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Effect of endurance training on lipid metabolism in women: a potential role for PPARα in the metabolic response to training

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Horowitz, Jeffrey F., Teresa C. Leone, Weqing Feng, Daniel P. Kelly, and Samuel Klein. Effect of endurance training on lipid metabolism in women: a potential role for PPARα in the metabolic response to training. *Am J Physiol Endocrinol Metab* 279: E348–E355, 2000.—Endurance training increases fatty acid oxidation (FAO) and skeletal muscle oxidative capacity. However, the source of the additional fat and the mechanisms for increasing FAO capacity in muscle are not clear. We measured whole body and regional lipolytic activity and whole body and plasma FAO in six lean women during 90 min of bicycling exercise (50% pretraining peak O_2 consumption) before and after 12 wk of endurance training. We also assessed skeletal muscle content of peroxisome proliferator-activated receptor-α (PPARα) and its target proteins that regulate FAO [medium-chain and very long chain acyl-CoA dehydrogenase (MCAD and VLCAD)]. Despite a 25% increase in whole body FAO during exercise after training ($P < 0.05$), training did not alter regional adipose tissue lipolysis (abdominal: 0.56 ± 0.26 and 0.57 ± 0.10 μmol · 100 $g^{-1} \cdot min^{-1}$; femoral: 0.13 ± 0.07 and 0.09 ± 0.02 μmol · 100 $g^{-1} \cdot min^{-1}$), whole body palmitate rate of appearance in plasma (168 ± 18 and 150 ± 25 μmol/min), and plasma FAO (554 ± 61 and 601 ± 45 μmol/min). However, training doubled the levels of muscle PPARα, MCAD, and VLCAD. We conclude that training increases the use of nonplasma fatty acids and may enhance skeletal muscle oxidative capacity by PPARα regulation of gene expression.

exercise; lipolysis; fatty acid; intramuscular triglyceride; stable isotopes

ENDURANCE TRAINING increases the use of endogenous fat as a fuel during exercise (13). The source of the additional triglyceride oxidized after training is controversial and may be affected by gender. Results from a recent study performed in women suggest that training increases the availability and oxidation of plasma fatty acids derived from adipose tissue triglycerides (8). In contrast, data from a study performed mostly in men (24) found that training did not increase the oxidation of plasma fatty acids, suggesting that the increase in fat oxidation after training is derived from intramuscular triglycerides. However, differences in preexercise feeding protocols between studies may have influenced the metabolic response to exercise and confounded potential gender comparisons. In the study involving men (24), exercise was performed in the postabsorptive state (after subjects fasted overnight), whereas in the study performed in women (8), exercise was performed 4–6 h after a meal, which can have a strong influence on substrate metabolism (27). The effects of training on whole body and regional lipolytic activity and fat oxidation during exercise in women during postabsorptive conditions (12 h fast) have not been studied.

The increase in fat oxidation during exercise induced by training is associated with an increase in skeletal muscle fatty acid oxidative capacity. Endurance training increases muscle levels of the mitochondrial enzymes involved in β-oxidation (26), tricarboxylic acid cycle (TCA) activity (14), and oxidative phosphorylation (12). However, little is known about the mechanisms responsible for the increase in mitochondrial proteins induced by training. Most mitochondrial proteins are encoded by nuclear genes, and their expression is regulated by nuclear transcription factors. The results of recent studies indicate that peroxisome proliferator-activated receptor-α (PPARα), a member of the nuclear receptor transcription factor superfamily, plays a critical role in the expression of genes involved in mitochondrial fatty acid oxidation (1, 10). However, the effect of endurance training on skeletal muscle PPARα and its target fatty acid oxidative enzymes has not been evaluated in either laboratory animals or humans.

The purpose of this study was to determine the effect of endurance exercise training on whole body regional adipose tissue and skeletal muscle fatty acid metabolism during exercise in women after an overnight fast. Specifically, we evaluated *1*) whole body plasma fatty acid availability and uptake, *2*) the relative contribution of plasma and nonplasma fatty acids to total energy production, and *3*) regional (abdominal and femoral subcutaneous adipose tissue) lipolytic activity

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during exercise performed at the same absolute intensity before and after 12–14 wk of endurance training. In addition, we assessed the effect of training on skeletal muscle levels of mitochondrial enzymes involved in fatty acid oxidation [medium-chain and very long-chain acyl-CoA dehydrogenase (MCAD and VLCAD, respectively)] and the TCA cycle [citrate synthase (CS)]. Levels of PPARα were also characterized in skeletal muscle before and after training to determine whether this regulatory factor might be involved in the known increase in mitochondrial oxidative capacity during exercise training.

Our results indicate that the increase in whole body fat oxidation after training is accompanied by an induction in levels of PPARα and its target proteins, but not by an increase in adipose tissue lipolysis or plasma fatty acid availability, uptake, and oxidation. These findings suggest that alterations in lipid metabolism that occur with endurance training are localized to skeletal muscle and may involve the nuclear receptor PPARα in these metabolic adaptations to training.

