Endurance training increases fatty acid turnover, but not fat oxidation, in young men

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Friedlander, Anne L., Gretchen A. Casazza, Michael A. Horning, Anton Usaj, and George A. Brooks. Endurance training increases fatty acid turnover, but not fat oxidation, in young men. *J. Appl. Physiol.* 86(6): 2097–2105, 1999.—We examined the effects of exercise intensity and a 10-wk cycle ergometer training program [5 days/wk, 1 h, 75% peak oxygen consumption ($\dot{V}O_{2peak}$)] on plasma free fatty acid (FFA) flux, total fat oxidation, and whole body lipolysis in healthy male subjects (n = 10; age = 25.6 ± 1.0 yr). Two pretraining trials (45 and 65% of $\dot{V}O_{2peak}$) and two posttraining trials (same absolute workload, 65% of old $\dot{V}O_{2peak}$; and same relative workload, 65% of new $\dot{V}O_{2peak}$) were performed by using an infusion of [1-^{13}C]palmitate and [1,1,2,3,3-^{2}H]glycerol. An additional nine subjects (age 25.4 ± 0.8 yr) were treated similarly but were infused with [1,1,2,3,3-^{2}H]glycerol and not [1-^{13}C]palmitate. Subjects were studied postabsorptive for 90 min of rest and 1 h of cycling exercise. After training, subjects increased $\dot{V}O_{2peak}$ by 9.4 ± 1.4%. Pretraining, plasma FFA kinetics were inversely related to exercise intensity with rates of appearance (R_a) and disappearance (R_d) being significantly higher at 45 than at 65% $\dot{V}O_{2peak}$ (R_a: 8.14 ± 1.28 vs. 6.64 ± 0.46, R_d: 8.03 ± 1.28 vs. 6.42 ± 0.41 mol·kg^{-1}·min^{-1}) ($P \leq 0.05$). After training, when measured at the same absolute and relative intensities, FFA R_a increased to 8.84 ± 1.1, 8.44 ± 1.1 and R_d to 8.82 ± 1.1, 8.35 ± 1.1 mol·kg^{-1}·min^{-1}, respectively ($P \leq 0.05$). Total fat oxidation determined from respiratory exchange ratio was elevated during exercise compared with rest, but did not differ among the four conditions. Glycerol R_a was elevated during exercise compared with rest but did not demonstrate significant intensity or training effects during exercise. Thus, in young men, plasma FFA flux is increased during exercise after endurance training, but total fat oxidation and whole-body lipolysis are unaffected when measured at the same absolute or relative exercise intensities.

stable isotopes; substrate utilization; free fatty acids; fat metabolism; glycerol; lipolysis; crossover concept

ALTHOUGH LIPID IS GENERALLY considered an important substrate for prolonged submaximal exercise, differences of opinion persist concerning the relative roles of lipids and carbohydrates as fuels for muscle contraction in humans. Most researchers agree that the relative contribution of lipids decreases with increasing exercise intensity above a power output eliciting 50% (2, 11), and that the contribution of lipid oxidation increases with the duration of exercise (26) as well as with time since last eating (24). In addition, results of biopsy studies of rat and human muscles suggest that training-induced increases in free fatty acid (FFA) binding proteins and mitochondrial density enhance the ability for FFA oxidation (10, 11, 16, 23, 28). However, because of the complex regulation of substrate utilization, increased enzymatic capacity does not necessarily imply increased utilization in vivo. For example, increased capacity of trained muscle for lipid oxidation in situ may be more important during recovery than during exercise in vivo (1, 17). Similarly, it has been proposed that, after training, lipid oxidation is favored in noncontracting tissue beds during exercise, thus permitting the blood glucose flux to be directed ("shunted") to working muscle (1).

When studies are conducted at a given absolute workload, an increase in total lipid oxidation is observed when compared with before training (7, 20, 23). This increase in whole body lipid oxidation after training is accompanied by an altered glucoregulatory hormonal responses (e.g., decreased glucagon and catecholamines and increased insulin), perhaps resulting in the removal of malonyl-CoA inhibition of carnitine palmitoyltransferase I, permitting FFA transport into muscle mitochondria for oxidation (19, 20, 23). Unfortunately, no direct evidence yet exists for this mechanism in human skeletal muscle. Fewer studies have evaluated the effects of training on lipid oxidation at given relative exercise intensities, and, given that hormonal responses are often tied closely to relative effort (6), one might predict that the balance of substrate utilization is related to relative exercise intensity, both before and after training (2).

