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Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content

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Bruce, Clinton R., A. Brianne Thrush, Valerie A. Mertz, Veronic Bezaire, Adrian Chabowski, George J. F. Heigenhauser, and David J. Dyck. Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. *Am J Physiol Endocrinol Metab* 291: E99–E107, 2006. First published February 7, 2006; doi:10.1152/ajpendo.00587.2005.—Muscle fatty acid (FA) metabolism is impaired in obesity and insulin resistance, reflected by reduced rates of FA oxidation and accumulation of lipids. It has been suggested that interventions that increase FA oxidation may enhance insulin action by reducing these lipid pools. Here, we examined the effect of endurance training on rates of mitochondrial FA oxidation, the activity of carnitine palmitoyltransferase I (CPT I), and the lipid content in muscle of obese individuals and related these to measures of glucose tolerance. Nine obese subjects completed 8 wk of moderate-intensity endurance training, and muscle biopsies were obtained before and after training. Training significantly improved glucose tolerance, with a reduction in the area under the curve for glucose ($P < 0.05$) and insulin ($P = 0.01$) during an oral glucose tolerance test. CPT I activity increased 250% ($P = 0.001$) with training and became less sensitive to inhibition by malonyl-CoA. This was associated with an increase in mitochondrial FA oxidation (+120%, $P < 0.001$). Training had no effect on muscle triacylglycerol content; however, there was a trend for training to reduce both the total diacylglcyerol (DAG) content (−15%, $P = 0.06$) and the saturated DAG-FA species (−27%, $P = 0.06$). Training reduced both total ceramide content (−42%, $P = 0.01$) and the saturated ceramide species (−32%, $P < 0.05$). These findings suggest that the improved capacity for mitochondrial FA uptake and oxidation leads not only to a reduction in muscle lipid content but also a to change in the saturation status of lipids, which may, at least in part, provide a mechanism for the enhanced insulin action observed with endurance training in obese individuals.

triacylglycerol; diacylglycerol; ceramide; insulin resistance

THE MECHANISMS LINKING OBESITY to the development of insulin resistance are not fully understood. However, it is becoming increasingly apparent that defects in skeletal muscle fatty acid (FA) metabolism are involved. In particular, a reduced capacity for FA oxidation (22, 23) and accretion of lipids in skeletal muscle seem to play a crucial role in the etiology of insulin resistance (1, 20, 36). Although the increase in muscle lipid content is manifested as an increase in triacylglycerol (TAG) (29), it is likely that elevated TAG stores may be only a marker of dysfunctional muscle FA metabolism and that accumulations of more reactive lipids, such as diacylglycerol (DAG) or ceramides, are actually responsible for the insulin resistance. Indeed, there are now plausible mechanistic links between the development of insulin resistance and accumulation of DAG and ceramide in muscle (7). Intramyocellular DAG levels are elevated in a number of models of insulin resistance (20, 32), and ceramide content is increased in muscle from obese insulin resistant humans (1). DAG can activate several isoforms of protein kinase C (PKC), which can impair insulin signal transduction to glucose transport via serine phosphorylation of insulin receptor substrate (IRS)-1 (39). In addition, ceramides can cause insulin resistance by preventing insulin-stimulated Akt serine phosphorylation and activation (33, 37).

In addition to lipid accumulation, the obese/insulin-resistant phenotype is characterized by an impaired capacity for FA oxidation, which is associated with a reduction in the activity of muscle carnitine palmitoyltransferase I (CPT I, EC 2.3.1.21), the rate-limiting step in the mitochondrial oxidation of FAs (23). Thus it is likely that an impaired ability to transport and oxidize FAs in skeletal muscle mitochondria of these individuals exacerbates lipid accumulation.

Therefore, it has been proposed that interventions that increase FA oxidation may exert an insulin-sensitizing effect on skeletal muscle, in part, by reducing the accumulation of cytosolic lipids (30). Endurance training, for example, enhances fat oxidation (16, 21, 31) and improves insulin sensitivity (8). Thus it is of interest to determine whether the improvement in muscle FA oxidation following endurance training in obese individuals is associated with a reduction in specific intramyocellular lipid pools, such as DAG and ceramide, that have a direct link with the development of insulin resistance. However, there is a paucity of data examining the effects of exercise training on skeletal muscle DAG and ceramide content. Recently, it was shown that training reduced skeletal muscle ceramide content in rats (10), whereas a cross-sectional study in humans was unable to demonstrate any differences in ceramide content in muscle from untrained and trained individuals (15). We are unaware of any study that has examined the effect of exercise on muscle DAG content.

Therefore, in the present study, we examined the effect of an endurance training program on rates of mitochondrial FA oxidation, as well as the activity of CPT I in skeletal muscle of obese individuals. Furthermore, we also determined the effect of training on skeletal muscle TAG, DAG, and ceramide content. We hypothesized that the training-induced increase in CPT I activity would enhance rates of mitochondrial FA

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Table 1. *Subject characteristics*

	Pre	Post
Age, yr	36±3	
Height, m	1.7±0.1	
Body mass, kg	103±8	102±7
BMI, kg/m^2	36±2	35±2
$\dot{V}O_{2\ peak}$, ml·min^{-1}·kg^{-1}	23.5±2.3	29.7±2.1†
Fasting blood glucose, mM	4.4±0.2	4.2±0.2
Fasting plasma insulin, pM	75.1±10.8	67.0±11.8*
Fasting plasma FA, mM	0.5±0.1	0.6±0.1
Fasting plasma adiponectin, μg/ml	14.0±4.1	9.6±2.9*

Data are means ± SE. Pre, pretraining; Post, posttraining; $\dot{V}O_{2\ peak}$, peak O_2 uptake; FA, fatty acid. *Significantly different from pretraining, $P < 0.01$; †significantly different from pretraining, $P < 0.001$.

oxidation, leading to a reduction in muscle lipid content, and would be associated with an improvement in glucose tolerance.

