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Coimmunoprecipitation of FAT/CD36 and CPT I in skeletal muscle increases proportionally with fat oxidation after endurance exercise training

Simon Schenk and Jeffrey F. Horowitz

Substrate Metabolism Laboratory, Division of Kinesiology, University of Michigan, Ann Arbor, Michigan

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Schenk, Simon, and Jeffrey F. Horowitz. Coimmunoprecipitation of FAT/CD36 and CPT I in skeletal muscle increases proportionally with fat oxidation after endurance exercise training. *Am J Physiol Endocrinol Metab* 291: E254–E260, 2006. First published May 2, 2006; doi:10.1152/ajpendo.00051.2006.—Although the increase in fatty acid oxidation after endurance exercise training has been linked with improvements in insulin sensitivity and overall metabolic health, the mechanisms responsible for increasing fatty acid oxidation after exercise training are not completely understood. The primary aim of this study was to determine the effect of adding endurance exercise training to a weight loss program on fat oxidation and the colocalization of the fatty acid translocase FAT/CD36 with carnitine palmitoyltransferase I (CPT I) in human skeletal muscle. We measured post-absorptive fat oxidation and acquired a muscle sample from abdominally obese women before and after 12% body weight loss through either dietary intervention with endurance exercise training (EX + DIET) or dietary intervention without endurance exercise training (DIET). Immunoprecipitation techniques were used on these muscle samples to determine whether the association between FAT/CD36 and CPT I is altered after DIET and/or EX + DIET. FAT/CD36 was found to coimmunoprecipitate with CPT I, and the amount of FAT/CD36 that coimmunoprecipitated with CPT I increased by ~25% after EX + DIET ($P < 0.005$) but was unchanged after DIET. In addition, the increase in the amount of FAT/CD36 that coimmunoprecipitated with CPT I in EX + DIET was strongly correlated with the increase in whole body fat oxidation ($R^2 = 0.857$, $P < 0.003$). In conclusion, the findings from this study indicate that exercise training alters the localization of FAT/CD36 and increases its association with CPT I, which may help augment fat oxidation.

carnitine palmitoyltransferase I; fatty acid translocase; obesity; weight loss; mitochondria

ABNORMALITIES IN THE REGULATION of fat metabolism in obesity are linked with impairments in metabolic health, especially insulin resistance. In particular, the high absolute rates of lipolysis and systemic fatty acid availability, which are a hallmark of abdominal obesity (17), have been shown to play a causative role in the insulin resistance commonly found in obesity (26, 27, 29). In addition, low rates of skeletal muscle fatty acid oxidation, in association with reduced mitochondrial number and/or function, have also been linked with insulin resistance in obesity (20, 24, 28). Accordingly, it has been hypothesized that improving skeletal muscle oxidative capacity (i.e., increasing mitochondrial density and/or function) may facilitate the oxidative disposal of fatty acids in the face of excess fatty acid availability (2, 14, 23). This increase in fat oxidation may subsequently reduce the accumulation of intracellular fatty acid intermediates (e.g., long-chain acyl-CoA,

diacylglycerol), which have been found to interfere with insulin signaling and induce insulin resistance (18, 34).

Regulation of fatty acyl-CoA entry into the mitochondria by carnitine palmitoyltransferase I (CPT I) has been identified as a rate-limiting step in the oxidation of fatty acids (6, 22). Moreover, CPT I activity has been found to be reduced in obesity (20, 28), and it has been hypothesized that abnormalities in the regulation at the step of fatty acid entry into the mitochondria may explain much of the impairments in metabolic health. However, more recent information suggests that CPT I may not act alone in the regulation of fatty acyl-CoA entry into the mitochondria. Recent studies have demonstrated that the fatty acid translocase FAT/CD36 is present on the mitochondrial membrane of both rodent (9) and human (3, 16) skeletal muscle, where it plays an important role in regulating fatty acid oxidation. Before these studies, FAT/CD36 was thought to play an important role only in regulating fatty acid metabolism by facilitating fatty acid transport into the cell across the plasma membrane and possibly the capillary endothelium (5, 12, 32). Interestingly, by use of immunoprecipitation techniques in rodent muscle, it was also found that mitochondrial FAT/CD36 was associated with CPT I, demonstrating that mitochondrial FAT/CD36 (in cooperation with CPT I), may be an important mediator of fatty acid oxidation (9). Therefore, because abnormalities in fatty acid metabolism are tightly linked with the development of metabolic disease in obesity, increasing the abundance of FAT/CD36 in the mitochondria in human skeletal muscle may enhance fat oxidation and subsequently may improve metabolic health.