The impact of training on plasma FFA flux is unclear. Whether training increases or decreases FFA uptake and disposal by working muscle appears partially dependent on the methodology used. Using tracers to measure whole body turnover rates, some investigators report that FFA flux is decreased after training (20, 23). In contrast, results of our report on women (7), as well as results of studies measuring arteriovenous FFA differences across working limbs of men, suggest that training may elevate plasma FFA disposal (7) and working muscle FFA uptake (1, 16, 26). Therefore, the purpose of this investigation was to determine training-induced changes in whole body lipolytic rate, FFA flux, and lipid oxidation in young men, measured at both the same absolute and relative exercise intensities after training. A longitudinal design was employed to avoid the confounding factor of inheritance in determining FFA flux and lipid oxidation during exercise. Results of the present investigation are similar in many respects

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to those contained in a similar report on women (7), but some differences are noted.

METHODS

Subjects. Twenty healthy, nonsmoking, sedentary male subjects between the ages of 18 and 35 yr were recruited from the University of California campus by flyers and mailings. Subjects were recruited in two groups of 10, but were treated identically, except that only the first set of 10 subjects received both labeled palmitate and glycerol infusions, whereas the second group received only labeled glycerol. One subject in the second group withdrew before posttraining testing because of illness, thus leaving data from only 19 subjects available for analysis. Subjects were considered sedentary if they had participated in fewer than 2 h of regular strenuous activity per week for the previous year and if they had a peak oxygen consumption ($\dot{V}O_{2peak}$) between 35 and 45 $ml \cdot kg^{-1} \cdot min^{-1}$, as determined by a continuous, progressive maximal stress test on a leg cycle ergometer. To qualify for participation in the study, subjects were required to be diet and weight stable, to have a body fat percentage of <30%, and to be disease and/or injury free, as determined by medical questionnaire and physical examination. Subjects provided informed consent, and the protocol was approved by the University of California Committee for Protection of Human Subjects (95–1–50).

General experimental design. After an initial interview and screening tests, two stable-isotope infusion trials were performed in subjects on a leg cycle ergometer for 1 h at 45 and 65% of $\dot{V}O_{2peak}$ (hereafter referred to as 45UT and 65UT, respectively). The order of trials was randomized and performed a minimum of 2 days apart. Subjects began training 2 days after their second isotope trial and continued for 10 wk. Anthropometric measurements and stress tests were repeated at 5 and 10 wk of training. After 10 wk of training, two more isotope trials were performed; one was at the same absolute workload that elicited 65% of pretraining $\dot{V}O_{2peak}$ (ABT); and the second was at a workload that elicited 65% of the new, posttraining $\dot{V}O_{2peak}$, or the same relative intensity (RLT). The two posttraining trials were ~1 wk apart and randomized, and training was continued between the two trials. All isotope trials were performed after 36–48 h without training.

Screening tests. Body composition was determined both by underwater weighing and skin-fold measurement (12). $\dot{V}O_{2peak}$ was determined to be the highest 1-min value obtained in subjects exercising on an electronically braked cycle ergometer (Monark Ergometric 829E) during a continuous, progressive protocol, which increased 25 or 50 W every 3 min until voluntary cessation. Respiratory gases were analyzed (AMETEK S-3A1 O_2 and Beckman LB-2 CO_2 analyzers) and recorded every minute by an online, real-time PC-based system. Each subject underwent two $\dot{V}O_{2peak}$ tests before commencement of the study, and the tests were evaluated on maximal heart rate, respiratory exchange ratio (RER) values (>1.15), and oxygen uptake ($\dot{V}O_2$) uniformity to ensure a true maximum effort both before and after training. Three-day dietary records were kept at the beginning and at 5 and 10 wk into training to monitor the subject's dietary composition and quantity of intake. Compositional analysis of food records was performed by using the Nutritionist III program (N-Squared Computing, Salem, OR).

Tracer protocols. Subjects were studied in a postabsorptive state in the morning, and dietary intake was monitored for the 24 h immediately preceding each of the four isotope trials. Dinner the night before each trial (timed to 12 h pretrial) was selected by the individual subject and repeated before every trial. Each subject was given a standardized snack (505 kcal: 16% protein, 52% carbohydrate, 32% fat) to consume before bedtime and a standardized breakfast (300 kcal: 17% protein, 83% carbohydrate; skim milk and cereal) to consume 1–2 h before reporting to the laboratory. We chose to test our subjects in a fed, postabsorptive state, so that the results would be typical of a nonlaboratory environment. Typically, subjects ate 1–2 h before reporting to the laboratory; subject preparation took a minimum of 1 h, and the rest ranged from 90 to 120 min. Thus we report data on resting subjects fed 3.5–5 h previously and on exercising subjects fed 4.5–6 h before study. We view these dietary practices as representative of habits in the general population. Furthermore, our controls of diet and exercise allowed subjects to complete exercise tasks with stable blood glucose concentrations (see Table 2).