RESEARCH DESIGN AND METHODS

Subjects. Nine obese (BMI >30 kg/m^2) volunteers (4 male and 5 female) were studied. The physical characteristics of all participants are presented in Table 1. For inclusion, volunteers were required to have maintained a stable weight (±2 kg) for ≥6 mo prior to the study. None of the volunteers had type 2 diabetes, nor were they participating in any regular form of exercise before the study. The experimental protocol was approved by the University of Guelph ethics committee. The purpose, nature, and potential risks of the study were explained to all subjects, and informed written consent was obtained prior to participation.

Determination of peak oxygen uptake. All subjects performed a progressive, incremental cycling test to volitional fatigue on an electronically braked ergometer (Lode, Groningen, The Netherlands) for determination of peak oxygen uptake ($\dot{V}O_{2\ peak}$) prior to, at the midpoint, and at the completion of the training program (see *Exercise training program*).

Oral glucose tolerance test. One week before, and 36–48 h after completion of the training program, an oral glucose tolerance test (OGTT) was performed. Subjects reported to the laboratory after a 10- to 12-h overnight fast. An anticubital vein was cannulated for blood sampling. After a baseline blood sample (5 ml), subjects ingested a 75-g glucose load (TRUTOL; Source Medical, Mississauga, ON, Canada), and blood (5 ml) was sampled every 20 min for the next 3 h. Area under the curve for insulin and glucose responses during the OGTT were calculated.

Skeletal muscle biopsy. One week prior to training, and 36–48 h after completion of the training program, three biopsies of the vastus lateralis muscle were obtained. Briefly, local anesthesia (1% lidocaine) was administered to the skin, subcutaneous tissue, and fascia of the vastus lateralis, and an incision was made. Three separate sites on the same leg were prepared. Resting muscle biopsies were obtained using a Bergström needle. Muscle biopsy specimens (~200 mg) from the first two biopsies were cleaned of any visible nonmuscle tissue and immediately placed in ice-cold *medium I* for isolation of mitochondria (see *Isolation of mitochondria from skeletal muscle*). Muscle obtained from the final biopsy (~100–150 mg) was immediately frozen and stored in liquid nitrogen until subsequent analysis.

Exercise training program. The exercise-training program consisted of 8 wk of ergometer cycling undertaken 5 days/wk under supervision in the laboratory. Subjects cycled for 60 min at 65–70% of pretraining $\dot{V}O_{2\ peak}$. The training intensity was verified by measuring $\dot{V}O_2$ during the training session in the first week. After 4 wk of exercise training, $\dot{V}O_{2\ peak}$ was reassessed and the training program adjusted, so that for the remaining 4 wk subjects cycled for 60 min at 70% of their new $\dot{V}O_{2\ peak}$. At the completion of the 8-wk training program, subjects performed a third and final $\dot{V}O_{2\ peak}$ test. During every training session, heart rate was monitored. Subjects were allowed free access to water during the training sessions. Whole body rates of substrate metabolism were determined from indirect calorimetry during a 20-min ride at 65% of pretraining $\dot{V}O_{2\ peak}$ during the first and last weeks of the program.

Diets. Subjects were required to complete a detailed 3-day food record prior to the pretraining OGTT and muscle biopsy procedures. For the posttraining testing, subjects were instructed to replicate those diets. The macronutrient composition of the diets was 45% carbohydrate, 38% fat, and 17% protein. Subjects were instructed not to make any dietary changes during the study, and this was confirmed by analysis of food records obtained after 4 and 8 wk of training. Body weight was stable during the study; thus the effects of training independent of weight loss were studied.

Blood biochemistry. Each blood sample was collected in a sodium-heparinized tube and immediately processed; 200 μl of blood were transferred into 1 ml of 0.6 N perchloric acid (PCA) and centrifuged for 2 min. The supernatant was then removed and stored at −80°C until the subsequent analyses of whole blood glucose, which was determined in duplicate fluorometrically. The remaining blood was centrifuged for plasma collection and stored at −80°C for subsequent analysis. Plasma insulin and adiponectin concentrations were determined by radioimmunoassay using commercially available kits (Linco Research, St. Charles, MO). Plasma FA concentration was measured using an enzymatic colorimetric method (NEFA C test kit, Wako, Richmond, VA).

Isolation of mitochondria from skeletal muscle. To obtain a pure and intact mitochondrial fraction, differential centrifugation was used (6). All procedures were performed at 0–4°C. Media used were as follows: *medium I*: 100 mM KCl, 5 mM $MgSO_4 \cdot 7H_2O$, 5 mM EDTA, and 50 mM Tris·HCl, pH 7.4; *medium II*: *solution I* and 1 mM ATP, pH 7.4; *medium III*: 220 mM sucrose, 70 mM mannitol, 10 mM Tris·HCl, and 1 mM EDTA, pH 7.4. Muscle was immediately placed in ice-cold *medium I* and then blotted and weighed. Muscle was minced with scissors in 1 ml of *medium II* and transferred to an ice-cold glass Potter-Elvehjem homogenizer (Tri-R Stir-R model S63C; Fisher, Toronto, ON, Canada). Tissue was homogenized in 20 volumes of *medium II* with a tight-fitting Teflon pestle (10 up-and-down strokes, 30% maximal speed). The homogenate was spun at 800 *g* for 10 min at 4°C. Subsarcolemmal (SS) mitochondria remained in the supernatant, which was removed and kept on ice. The intermyofibrillar (IMF) mitochondria were pulled down in the pellet, which was resuspended in 5 volumes of *medium II* and treated with a protease (Sigma P5380, 0.025 ml/g) for 5 min to digest the myofibrils. Addition of 15 ml of ice-cold *medium II* was used to diminish the action of the protease. Samples were spun at 5,000 *g* for 5 min, and the supernatant was removed. The pellet was resuspended in 10 volumes of *medium II* and spun at 800 *g* for 10 min. The IMF mitochondria found in the supernatant were combined with the SS supernatant from the first 800-*g* spin to increase the mitochondrial yield and was spun at 10,000 *g* for 10 min. The pellet was washed twice in *medium II* and spun at 10,000 *g* for 10 min at 4°C. The pellet was resuspended in 1 μl *medium III*/mg tissue and used for CPT I activity measurements. The remaining mitochondria were further diluted for FA oxidation measurements.