METHODS

Subjects. Six lean, premenopausal women (28 ± 3 yr of age) participated in this study (Table 1). No subjects were taking any medications or smoked tobacco, and none had evidence of medical illness after a comprehensive examination, which included a history and physical examination, routine blood tests, and an electrocardiogram. All subjects were weight stable for ≥2 mo before the study. No subjects were involved in any regular exercise program for ≥6 mo before the study, and none had ever been endurance-trained athletes. All experimental protocols were performed within the first 2 wk of the follicular phase of their menstrual cycle. Written informed consent was obtained before participation in the study, which was approved by the Human Studies Committee and the General Clinical Research Center (GCRC) Scientific Advisory Committee of Washington University School of Medicine.

Preliminary testing. Peak aerobic capacity was measured before and after 12–14 wk of endurance training. Peak oxygen consumption ($\dot{V}O_{2\,peak}$) was measured with a Vmax 29 metabolic cart (SensorMedics, Yorba Linda, CA) during upright cycle ergometer exercise. The protocol consisted of a 4-min warm-up, after which the work rate was progressively increased every minute until at least two of the following three criteria were met: *1*) a leveling off of the rate of oxygen consumption ($\dot{V}O_2$) despite increases in workload, *2*) respiratory exchange ratio ≥1.15, and *3*) attainment of age-predicted maximal heart rate.

Table 1. *Characteristics of the study subjects*

	Pretraining	Posttraining	Percent Change
Body weight, kg	57.5 ± 2.1	56.7 ± 1.6	1 ± 1
Body mass index, kg/m^2	21.2 ± 0.4	20.9 ± 0.4	1 ± 1
$\dot{V}O_{2peak}$, l/min	1.9 ± 0.1	2.2 ± 0.1*	15 ± 3
$\dot{V}O_2$ during exercise trial, l/min	1.03 ± 0.09	1.06 ± 0.08	4 ± 5
HR during exercise trial, beats/min	128 ± 5	113 ± 4*	11 ± 2

Values are means ± SE. $\dot{V}O_2$, O_2 consumption; $\dot{V}O_{2peak}$, peak $\dot{V}O_2$; HR, heart rate. *Significantly different from pretraining value, $P < 0.05$.

Experimental protocol. Subjects were admitted to the GCRC at Washington University School of Medicine on four occasions: twice before and twice after 12–14 wk of endurance training. At 1900 on each day of admission, subjects ingested a standard meal (60% carbohydrate, 25% fat, and 15% protein) containing 12 kcal/kg body weight. Subjects were randomized to perform an exercise study with isotope tracer infusion (i.e., exercise-infusion trial) or an exercise study without tracer infusion (i.e., background trial) the following morning. One week later, subjects were readmitted to the GCRC to perform the other study (exercise-infusion or background trial).

The exercise-infusion trials began at ~0700, after subjects had fasted overnight (12 h). Catheters were placed into a forearm vein for isotope infusion and into a radial artery for blood sampling while subjects were lying in bed. Blood and breath samples were taken to determine background substrate isotopic enrichments. Subjects then moved from bed to the chair of a cycle ergometer (Ergometrics 800, Ergoline, Germany) that was modified for recumbent cycling. At ~0815 (75 min before exercise), a priming dose (1.05 μmol/kg) of [1-^{13}C]bicarbonate was given, and a constant infusion (0.035 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) of [1-^{13}C]palmitate [98% atom percent excess (APE); Cambridge Isotopes, Andover, MA] bound to albumin (Centeon, LLC, Kankakee, IL) was started and continued throughout the study.

Four blood samples were obtained 5 min apart during the last 15 min of the basal resting period to determine basal plasma substrate and hormone concentrations and substrate kinetics. After the last basal samples were collected, subjects exercised for 90 min at ~50% of their pretraining $\dot{V}O_{2\,peak}$ on a recumbent cycle ergometer. Blood samples were obtained every 10 min during exercise to determine substrate kinetics, and heart rate was measured every 10 min using a telemetry heart rate monitor (Cardiochamp Sensor, Dynamics; Freemont, CA). Evacuated test tubes were used to collect expired breath samples in quadruplicate from a mixing chamber at 60, 70, 80, and 90 min of exercise to determine plasma fatty acid oxidation rate. $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) rates were measured from 60 through 90 min of exercise with a Vmax 29 metabolic cart (SensorMedics) to determine whole body fat and carbohydrate oxidation rates.

Two microdialysis probes (30 × 0.62 mm, molecular cut-off 20,000 Da; CMA/60; CMA Microdialysis, Stockholm, Sweden) were placed in subcutaneous adipose tissue with subjects under local anesthesia. One probe was placed in the abdominal region, ~5 cm lateral to the umbilicus, and the other probe was placed in the femoral region, ~25 cm above the patella. Throughout the study, the probes were perfused at 0.3 μl/min with Ringer solution containing 3% dextran. It has been shown that almost 100% of interstitial glycerol is recovered from these probes when they are perfused at this slow rate (32). No measurement of glycerol recovery was made in the present study. Microdialysis samples were not collected for ≥1 h after insertion to avoid any influence of placement trauma on glycerol measurements. Microdialysis samples were collected during the 25-min period immediately before exercise and during three 25-min periods (15–40, 40–65, and 65–90 min) of exercise. To prevent evaporation, the samples were collected in sealed microvials (CMA Microdialysis), flash-frozen in liquid nitrogen, and stored at −70°C until glycerol analyses were performed.