On the morning of the trial, a catheter was placed into the radial artery for sampling, and an antecubital venous catheter was placed in the opposite arm for infusion of tracers for 90 min of rest and 1 h of exercise. After the collection of background blood and expired air samples, a priming bolus of glycerol (150 times the resting minute infusion rate) was given, and the subjects rested semisupine for 90 min while the glycerol and palmitate (no priming) tracers were infused continuously (Baxter Travenol 6200 infusion pump). The resting infusion rate was set at 0.4 mg/min for [1,1,2,3,3-^{2}H]glycerol and 0.8 mg/min for [1-^{13}C]palmitate during rest. On initiation of exercise, the palmitate infusion rate was doubled. The glycerol infusion rate was increased three times for the two pretraining isotope trials and for the 65% of the old $\dot{V}O_{2peak}$ posttraining trial (same absolute workload) at the start of exercise. Because the glycerol tracer was prepared in the same infusion cocktail as [6,6-^{2}H]glucose (9) and because of the increased glucose flux anticipated for the 65% of the new $\dot{V}O_{2peak}$ posttraining, the exercise infusion rate for the glucose and glycerol cocktail was increased four times the resting value. Isotopes were obtained from Cambridge Isotope Laboratories (Woburn, MA). Glycerol was diluted in 0.9% sterile saline, pharmaceutically tested for sterility and pyrogenicity (Univ. of California, San Francisco, School of Pharmacy), and, on the day of the experiment, passed through a 0.2-μm Millipore filter (Nalgen, Rochester, NY). Tracer palmitate was combined with 100 ml of 25% human albumin and suspended in 0.9% saline by the University of California, San Francisco, School of Pharmacy. The palmitate tracer cocktail was tested for sterility and pyrogenicity, and all palmitate/albumin infusates were used within 5 days after completion of sterility testing.

At each of the blood sampling time points, respiratory gas exchange was determined by using the calorimetry system described above, and a sample of expired air was collected in a 10-ml vacuum Exetainer tube to determine $^{13}CO_2$ isotopic enrichment. The expired air samples were stored at room temperature until they were analyzed by using isotope ratio mass spectrometry (IRMS) by Metabolic Solutions (Merrimack, NH). Heart rate was recorded throughout rest and exercise by using a Quinton Q750 electrocardiogram (Seattle, WA).

Blood sample collection and analysis. Blood samples were taken at 0, 75, and 90 min of rest and at 5, 15, 30, 45, and 60 min of exercise and immediately placed on ice, centrifuged for 10 min at 2,500 *g*, decanted, and frozen. Blood samples for the analyses of glucose and lactate concentrations were collected in 8% perchloric acid and vortex mixed before chilling and centrifugation. Plasma glucose concentration was determined enzymatically (Sigma Chemical, St. Louis, MO). Plasma levels for FFA and glycerol concentration determinations

were collected in EDTA-treated tubes and measured by using WAKO (Richmond, VA) and Sigma Chemical kits, respectively. Palmitate isotopic enrichments were measured by mixing a 1-ml aliquot of plasma with a solution of heptane, isopropanol, and H_2SO_4 that contained 100 nmol pentadecanoic acid as an internal standard. The solution was stored frozen for subsequent TLC separation of blood lipids. After isolation by TLC, FFA were derivatized to the fatty acid methyl ester to allow easy volatilization by gas chromatography. The instrumentation was equipped to detect simultaneously both the total FFA concentration by flame ionization detector and isotopic enrichment of palmitic acid by gas chromatography-mass spectrometry (Hewlett-Packard, gas chromatography model 5890 series II and mass spectrometry model 5989A). Glycerol enrichments were determined on neutralized perchloric acid extracts by using ion-exchange chromatography, and the isotopic enrichment of the trimethylsilyl derivative of [1,1,2,3,3-^{2}H]glycerol and of an internal standard of [2-^{13}C]glycerol was determined by gas chromatography-mass spectrometry.

Training protocol. Subjects were required to exercise with a personal trainer in our facility 5 days/wk for 1 h each day on the cycle ergometer. In addition to the supervised training, subjects were required to exercise an additional 1 h on the weekend in any manner they desired. The personal trainers were current undergraduate students in, or recent graduates of, the Department of Human Biodynamics and, for the most part, were competitive or recreational athletes themselves. During the first 3 wk of training, exercise intensity was gradually increased from 50% of each participant's $\dot{V}O_{2peak}$ to 75% of $\dot{V}O_{2peak}$. Subjects were asked to warm up for 5 min and stretch before their hour of exercise. Personal trainers used heart rate monitors and data from periodic evaluations of $\dot{V}O_{2peak}$ to adjust workloads as the subjects improved. Throughout the training intervention, subjects were weighed daily and instructed to increase their energy intake without altering their normal dietary composition to compensate for increased energy expenditure and to ensure weight and body fat stability. Because of the extensive work by Schutz and associates (25), it was deemed necessary to prevent large changes in total body or fat mass, as changes in tissue mass are likely to affect insulin action and the balance of substrate utilization, independent of training.