CPT I activity. The forward radioisotope assay for the determination of CPT I activity was used as described by McGarry et al. (27), with minor modifications (2). Briefly, the assay was conducted at 37°C and was initiated by the addition of 10 μl of 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 $MgCl_2$, 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 of L-[^{3}H]carnitine and a final pH of 7.1. The sensitivity of CPT I to malonyl-CoA (M-CoA) was also determined with the addition of M-CoA in concentrations of 0.2, 0.7, and 2.0 μM. The

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reaction was stopped after 6 min with the addition of ice-cold 1 N HCl. Palmitoyl-[^{3}H]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.

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 citrate synthase activity in intact mitochondrial suspensions to total muscle citrate synthase activity to account for the quality of the mitochondrial preparation (see below).

Skeletal muscle mitochondrial FA oxidation. Muscle FA oxidation rate was determined in intact isolated mitochondria from the sum of $^{14}CO_2$ production and ^{14}C-labeled acid-soluble metabolites following a 30-min incubation in a sealed system. A volume of 900 μl of pregassed (37°C for 15 min, 5% CO_2-95% O_2 and constantly shaking) modified Krebs-Ringer buffer (MKR: 115 mM NaCl, 2.6 mM KCl, 1.2 mM KH_2PO_4, 10 mM $NaHCO_3$, 10 mM HEPES, pH 7.4) supplemented with 5 mM ATP, 1 mM NAD^+, 0.5 mM DL-carnitine, 0.1 mM coenzyme A, 25 μM cytochrome *c*, and 0.5 mM malate was added to a 20-ml vial. The 20-ml glass scintillation vial contained a microcentrifuge tube with 300 μl of 1 M benzethonium hydroxide inserted into a 1.5-ml centrifuge tube to capture $^{14}CO_2$ produced during the oxidation reaction. Viable mitochondria (100 μl) were added to the system, which was then sealed with a rubber cap and secured with parafilm. The reaction was initiated by the addition of a 6:1 palmitate-BSA complex (containing 10 μCi of [1-^{14}C]palmitate) administered 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 PCA.

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

Gaseous CO_2 produced from oxidation of [1-^{14}C]palmitate was measured by acidifying the remaining reaction mixture in the 20-ml glass scintillation vial with 1 ml of 1 M H_2SO_4. Liberated $^{14}CO_2$ was trapped by benzethonium hydroxide over a 90-min incubation period at room temperature. The microcentrifuge tube containing the $^{14}CO_2$ was put in a scintillation vial and radioactivity was counted.

Oxidative enzymes. Citrate synthase activity was determined in isolated mitochondria as well as in aliquots of homogenized whole muscle, according to Srere (34). Citrate synthase activity in intact mitochondria was determined by first assaying the extramitochondrial fraction in the suspension (1:20 dilution) and then assaying the total citrate synthase activity of the suspension (1:20 dilution) after lysing the mitochondria with 0.04% Triton X-100 and repeated freeze-thawing. The net difference provides a measure of activity in the intramitochondrial fraction. β-Hydroxyacyl-CoA dehydrogenase (β-HAD) activity was assayed spectrophotometrically at 37°C by measuring the disappearance of NADH, using the whole muscle homogenate as for citrate synthase (25).

Skeletal muscle lipids. Muscle samples (~100 mg) were freeze-dried and cleaned of any visible nonmuscle tissue, including adipose tissue. Lipids were extracted in chloroform-methanol (2:1, vol/vol) according to the method of Folch et al. (11), with addition of butylated hydroxytoluene (0.01%; Sigma-Aldrich, St. Louis, MO) as an antioxidant. Muscle lipids were separated by thin-layer chromatography on silica gel plates (0.22 mm Kieselgel 60; Merck, Darmstadt, Germany). To isolate TAG and DAG, the total lipid extract was separated using heptane-isopropyl ether-acetic acid (60:40:3, vol/vol/vol). Ceramide content was assayed as described previously (9). Briefly, samples were developed to one-third of the total length of the plate in chloroform-methanol-25% NH_3 (20:5:0.2, vol/vol/vol), dried, and rechromatographed in heptane-isopropyl ether-acetic acid (60:40:3, vol/vol/vol). Plates for were then dried, sprayed with dichlorofluorescein dye (0.2% wt/vol in methanol), and visualized under long-wave ultraviolet light. Standards for TAG, DAG, and ceramide were run along with the samples to facilitate the identification of lipid bands. The individual lipid bands were marked on the plate and scraped into vials. After the lipid separation, FAs, together with methylpentadecanoic acid (Sigma) used as an internal standard, were transmethylated in the presence of 1 ml of 14% boron fluoride in methanol at 100°C for 90 min (28). The samples were cooled to room temperature, and 1 ml of pentane and 0.5 ml of water were added. After centrifugation, the upper pentane phase was dried under nitrogen. The methyl esters were dissolved in 40 μl of hexane and analyzed by gas-liquid chromatography. A Hewlett-Packard 5890 Series II and a fused HP-INNOWax (50 m × 0.53 mm) capillary column were used. Injector and detector temperatures were set at 250°C each. The oven temperature was increased linearly from 160 to 230°C at a rate of 5°C/min. Individual FA methyl esters were quantified using the area corresponding to the internal standards (Sigma). Total TAG, DAG, and ceramide content was estimated as the sum of the particular FA content of the assessed fraction and was expressed in nanomoles per gram of tissue.