Weight loss combined with endurance exercise training is often recommended as the preferred means of improving metabolic health in obesity, perhaps in part through an increase in fatty acid oxidation (2, 14). However, the mechanisms responsible for increasing fat oxidation after exercise training are still not completely understood. Although chronic muscle contraction has been found to augment the localization of FAT/CD36 to the mitochondrial membrane in rodent skeletal muscle, with a resultant increase in fatty acid oxidation (9), the effect of exercise training on mitochondrial FAT/CD36 localization in human muscle is unknown. Therefore, the primary aim of the present study was to evaluate the effects of adding endurance exercise training to a weight loss program on the localization of FAT/CD36 in skeletal muscle from obese humans and whether such changes are associated with changes in fat oxidation.

MATERIALS AND METHODS

Subjects. Fifteen abdominally obese women [body mass index (BMI) 30–40 kg/m²; waist circumference >100 cm] participated in

Address for reprint requests and other correspondence: J. F. Horowitz, Substrate Metabolism Laboratory, Div. of Kinesiology, Univ. of Michigan, 401 Washtenaw Ave., Ann Arbor, MI 48109-2214 (e-mail: jeffhor@umich.edu).

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this study. All subjects were premenopausal and were considered to be in good health after a comprehensive medical examination, which included a history and physical examination, a 12-lead electrocardiogram, standard blood and urine tests, and an aerobic fitness test (i.e., peak oxygen uptake). No subject was taking regular medications (except birth control). All subjects were nonsmokers, weight stable (± 2 kg) for 4–6 mo and had not exercised regularly for ≥ 6 mo before beginning the study. Subjects with type 2 diabetes, coronary heart disease, clinically significant hypertriglyceridemia (i.e., plasma triglycerides >150 mg/dl), or hypertension were excluded. Subject characteristics are presented in Table 1. All subjects were fully informed of the possible risks associated with the study and signed an informed consent document, which was approved by the University of Michigan Institutional Review Board.

Experimental trials. Both before and after weight loss, subjects were admitted to the General Clinical Research Center (GCRC) at the University of Michigan hospital at 1800 and stayed overnight. On both occasions, a standardized meal [15 kcal/kg fat-free mass (FFM)] was provided at 2000, and subjects remained fasted until completion of the trial the next day. At 0700 the next morning, a muscle biopsy was taken from the vastus lateralis muscle with the percutaneous muscle biopsy technique. Muscle samples were dissected free of adipose and connective tissue, rinsed in saline, blotted dry, and then frozen in liquid nitrogen. Muscle samples were stored at -80°C until later analysis. At 0800, resting oxygen consumption ($\dot{V}\text{O}_2$) and carbon dioxide production ($\dot{V}\text{CO}_2$) were measured (DeltaTrac II, Sensor-medics) for 20–30 min to assess whole body fat oxidation. Both trials were conducted during the first 2 wk of the subjects' menstrual cycles.

Dietary intervention and weight loss. After completing the first experimental trial, subjects were assigned to one of two weight loss treatment groups: 1) diet only (DIET; $n = 7$) or 2) exercise plus diet (EX + DIET; $n = 8$). Group assignments were counterbalanced. Subjects in both groups adhered to the same dietary treatment, in which they were instructed to reduce their caloric intake 500–800 kcal/day below that required to maintain body weight, with the goal to achieve a rate of weight loss of 0.5 to 1.0 kg/wk. The caloric intake necessary to maintain body weight was based on the basal metabolic rate as determined by the Weir equation (33) from indirect calorimetry data collected during their first hospital visit. The macronutrient composition of the prescribed diet consisted of $\sim 55\%$ carbohydrate, 25% fat, and 20% protein (i.e., percentage of total energy intake). Subjects completed daily food diaries and met with our research dietitian weekly to help monitor and facilitate their weight loss progress. Dietary recommendations were based on the guidelines outlined by the National Institutes of Health (31). After subjects lost 12% of their initial weight, dietary caloric intake was increased to meet the energy needs of weight maintenance. Subjects were required to be in weight maintenance (± 0.5 kg) for 3–4 wk before being admitted to the hospital for their follow-up experimental trial. Overall, the time taken to complete the study was significantly shorter in EX + DIET compared with DIET (21 ± 2 vs. 31 ± 3 wk, $P < 0.01$).

Exercise training. In addition to adhering to the same dietary intervention described above, subjects in the EX + DIET group performed supervised exercise training on a stationary bicycle ergometer for 45 min 3 days a week, with one additional, unsupervised exercise training session per week. The exercise intensity was based on the percentage of each subject's maximal heart rate (HR_{max}), determined during the initial aerobic fitness test. During the first 6 wk of the training period, exercise intensity was increased progressively from 70 to 85% HR_{max} , and exercise duration was progressively increased from 35 to 45 min. Exercise training continued throughout the weight maintenance period. After weight loss and the weight maintenance period, the follow-up experimental trial was completed exactly 3 days after the last exercise session. Subjects in the DIET group were specifically instructed not to engage in an exercise fitness program, and they maintained their sedentary lifestyle throughout the weight loss intervention.