Calculations and statistics. Palmitate and glycerol rates of appearance (R_a) and disappearance (R_d) and metabolic clearance rate (MCR) were calculated by using equations defined by Steele and modified for use with stable isotopes (27). A detailed description of the equations has been reported previously (9). The volumes of distribution (V) for palmitate and glycerol were set at 40 and 270 ml/kg, respectively. For glycerol, values of V used in the literature range between 200 and 270 ml/kg (18, 23, 24, 28). Calculation of glycerol flux rates with V values anywhere in that range changed results by only 0.5–4%, depending on the exercise trial, and had no significant impact on relationships among trials. Palmitate rate of oxidation for the pretraining tests was calculated by using the IRMS analysis of the expired air samples. Posttraining values for palmitate rate of oxidation are not available because of the contamination of the $^{13}CO_2$ samples in the IRMS analysis process. From a previous study in our laboratory (21), correction factors of 0.65 and 0.9 were determined to account for labeled CO_2 retained in the body compartments during rest and exercise, respectively. FFA kinetics and oxidation were calculated by dividing the value for palmitate kinetics by the fraction of plasma palmitate concentration to total plasma FFA concentration, as determined by flame ion detection. Rates of total FFA oxidation ($R_{ox_{tot}}$) were calculated by using the RER and the $\dot{V}O_2$ (assuming 4.7 kcal/l O_2, 9.5 kcal/g triglyceride, and an average of 860 g/mol for triglyceride) (8). Percentage of oxidative energy from FFA and lipid was calculated from RER. Calculations of steady-state FFA and glycerol kinetics were made by using the last two (75, 90 min) and three (30, 45, 60 min) isotopic enrichment measurements obtained during rest and exercise, respectively. Due to the split design of the study, glycerol flux rates and concentrations were calculated by using the full contingent of 19 subjects, whereas the data for FFA flux and oxidation were determined by using 9 subjects. For that reason, we did not calculate reesterification rates. Data are represented as means ± SE. To assess significance of mean differences in metabolite concentration and flux rates among the four isotope trials, ANOVA with repeated measures was used, and, where appropriate, Fisher least significant difference tests were used for post hoc analyses. Statistical significance was set at $\alpha = 0.05$.

RESULTS

Subject characteristics. Pre- and posttraining characteristics of the 19 men who completed the study are listed in Table 1. Subjects were weight stable throughout the study period but did lose a significant amount of body fat whether measured by skin folds or underwater weighing. $\dot{V}O_{2peak}$ improved by 9.4 ± 1.4% over the training period. The workload characteristics for the four isotope trials are presented in Table 2. Due to the training-induced increase in aerobic capacity, the posttraining trial at the same absolute workload approximated 56% of the subject's new $\dot{V}O_{2peak}$. There was a significant exercise intensity and training effect on the average exercising heart rate. Training resulted in a significantly reduced heart rate during exercise at the ABT but not RLT workload (Table 2).

Metabolite concentrations and isotopic enrichments. Plasma glycerol concentration demonstrated a significant increase throughout exercise in all four trials. Pretraining, glycerol concentration was significantly elevated in the 65UT trial compared with the 45UT trial during the last 30 min of exercise. However, plasma glycerol concentrations were not significantly affected by training during rest or exercise, when measured at either the same absolute or relative exercise intensities (Fig. 1*A*). Plasma FFA concentrations were stable during the last 15 min of rest and increased

Table 1. *Physical characteristics of young men before and after 10 wk of endurance training*

Variable	Pretraining	Posttraining	%Difference
Age, yr	25.5 ± 0.7		
Height, cm	179.8 ± 1.3		
Weight, kg	78.6 ± 2.0	78.2 ± 1.9	−0.4 ± 0.5
Body fat, %			
UW weighing	15.5 ± 1.0	13.8 ± 0.9*	−9.6 ± 4.0
Skin folds	14.4 ± 0.9	12.7 ± 0.8*	−11.3 ± 3.5
$\dot{V}O_{2peak}$			
ml·kg^{-1}·min^{-1}	46.5 ± 1.1	50.7 ± 1.3*	9.4 ± 1.4
l/min	3.6 ± 0.1	3.9 ± 0.1*	8.5 ± 1.3
ml·kg LM^{-1}·min^{-1}	52.2 ± 1.9	56.7 ± 2.3*	8.7 ± 1.1

Values are means ± SE; $n = 19$ subjects. UW, underwater; $\dot{V}O_{2peak}$, peak O_2 uptake; LM, lean mass. *Significantly different from pretraining values, $P < 0.05$.