Abdominal and femoral subcutaneous adipose tissue blood flow (ATBF) was measured by the ^{133}Xe clearance technique (21). At least 90 min before exercise, ~100 μCi of ^{133}Xe

dissolved in 0.1 ml of normal saline were slowly injected over 60 s into abdominal and femoral subcutaneous adipose tissue. A cesium iodide detector (Oakfield Instruments, Eynsham, UK) was placed directly over the injection sites and secured to the skin by use of a standard ostomy appliance and tape. The decline in ^{133}Xe was determined by collecting 10-s counts (33) beginning 15 min before exercise and throughout the exercise bout.

The background trial was performed on a separate occasion to account for the increase in background breath $^{13}CO_2$ that is produced during exercise because of increased oxidation of naturally occurring ^{13}C-enriched carbohydrate. After subjects fasted overnight (12 h), they exercised on a recumbent cycle ergometer for 90 min at ~50% of their pretraining $\dot{V}O_{2\ peak}$, just as in the exercise-infusion trial. Breath samples were collected at 60, 70, 80, and 90 min of exercise; $\dot{V}O_2$ and $\dot{V}CO_2$ were measured from 60 through 90 min of exercise.

Muscle biopsies. Muscle tissue samples were obtained before and after training ~1 wk after the exercise studies. After subjects fasted overnight, ~100 mg of tissue were obtained from the vastus lateralis muscle by needle biopsy (3). Samples were immediately frozen in liquid nitrogen and stored at −70°C. The pretraining sample was obtained ≥7 days after the pretraining exercise study but before the start of exercise training. The posttraining sample was obtained exactly 3 days after the last exercise training bout.

Exercise training. After the pretraining studies were completed, all subjects participated in an endurance training program, which consisted of cycling on an ergometer for 35–45 min, 4 days/wk, for 12–14 wk under direct supervision. Exercise intensity was based on the percentage of each subject's maximal heart rate (HR_{max}), determined during the initial aerobic capacity 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 increased from 35 to 45 min. To prevent weight loss during the 12- to 14-wk training period, energy balance was maintained by feeding the subjects a defined liquid formula supplement (Ensure, Ross Laboratories, Columbus, OH) at the end of each training session to replenish the calories expended during exercise. We have previously shown that this refeeding regimen prevented changes in body weight and body composition in lean men who completed 16 wk of endurance training (15).

Posttraining studies. All studies performed before training were repeated after 12–14 wk of exercise training. During the exercise studies, each subject exercised at the same absolute intensity before and after training. This allowed us to compare the absolute rates of substrate oxidation before and after training, as well as the relative contribution of plasma fatty acids, nonplasma fatty acids, and carbohydrate to total energy expenditure. The posttraining exercise studies and muscle biopsy were performed exactly 3 days after the end of exercise training. To ensure a 3-day rest interval before each study, subjects resumed training for several days after each procedure until all trials were completed. Therefore, the total duration of training ranged from 12 to 14 wk.

Analytical procedures. Plasma insulin concentration was measured by radioimmunoassay (antibody raised to porcine insulin; coefficient of variation = 11.8; Linco Research, St. Louis, MO). Plasma catecholamine concentrations were determined by a radioenzymatic method (34). Plasma fatty acid concentrations were quantified by gas chromatography by adding heptadecanoic acid to plasma as an internal standard (39). Microdialysis glycerol concentration was measured using a CMA 600 Microdialysis Analyzer (CMA Microdialysis).

The tracer-to-tracee ratio (TTR) for plasma palmitate was determined by gas chromatography-mass spectrometry (GC-MS) with an MSD 5971 system (Hewlett-Packard, Palo Alto, CA) with capillary column (30). Acetone was used to precipitate plasma proteins, and hexane was used to extract plasma lipids. Free fatty acids were converted to their methyl esters with iodomethane and isolated by using solid phase extraction cartridges. Samples were dried in a Speed-Vac concentrator (Savant Instruments, Farmingdale, NY), reconstituted in heptane, and transferred to auto sampler vials for GC-MS analysis. Ions at mass-to-charge ratios 270.2 and 271.2, produced by electron impact ionization, were selectively monitored.

The ratio of $^{13}CO_2$ to $^{12}CO_2$ in expired breath was determined by isotope ratio mass spectrometry (IRMS; Sira II, dual-inlet triple collector, VG Fisons, Cheshire, UK) as described previously (35). Briefly, CO_2 was isolated from breath by passing the sample through a series of traps to remove water vapor, nitrogen, and oxygen. The purified sample was then ionized by electron bombardment and repelled past a series of focusing lenses toward the detector. The ratio of masses 45 and 44 were determined, representing $^{13}CO_2$ and $^{12}CO_2$, respectively.