Body composition analysis. Percent body fat and FFM were determined before and after weight loss using dual-energy X-ray absorptiometry (DPX DEXA Scanner; Lunar, Madison, WI).

Peak oxygen uptake. Peak oxygen consumption ($\dot{V}\text{O}_{2\text{peak}}$) was measured (PhysioDyne Technologies, Quogue, NY) during cycle ergometer exercise to assess cardiorespiratory fitness. The protocol consisted of a 4-min warm-up, after which the work rate was progressively increased every minute until volitional fatigue.

Whole body fat oxidation. Resting whole-body fat oxidation was calculated from resting $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ measurements (DeltaTrac II) using the equations of Frayn (10).

Immunoprecipitation. First, to determine whether FAT/CD36 coimmunoprecipitates with CPT I, we performed immunoprecipitation experiments in muscle from two subjects. Muscle was homogenized [150 mM NaCl, 20 mM Tris·HCl, pH 7.4, 10 mM EDTA, 1% Nonidet P-40 (NP-40), 1 mM NaF, phosphate inhibitor cocktail (P-5726; Sigma, St. Louis, MO)] and then rotated for 60 min at 4°C . Samples were centrifuged at 5,000 g for 10 min, the protein concentration of the supernatant [whole cell lysate (WCL)] was determined (Pierce, Rockford, IL), and then samples were aliquoted and stored at -80°C . Samples were immunoprecipitated with an antibody specific for the muscle isoform of either CPT I or FAT/CD36 and then immunoblotted for FAT/CD36 or CPT I, respectively. Specifically, WCL was rotated overnight at 4°C with 4 μg of a CPT I (sc-20670, Santa Cruz Biotechnology) or FAT/CD36 (sc-9154, Santa Cruz Biotechnology) antibody with protein A-agarose beads (cat. no. 16–156, Upstate). The next day, the agarose beads were washed four times in a buffer solution containing 1% NP-40 and 0.1 mM Na_3VO_4 in PBS and were then washed three times in a second buffer solution, containing 0.01 M Tris, pH 7.5, 1 mM EDTA, 0.1 M NaCl, and 0.1 mM Na_3VO_4 in PBS. Antigens were eluted from the beads with 60 μl of $2\times$ Laemmli SDS buffer and were boiled for 5 min before separation using SDS-PAGE.

To determine whether the amount of FAT/CD36 that coimmunoprecipitates with CPT I was altered by our diet- and/or exercise-induced weight loss interventions, we immunoprecipitated for CPT I

Table 1. Subject characteristics before and after losing 12% body weight

	DIET			EX + DIET		
	Before	After	Δ , %	Before	After	Δ , %
Age, yr	30 \pm 3			30 \pm 2		
Weight, kg	88.6 \pm 2.9	78.0 \pm 2.5*	-12.0 \pm 0.1	89.2 \pm 2.4	78.5 \pm 2.1*	-12.0 \pm 0.1
BMI, kg/m ²	34.9 \pm 1.8	30.7 \pm 1.5*	-11.9 \pm 0.1	31.7 \pm 0.9	27.9 \pm 0.7*	-12.0 \pm 0.1
%Body fat	47.9 \pm 1.5	44.1 \pm 2.2*	-7.6 \pm 2.3	47.4 \pm 0.9	43.1 \pm 1.3*	-9.1 \pm 2.0
Fat-free mass, kg	45.9 \pm 1.1	43.4 \pm 1.3*	-5.6 \pm 1.3	46.9 \pm 1.3	44.7 \pm 1.5*	-4.9 \pm 1.5
$\dot{V}\text{O}_{2\text{peak}}$, l/min	2.07 \pm 0.09	2.02 \pm 0.12	-2.9 \pm 2.9	2.12 \pm 0.13	2.57 \pm 0.13*†	+22.5 \pm 4.0

Values are means \pm SE; $n = 7$ for DIET and $n = 8$ for Exercise (EX) + DIET. BMI, body mass index. * $P < 0.001$ within each group compared with Before. † $P < 0.005$ EX + DIET vs. DIET within After.