Table 2. *Ergometric and physiological characteristics of male subjects during rest and exercise, before and after training*

Variable	Rest		Exercise			
	Pretraining	Posttraining	45UT	65UT	ABT	RLT
Workload, W	0	0	90.0 ± 6.2	152.2 ± 6.4*	152.7 ± 6.4*	176.7 ± 6.1*‡
$\dot{V}O_2$, ml·kg^{-1}·min^{-1}	4.1 ± 0.4	4.3 ± 0.2	21.6 ± 0.7	30.1 ± 0.8	29.7 ± 0.9	33.1 ± 0.8*†‡
Heart rate, beats/min	67.7 ± 1.8	63.6 ± 1.7	128.2 ± 2.8	157.8 ± 3.1*	138.9 ± 2.6*†	156.2 ± 2.6*‡
Respiratory exchange ratio	0.86 ± 0.01	0.85 ± 0.01	0.89 ± 0.01	0.94 ± 0.02*	0.92 ± 0.01*	0.95 ± 0.01*‡
Total fat R_{ox}, μmol·kg^{-1}·min^{-1}	3.3 ± 0.6	3.7 ± 0.7	13.3 ± 1.7	12.3 ± 2.8	13.0 ± 2.1	11.4 ± 2.1
%Energy from lipid	49.5 ± 7.5	51.1 ± 6.6	36.4 ± 3.7	23.7 ± 4.4*	26.7 ± 3.5*	19.0 ± 4.1*
Plasma glucose, mM	4.9 ± 0.1	4.8 ± 0.1	4.6 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.6 ± 0.1

Values are means ± SE; n = 9 subjects for respiratory exchange ratio, total fat rate of oxidation (R_{ox}), and %energy from lipid; n = 19 for all others. 45UT, 45% of $\dot{V}O_{2peak}$ pretraining; 65UT, 65% of $\dot{V}O_{2peak}$ pretraining; ABT, same absolute workload (65% of old $\dot{V}O_{2peak}$); RLT, same relative workload (65% of new $\dot{V}O_{2peak}$); $\dot{V}O_2$, O_2 uptake. All exercise values are significantly different from rest, except posttraining plasma glucose concentration. *Significantly different from 45UT at $P < 0.05$; †significantly different from 65UT at $P < 0.05$; ‡significantly different from posttraining (ABT) at $P < 0.05$.

steadily throughout the 1-h exercise period. Circulating FFA levels tended to be lower after training; however, there were no significant differences between any of the four exercise trials (Fig. 1*B*). Glycerol and palmitate isotopic enrichments for the four isotope trials are presented in Fig. 2, *A* and *B*, respectively. There were no differences between trials in the measured mean value for palmitate as a percentage of total FFA concentration; thus the average value of 28% was used to calculate the FFA kinetics. Pretraining, blood glucose concentrations fell significantly during the first 15 min of exercise; however, there were no significant differences in blood glucose concentrations among the four

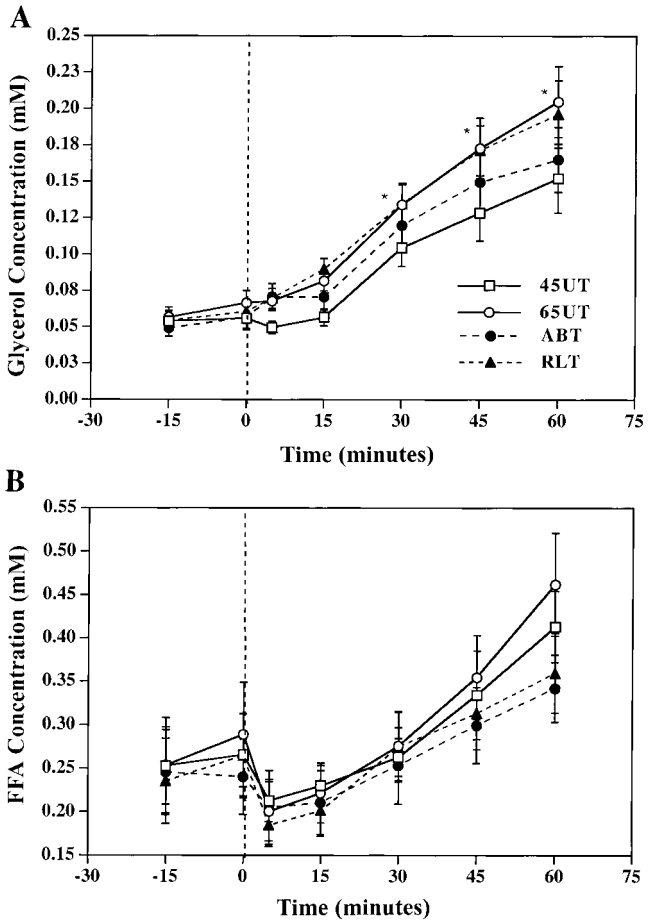

Fig. 1. Plasma concentrations of glycerol (*A*) and free fatty acids (FFA) (*B*) over time in men studied twice before and twice after training. Pretraining exercise tasks were at 45 and 65% peak oxygen consumption ($\dot{V}O_{2peak}$) (45UT and 65UT, respectively). After 10 wk of training, subjects were studied at the power output eliciting 65% of pretraining $\dot{V}O_{2peak}$ [absolute (ABT)], and power output eliciting 65% of posttraining $\dot{V}O_{2peak}$ [relative (RLT)]. Values are means ± SE, n = 19 and 9 subjects for glycerol and FFA, respectively. *Significantly different from 45UT; # significantly different from ABT ($P < 0.05$).