Skeletal muscle protein content of PPARα, MCAD, VLCAD, CS, and actin was assessed by protein immunoblot (Western) analysis (4) by use of the Enhanced Chemiluminescence Detection System (Amersham Pharmacia Biotech, Piscataway, NJ). Total cellular protein was extracted from skeletal muscle by homogenization in lysis buffer [1% NP-40, 1% SDS, and Complete protease inhibitor cocktail (Roche Molecular Biochemicals, Indianapolis, IN)], and insoluble cellular debris was removed by centrifugation. Western analysis was performed using specific polyclonal antibodies raised to porcine MCAD (17), D-domain of the murine PPARα (a gift from Michael Arand) (9), human VLCAD (a gift from Arnold Strauss), CS (Alpha Diagnostic International, San Antonio, TX), and an affinity-purified antigen-specific antibody to the COOH terminus of actin (A2066, Sigma, St. Louis, MO). This was followed by incubation with horseradish peroxidase-conjugated secondary antibody to rabbit immunoglobulin G (Amersham Pharmacia Biotech). Western blots were visualized on the Molecular Imager GS525 (Bio-Rad Laboratories, Hercules, CA) and quantified using the "Multi-Analyst" software. All values were expressed normalized to the actin signal.

Calculations. Steady-state substrate concentrations and TTRs were achieved during basal conditions, so basal palmitate rate of appearance (R_a) in plasma and disappearance from plasma (R_d) were calculated using Steele's equation for steady-state conditions (36). During exercise, however, the non-steady-state equation of Steele (36) was used to calculate palmitate R_a and R_d. The effective volume of distribution for palmitate was estimated to be 50 ml/kg body weight.

The oxidation of plasma fatty acids was calculated as (35)

$$\text{plasma fatty acid oxidation} = (E_{CO_2} \cdot \dot{V}CO_2)/(TTR_p \cdot AR)$$

where E_{CO_2} is the enrichment in breath CO_2, TTR_p is the palmitate TTR in plasma, and AR is the acetate carbon recovery factor calculated from $\dot{V}O_2$ by use of a single exponential model (35)

$$\text{acetate recovery} = 92.68 \cdot \{1 - [0.5338 \cdot e^{-(0.0753 \cdot \dot{V}O_2)}]\}$$

Whole body fatty acid and carbohydrate oxidation were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ (7). The oxidation rate of nonplasma fatty acids was calculated as the difference between the rates of whole body fatty acid oxidation and plasma fatty acid oxidation.

Subcutaneous ATBF was calculated from ^{133}Xe clearance (21)

$$\text{ATBF} = -k \cdot \lambda \cdot 100 \ (\text{ml} \cdot 100\ \text{g adipose tissue}^{-1} \cdot \text{min}^{-1})$$

where k is the rate constant of the ^{133}Xe monoexponential washout curve, and λ is the adipose tissue-to-blood partition coefficient for xenon. The values for k were determined experimentally as $(\ln y_2 - \ln y_1)/(t_2 - t_1)$, where y_1 and y_2 were the counting rates at times t_1 and t_2, respectively. The value for λ was assumed to be 10 ml/g (40) for all subjects.

Regional glycerol release was estimated using the Fick principle

$$\text{regional glycerol release} = (\text{V} - \text{A}) \times \text{ATBF} \ (\mu\text{mol} \cdot 100\ \text{g}^{-1} \cdot \text{min}^{-1})$$

where A and V are the arterial and adipose tissue venous glycerol concentrations, respectively. Adipose tissue venous glycerol concentration was approximated as: $\text{V} = [(\text{I} - \text{A}) \times (1 - e^{-\text{PS/ATBF}})] + \text{A}$, where I is the interstitial glycerol concentration (calculated from microdialysis glycerol concentration), and PS is the permeability-surface area product for glycerol [assumed to be 5 ml $\cdot$ 100 g^{-1} $\cdot$ min^{-1} (29)].

Statistical analysis. A two-way ANOVA (training $\times$ time) with Tukey's post hoc analysis was used to test significance of differences for palmitate R_a, palmitate R_d, and the rates of abdominal and femoral subcutaneous adipose tissue glycerol release. A two-way ANOVA (anatomical site $\times$ time) with Tukey's post hoc analysis was used to assess the significance of differences in glycerol release between abdominal and femoral regions. Values for pre- and posttraining regional glycerol kinetics were combined, because there was no effect of endurance training on regional lipolysis. Student's t-test for independent samples was used to test the significance of differences for all of the other parameters. A value of $P \leq 0.05$ was considered to be statistically significant. All data are expressed as means ± SE.

RESULTS

Twelve weeks of endurance exercise training increased aerobic capacity by 15% ($P < 0.05$) but did not alter body weight (Table 1). Subjects cycled at the same absolute workload before and after training (51 ± 4 W), which elicited similar rates of $\dot{V}O_2$ (Table 1). However, mean HR response during exercise was >10% lower after training (Table 1) ($P < 0.05$).

