Identification of fatty acid translocase on human skeletal muscle mitochondrial membranes: essential role in fatty acid oxidation


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Fatty acid translocase (FAT/CD36) is a multiligand scavenger receptor involved in LCFA transport in adipocytes, heart, and skeletal muscle. Binding of the reactive oleate ester sulfo-N-succimidyl-oleate (SSO) to a cell surface protein led to the identification of the LCFA transporter FAT/CD36 (1, 23). Since then, SSO has been a valuable tool in examining the role of FAT/CD36 on cell membranes, since it binds specifically to FAT/CD36 and prevents transport of LCFA (21). CAT and CPT II are located on the outer and inner leaflets of the mitochondrial inner membrane (MIM), respectively [see McGarry and Brown for review (35)]. CPT I catalyzes the trans-esterification of LCFA-CoA to LCFA-carnitine. CAT accepts LCFA-carnitine in exchange for carnitine, the latter being recycled in the intermembrane space (IMS). LCFA-carnitine is then translocated to the MIM where it is reconverted to LCFA-CoA by CPT II. Taken altogether, this system translocates LCFA-CoA from the cytosol into the mitochondrial matrix.

Evidence for regulation of the CPT system was first identified in rat skeletal muscle (53, 54). Briefly, malonyl-CoA (M-CoA), the product of the acetyl-CoA carboxylase (ACC) reaction, inhibits CPT I activity at rest. When aerobic exercise duration (53, 54) and/or intensity (40) increases in rat skeletal muscle, M-CoA levels decrease, thereby relieving CPT I inhibition and purportedly allowing greater LCFA oxidation rates to take place. The observed decrease in M-CoA levels during exercise is mainly caused by the AMP-activated protein kinase phosphorylation of ACC, which results in its inactivated state (40). However, the regulation of CPT I activity appears to differ in human skeletal muscle. A large portion of CPT I activity in human skeletal muscle appears to be insensitive to M-CoA inhibition (6, 7, 46). Most research has shown that, unlike M-CoA levels in rodent muscles, M-CoA levels in human muscle do not decrease during moderate exercise despite a marked increase in LCFA oxidation (16, 37, 38). One group did report a statistically significant decrease in M-CoA in human skeletal muscle after 60 min of moderate-intensity exercise (41) but still concluded that the decrease in M-CoA levels could not explain the increase in LCFA oxidation during exercise. The search for other regulators of CPT I activity, such as cofactor coenzyme A and intermediates acetyl-CoA/acyetyl-carnitine (46) as well as exercise-related metabolites AMP, ADP, and Ca²⁺ (7), has not been successful (7, 46).

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in adipocytes, heart, and skeletal muscle (8, 14, 33, 47). Recently, FAT/CD36 was identified as having a role in regulating LCFA oxidation in rat skeletal muscle mitochondria (12). Mitochondrial FAT/CD36 protein content correlated with oxidative capacity of different muscle tissues, and FAT/CD36 coprecipitated with CPT I. Moreover, in isolated mitochondria, blocking FAT/CD36 with SSO inhibited fatty acid (FA) oxidation by 86%. With acute muscle electrical stimulation (30 min), LCFA oxidation increased and was associated with the translocation of FAT/CD36 to the mitochondria. Blocking the increased mitochondrial FAT/CD36 inhibited the contraction-induced increase in LCFA oxidation. These studies indicated that FAT/CD36 may act in concert with CPT I to regulate LCFA oxidation in rodent muscle (12).

Because the regulation of CPT I in rodent and human muscle appears to differ (16, 37, 38), the aim of the present study was to investigate the presence and potential role of FAT/CD36 in LCFA oxidation in human skeletal muscle mitochondria. Our studies indicate that FAT/CD36 may work in conjunction with CPT I to regulate LCFA oxidation in human skeletal muscle. FAT/CD36 may be the missing link in understanding the regulation of LCFA transport and oxidation in human skeletal muscle.

RESEARCH DESIGN AND METHODS

Subjects. Twenty-three healthy individuals volunteered for this study [n = 15 males; n = 8 females; age: 23 ± 1 yr, weight: 78 ± 5 kg, body mass index (BMI): 25 ± 1 kg/m², and peak pulmonary O₂ uptake (V˙O₂ peak): 42 ± 2 (SE) ml·min⁻¹·kg body wt⁻¹]. Activity and aerobic fitness level varied among these individuals, which enabled us to obtain muscle samples over a wide range of aerobic capacities. A subset of 14 subjects (11 males and 3 females) was used for the correlation studies [age: 23 ± 1 yr, weight: 84 ± 7 kg, BMI: 26 ± 2 kg/m², and V˙O₂ peak: 41 ± 3 (SE) ml·min⁻¹·kg body wt⁻¹]. Female subjects were in the early follicular phase of their menstrual cycle phase at the time of muscle biopsies. Subjects were fully informed of the purpose of the experiments and of any possible risk before giving written consent to participate. The study conformed to the standards set by the Declaration of Helsinki, and procedures were approved by the University of Guelph Ethics Committee.