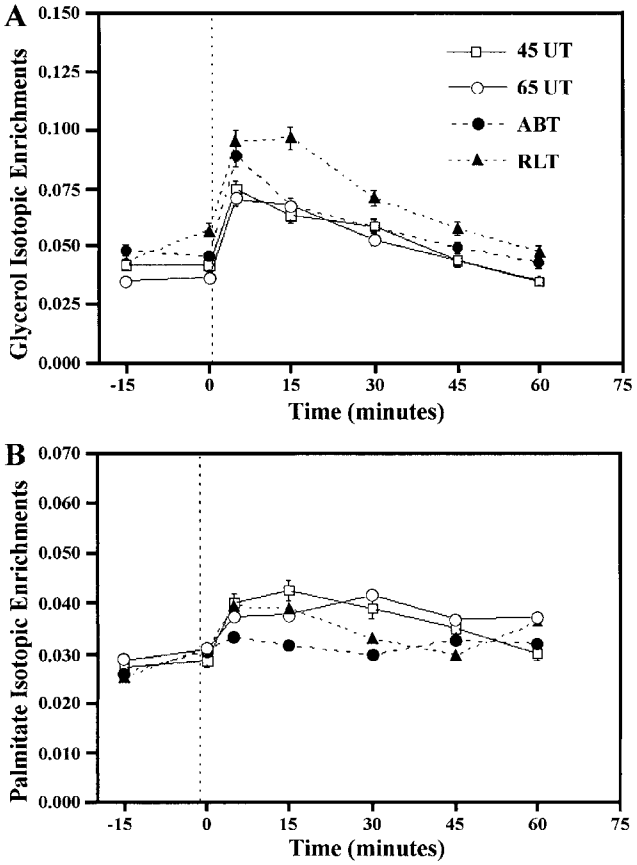

Fig. 2. Isotopic enrichments of [1,1,2,3,3-^{2}H]glycerol (*A*) and [1-^{13}C]palmitate (*B*) over time in men studied twice before and twice after training. See Fig. 1 legend for details. Values are means ± SE, n = 19 and 9 subjects for glycerol and palmitate, respectively.

trials during steady-state exercise, and concentrations remained steady at ~4.6 mM (Table 2).

Glycerol kinetics. Glycerol R_a was significantly lower at rest posttraining than at rest pretraining. During all four exercise trials, glycerol R_a was significantly elevated above resting values (Fig. 3). There was no pretraining intensity effect on glycerol R_a between the 45UT and 65UT trials, nor was there a training effect at either workload relative to the 65UT trial (Fig. 3). The pattern of glycerol R_d was similar to that of R_a during rest and exercise for all workloads, although R_d values were slightly, but not significantly, lower.

FFA kinetics. Resting FFA R_a and R_d were unaffected by training (Fig. 4); FFA R_a and R_d were elevated in exercise compared with rest during all four trials. Pretraining FFA R_a was significantly higher (23%) during exercise in the 45UT trial than in the 65UT trial (Fig. 4*A*). In addition, there was a significant training effect with FFA R_a in the ABT and RLT trials being 38 and 31% higher than the 65UT trial, respectively (Fig. 4*A*). Responses of FFA R_d to exercise and training were similar to those of R_a and are presented in Fig. 4*B*. In all trials, FFA R_d was slightly lower than R_a, thus explaining the gradual, but consistent, rise in FFA concentration during exercise in all trials. The MCR was higher at rest after training, and it did increase during exercise relative to rest in all trials except 65UT. In addition, during exercise, MCR was significantly higher after training at the same absolute and relative workloads (Fig. 4*C*).

FFA oxidation. $R_{ox_{tot}}$, as determined from indirect calorimetry, is presented in Table 2. Values for $R_{ox_{tot}}$ during exercise were significantly elevated above rest for all four trials. However, there was no significant difference in $R_{ox_{tot}}$ at rest before and after training, nor were there differences between trials during exercise.

