitochondria without contamination from other sources.

Tissue Comparison

Examination of the presence of FAT/CD36 in the mitochondria of various muscle tissues demonstrated that the quantity of this protein corresponded to differences in the oxidative capacity of these three types of muscle tissues (Fig. 2). The SS and IMF FAT/CD36 in each tissue did not differ (p = 0.11), but FAT/CD36 protein was lower in red (52.5 and 50.7% lower, SS and IMF) and white (67.2 and 68.3% lower, SS and IMF) gastrocnemius when compared with the heart (p < 0.05). Additionally, FAT/CD36 protein was lower in white gastrocnemius (30.9 and 36.4% lower, SS and IMF) when compared with red gastrocnemius (p < 0.05).

Functional Assessments

Fatty Acid Binding—In the absence of carnitine, the mitochondria are unable to transport the LCFA into the matrix; hence, further metabolism can not occur. Therefore, by omitting carnitine from the incubating medium, we were able to determine whether the mitochondria could bind LCFA and its sulfo-N-succinimidy esters, SSO and SSP. The mitochondria were capable of binding both 14C-labeled palmitate and 14C-labeled SSP in a similar manner. The quantity of mitochondria present directly impacted upon the quantity of the substrate bound (Fig. 3). Under optimal conditions (data not shown), binding of 14C-palmitate remained stable between 15 and 60 min (Fig. 3C), while no CO2 was produced (Fig. 3C), indicating that the palmitate could be bound but not oxidized by the mitochondria. Palmitate binding was inhibited by the addition of SSO to the medium (Fig. 3D, 77.0 and 76.9% inhibition in SS and IMF, respectively; p < 0.05). Furthermore, binding of 14C-SSP to the mitochondria could be competitively inhibited by the presence of excess unlabeled SSO (Fig. 3E, 96.7 and

FIG. 1. Representative Western blot performed on highly purified mitochondrial fractions (SS and IMF) obtained from skeletal muscle. Standards (Std) consisted of crude membranes obtained from the red gastrocnemius muscle. Similar results were obtained in 12 independent experiments. A, fatty acid translocase (FAT/CD36). B, cytochrome c oxidase IV. C, sodium potassium ATPase α1 subunit. D, sarcoplasmic reticulum calcium ATPase 1. E, transferrin receptor.

FIG. 2. Representative Western blot performed on highly purified mitochondrial fractions (SS and IMF) from heart, red gastrocnemius, and white gastrocnemius. Results are expressed in arbitrary units and were normalized for the amount of cytochrome c oxidase IV present for each blot. Data are represented as means ± S.E. (n = 8 for each tissue). *, p < 0.05 (different from heart); #, p < 0.05 (different from red gastrocnemius).
96.9% inhibition in SS and IMF, respectively; \( p < 0.05 \)) in a manner similar to that demonstrated previously in studies of FAT/CD36 in the plasma membrane (11, 16).

**Fatty Acid Oxidation**—Mitochondria incubated with palmitate only oxidized the fatty acid in the presence of carnitine (Fig. 4A, \( p < 0.05 \)), similarly to previous studies (27). However, in the presence of SSO, a known inhibitor of FAT/CD36, palmitate oxidation was also dramatically reduced (Fig. 4B, 87.2 and 85.9% inhibition in SS and IMF, respectively; \( p < 0.001 \)). Importantly, mitochondrial function was not affected by the addition of SSO to the medium, as pyruvate oxidation and octanoate oxidation were similar in control and SSO-treated mitochondria (Fig. 4, C and D, respectively). However, maximal CPTI activity in vitro was inhibited by the presence of SSO (Fig. 4E, 42.1 and 47.5% inhibition in SS and IMF, respectively; \( p < 0.05 \)).

**Immunoprecipitation**

Mitochondrial extracts immunoprecipitated with the monoclonal antibody 1207 for FAT/CD36 revealed the presence of CPTI (Fig. 5), but not cytochrome c oxidase.

**Effects of Increased Rates of Fatty Acid Oxidation**

To determine whether mitochondrial FAT/CD36 was altered when the capacity for fatty acid oxidation was increased, two different models were used: (i) chronic muscle stimulation designed to increase FAT/CD36 expression and capacity for fatty acid oxidation, and (ii) acute electrical stimulation to increase oxidation of fatty acids while the expression of FAT/CD36 remained unaltered.