Experimental protocols. Subjects visited the laboratory on two occasions and were asked to refrain from exercise in the 48 h before each visit. On the first occasion, V˙O₂ peak was measured with a metabolic cart (SensorMedics model) during an incremental exercise test on a cycle ergometer (LODE Instrument, Groningen, The Netherlands). On the second occasion, three resting muscle samples were obtained from the vastus lateralis under local anesthesia (2% lidocaine without epinephrine) using the percutaneous needle biopsy technique described by Bergstrom (5). Visible fat and connective tissue were dissected free from the muscle, and the sample was blotted to remove excess blood. The muscle sample (∼600 mg) was divided into the following two portions: the first (∼590 mg) was used for the immediate isolation of mitochondria for the determination of CPT I activity and palmitate oxidation rates. A portion of the mitochondria was frozen until analyzed for selected proteins with Western blotting. A small section of the muscle biopsy sample (∼10 mg) was frozen in liquid N₂ for the subsequent analysis of citrate synthase (CS; EC 4.1.3.7) and 3-hydroxyacyl-CoA dehydrogenase (β-HAD; EC 1.1.1.13) activities.

Isolation of mitochondria from skeletal muscle. Differential centrifugation was used to obtain intact mitochondria containing both intermyofibrillar (IMF) and subsarcolemmal (SS) subfractions, as previously described (12). IMF and SS pellets were pooled and resuspended in 1 μl medium III/mg tissue and used for CPT I activity measurements. The remaining mitochondria were further diluted for palmitate oxidation measurements.

Leftover mitochondria from activity and oxidation measurements were further purified using a Percoll gradient (Sigma-Aldrich) and used for Western blotting analysis. Samples were centrifuged at 20,000 g for 1 h, and the mitochondrial layer was removed. The Percoll was removed from the sample by further centrifuging at 21,000 g for 5 h. At this point the mitochondria are no longer metabolically viable, but they are suitable for Western blotting (12).

Mitochondrial lipid oxidation measurements. Labeled CO₂ production and acid-soluble trapped 14C from palmitate oxidation were measured after a 30-min incubation of viable mitochondria in a sealed system. A 900-μl aliquot of pregassed (37°C for 15 min with 5% CO₂-95% O₂ and constant skaking) modified Krebs Ringer buffer (MKR; 115 mM NaCl, 2.6 mM KCl, 1.2 mM KH₂PO₄, 10 mM NaHCO₃, and 10 mM HEPS, pH 7.4) supplemented with 5 mM ATP, 1 mM NAD⁺, 0.5 mM mt-carnitine, 0.1 mM coenzyme A, 25 μM cytochrome c, and 0.5 mM malate was added to a 20-ml vial. The 200-ml glass scintillation vial contained a microcentrifuge tube with glass wool to trap 14CO₂ produced during the oxidation reaction. Viable mitochondria (100 μl) were added to the system, which was then sealed with a rubber cap and further sealed with parafilm. The reaction was initiated by the addition of a 6:1 palmitate-BSA complex (containing 10 μCi [1-14C]palmitate) administrated by syringe through the rubber cap. The reaction ran for 30 min at 37°C and was terminated with the addition of ice-cold 12 N perchloric acid by syringe through the rubber cap.

A fraction of the reaction medium was removed through the cap and analyzed for isotopic fixation. Briefly, 500 μl reaction medium were transferred to a 14-ml centrifuge tube, combined with 3 ml of 2:1 chloroform-methanol mixture (vol/vol), and shaken for 15 min before the addition of 1.2 ml of 2 M KCl-HCl. Samples were shaken again and centrifuged at 5,000 g for 15 min. A 1-ml aliquot of the aqueous phase was removed and quantified by liquid scintillation determination.