DISCUSSION

Our data obtained in young men suggest that plasma FFA flux is increased after 10 wk of endurance training

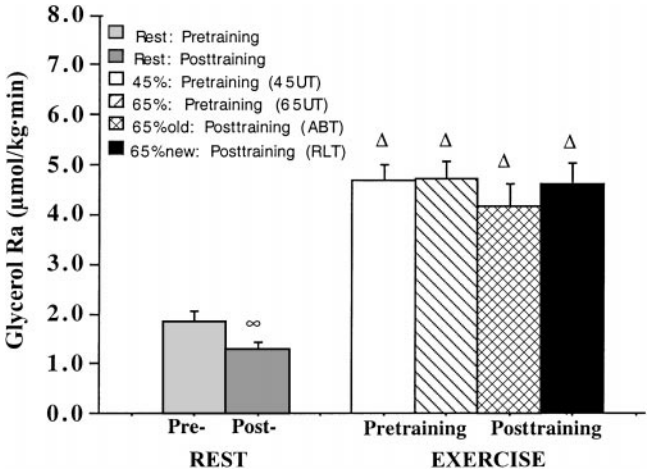

Fig. 3. Effect of exercise intensity and training on plasma glycerol rate of appearance (R_a). Values are means ± SE of the last 15 and 30 min for rest and exercise, respectively; $n = 19$ subjects. $^{\Delta}$Significantly different from rest at $P < 0.05$; $^{\infty}$significantly different between resting conditions at $P < 0.05$.

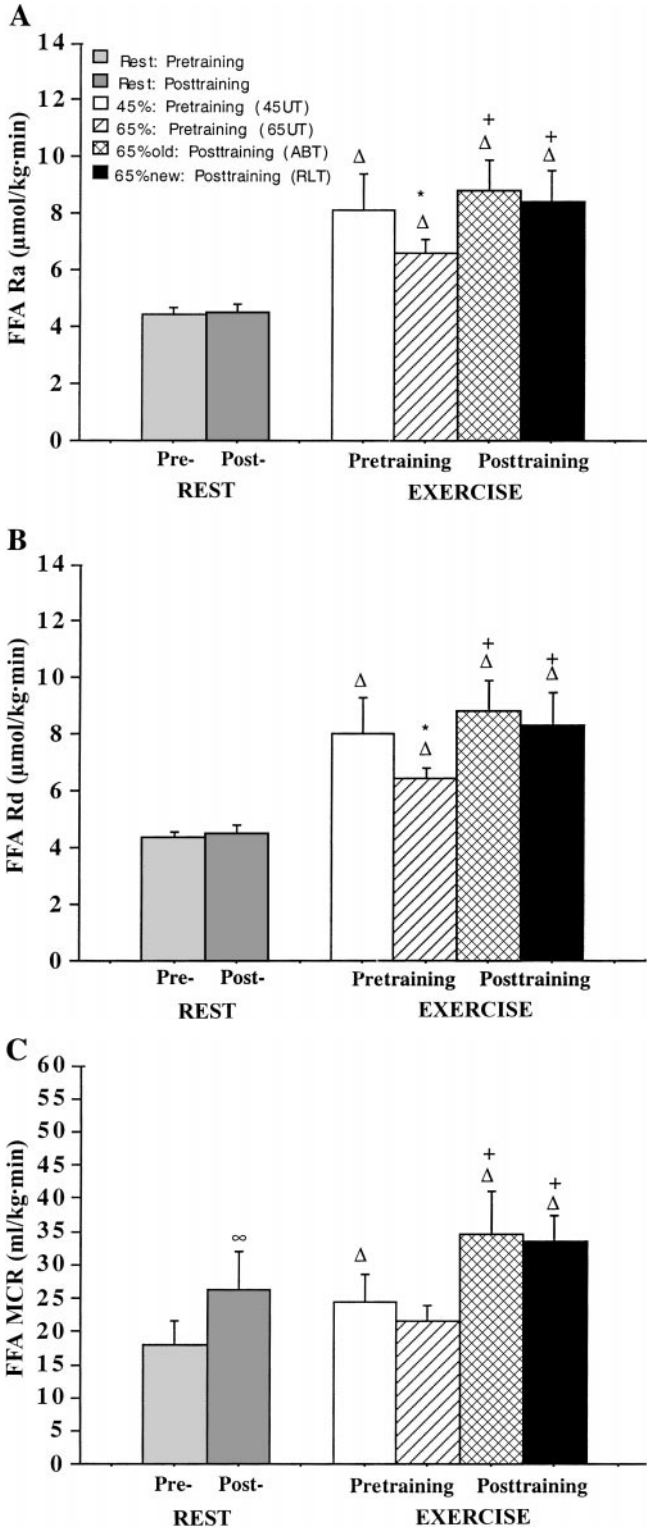

Fig. 4. Effect of exercise intensity and training on plasma FFA R_a (*A*), rate of disappearance (R_d) (*B*), and metabolic clearance rate (MCR) (*C*). Values are means ± SE of the last 15 and 30 min for rest and exercise, respectively; $n = 9$ subjects. *Significantly different from pretraining (45UT) at $P < 0.05$; $^{\Delta}$significantly different from rest at $P < 0.05$; $^{+}$significantly different from 65UT at $P < 0.05$; $^{\infty}$significantly different between resting conditions at $P < 0.05$.