Western blot anaylsis. Muscle tissue (~20 mg) was homogenized (Polytron; Brinkman Instruments, Westbury, NY) in ice-cold buffer containing 50 mM HEPES, 150 mM NaCl, 10 mM NaF, 1 mM Na_3VO_4, 5 mM EDTA, 0.5% Triton X-100, 10% glycerol (vol/vol), 2 mg/ml leupeptin, 100 mg/ml phenylmethylsulfonyl fluoride, and 2 mg/ml aprotinin. Homogenates were spun at 16,000 *g* for 60 min at 4°C, and the supernatant was removed and protein content determined. Muscle lysates were solubilized in Laemmli buffer and boiled for 5 min, resolved by SDS-PAGE on 5–10% polyacrylamide gels, transferred to a nitrocellulose membrane, and blocked with either 5% nonfat milk powder or 7% BSA. Membranes were immunoblotted overnight with antibodies specific for Thr^{172}-phosphorylated AMP-activated protein kinase-α (AMPKα 1:1,000; Cell Signaling Technology, Beverly, MA), total AMPK (1:1,000; Upstate Cell Signaling Solutions, Waltham, MA), Ser^{79}-phosphorylated acetyl-CoA carboxylase (ACC), which most likely recognizes the equivalent Ser^{221} in human ACCβ in the phosphorylated state (1:1,000; Cell Signaling Technology), total ACC (1:1,000; Cell Singaling Technology), peroxisome proliferator-activated receptor (PPAR)α (1:1,000; Chemicon, Temecula, CA), and PPARδ (1:800; Santa Cruz Biotechnology, Santa Cruz, CA). After incubation with horseradish peroxidase-conjugated secondary antibody (1:1,000; Cell Signaling Technology), the immunoreactive proteins were detected with enhanced chemiluminescence and quantified by densitometry.

Statistics and calculations. The insulin sensitivity index was calculated from the following formula [10,000/square root of (fasting glucose × fasting insulin) × (mean glucose × mean insulin during OGTT)] from Matsuda and DeFronzo (26). All data are reported as means ± SE. Differences between pre- and posttraining were analyzed with paired *t*-tests. The sensitivity of CPT I to M-CoA was examined using a two-way ANOVA. Associations between variables were investigated using Pearson correlation analysis. Statistical significance was accepted at $P < 0.05$.

RESULTS

Subject characteristics. Subject characteristics are presented in Table 1. Body mass and BMI did not change following training. $\dot{V}O_{2\,peak}$ increased by 26% ($P < 0.001$). No change was observed in fasting FA levels. Circulating adiponectin levels were significantly decreased with training ($P < 0.01$).

Glucose tolerance. Exercise training had no effect on fasting blood glucose concentration but reduced fasting plasma insulin ($P < 0.005$; Table 1). Blood glucose and plasma insulin concentrations during the OGTT are shown in Fig. 1, *A* and *B*.

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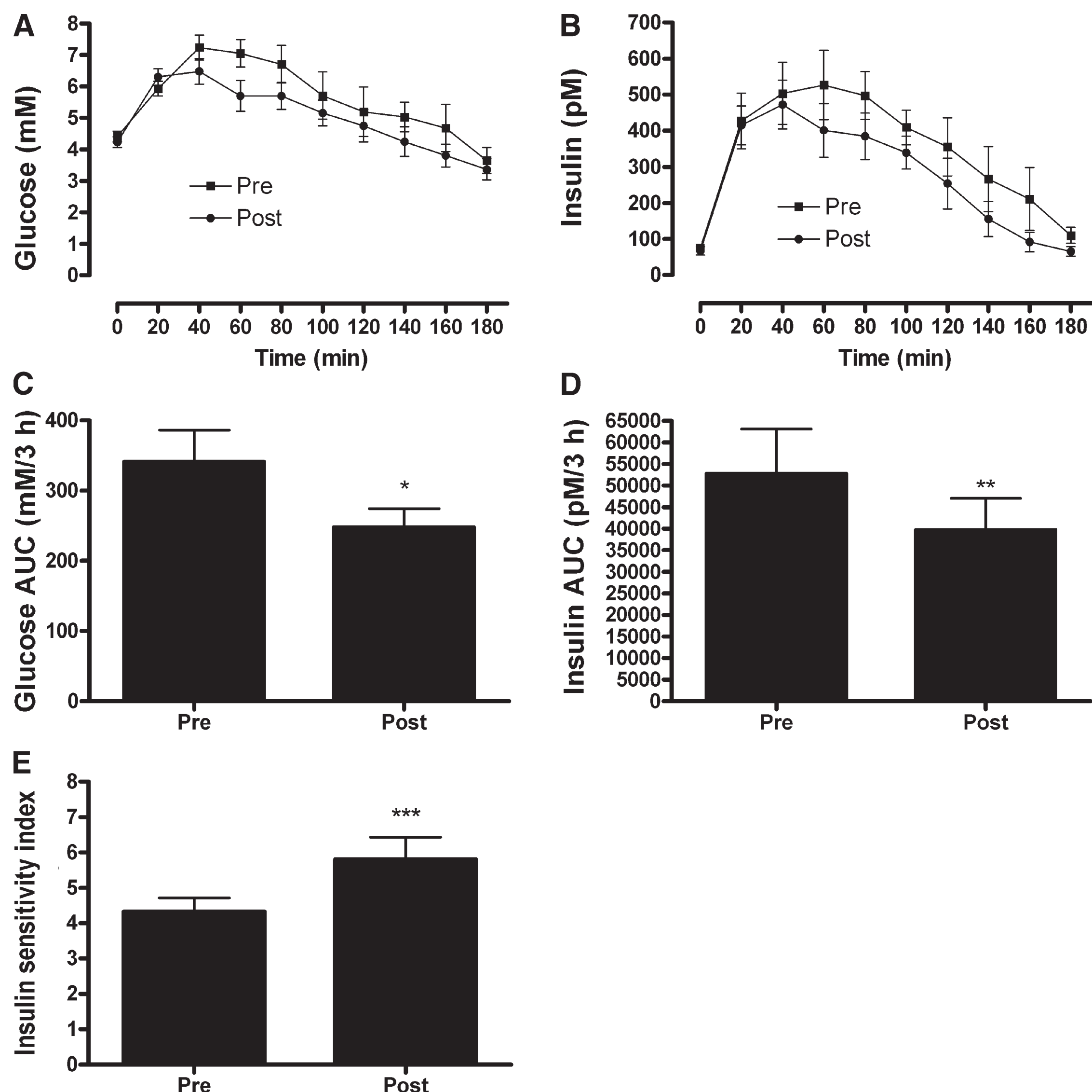