**Chronic Stimulation**—Chronic electrical stimulation for 7 days resulted in an up-regulation in the total amount of FAT/CD36 present in the total homogenate and in the plasma membrane fraction of skeletal muscle (data not shown), as has been demonstrated previously (29). Interestingly, an increase in the amount of FAT/CD36 protein in the mitochondrial fraction was also observed in the mitochondria obtained from chronically stimulated muscles when compared with the mitochondrial fraction of the contralateral control muscles (Fig. 6A, 46.2 and 46.9% increase in SS and IMF, respectively; \( p < 0.05 \)). Concomitantly, mitochondrial palmitate oxidation was increased in the chronically stimulated muscles compared with the control muscles (Fig. 6B, 32.2 and 36.4% increase in SS and IMF, respectively; \( p < 0.05 \)). Interestingly, in both limbs, SSO reduced fatty acid oxidation to the same rate (Fig. 6B, \( p < 0.05 \)).

**Acute Stimulation**—In accord with previous observations (11), acute electrical stimulation for 30 min did not increase the expression of FAT/CD36 (data not shown), but the amount of FAT/CD36 at the plasma membrane fraction was increased (data not shown), indicating that FAT/CD36 was translocated within the cell. Interestingly, the present data demonstrate that acute stimulation also increases the amount of FAT/CD36 protein in the mitochondrial fraction, compared with the mitochondrial fraction of the contralateral control muscles (Fig. 7A, 34.5 and 37.0% increase in SS and IMF, respectively; \( p < 0.05 \)). This finding suggested that the FAT/CD36 protein was translocated to the mitochondria. Palmitate oxidation was increased in isolated mitochondria from acutely stimulated muscles, compared with control muscles (Fig. 7B, 32.2 and 34.5% increase in increased amounts of mitochondria (6 for each concentration). *, \( p < 0.05 \) (different from 25 mg of mitochondria); \( \# \), \( p < 0.05 \) (different from 50 mg of mitochondria). C, effect of time on palmitate binding (6 at each time point). D, effect of 50 \( \mu \)M SSO on palmitate binding (6). *, \( p < 0.05 \) (different from 25 mg of mitochondria). E, displacement of 50 \( \mu \)M SSP with 100-fold higher concentration of unlabelled 5 \( \mu \)M SSO (6). **, \( p < 0.05 \) (different from SSP).
SSO and IMF, respectively; \( p < 0.05 \). Additionally, SSO reduced both stimulated and control mitochondrial oxidation to a similar level (Fig. 7B, \( p < 0.05 \)).

**DISCUSSION**

In the present studies, we investigated the presence of FAT/CD36 and its functional significance in both the SS and IMF fractions of skeletal muscle mitochondria. Contrary to our hypothesis, in the parameters we investigated, there were no differences between the SS and IMF mitochondria. However, we have demonstrated for the first time a potential role for FAT/CD36 in mitochondrial uptake of long chain fatty acids. The results from this study revealed that palmitate binding and palmitate oxidation by the mitochondria can be significantly suppressed by SSO, a specific inhibitor of FAT/CD36. This inhibitor alters only the mitochondrial uptake and oxidation of LCFAs, as demonstrated by the normal octanoate and pyruvate oxidation in the presence of SSO. Interestingly, the quantity of FAT/CD36 protein present in the mitochondria
followed similar trends to oxidative capacity of the tissue (ie: heart > red muscle > white muscle). Finally, we have demonstrated that in muscle tissues, when the rates of fatty acid oxidation were increased, there were concomitant increases in mitochondrial FAT/CD36, induced either by increased expression of FAT/CD36 (chronic stimulation model), or by the apparent translocation of FAT/CD36 to the mitochondrial membrane (acute stimulation model).

These studies are the first to demonstrate that FAT/CD36 may play a role in LCFA transfer across the mitochondrial membrane, likely in conjunction with CPTI. Previous studies have demonstrated that although CPTI plays a pivotal role in the movement of LCFA into the mitochondria, its regulation cannot fully explain the increases seen in fatty acid oxidation during rapid increases in oxidative demand, such as those that occur during exercise (2–4). Therefore, it is not completely surprising that another protein could be involved in regulating LCFA oxidation.