and then immunoblotted for FAT/CD36. This immunoprecipitation procedure, as described above, was performed on 150 μ g of crude membrane fraction (CMF). To obtain the CMF fraction, muscle was homogenized in *buffer 1* (20 mM HEPES, pH 7.4, 1 mM EDTA, 250 mM sucrose, PIC) with a glass-on-glass homogenizer. An aliquot of this homogenate was rotated for 60 min at 4°C in 1% NP-40 and then centrifuged at 5,000 *g* for 20 min. The protein concentration of the supernatant was determined, and then samples were aliquoted and stored at -80°C. This represents the WCL fraction, which was used for analysis of FAT/CD36 and NADH-ubiquinol oxidoreductase (COX-I) protein abundance. The remainder of the homogenate was centrifuged at 150,000 *g* for 150 min, and the resultant pellet was homogenized in *buffer 2* (*buffer 1* + 1% NP-40). The homogenate was then rotated for 60 min at 4°C and centrifuged at 5,000 *g* for 20 min. The protein concentration of the supernatant, which represents the CMF, was determined, and then samples were aliquoted and stored at -80°C for later use in CPT I immunoprecipitations, as described above. Separation of the CMF fraction from the cytosolic fraction was confirmed by immunoblotting both fractions for COX-I and caveolin-3 (CAV-3), which are known mitochondrial and plasma membrane proteins, respectively. Both COX-I and CAV-3 were found in the CMF fraction, but they were not present in the cytosolic fraction, confirming that the CMF fraction was isolated successfully (data not shown). In addition, to ensure that the CPT I immunoprecipitations were bringing down only FAT/CD36 and not other membrane-associated proteins, in preliminary experiments we immunoblotted for COX-I and CAV-3 in immunoprecipitates and also in the supernatant of the immunoprecipitate. We found that these proteins were not present in the immunoprecipitates, but they were found in supernatant from the immunoprecipitations. This verifies that our CPT I immunoprecipitations were specifically bringing down FAT/CD36, and not simply bringing down all membrane-associated proteins.

SDS-PAGE and immunoblotting. Samples (immunoprecipitates or WCL) were separated by 8% SDS-PAGE and transferred to nitrocellulose at 200 mA at 4°C. For WCL, 25 μ g of protein from each sample were loaded in each lane. Membranes were blocked and then probed with a FAT/CD36 antibody (sc-7309, Santa Cruz Biotechnology), COX-I (Molecular Probes, cat. no. A21344), or CAV-3 (sc-5310, Santa Cruz Biotechnology). Membranes were washed, incubated in the appropriate secondary for 60 min, and washed again. Membranes were developed with enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ) and quantified by densitometry (Fluor Chem SP; Alpha Innotech, San Leandro, CA). Band densities on each blot were expressed relative to a control (WCL from human skeletal muscle), which was run in duplicate on all blots. Because of difficulties in measuring CPT I by Western blot in human muscle, the CPT I abundance before and after DIET and EX + DIET was determined by stripping blots from the CPT I immunoprecipitations and reprobing for CPT I using a rabbit polyclonal antibody (a gift from Dr. Gebre Woldegiorgis).

Statistics. A two-way ANOVA for repeated measures (one-factor repetition) and Tukey's post hoc analysis was used to assess differences before vs. after weight loss in the DIET and EX + DIET groups and to assess differences between these groups. Simple linear regression analysis was used to assess the relationship between the changes in the amount of FAT/CD36 that coimmunoprecipitates with CPT I and changes in fat oxidation, as well as changes in other factors that are thought to be associated with an increase in fatty acid oxidative capacity and improved physical fitness (i.e., protein abundance of COX-I and CPT I, and $\dot{V}O_{2\text{ peak}}$). Regression analysis was also used to assess relationships between the increase in fat oxidation and changes in $\dot{V}O_{2\text{ peak}}$ and abundance of CPT I and COX-I. Statistical significance was set at $P < 0.05$. All values are presented as means \pm SE.

RESULTS

Body weight, body composition, and aerobic fitness. Before weight loss, body weight, BMI, body composition, and aerobic fitness were all similar between the DIET and EX + DIET groups. As designed, subjects in both groups lost 12% of their initial body weight after the weight loss intervention (Table 1). In addition, the magnitude of reductions in percent body fat and FFM were the same in both groups (Table 1). Endurance exercise training improved aerobic fitness as indicated by a \sim 25% increase in $\dot{V}O_{2\text{ peak}}$ after weight loss in EX + DIET ($P < 0.005$), whereas $\dot{V}O_{2\text{ peak}}$ was not changed in DIET [$P =$ non-significant (NS); Table 1].

Coimmunoprecipitation of FAT/CD36 with CPT I in human skeletal muscle. We found FAT/CD36 to coimmunoprecipitate with the mitochondrial protein CPT I (Fig. 1A). When run side by side, immunoprecipitation with an antibody for either CPT I or FAT/CD36 pulled down an 88-kDa protein that was immunoreactive with a FAT/CD36 antibody in an immunoblot assay (Fig. 1B). Similar to results in rat skeletal muscle (9), reverse immunoprecipitations experiments, whereby we immunoprecipitated for FAT/CD36 and immunoblotted for CPT I afterward, verified an association between FAT/CD36 and CPT I (data not shown).