Plasma hormone and substrate concentrations. Exercise increased plasma epinephrine and norepinephrine concentrations and reduced plasma insulin concentration compared with resting values (all $P < 0.05$) (Table 2). Endurance training reduced the plasma epinephrine response to exercise ($P < 0.05$) but did not significantly alter plasma norepinephrine or insulin concentrations (Table 2). Basal plasma fatty acid concentration was almost identical before and after training (0.45 ± 0.02 and 0.44 ± 0.01 μmol/ml, respectively). Although exercise increased ($P < 0.05$) plasma fatty acid concentration above basal values, endurance training did not affect fatty acid concentration during exercise (0.72 ± 0.04 and 0.67 ± 0.06 μmol/ml, mean concentration during the 60- to 90-min period of exercise before and after training, respectively).

Table 2. *Plasma hormone concentrations*

	Pretraining		Posttraining	
	Rest	Exercise	Rest	Exercise
Epinephrine, pg/ml	59 ± 11	93 ± 10*	56 ± 19	75 ± 10*†
Norepinephrine, pg/ml	297 ± 58	472 ± 58*	264 ± 41	439 ± 34*
Insulin, μU/ml	7.1 ± 0.5	4.1 ± 0.4*	5.9 ± 1.4	3.4 ± 0.5*

Values are means ± SE. *Significantly different from rest value, $P < 0.05$. †Significantly different from pretraining value, $P < 0.05$.

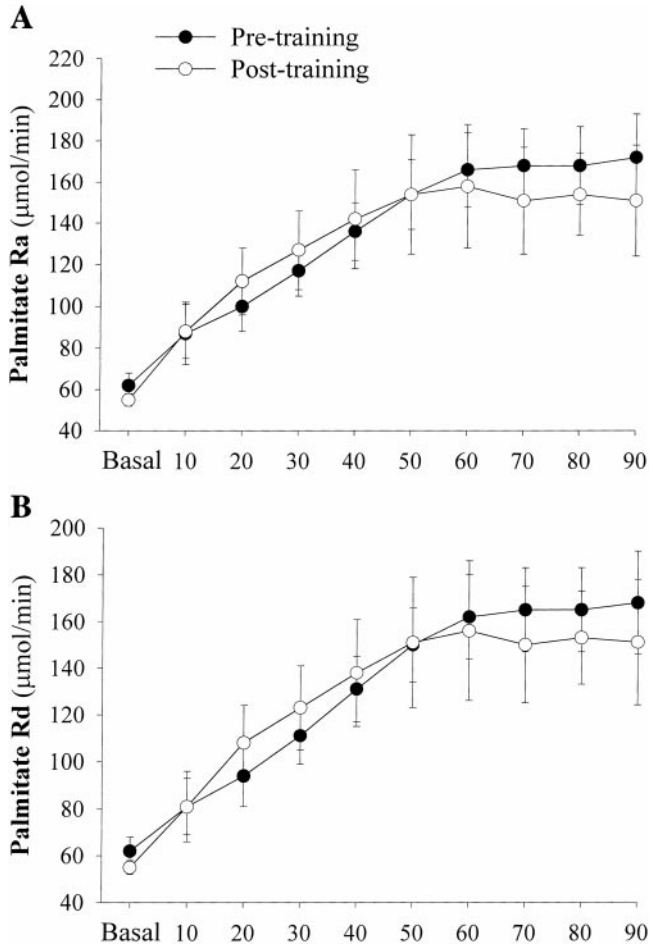

Fig. 1. Mean palmitate appearance in plasma (R_a, *A*) and palmitate disappearance from plasma (R_d, *B*) in the basal state and during 90 min of cycling exercise performed at 50% pretraining peak O_2 consumption ($\dot{V}O_{2peak}$), before (●) and after (○) training.

Plasma lipid kinetics. Plasma palmitate R_a and R_d increased more than twofold during exercise ($P < 0.05$) (Fig. 1). Endurance training did not alter palmitate or total fatty acid kinetics during the last 30 min of exercise (fatty acid R_a: 589 ± 65 and 545 ± 107 μmol/min; fatty acid R_d: 576 ± 62 and 537 ± 104 μmol/min, before and after training, respectively). The contribution of palmitate to total plasma fatty acids during exercise also remained the same before and after training (26 ± 1 and 25 ± 1%, respectively).

Regional lipolysis. Glycerol release from abdominal subcutaneous adipose tissue increased progressively during exercise, whereas glycerol release from femoral subcutaneous adipose tissue did not change (Fig. 2).

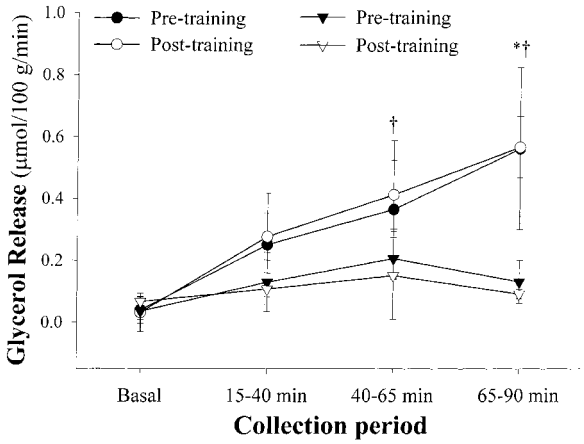

Fig. 2. Mean regional lipolytic activity during 90 min of cycling exercise performed at 50% pretraining $\dot{V}O_{2peak}$ in abdominal (circles) and femoral (triangles) adipose tissue, before (closed symbols) and after (open symbols) training. $P < 0.05$: *Significantly different from corresponding femoral adipose tissue; †significantly different from basal value.