Traditionally, the carnitine palmitoyl transferase system was thought to be the only access point for LCFA entrance into the mitochondria. Our studies support this hypothesis, as the removal of carnitine from the reagent mixture prevents palmitate oxidation. However, our studies also demonstrate that FAT/CD36 is actively involved in LCFA transport into the mitochondria. Previous studies using SSO have demonstrated its specificity for FAT/CD36 (14, 17). Indeed, this reactive ester was used in characterizing and identifying the FAT/CD36 protein (13). Although addition of SSO to the reagent mixture dramatically inhibits palmitate oxidation, it has no effect upon either pyruvate oxidation or octanoate oxidation. This is an important observation, as neither pyruvate nor octanoate require fatty acid transporters to facilitate their entry into the mitochondria. Hence, normal pyruvate and octanoate oxidation in the presence of SSO indicate that mitochondrial function has remained intact. Unexpectedly, the treatment of intact mitochondria with SSO did result in a 42% inhibition of the maximal activity of CPTI in vitro. The manifestation of this effect upon palmitate oxidation is difficult to interpret, as it is unlikely that CPTI is acting at full capacity under normal physiological conditions. Interestingly, using isolated mitochondria from rat heart, CPTI seemed not to be rate-limiting, as CPTI activity could be blocked by −50% without affecting β-oxidation (33). Thus, the 42–47% inhibition of CPTI in the presence of SSO would still allow for maximal β-oxidation. Therefore, the 86–87% reduction of β-oxidation in the presence of SSO would be due to FAT/CD36. However, it is important to remember that cardiac tissue contains a mixture of the muscle and the liver isoforms of CPTI; therefore, isolated mitochondria from skeletal muscle may not respond identically. Regardless, it is important to consider these results when establishing the role of FAT/CD36 in LCFA transfer into the mitochondria. It is possible that the actions of FAT/CD36 and CPTI are synchronized, such that the inhibition of FAT/CD36 by SSO has a deleterious effect upon the maximal capacity of CPTI. Indeed, by using immunoprecipitation, we have demonstrated a physical link between FAT/CD36 and CPTI, thus suggesting that the inhibitory action of SSO is conceivably achieved through FAT/CD36.

Given the above observations, we have hypothesized the following model as a working interaction between FAT/CD36 and CPTI for transferring LCFA into the mitochondria (Fig. 8). In this model, FAT/CD36 acts as an LCFA acceptor (possibly in an inverse manner to its action at the plasma membrane), receiving LCFA from cytosolic-binding proteins and handing them off to the long chain acyl CoA synthetase. The acyl CoA synthetase then activates the LCFA, which CPTI can then accept. Acyl CoA synthetase and CPTI have previously been demonstrated to be immunoreactive proteins (34), further supporting this hypothesis. Importantly, this model could allow for another key regulatory step in fatty acid oxidation, which could give a significant flux control coefficient in the regulation of β-oxidation.

Interestingly, both Western blotting and in vitro assessment...
of mitochondrial oxidation demonstrated system flexibility in FAT/CD36 on the mitochondria. Chronic electrical stimulation of the peroneal nerve for 7 days resulted in a significant increase in the amount of protein in the mitochondrial fraction, as well as increased palmitate oxidation under identical conditions. Chronic electrical stimulation also resulted in an increase in the amount of FAT/CD36 protein in the plasma membrane fractions and in the total homogenate, as has been demonstrated previously (29). Thus, in response to a chronic increase in the oxidative demand of muscles, FAT/CD36 protein expression is increased, and the additional protein is located both at the plasma membrane and at the mitochondria.

Conversely, after 30 min of acute electrical stimulation, the total amount of FAT/CD36 protein was not increased; however, in the mitochondria, there was a significant increase in FAT/CD36. This result parallels the increase that has been demonstrated under identical conditions in the plasma membrane fraction (11). Taken together, these results indicate that it is possible to translocate FAT/CD36 to the mitochondria. This translocation could potentially occur from previously identified intracellular pools of FAT/CD36 (11, 15), or possibly FAT/CD36 is trafficked directly from the plasma membrane to the mitochondria. This is a novel hypothesis, which provides new avenues for further understanding the role of LCFA and the mitochondria in cellular energy homeostasis. Translocation of FAT/CD36 to the plasma membrane and the mitochondria in a coordinated fashion during times of increased energy demands could perhaps be responsible for the rapid up-regulation of lipid metabolism previously observed during exercise (35).

The mechanism of action of FAT/CD36 in the mitochondria remains to be determined. It is possible that it acts in concert with CPTI in transporting LCFA across the mitochondrial membrane. Little is known about the mechanisms of LCFA transport by FAT/CD36, even at the plasma membrane. It is possible that FAT/CD36 acts as an acceptor or docking protein, either handing LCFA off to other proteins (36, 37), or simply assisting their movement through lipophbic environments. Conversely, it is possible that FAT/CD36 acts as a carrier protein, delivering cytosolic LCFA to the mitochondria for further processing. Indeed, recent studies in our laboratory have demonstrated that other fatty acid-binding proteins besides FABPc may be involved in the trafficking of fatty acids in the cytosol (38). A better understanding of the role of FAT/CD36 in the mitochondria will increase our comprehension of cellular handling of LCFA and their effect upon lipid homeostasis. Furthermore, it is important to ascertain whether impairments in fatty acid oxidation in insulin-resistant skeletal muscle are associated with disturbances in FAT/CD36 at the mitochondrial membrane.

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