Changes in abundance of COX-I, FAT/CD36, and CPT I. Neither weight loss program affected the total FAT/CD36 abundance (change of 0 ± 4 and $3 \pm 7\%$ relative to before weight loss, for DIET and EX + DIET, respectively; both $P =$ NS; Fig. 2A). However, COX-I abundance increased $32 \pm 4\%$ after EX + DIET ($P < 0.01$), indicative of an increase in

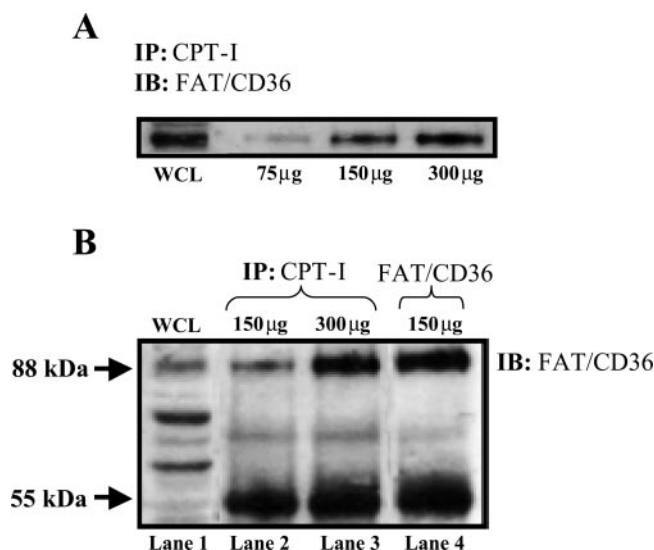


Fig. 1. Representative immunoblots (IB) demonstrate that fatty acid translocase (FAT)/CD36 is associated with carnitine palmitoyltransferase (CPT I) in human skeletal muscle. *A*: whole cell lysate (WCL) protein (i.e., 75, 150, and 300 μ g of WCL protein) from the same homogenate were immunoprecipitated with a polyclonal CPT I antibody specific for the muscle isoform of CPT I. Immunoprecipitates (IP) were separated by SDS-PAGE and membranes probed for FAT/CD36. Similar results were found for $n = 2$. *B*: different amounts of WCL were immunoprecipitated with either a CPT I (lanes 2 and 3) or FAT/CD36 (lane 4) antibody. These immunoprecipitates and 25 μ g of WCL (lane 1) were separated by SDS-PAGE and immunoblotted for FAT/CD36. Results demonstrate that immunoprecipitation with an antibody for CPT I or FAT/CD36 pulls down an 88-kDa protein that is immunoreactive with a FAT/CD36 antibody. 55 kDa represents IgG band.