Training did not alter the rate of glycerol release from either abdominal or femoral adipose tissue sites. During the last 25 min of exercise (65–90 min), the rate of glycerol release was greater in the abdominal than in the femoral region ($P < 0.05$).

Substrate oxidation. The mean rate of whole body fatty acid oxidation during the final 30 min of exercise was 25% greater after than before training (1,024 ± 114 and 789 ± 62 μmol/min, respectively; $P < 0.05$). However, endurance training did not affect the rate of plasma fatty acid oxidation (554 ± 61 and 601 ± 45 μmol/min, before and after training, respectively; Fig. 3) or the mean calculated acetate correction factor (0.80 ± 0.01 and 0.80 ± 0.01). Therefore, the increase in total fatty acid oxidation after training was due to an increase in the oxidation of nonplasma fatty acids. Training also caused a decrease in the relative contribution of carbohydrate to total energy production from 58 ± 2 to 47 ± 4% during the last 30 min of exercise ($P < 0.05$; Fig. 3).

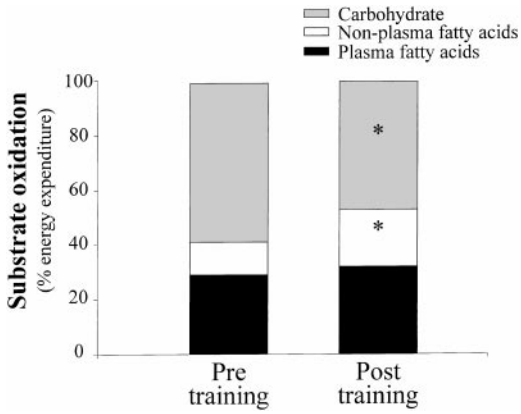

Fig. 3. Relative oxidation of plasma fatty acids (black bar), nonplasma fatty acids (white bar), and carbohydrate (gray bar) during 60–90 min of exercise in the pretraining and posttraining conditions. *Significantly different from pretraining value, $P < 0.05$.

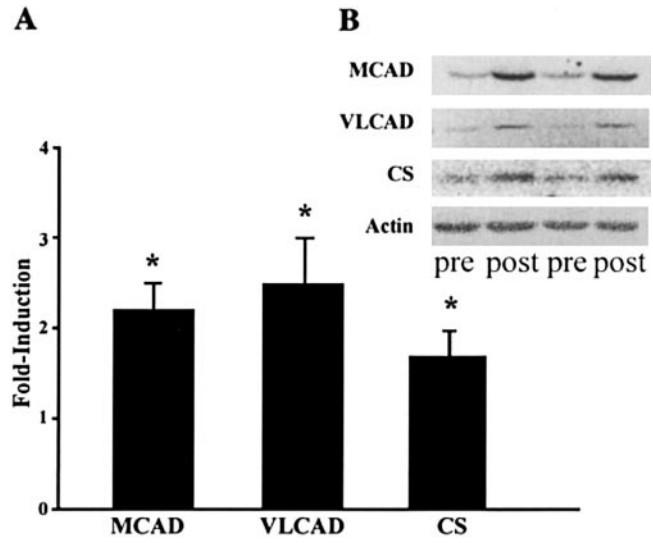

Fig. 4. *A*: mean induction of medium-chain acyl-CoA dehydrogenase (MCAD), very long chain acyl-CoA dehydrogenase (VLCAD), and citrate synthase (CS) protein content in skeletal muscle after 12–14 wk of endurance training. *B*: an autoradiogram of a representative Western blot in 2 subjects comparing protein levels before (pre) and after (post) training. All values are normalized to actin protein content. *Significantly different from 1, $P < 0.05$.

PPARα and fatty acid oxidative enzymes in skeletal muscle. As shown in Fig. 4, endurance exercise training resulted in a twofold increase in the mean immunodetectable levels of MCAD, VLCAD, and CS in skeletal muscle (all $P < 0.05$). Mean skeletal muscle PPARα protein content was also twofold higher ($P < 0.05$) in trained compared with untrained skeletal muscle (Fig. 5). Therefore, the increase in enzymes involved in fatty acid oxidation was associated with an increase in PPARα.