Gaseous CO₂ produced from oxidation of [1-14C]palmitate was measured by acidifying the remaining reaction mixture in the 20-ml glass scintillation vial with 1.0 ml of 1 M H₂SO₄. Liberated 14CO₂ was trapped by benzethonium hydroxide over a 90-min incubation period at room temperature. The microcentrifuge tube containing the 14CO₂ was put in a scintillation vial, and radioactivity was counted.

Final palmitate concentration was 77 μM (15 nCi [1-14C]palmitic acid; Amersham Biosciences). By substituting palmitate with the equivalent concentration of palmitoyl carnitine and the short-chain FA octanoate, we were able to measure the rate of palmitoylcarnitine (15 nCi [1-14C]palmitoylcarnitine; Perkin-Elmer) and octanoate (15 nCi [1-13C]octanoic acid; MP Biomedicals) oxidation.

Inhibition studies with SSO were performed by preincubating mitochondria with SSO dissolved in DMSO in supplemented MKR (containing 0.1% albumin) for 30 min. After the preincubation, mitochondria were centrifuged at 10,000 g for 10 min and washed two times in medium III to remove traces of SSO/DMSO before being resuspended in their original volume of supplemented MKR. Vials were sealed, and the reaction was initiated with the addition of 6:1 palmitate-BSA complex (containing 10 μCi [1-14C]palmitate) by syringe through the rubber cap. On the basis of dose-response experiments, the final SSO concentration was set at 200 μM. For control purposes, the same volume (1 μl) of DMSO was added to vials that were not supplemented with SSO.

CPT I activity. The forward radioisotope assay was used for the determination of CPT I activity as described by McGarry et al. (36) with minor modifications (6). Briefly, the assay was conducted at 37°C and initiated by the addition of 10 μl mitochondrial suspension (1:3 dilution) to 90 μl of the following standard reaction medium: 117 mM Tris·HCl (pH 7.4), 0.28 mM reduced glutathione, 4.4 mM ATP, 4.4 mM MgCl₂, 16.7 mM KCl, 2.2 mM KCN, 40 mg/l rotenone, 0.5%
BSA, 300 μM palmitoyl-CoA, and 5 mM l-carnitine with 1 μCi l-[3H]carnitine and a final pH of 7.1. The reaction was stopped after 6 min with the addition of ice-cold HCl. Palmitoyl-[3H]carnitine was extracted in water-saturated butanol in a process involving three washes with distilled water and subsequent recentrifugation steps to separate the butanol phase in which the radioactivity was counted.

Inhibition studies were performed by the addition of SSO to the reaction mixture before initiation of the reaction with mitochondria. The final concentration of SSO was set at 200 μM. As a control, the same volume of DMSO was added to the control tubes. CPT I activity was expressed in terms of the whole muscle (nmol·min⁻¹·kg wet muscle⁻¹) and was normalized to the ratio of CS activity in intact mitochondrial suspensions to total muscle CS activity to account for the quality of the mitochondrial preparation (see below).

**CS and β-HAD activity:** CS activity was determined in isolated mitochondria and in aliquots of homogenized whole muscle. Total muscle CS activity was assayed in a portion of quadriceps muscle (~10 mg) that was homogenized in 100 vol/wt of a 100 mM potassium phosphate buffer solution (45). Mitochondrial recovery and quality were calculated as follows:

\[
\text{recovery} = \frac{\text{CS}_\text{M} - \text{CS}_\text{EM}}{\text{CS}_\text{M}} \times 100
\]

\[
\text{quality} = \frac{\text{CS}_\text{M} - \text{CS}_\text{EM}}{\text{CS}_\text{M}} \times 100
\]

where CS activity in TS is total mitochondrial suspension (0.04% Triton X-100 and 3 freeze-thaw cycles), EM is extramitochondrial suspension in intact mitochondria, and MH is muscle homogenate. β-HAD activity was assayed spectrophotometrically at 37°C by measuring the disappearance of NADH using the whole muscle homogenate as for CS (4).

**Western blotting.** Purified isolated mitochondrial fractions were analyzed for total protein (bicinchoninic acid protein assay), and 25 μg denatured protein from each sample were separated by electrophoresis on 8% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. The monoclonal antibody MO25 was used to detect FAT/CDC36 (34). MO25 was obtained by immunizing mice with ultrapure (99%) SDS denatured human CDC36 (34). This monoclonal antibody has been used to detect human skeletal muscle FAT/CDC36 (9–11, 27, 29, 42, 43, 52). Commercially available antibodies were used to detect cytochrome c oxidase IV (Molecular Probes), Na⁺-K⁺-ATPase-1 (Upstate Biotechnology), and sarcoplasmic reticulum (SERCA1) ATPase (Affinity Bioreagents). An internal control of previously extracted human muscle crude membrane was used in each gel to normalize for variation in signal observed across the membranes. Blots were quantified using chemiluminescence and the ChemiGenius 2 Bioimaging system (SynGene).