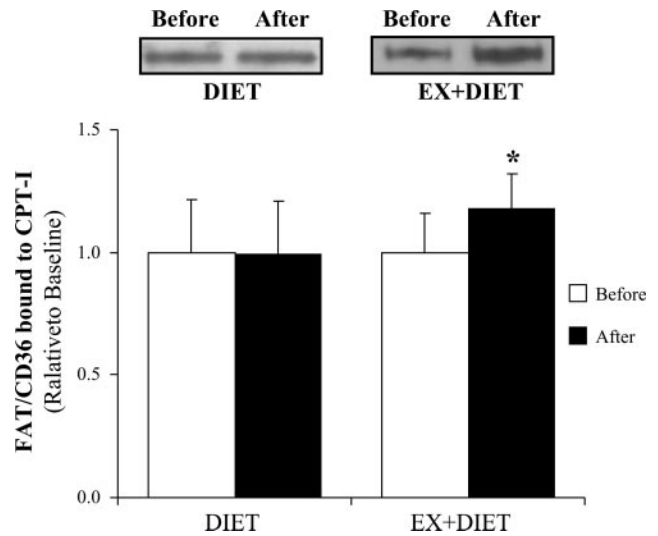
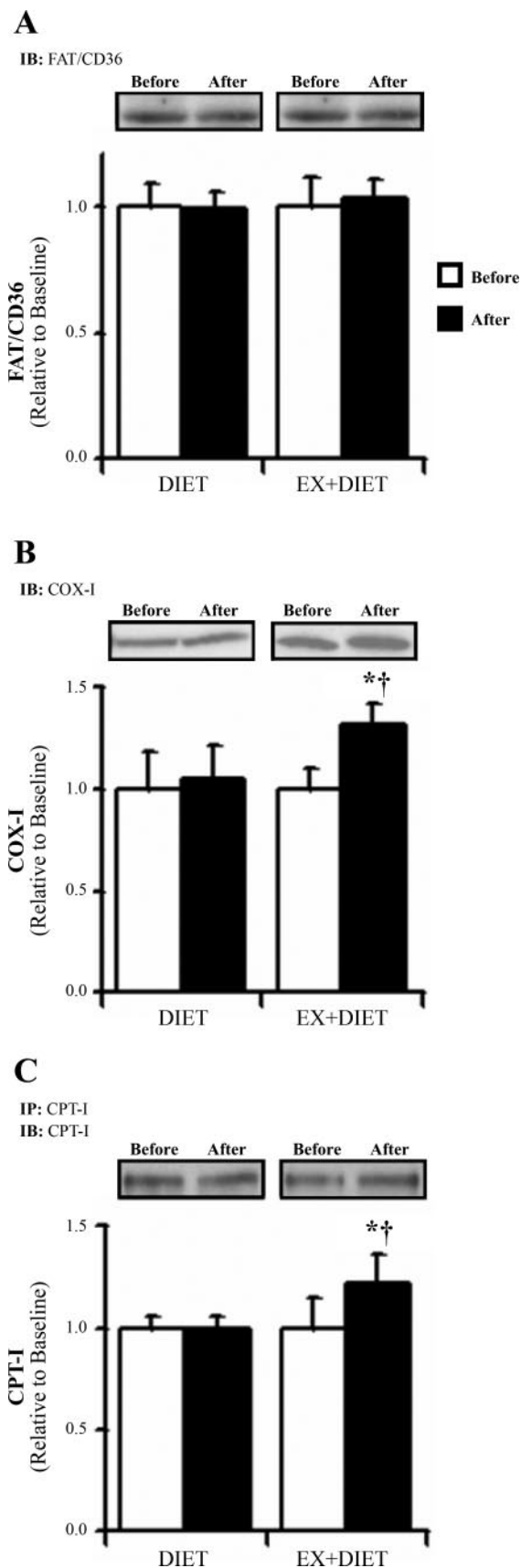


Fig. 3. Amount of FAT/CD36 that coimmunoprecipitated with CPT I in crude membrane protein before and after losing 12% of body weight via DIET or EX + DIET intervention. * $P < 0.005$ vs. before weight loss; $n = 7$ for DIET and $n = 8$ for EX + DIET.

mitochondrial mass, and was unchanged in DIET (Fig. 2B). Similarly, the amount of CPT I that was immunoprecipitated after weight loss was significantly increased in EX + DIET (change of $20 \pm 4\%$ relative to before weight loss, $P < 0.01$; Fig. 2C) but was unchanged in DIET.

Changes in amount of FAT/CD36 that coimmunoprecipitates with CPT I after weight loss interventions. The amount of FAT/CD36 that coimmunoprecipitated with CPT I increased $\sim 25\%$ ($P < 0.005$) after weight loss in EX + DIET (Fig. 3). This increase appeared to be due to exercise training per se, because there was no change in the amount of FAT/CD36 that coimmunoprecipitated with CPT I after the same magnitude of weight loss in the DIET group (Fig. 3). The increase in the amount of FAT/CD36 that coimmunoprecipitated with CPT I seen in the EX + DIET group was not associated with changes in CPT I abundance ($R^2 = 0.225$, $P = 0.24$), COX-I abundance ($R^2 = 0.0004$, $P = 0.963$), or $\dot{V}O_{2\text{ peak}}$ ($R^2 = 0.197$, $P = 0.27$). Therefore, the increase in the coimmunoprecipitation of FAT/CD36 and CPT I was apparently not simply a consequence of an increased CPT I content or due to an increase in mitochondrial protein content in general.

Whole body fat oxidation. Resting fat oxidation was not different before and after weight loss in DIET (1.93 ± 0.24 vs. $1.90 \pm 0.12 \mu\text{mol}\cdot\text{kg FFM}^{-1}\cdot\text{min}^{-1}$, $P = \text{NS}$; Fig. 4A). In contrast, resting fat oxidation was significantly increased by $23 \pm 9\%$ after EX + DIET (1.79 ± 0.07 vs. $2.19 \pm 0.12 \mu\text{mol}\cdot\text{kg FFM}^{-1}\cdot\text{min}^{-1}$, $P < 0.01$) and was also significantly greater than DIET after weight loss ($P < 0.05$). Interestingly, there was a significant correlation between the percent increase

Fig. 2. FAT/CD36 (A), NADH-ubiquinol oxidoreductase (COX-I; B), and CPT I (C) abundance in skeletal muscle before and after losing 12% of body weight via a diet-only (DIET) or exercise + diet (EX + DIET) intervention. FAT/CD36 and COX-I abundance were measured by immunoblot in the WCL fraction ($25 \mu\text{g}$ loaded in each lane). CPT I was measured in CPT I immunoprecipitates of the crude membrane fraction (CMF). † $P < 0.01$ vs. before weight loss; * $P < 0.05$ vs. same time point in DIET; $n = 7$ for DIET and $n = 8$ for EX + DIET.