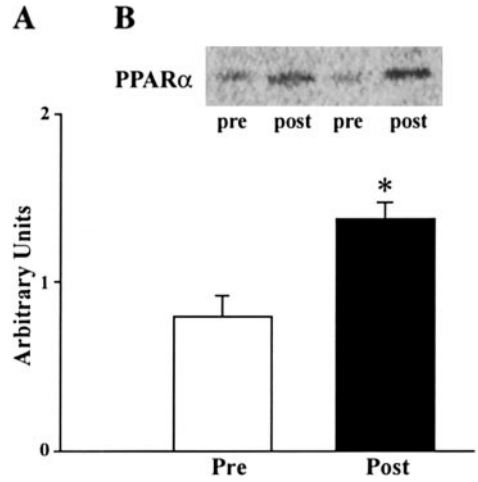

Fig. 5. *A*: skeletal muscle peroxisome proliferator-activated receptor-α (PPARα) levels pre- and posttraining. *B*: an autoradiogram of a representative Western blot in 2 sujects comparing protein levels before (pre) and after (post) training. All values are normalized to actin protein content. *Significantly different from pretraining value, $P < 0.05$.

DISCUSSION

An important adaptation to endurance training is a shift in skeletal muscle energy metabolism from carbohydrate to fat and increased skeletal muscle mitochondrial capacity to oxidize fatty acids during exercise. However, the source of the increased fat used during exercise and the mechanism for mitochondrial fatty acid oxidative enzyme induction remain unclear. In the present study, we found that 12–14 wk of endurance exercise training in lean women caused a 25% increase in total fat oxidation during exercise. The additional fat oxidized during exercise was derived from nonplasma fatty acids because plasma fatty acid availability, uptake, and oxidation did not change. It is likely that the majority of these nonplasma fatty acids originated from intramuscular triglycerides (IMTG), because plasma triglycerides are probably not an important fuel during exercise (28). In addition, we found that endurance training increased skeletal muscle protein content of PPARα and its target mitochondrial fatty acid oxidative enzymes. These data suggest that the adaptations in lipid metabolism that occur with endurance exercise training are intrinsic to skeletal muscle.

The effect of training on fatty acid kinetics in our female subjects is consistent with results reported previously in men (24, 31) but not in women (8). Data reported by Friedlander et al. (8) suggest that endurance training increases plasma fatty acid availability and oxidation during exercise in women. In contrast, we found that training increased the oxidation of nonplasma fatty acids during exercise. The reason(s) for the discrepancy between our findings and those reported by Friedlander et al. are unclear, but they may be related to differences in the contribution of palmitate to total plasma fatty acids rather than differences in palmitate kinetics itself. In fact, palmitate R_a (i.e., availability) during exercise was the same before and after training in both studies. However, Friedlander et al. found that the percentage of total plasma fatty acids derived from palmitate decreased after training, which caused an increase in the calculated rate of plasma fatty acid availability and oxidation. In contrast, the percentage of total plasma fatty acids derived from palmitate remained the same in our subjects. Another possible factor that may have caused differences between studies is the feeding protocol used before the exercise infusion protocol. The interval between feeding and an exercise bout can have a considerable effect on substrate metabolism during exercise (27). The subjects in our study performed the exercise-infusion protocol after they fasted overnight (12 h), whereas the subjects studied by Friedlander et al. ingested a meal 4–6 h before their exercise study.

It is possible that our calculated values for the rate of plasma fatty acid oxidation during the last 30 min (60–90 min) of exercise slightly overestimated the true values. In calculating plasma fatty acid oxidation rates, we used an acetate correction factor established during 50–60 min of exercise to estimate the amount of palmitate tracer lost to label fixation (35). However, acetate recovery increases with time (25), so it is possible that we underestimated the acetate correction factor in our subjects during the 60- to 90-min period of exercise, which would cause an artifactual increase in plasma fatty acid oxidation rates. Indeed, our observation that nearly all fatty acids cleared from plasma during exercise were oxidized supports this possibility. An overestimation of plasma fatty acid oxidation suggests that the rate of nonplasma fatty acid oxidation may have been greater than we reported. However, this potential error does not affect our conclusion that endurance training does not alter plasma fatty acid oxidation rates during exercise. Acetate recovery depends on the rate of $\dot{V}O_2$ (35), and our subjects were exercising at the same absolute intensity (i.e., same rate of $\dot{V}O_2$) before and after training.

We assumed that IMTG, rather than plasma triglycerides, provided the additional source of nonplasma fatty acids oxidized during exercise after training. Although our tracer methods cannot distinguish between triglyceride sources, it is unlikely that fatty acids derived from circulating triglycerides were responsible for the increase in fat oxidation, because plasma triglycerides are normally not an important source of energy production during exercise (28). Human skeletal muscle contains ~300 mmol of triglyceride (6), representing nearly 2,500 kcal of potential energy, which can serve as a considerable source of fuel during exercise. Moreover, the use of IMTG during exercise is efficient, because fatty acids released during lipolysis of IMTG are in close proximity to their site of oxidation (muscle mitochondria) and do not require transport from an extramuscular depot. By measuring IMTG content in muscle samples taken before and after exercise, Hurley et al. (16) found that 12 wk of endurance training doubled the use of IMTG during exercise. In our study, we also found that 12–14 wk of endurance training nearly doubled nonplasma fatty acid oxidation during exercise, presumably from IMTG. Nonetheless, an increased use of IMTG after endurance training is controversial because of conflicting results from some studies (8, 19), which may be related to differences in exercise protocols between studies, variability in IMTG concentration determined by the muscle biopsy technique, and differences in the interval between the last exercise bout and the experimental trial.