**Statistics.** All data are presented as means ± SE. Differences between the control and SSO treatments were analyzed with Student’s paired t-tests. One-way ANOVA was used to determine significance between all other treatments. When a significant F-ratio was obtained, a Dunnett’s post hoc analysis was completed using 100% activity as control. Associations between variables were investigated using simple or multiple regression analyses as appropriate. Statistical significance was accepted at P < 0.05.

**RESULTS**

**Identification of FAT/CDC36 in pure human skeletal muscle mitochondria.** FAT/CDC36 was identified in isolated mitochondria from human skeletal muscle (Fig. 1A). Figure 1B shows the presence of mitochondrial marker cytochrome c oxidase (cytochrome c oxidase IV) in our preparation, demonstrating its mitochondrial origin. To eliminate the possibility of non-mitochondrial membrane contamination, we probed our purified mitochondrial preparations for plasma (Na⁺-K⁺-ATPase) and sarcoplasmic reticulum (SERCA1) membrane markers. Figure 1, C and D, demonstrates the absence of Na⁺-K⁺-ATPase and SERCA1 in our mitochondrial preparations compared with a crude membrane positive control, suggesting that the detected FAT/CDC36 was located at the mitochondria.

**Assessment of FAT/CDC36 function in FA mitochondrial transport.** Assessment of human mitochondrial FAT/CDC36 function was performed in isolated mitochondria obtained from muscle biopsies. Mitochondrial preparation recovery was assessed at 31.2 ± 2.3%, whereas quality of the mitochondrial preparation was 88.4 ± 1.6%.

We used increasing concentrations of SSO, a specific inhibitor of FAT/CDC36, to examine the relationship between FAT/CDC36 and palmitate oxidation (Fig. 2). We demonstrate a strong inverse relationship between LCFA oxidation occurred at 50 μM SSO, whereas at 200 μM SSO palmitate oxidation was reduced by 95% (P < 0.001). SSO had no effect (control: 100 ± 0% vs. SSO: 94 ± 3%; P = 0.16) on short-chain FA octanoyl oxidation, demonstrating SSO specificity to inhibit FAT/CDC36-mediated LCFA oxidation.

To ascertain the possible interaction of FAT/CDC36 with CPT I and its product in the mitochondria, we examined the effects of SSO on CPT I activity and palmitoylcarnitine oxidation. Maximal and submaximal CPT I activities were unchanged in
oxidation and CPT I activity was weakened (smaller subset of subjects, the correlation between palmitate oxidation studies, correlation measurements were performed on a subset of subjects (n = 14) chosen arbitrarily from the group. Rates of mitochondrial palmitate oxidation were correlated with VO2 peak (r = 0.73, P = 0.002) and CPT I activity (r = 0.97, P = 0.004). β-HAD activity was not associated with rates of palmitate oxidation (r = 0.22, P = 0.280); however, there was a significant correlation between palmitate oxidation and CS activity (r = 0.61, P = 0.011).

Linear regression analysis between palmitate oxidation and FAT/CD36 could only be performed in a smaller subset of subjects (n = 8) where no correlation was found. On this smaller subset of subjects, the correlation between palmitate oxidation and CPT I activity was weakened (r = 0.60, P = 0.06). However, to further assess the relationship between CPT I and FAT/CD36 and their impact on mitochondrial palmitate oxidation, a multiple regression analysis was performed on these eight subjects. In combination with each other, CPT I and FAT/CD36 strongly predicted mitochondrial palmitate oxidation (r = 0.90, P = 0.017). To illustrate the strength of the relationship between CPT I and FAT/CD36 and its impact on mitochondrial oxidation, the calculated rate of mitochondrial oxidation was plotted against the predicted rate of mitochondrial oxidation (Fig. 4).

**DISCUSSION**

Identifying the mechanisms controlling LCFA transport across the mitochondrial membranes is key in understanding the mechanisms that regulate skeletal muscle LCFA oxidation. The upregulation of human skeletal muscle CPT I activity and increased LCFA oxidation during moderate-intensity exercise cannot exclusively be explained by M-CoA control (16, 37, 38). In this study, we provide the first report of the presence of the LCFA transporter protein FAT/CD36 in the membranes of human skeletal muscle mitochondria. Functional assessment of FAT/CD36 using a specific inhibitor demonstrated that this LCFA transporter protein is required for palmitate oxidation. Blocking of FAT/CD36 had no effect on CPT I activity but did inhibit palmitoylcarnitine oxidation, suggesting that FAT/CD36 is playing a role downstream of CPT I activity.