Fig. 1. Blood glucose and plasma insulin responses during an oral glucose tolerance test performed pre- and posttraining. *A*: blood glucose concentration. *B*: plasma insulin concentration. *C*: blood glucose area under the curve (AUC). *D*: plasma insulin AUC. *E*: calculated insulin sensitivity index. Date are means ± SE. *$P < 0.05$ vs. pretraining; **$P = 0.01$ vs. pretraining; ***$P = 0.001$ vs. pretraining.

The area under the curve for both glucose ($P < 0.05$; Fig. 1*C*) and insulin ($P = 0.01$; Fig. 1*D*) during the OGTT was reduced with training. The insulin sensitivity index improved by 34% with training ($P = 0.001$; Fig. 1*E*).

CPT I activity and oxidative enzymes. Maximal CPT I activity increased by ~250% with training ($P = 0.001$; Fig. 2*A*). After training, CPT I was less sensitive to inhibition by M-CoA (main effect $P < 0.05$; Fig. 2*B*). The mitochondrial yield was 29 ± 3%, and the quality was 89 ± 1% and was not different pre- vs. posttraining. There was an increase in the activity of citrate synthase (68%, $P < 0.001$; Table 2) and β-HAD (36%, $P < 0.001$; Table 2) with training.

Mitochondrial FA oxidation. The rate of mitochondrial FA oxidation increased by 120% following training ($P < 0.001$; Fig. 3). The change in mitochondrial FA oxidation did not significantly correlate with any changes in glucose tolerance. The exercise training program increased whole body fat oxidation during a 20-min standardized ride at 65% of pretraining $\dot{V}O_{2\ peak}$, as shown by the reduction in respiratopry exchange ratio (0.96 ± 0.03 vs. 0.91 ± 0.02 for pre- and posttraining, respectively; $P < 0.001$).

Skeletal muscle lipids. Training did not alter the FA composition or the content of skeletal muscle TAG (Table 3). Training resulted in an ~15% reduction in total DAG content in muscle ($P = 0.06$; Table 4). There was a significant decrease in the C16:0 DAG species following training ($P < 0.05$; Table 4). There were also reductions in C18:0 (−44%, $P = 0.07$), C18:1 (−14%, $P = 0.07$), and the sum of the saturated DAG FAs with training (−27%, $P = 0.06$). Interestingly, there were correlations between the change in the area under the curve for plasma insulin during the OGTT and the change in C16:0 DAG ($r^2 = 0.50$, $P < 0.05$), C18:0 DAG ($r^2 = 0.80$, $P < 0.05$), and total saturated DAG FAs ($r^2 = 0.46$, $P < 0.05$). Training resulted in a 42% decrease in total ceramide content in muscle ($P = 0.01$; Table 5). The decrease was mostly attributable to changes in C16:0 (−40%, $P < 0.05$), C16:1 (−46%, $P =$

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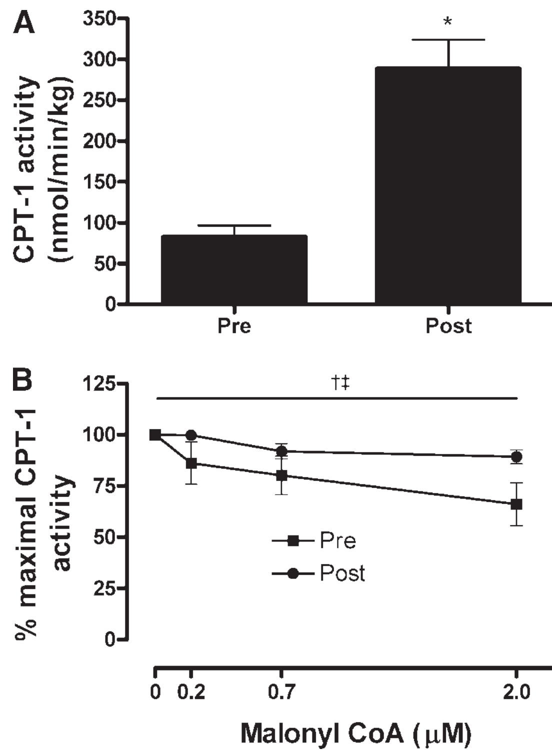

Fig. 2. Effects of training on skeletal muscle carnitine palmitoyltransferase I (CPT I) activity. *A*: maximal activity of CPT I pre- and posttraining. *B*: effect of training on the sensitivity of CPT I to inhibition by malonyl-CoA. Data are means ± SE. *$P = 0.001$ vs. pretraining; †main effect for training, $P < 0.05$; ‡main effect for malonyl-CoA, $P < 0.01$.

0.001), C18:1 (−52%, $P = 0.001$), C18:2 (−58%, $P < 0.05$), and C20:0 ceramide (−40%, $P < 0.05$). There was also a tendency for C18:0 ceramide to decrease with training (−52%, $P = 0.06$). Training resulted in a reduction in the total saturated (−32%, $P < 0.05$) and monounsaturated ceramide FAs (−40%, $P < 0.01$). The changes in ceramide content were not associated with any changes in glucose tolerance

Skeletal muscle signaling. Training did not affect total protein expression or the phosphorylation state of AMPK (Fig. 4, *A* and *B*). Furthermore, the protein content and phosphorylation of ACC, a target of AMPK, was unaltered by training (Fig. 4, *C* and *D*). PPARα (Fig. 4*E*) and PPARδ (Fig. 4*F*) were not changed with training.