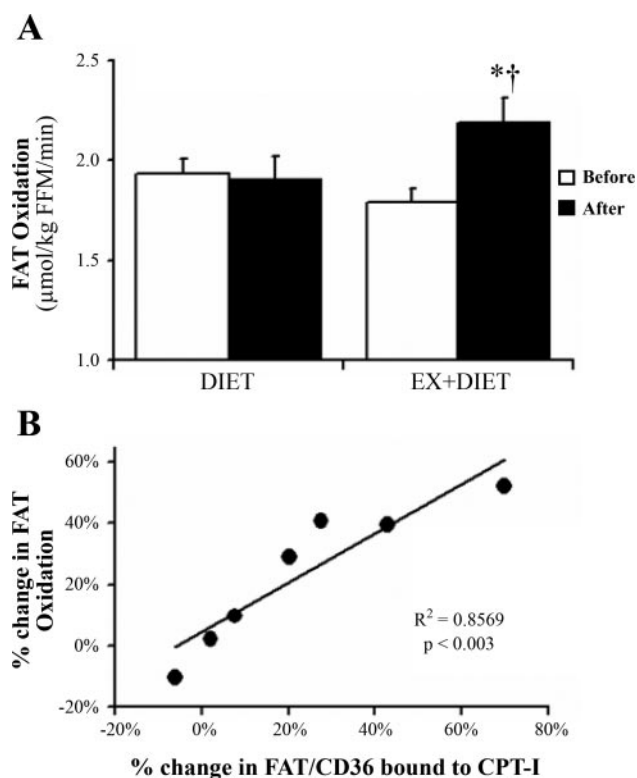


Fig. 4. A: whole body fat oxidation before and after losing 12% of body weight via DIET or EX + DIET intervention. B: simple linear regression demonstrates that the %change in resting fat oxidation after EX + DIET is strongly correlated with the %change in the amount of FAT/CD36 that coimmunoprecipitated with CPT I. $\dagger P < 0.01$ vs. before weight loss; $*P < 0.05$ vs. same time point in DIET; $n = 7$ for DIET and $n = 7$ for EX + DIET (due to technical problems with indirect calorimetry, data were not collected on 1 subject in the EX + DIET group).

in fat oxidation that occurred in EX + DIET and the percent increase in the amount of FAT/CD36 that coimmunoprecipitates with CPT I ($R^2 = 0.857$, $P < 0.003$; Fig. 4B). The increase in fat oxidation was not correlated with changes in $\dot{V}O_{2\text{ peak}}$ ($R^2 = 0.182$, $P = 0.34$), CPT I ($R^2 = 0.159$, $P = 0.376$), or COX-I abundance ($R^2 = 0.077$, $P = 0.55$).

DISCUSSION

Among the metabolic benefits of adding exercise to a weight loss program, it has been hypothesized that a training-induced increase in the capacity of skeletal muscle to oxidize fatty acids may reduce the deleterious effects of excess fat on intracellular insulin-signaling pathways (2, 14, 23). Nevertheless, the mechanisms responsible for increasing fatty acid oxidation after exercise training are not completely understood. Although the capacity of skeletal muscle to oxidize fat is traditionally thought to be limited by the activity of CPT I (22) and/or mitochondrial density (15), recent studies have implicated a necessary role for mitochondrial FAT/CD36 (in cooperation with CPT I) in regulating fatty acid oxidation (3, 9, 16). The results from the present study confirm that FAT/CD36 is physically associated with CPT I (as determined by coimmunoprecipitation) and demonstrate that the amount of FAT/CD36 that coimmunoprecipitated with CPT I was significantly increased by a physiological stimulus that is known to increase fat oxidation (i.e., endurance exercise training). Indeed, we

found that the increase in the amount of FAT/CD36 that coimmunoprecipitated with CPT I after EX + DIET was strongly correlated with the increase in resting fat oxidation.