*Mitochondrial LCFA transport: roles for the CPT I complex and FAT/CD36?* These studies are the first to identify the presence of FAT/CD36 in highly purified human skeletal muscle mitochondria. This is of considerable importance given the uncertainty regarding the regulation of the CPT I complex in human skeletal muscle (7, 30, 37, 38, 41, 46). In both human and rodent skeletal muscle, M-CoA levels are elevated at rest, inhibiting CPT I activity and minimizing fat oxidation. During moderate-intensity exercise in rodent skeletal muscle, M-CoA levels decrease, relieving CPT I inhibition (53, 54). However, the majority of studies in human skeletal muscle show no change in M-CoA levels during moderate-intensity exercise (16, 37, 38). Despite this, there was a large increase in LCFA oxidation rates, as determined by open-circuit calorimetry (16). Only one group has been successful in showing a significant but very modest (~13%) decrease in M-CoA levels after 60 min of moderate-intensity exercise (41), which the authors
concluded could not explain the increase in LCFA oxidation during exercise. These results suggest that the regulation of CPT I activity is complex and likely involves more than one modulator. Thus the following points should be considered. First is the possibility that there are other as yet unknown regulator(s) of CPT I. In this regard, the search for potential modulators, including factors known to upregulate metabolism in many other metabolic pathways, has been thus far unsuccessful (7, 46). However, a CPT I regulator other than M-CoA would support data reported by Kim et al. (30), who have demonstrated the presence of an M-CoA-insensitive CPT I isoform. In addition, several reports have been unable to account for the 50–70% residual CPT I activity that remains after in vitro M-CoA inhibition (7, 20, 30, 36, 46).

Second, it is possible that another LCFA transport/binding protein is associated with the mitochondrial membranes of human skeletal muscle, providing an additional level of regulation to mitochondrial LCFA uptake. Kerner and Hoppel (28) previously described the potential requirement for a mitochondrial protein to transport LCFA-carnitine units from the cytosolic face of CPT I to the MIM where LCFA-carnitine units can be exposed to CAT. Among the candidates for LCFA-carnitine transporter proteins are the mitochondrial porins (3) and an unknown protein with a molecular weight equivalent to that of FAT/CD36 found to be enriched in mitochondrial membrane contact sites (28). This hypothesis is more directly supported by the recent identification and characterization of the well-known LCFA transport protein FAT/CD36 in rat skeletal mitochondria membranes (12). Our present work confirms those findings to human skeletal muscle mitochondria, and we extend the work of Campbell et al. (12) by showing that FAT/CD36 appears to interact with LCFA transport in mitochondria downstream of CPT I.

**FAT/CD36 and mitochondrial LCFA transport.** Our functional assessment studies demonstrated that mitochondrial FAT/CD36 plays an essential role in the transport of LCFA in the mitochondria for oxidation in human skeletal muscle. Inhibition studies with SSO, which blocks FAT/CD36, suggest that FAT/CD36 must participate in the shuttling of LCFA in the mitochondria because otherwise a significant residual palmitate oxidation would have been observed after SSO preincubation. Indeed, we demonstrated a strong causal relationship between FAT/CD36 inhibition and palmitate oxidation using increasing concentrations of SSO and almost complete inhibition of palmitate oxidation when using 200 μM SSO (Fig. 2). Importantly, the maximal SSO concentration had no effect on octanoate oxidation. This short-chain FA is known to enter the mitochondrion by passive diffusion (2). SSO has previously been shown to have no effect on palmitate uptake of liver (contains little or no FAT/CD36) giant vesicles, highlighting its specificity to FAT/CD36 (33). Our intact octanoate oxidation rates confirm the specificity of SSO to inhibit FAT/CD36 in humans and the proper functioning of mitochondrial FA oxidation, as previously demonstrated (12).