The increase in lipolysis during endurance exercise is largely due to catecholamine-mediated stimulation of adipocyte β-adrenergic receptors (2). In the present study, we found that the mobilization of adipose tissue triglycerides during exercise was heterogeneous (2, 11, 38). Lipolytic rate increased progressively in abdominal, but not femoral, adipose tissue during exercise in our subjects. Therefore, it is likely that most of the fatty acids released into the circulation during exercise were derived from upper-body rather than lower-body subcutaneous fat. This observation is consistent with and extends the data reported by Arner et al. (2), who found that adipose tissue interstitial glycerol concentration increased more in abdominal than in femoral

sites during cycle ergometer exercise. Our results are also consistent with studies demonstrating regional differences in lipolytic sensitivity to catecholamines in vivo (11) and in vitro (38). It is likely that regional heterogeneity in catecholamine-mediated lipolysis of adipose tissue triglycerides is determined by regional variations in α_2- and β-adrenergic receptor affinity and density (38).

The results of the present study demonstrate that endurance training does not affect the lipolytic response to moderate-intensity exercise in either upper (abdominal) or lower (femoral) subcutaneous adipose tissue in lean women. Therefore, the similar values we observed in whole body fatty acid kinetics during exercise before and after training were caused by similar upper- and lower-body regional lipolytic rates rather than a combination of increased and decreased rates of lipolysis within different adipose tissue sites. Measurement of regional lipolytic activity by microdialysis has limitations, because it requires accurate measurement of ATBF and appropriate assessment of glycerol recovery. Although we measured blood flow in the identical contralateral adipose tissue region of each microdialysis probe by use of ^{133}Xe clearance, it is possible that this may not accurately reflect nutritive blood flow at the exact site of each probe. We also assumed that 100% of interstitial glycerol was recovered by each probe, because others have demonstrated near 100% recovery when using the same probes and same perfusion rate (32). Therefore, incomplete glycerol recovery would affect our assessment of interstitial glycerol concentration. Although these limitations of the microdialysis method can be difficult to correct, they do not affect our qualitative conclusions regarding regional glycerol release, because the potential errors should be similar before and after training.

This is the first study to demonstrate that endurance exercise training increases PPARα content in skeletal muscle. PPARα regulates the expression of genes encoding several key muscle enzymes involved in fatty acid oxidation, including MCAD (10) and VLCAD (1), which also increased in our subjects after training. Our data are consistent with the results obtained in a dog model, which found that chronic electrical stimulation of latissimus dorsi muscle increased muscle PPARα content and MCAD gene expression (5). Although measurement of cellular protein content and gene expression does not necessarily reflect enzyme activity, these observations suggest that PPARα may be an important component of the adaptive response to endurance training by transducing physiological signals related to exercise training to the expression of nuclear genes encoding in skeletal muscle mitochondrial fatty acid oxidation enzymes. In fact, PPARα could increase the production of mitochondrial fatty acid oxidative enzymes without changing cellular PPARα content. Unsaturated long-chain fatty acids, which are released from adipose tissue during exercise, serve as ligands for PPARα and stimulate PPARα-activated gene transcription (20). Therefore, increase in muscle PPARα content and the intermittent increase in fatty acid delivery to muscle during exercise training may both be important factors in enhancing muscle fatty acid oxidative capacity. Studies performed in PPARα-deficient mice found that PPARα is also critical for the adaptation to other physiological conditions that increase the demand for long-chain fatty acid oxidation, such as fasting (18, 23) and high-fat feeding (18).

The mechanism responsible for the training-induced increase in PPARα in skeletal muscle is not known. One possibility is that fatty acids regulate their own oxidation by serving as a PPARα ligand and by stimulating PPARα expression directly. Indeed, fatty acids have been shown to increase hepatic PPAR mRNA expression (37). In addition, glucocorticoids, which are released during prolonged exercise, are a potent stimulator of PPARα mRNA induction in cultured hepatocytes (22). Moreover, the effects of elevated fatty acid concentration in combination with the synthetic glucocorticoid dexamethasone on PPAR expression are synergistic (37). Therefore, regular exposure to elevated plasma glucocorticoids, such as cortisol, together with a high rate of fatty acid flux during endurance training, may increase PPARα mRNA expression and content in skeletal muscle.

In summary, we found that 12–14 wk of endurance exercise training in lean women increased skeletal muscle oxidative capacity and the oxidation of fatty acids during exercise. The increase in skeletal muscle PPARα content and its target fatty acid oxidative enzymes after training suggests a candidate gene regulatory pathway that is activated by training. The additional fatty acids oxidized during exercise after training were derived from nonplasma sources, possibly from IMTG stores. Therefore, the adaptations in fat metabolism that occur in response to endurance training are localized to skeletal muscle: endurance training may increase the mobilization of endogenous triglyceride present within working muscles and increase mitochondrial oxidative enzyme content to enhance skeletal muscle fatty acid oxidative capacity.

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