By use of a variety of different techniques, FAT/CD36 has been found to be present in the cytosol, on endothelial and plasma membranes (5, 11, 19, 32), and in isolated skeletal muscle mitochondria (3, 16). However, the presence of FAT/CD36 in skeletal muscle mitochondria has been controversial (19, 32). It is likely that methodological differences (i.e., isolation of purified mitochondria by differential centrifugation vs. immunofluorescence) are responsible for this discrepancy. In particular, it is possible that mitochondria-bound FAT/CD36 cannot be detected by immunofluorescence because the epitope that is recognized by the FAT/CD36 antibody may not be exposed when FAT/CD36 is embedded in the mitochondrial membrane (21). Our findings agree with those of others (3) that FAT/CD36 is physically associated with CPT I. However, further research is required to determine whether these two proteins are in direct physical contact with one another, as it is possible that bridging and/or adaptor proteins may link these proteins.

Consistent with previous research in rodent skeletal muscle demonstrating that the abundance of FAT/CD36 at the mitochondrial membrane is increased by muscle contraction (9), we found that the amount of FAT/CD36 that coimmunoprecipitated with CPT I increased after endurance exercise training in muscle samples from our subjects in the EX + DIET group. The increase in the amount of FAT/CD36 that coimmunoprecipitated with CPT I after EX + DIET appears to be due to the endurance exercise training per se, because the amount of FAT/CD36 that coimmunoprecipitated with CPT I did not change in our subjects who lost the same amount of weight without exercise training. Although a single session of exercise (or muscle contraction) has been found to increase the amount of FAT/CD36 present in the mitochondria in skeletal muscle (9, 16), the increase in CPT I-bound FAT/CD36 that we found after EX + DIET was likely not a residual effect of an acute bout of exercise, because the post-weight-loss muscle biopsy was taken 3 days after the last exercise session in all subjects. The increase in CPT I abundance that we found after training may have facilitated the increased interaction of FAT/CD36 and CPT I. However, the correlation between changes in FAT/CD36 bound to CPT I and the change in CPT I abundance after training was not statistically significant. Regardless of whether increased CPT I protein abundance helped mediate the increased interaction of these proteins, because total FAT/CD36 abundance did not increase in our subjects after EX + DIET we hypothesize that the exercise training induced a new baseline level of FAT/CD36 present on the mitochondrial membrane (i.e., associated with CPT I). Although our finding that FAT/CD36 abundance did not change after exercise training conflicts with some previous studies (4, 9, 30), a number of studies have also found that exercise training does not increase skeletal muscle FAT/CD36 gene or protein expression (1, 7, 8). Although the reasons for these discrepancies are not readily apparent, it is likely related to the interactive effects of exercise and dietary fat intake on FAT/CD36 expression (1, 8, 25).

The mechanism for increased fat oxidation after exercise training is generally attributed to changes in mitochondrial density and/or function (14, 23). It is notable, however, that mitochondrial enzyme activity (e.g., citrate synthase) and max-



imal exercise capacity are generally poor predictors of resting fat oxidation in endurance-trained individuals (13). In the present study, we found that the increase in resting fat oxidation after EX + DIET was strongly correlated with the increase in the amount of FAT/CD36 that coimmunoprecipitated with CPT I. Traditionally, the entry into and subsequent metabolism of fatty acids by skeletal muscle mitochondria are thought to be limited by the activity of CPT I (22). However, the fact that FAT/CD36 is associated with CPT I is suggestive of an additional mechanism whereby fatty acid oxidation may be regulated (3, 9, 16). Indeed, palmitate oxidation in purified rodent and human skeletal muscle mitochondria is significantly reduced by pharmacological inhibition of FAT/CD36 (3, 9, 16). As such, given the important role of mitochondrial FAT/CD36 in regulating fatty acid oxidation, our finding that the association of FAT/CD36 and CPT I increases in proportion with an increase in resting fat oxidation after endurance exercise training provides additional evidence for an important role of mitochondrial FAT/CD36 in the regulation of resting fat oxidation.

It should be noted that two of our subjects did not demonstrate an appreciable increase in resting fat oxidation after exercise plus weight loss (Fig. 4B). This finding is comparable to a recent study from Goodpaster et al. (14) who found that about one-third of their subjects did not increase resting fat oxidation after an exercise/weight loss intervention. These findings highlight the fact that endurance exercise training is not always found to increase resting fat oxidation. Importantly, our findings suggest that resting fat oxidation may not increase in some individuals after exercise plus weight loss, because the colocalization of FAT/CD36 with CPT I at the mitochondria did not increase.

In summary, our results suggest that mitochondrial FAT/CD36 localization is increased by exercise training and therefore could play an important role in regulating fatty acid oxidation in human skeletal muscle. Because abnormalities in the regulation of fat metabolism in obesity are linked with impairments in metabolic health, especially insulin resistance, it will be of great interest to design future studies to determine whether the abundance of FAT/CD36 at the mitochondrial membrane plays an important role in regulating skeletal muscle insulin sensitivity.

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