DISCUSSION

It has been proposed that interventions that lead to an increased capacity for skeletal muscle FA oxidation, such as endurance training, may improve insulin action through a reduction in intramyocellular lipids. However, very little is known regarding the effect of endurance training on skeletal

Table 2. *Citrate synthase and β-HAD activity*

	Pre	Post
Citrate synthase	23.0±2.3	38.6±3.1*
β-HAD	12.7±1.4	17.2±1.7*

Data are means ± SE expressed as $\mu mol \cdot min^{-1} \cdot g$ wet wt^{-1}. β-HAD, β-hydroxyacyl-CoA dehydrogenase. *Significantly different from pretraining, $P < 0.001$.

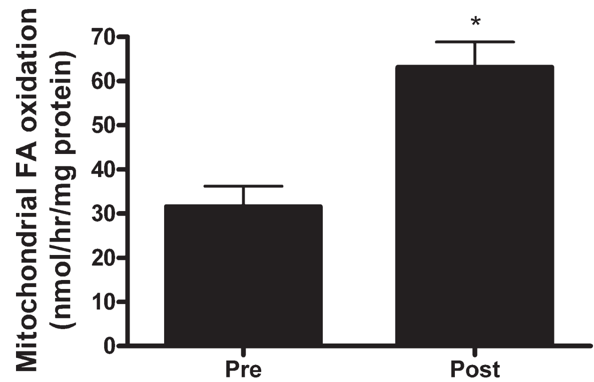

Fig. 3. Effect of training on rates of mitochondrial fatty acid (FA) oxidation. Data are means ± SE. *$P < 0.001$ vs. pretraining.

muscle lipids, in particular DAG and ceramide content. Therefore, in the present study we examined whether the anticipated improvement in the capacity for muscle to oxidize FA following training would be associated with a reduction in intramuscular DAG and ceramide content in obese individuals. Here, we report that endurance training increases CPT I maximal activity and reduces its sensitivity to inhibition by M-CoA, leading to increased rates of mitochondrial FA oxidation. This was associated with a reduction in the total content of ceramide and altered composition of ceramide-FAs in muscle. Furthermore, there was a trend toward a reduction in total DAG content in muscle as well as certain saturated DAG-FA species. Importantly, these changes in intramuscular lipids were correlated with the observed improvement in glucose tolerance.

Effect of training on CPT I activity and mitochondrial FA oxidation. CPT I is considered the rate-limiting step in the oxidation of FAs. CPT I activity is reduced in skeletal muscle from obese individuals and is likely to contribute to the suppressed rates of FA oxidation in obesity (23). Thus obese muscle appears to be prone to partitioning FAs toward storage. Although studies have shown that CPT I activity is increased in muscle of trained individuals (21,35), no studies have examined the effect of endurance training on CPT I activity in muscle of obese individuals. Furthermore, few studies have directly linked changes in CPT I activity with an alteration in mitochondrial FA oxidation. In the present study, CPT I

Table 3. *Effect of exercise training on skeletal muscle triacylglycerol content*

Fatty Acid	Pre	Post
14:0	0.42±0.11	0.33±0.45
16:0	4.13±1.17	3.37±4.82
16:1	0.73±0.14	1.04±0.37
18:0	0.79±0.30	0.62±0.23
18:1	6.53±1.18	5.86±0.81
18:2	1.57±0.20	1.99±2.71
18:3	0.12±0.02	0.26±0.11
20:0	0.02±0.01	0.02±0.01
20:4	0.05±0.01	0.06±0.01
20:5	0.01±0.01	0.01±0.01
22:0	0.02±0.01	0.01±0.01
22:6	0.02±0.01	0.02±0.01
24:1	0.01±0.01	0.01±0.01
Total	14.40±2.55	13.59±1.52

Data are means ± SE expressed as μmol/g dry wt.

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Table 4. *Effect of exercise training on skeletal muscle DAG content*

Fatty Acid	Pre	Post
14:0	61.8±10.0	54.8±10.8
16:0	452.7±75.8	330.2±34.5*
16:1	70.1±12.5	72.5±17.3
18:0	75.3±16.9	42.3±7.7
18:1	487.4±61.6	418.9±50.9
18:2	119.6±12.2	118.7±12.3
20:4	48.5±19.8	41.6±4.5
20:5	9.5±1.9	6.0±0.1
22:0	5.8±0.9	6.0±1.5
22:6	165.9±30.5	171.6±42.7
24:1	20.9±7.3	8.1±2.1
Total	1,517.6±168.1	1,270.6±150.6§

Data are means ± SE expressed as nmol/g dry wt. *$P < 0.05$ vs. pretraining, §$P = 0.06$ vs. pretraining.

activity was increased with training, and this was associated with enhanced rates of mitochondrial FA oxidation. It is possible that the increase in both CPT I activity and mitochondrial FA oxidation could be due solely to an increase in mitochondrial density, which occurs with training. To account for this possibility, we measured citrate synthase activity, which is in direct proportion to muscle mitochondrial content (12). Training resulted in a 68% increase in citrate synthase activity, whereas CPT I activity increased by ~250% and mitochondrial FA oxidation increased 120% with training. Thus an increase in mitochondrial density cannot entirely account for the improved fat oxidation. It is difficult to reconcile the mismatch in the relative increase in CPT I activity and mitochondrial FA oxidation, as it would be expected that changes in CPT I activity be closely mirrored by changes in mitochondrial FA oxidation. It should be noted that the post-training CPT I activities reported here are similar to those reported in young, healthy, untrained individuals (35) and that the large increase in CPT I with training could be a function of the very low pretraining levels in our subjects. Nevertheless, these adaptations to training appear to reflect both an increase in mitochondrial density and a change in mitochondrial function.