A major difference between our results and those reported by Campbell et al. (12) in rat skeletal muscle was that FAT/CD36 was not reduced by preincubating mitochondria with SSO (Fig. 3A). This finding changes the interpretation of the role of mitochondrial FAT/CD36, suggesting that FAT/CD36 may be located downstream of CPT I activity, rather than upstream, as previously hypothesized. This interpretation is supported by the results showing that LCFA oxidation was inhibited by SSO when the product of CPT I activity, palmitoylcarnitine, was the substrate (Fig. 3B). It has previously been established that FAT/CD36 is a multiligand scavenger protein. It binds to a variety of ligands that structurally can vary significantly from each other (15, 19, 44, 48). Thus these findings do not allow us to necessarily conclude that palmitoylcarnitine is the bona fide substrate of FAT/CD36, but our results do point strongly toward a functional interaction between FAT/CD36 and palmitoylcarnitine. To this end, we have modified our initial hypothesis (12) regarding the functioning of mitochondrial FAT/CD36 (Fig. 5). We suggest, based on our present data, that FAT/CD36 facilitates the transport of LCFA-carnitine from CPT I to CAT across the MOM and IMS.

**CPT and FAT/CD36 cooperation: structural feasibility.** The structure of the CPT complex has been studied extensively [see McGarry and Brown for review (35)]. It is widely accepted that CPT I is tightly inserted in the MOM, whereas CAT and CPT II are loosely attached to the outer and inner leaflet of the MOM, respectively. Studies examining the structure of FAT/CD36 are more recent and rely on the amino acid sequence of FAT/CD36 and a mere handful of chimeric experiments (17, 22, 39). The consensus, at the sarcolemma, is that FAT/CD36 has two transmembrane domains and is largely extracellular (17, 22). Four palmitoylation sites near the cytosolic interface of FAT/CD36 have been identified (25, 49) and associated with a change in subcellular localization (24, 32). These findings support the recent demonstration that FAT/CD36 translocates from a cytosolic pool to mitochondria and plasma membranes.
in response to muscle contraction (8) and insulin (31). Assuming an identical structure and translocation process as seen at the sarcolemma, mitochondrial FAT/CD36 could potentially be located on the MOM with both terminals in the IMS and the bulk of the protein facing the cytosol. In this arrangement, mitochondrial FAT/CD36 could become a MOM receptor for palmitoylcarnitine and facilitate its translocation from CPT I to CAT. Despite evidence of mitochondrial LCFA uptake by FAT/CD36 (12), the specific location of an LCFA binding site on this protein has yet to be established. However, the reactive oleate ester SSO is too large to cross the MOM and thus must inhibit FAT/CD36 from the cytosol, suggesting a cytosolic LCFA binding site.

Correlation studies of FAT/CD36 and oxidative capacity. Few studies have performed complete correlations between markers of oxidative capacity in human skeletal muscle because of the large amount of tissue required for analysis. We have examined several markers of oxidative capacity at the whole body, muscle homogenate, and mitochondrial levels in both the carbohydrate and fat oxidation pathways. We demonstrate that rates of mitochondrial palmitate oxidation are correlated with maximal aerobic power and CS and CPT I activity, which corroborate previous findings (6, 26). The exception appeared to be β-HAD, which did not correlate with palmitate oxidation but did correlate with CS activity. Despite being a marker of β-oxidation, β-HAD activity has not consistently been shown to increase in response to long-term aerobic training (13, 50, 51).

We found that mitochondrial FAT/CD36 protein content did not correlate with palmitate oxidation on its own. Campbell et al. (12) have previously demonstrated that FAT/CD36 levels from various rat tissues followed oxidative potential hierarchy. However, the variations in oxidative potential between rat heart and red and white gastrocnemius muscles far exceed the differences that would be expected in recreationally active human vastus lateralis muscle. Thus not being able to detect differences in FAT/CD36 content across our relatively homogenous sample population is not startling. Importantly, in combination with each other, FAT/CD36 and CPT I provided a very strong prediction of mitochondrial palmitate oxidation on a limited sample size (Fig. 4). This finding supports our current hypothesis that FAT/CD36 may be working in conjunction with CPT I to allow proper transport of LCFA-carnitine to CAT to ultimately reach the matrix for oxidation.

In summary, we provide the first evidence of the presence of FAT/CD36 in the membranes of mitochondria isolated from human skeletal muscle. Our in vitro functional studies demonstrate that FAT/CD36 is necessary for mitochondrial LCFA transport and subsequent oxidation. We hypothesize that FAT/CD36 is functioning downstream of CPT I, possibly playing a role in the transfer of palmitoylcarnitine from CPT I to CAT in the mitochondrial membranes. These data provide new insight regarding the LCFA transport system across the mitochondrial membranes in human skeletal muscle and could be involved in mechanisms leading to states of altered LCFA oxidation rates.

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