We also measured the effect of training on several regulators of skeletal muscle FA metabolism. Training did not influence the protein content or phosphorylation state of AMPK and its target ACC, nor did it affect the protein content of PPARα and -δ. The lack of change in PPARα protein expression is in contrast to a previous report by Horowitz et al. (17), who showed a doubling of PPARα protein following 12 wk of training. The difference in studies could be due to differences in subject characteristics. Horowitz et al. (17) used female subjects who were leaner and younger and also trained for a longer period of time than the subjects in the present study. Surprisingly, we did find a reduction in circulating adiponectin levels. Other studies examining the effect of endurance training on circulating levels of adiponectin have reported no change (19) or an increase (24), which is in contrast to our findings. Adiponectin increases skeletal muscle FA oxidation by stimulating AMPK, leading to inhibition of ACC and a reduction in M-CoA content (5, 38). Thus it seems paradoxical that endurance training, which increases FA oxidation, would lead to a reduction in adiponectin levels.

Effect of training on the sensitivity of CPT I to M-CoA. CPT I is reversibly inhibited by M-CoA, thereby reducing FA oxidation (27, 35). The increase in CPT I activity and the concomitant increase in mitochondrial FA oxidation observed with exercise training could be mediated by either a change in M-CoA levels or a change in the sensitivity of CPT I to inhibition by M-CoA. Due to limited sample size, we were unable to determine M-CoA concentration. However, we were able to demonstrate that with training CPT I became less sensitive to inhibition by M-CoA. This may be a potential mechanism to explain the increase in CPT I activity and subsequent enhancement of mitochondrial FA oxidation with training. However, this finding differs from a previous report that found an increased sensitivity of CPT I to M-CoA in trained individuals (35). This seems paradoxical, considering that endurance-trained individuals have a greater capacity to oxidize FA (21). These differences between studies could be related to differences in the subject populations. The subjects in the current study were vastly different from those of Starritt et al. (35) in that they were obese, less fit, and had lower levels of CPT I activity pretraining. Nonetheless, the results of the present study offer new insight into the regulation of CPT I activity by M-CoA and subsequent mitochondrial FA oxidation in skeletal muscle following training.

Effect of training on skeletal muscle lipids. The major finding of the present study was that total ceramide content of skeletal muscle decreased in conjunction with enhanced glucose tolerance and insulin sensitivity in obese individuals after a short-term training program. These findings differ from those of Helge et al. (15), who reported no difference in the ceramide content of muscle from trained and untrained individuals. However, similar observations to the present study have been reported in rat skeletal muscle, where training reduced total ceramide content (10). This finding is of particular importance in determining the mechanisms by which endurance training enhances insulin action, especially given that ceramide levels are negatively associated with insulin sensitivity (36) and are elevated in skeletal muscle from obese, insulin-resistant humans (1). Furthermore, ceramide has been shown to play a mechanistic role in the development of skeletal muscle insulin

Table 5. *Effect of exercise training on skeletal muscle ceramide content*

Fatty Acid	Pre	Post
14:0	75.6±21.4	40.9±10.8
16:0	162.4±20.0	97.6±7.4*
16:1	16.9±2.0	9.2±1.4‡
18:0	117.7±18.4	77.3±2.8§
18:1	71.2±8.7	34.2±5.3‡
18:2	70.5±21.8	31.1±11.6*
18:3	2.8±0.7	2.4±0.5
20:0	8.2±1.4	4.8±0.6*
20:4	23.8±3.9	19.4±5.2
20:5	12.6±3.4	13.9±2.2
22:0	19.6±3.2	22.6±2.8
22:6	72.1±20.0	61.4±11.6
24:0	50.3±11.5	51.8±5.2
24:1	30.3±7.2	27.9±5.8
Total	734.0±98.8	494.2±50.8†

Data are means ± SE expressed as nmol/g dry wt. *$P < 0.05$ vs. pretraining; †$P = 0.01$ vs. pretraining; ‡$P = 0.001$ vs. pretraining; §$P = 0.06$ vs. pretraining.

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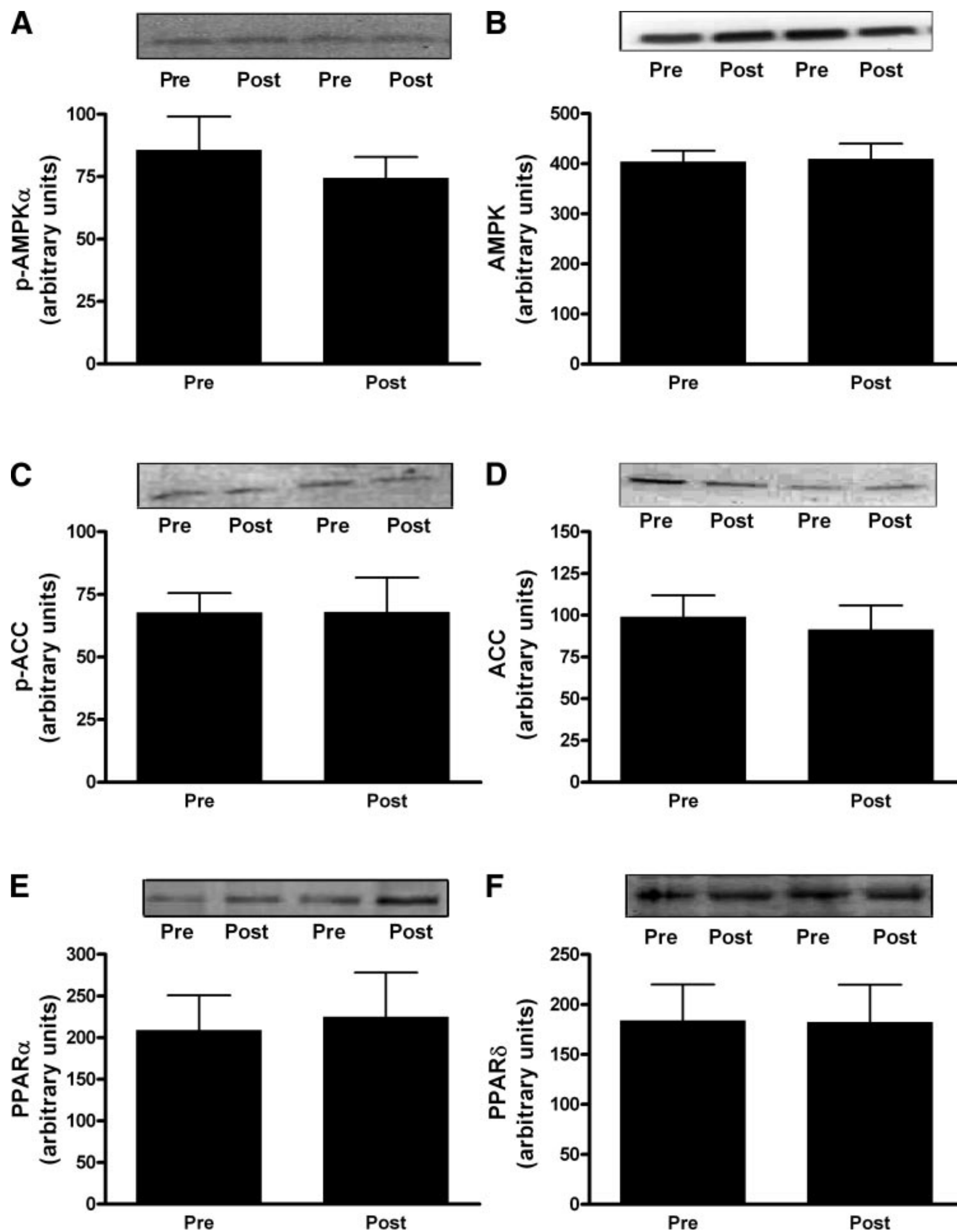

Fig. 4. Effect of training on phosphorylation and expression of proteins involved in regulating skeletal muscle FA metabolism. *A*: phosporylation (p) of AMP-activated protein kinase-α (AMPKα). *B*: total AMPK expression. *C*: phosporylation of acetyl-CoA carboxylase (ACC). *D*: total ACC expression. *E*: peroxisome proliferator-activated receptor (PPAR)α expression. *F*: PPARδ expression. Data are means ± SE.

resistance. In C_2C_{12} muscle cells, ceramide induces insulin resistance and inhibits insulin-stimulated Akt serine phosphorylation and activation (33). Therefore, these findings suggest that a reduction in muscle ceramide content would, at least in part, contribute to enhanced insulin action with training.

The accumulation of DAG in skeletal muscle has also been shown to be associated with the development of insulin resistance in rodents (39) and humans (1). DAG is a potent activator of a number of protein kinase C isoforms that can impair insulin-stimulated glucose uptake through inhibition of insulin receptor signaling at the level of IRS-1 (39). To our knowledge, this is the first study to investigate the effect of endurance training on muscle DAG content in humans. Thus we hypothesized that the increased capacity for FA oxidation following training would be associated with a reduction in skeletal muscle DAG content. Despite being unable to detect any significant change in the total DAG content with training, there was a strong trend for training to reduce DAG levels.

Apart from the content of lipid in the muscle, the composition of the lipids may also affect insulin action (14). Indeed, in addition to the reduction in total ceramide content, we also found that training altered the composition of individual ceramide-FA species in skeletal muscle. After training, there was reduction in the C16:0, C16:1, C18:1, C18:2, and C20:0, and there was a trend for the C18:0 species to also decrease with training. This is consistent with the findings of Dobrzyń et al. (10), who also reported changes in the composition of ceramide-FA in several muscle types of rats who were exercise trained for 6 wk. It is of particular interest that there was a decrease in the total saturated ceramide-FA species with training. In addition, there was decrease in the C16:0 DAG FA with training, and there was also a tendency for the other saturated species of DAG, C18:0 (P = 0.07), and the total saturated DAG-FA (P = 0.06) to decrease with training. Furthermore, there were significant correlations between the change in these saturated species of DAG and the change in the area under the

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curve for insulin determined during the OGTT. These findings are similar to those of Houmard et al. (18) who reported that the saturated species of long-chain acyl-CoAs (palmitoyl-CoA and stearate-CoA) decreased significantly with weight loss. It has been suggested that saturated FA and their derivatives are potent in terms of functioning as intracellular signaling molecules that induce insulin resistance (18). Therefore, the findings of the present study suggest that exercise training can modify the FA profile of skeletal muscle lipids in favor of enhanced insulin sensitivity.

Interestingly, we found that the reduction in muscle ceramide and DAG content with training was independent of any change in muscle TAG content. It has previously been shown that well-trained individuals have increased muscle TAG content despite being insulin sensitive (13). Thus it has been hypothesized that intramyocellular TAG per se does not directly influence insulin action in skeletal muscle (13). However, recent studies have demonstrated that, in contrast to Goodpaster et al. (13), muscle TAG concentrations are not elevated in endurance-trained individuals (3) and that training does not alter muscle TAG levels in healthy individuals (4). Therefore, the effect of exercise training on muscle TAG content is equivocal. Nonetheless, taken together, the results from this study suggest that muscle DAG and ceramide play a more important role in the regulating insulin sensitivity than muscle TAG.

In conclusion, we show that endurance training increases maximal CPT I activity, leading to enhanced rates of mitochondrial FA oxidation in vitro. This was associated with a reduction in the total content of ceramide and changes in the composition of ceramide-FA in skeletal muscle of obese individuals. There was also a tendency for a reduction in total DAG content and the saturated DAG-FA species with training. These findings suggest that the improved capacity for mitochondrial FA uptake and oxidation not only leads to a reduction in muscle lipid content but also a change in the saturation status of lipids, which may, at least in part, provide a mechanism for the enhanced insulin action observed with endurance training in obese individuals.

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GRANTS

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