when measured at either the same absolute or relative exercise intensity. In contrast, total lipid oxidation (determined from indirect calorimetry) and whole body lipolytic rate (estimated from the glycerol R_a) were unaffected by the training regimen imposed. Results are consistent with those of a parallel investigation from our laboratory in men (1), showing increased working limb (leg) net FFA uptake after training. As well, the present results showing increased FFA R_d in men after training are similar to those obtained in a parallel study conducted in women (7). Despite similar results of three studies showing a training effect increasing FFA uptake and R_d, the changes are quantitatively small, and carbohydrate energy sources remain predominant during moderate- and greater-intensity exercises after training.

FFA flux (effects of training). The increased FFA flux rates we observed in our subjects after training differed from results of previous reports, which demonstrated reduced FFA R_d after training at the same absolute workload (20, 23) or no difference in values at the same relative exercise intensity (13). However, our finding of increased FFA R_d after training fits well with data from limb arteriovenous difference studies comparing trained vs. untrained men (1, 26) or using one-legged training protocols comparing trained vs. untrained legs (10, 16). In addition, the working limb arteriovenous difference studies by Kiens et al. (16) and Turcotte et al. (26) showed increased uptake in trained working muscles and individuals, respectively, despite similar FFA delivery in trained and untrained conditions. Thus, on the whole body level, our findings of increased FFA MCR after training in our subjects are consistent with data obtained across working limbs.

Differences between the present investigation showing a training effect increasing FFA flux rates (Fig. 4, *A* and *B*) and previous studies in men using tracers to estimate FFA disposal may be more related to the subject populations studied and details of protocol rather than physiology. For instance, in their report, Martin et al. (20) observed training to decrease FFA R_d for exercise at a given power output. However, in their study, circulating FFA concentration was significantly lower by 30% during exercise after training, whereas in our study arterial FFA concentration rose throughout exercise and did not significantly differ between exercise conditions (Fig. 1*B*). Based on extensive biochemical studies demonstrating training to increase muscle capillarity, mitochondrial and fatty acid-binding protein contents, if blood flow and arterial FFA concentration are maintained to working trained muscles, increased net FFA uptake is the expected result (J. O. Holloszy, personal communication).

FFA flux (effects of intensity). Pretraining, the FFA flux values were significantly reduced at the 65UT, relative to the 45UT, workload. Posttraining, the flux values were not different between the two workloads. Jones et al. (15) also observed decreased FFA R_a at 70 vs. 36% of maximal oxygen uptake ($\dot{V}O_{2max}$), which was accompanied by an elevated glycerol concentration at the higher workload. They suggested that fat oxidation was more dependent on nonplasma sources at the higher workloads, because heavy exercise limits lipolysis in adipose tissue, thus reducing plasma FFA availability. Romijn et al. (24) reached a similar conclusion after observing that FFA turnover did not change despite elevated fat oxidation at a workload of 65 vs. 25% of $\dot{V}O_{2max}$. Whereas our data and those of Jones et al. (15) demonstrated reduced FFA flux at the higher workloads, Romijn et al. (24) showed no difference. However, comparisons between our study and that of Romijn et al. are difficult to make because of the differences in experimental workloads and dietary treatments. It is possible that the peak FFA flux rates are closer to 50%, as suggested by Hultman (11). If Romijn et al. (24) had included an additional workload closer to 45%, they, too, might have demonstrated a decrease in flux values at 65% relative to 45% of $\dot{V}O_{2max}$.

Viewed from the perspective that the relationship between plasma FFA flux and exercise intensity is described by an inverted hyperbola with apogee at ~45% $\dot{V}O_{2max}$ (3), the present and previously published results indicate good general agreement. Between power outputs eliciting 40–70% $\dot{V}O_{2max}$, the curve describing FFA R_d and relative exercise intensity is essentially flat. Thus small differences among reports are likely due to differences in experimental treatment as well as expected variations among subject populations and normal experimental errors. In the aggregate, the literature supports the conclusion that during exercises eliciting 65% and greater percentages of $\dot{V}O_{2max}$, carbohydrate energy sources will predominate over lipid.

FFA oxidation. In our study, endurance training tended to increase total lipid oxidation at a given absolute workload. These findings are similar to those of other studies, which demonstrated significant increases in total body lipid oxidation after training when measured at a given absolute workload in men (9, 20, 23). When measured at the same relative workload, total lipid oxidation tended to be lower in our subjects. In addition, total lipid oxidation presented as a percentage of total energy expenditure also tended to be lower after training during the RLT trial than the 65UT trial (Table 2).

Studies that measure percent of R_d oxidized, such as the training studies presented by Martin et al. (20) and Turcotte at al. (26), suggest that only a percentage of those FFA taken up are oxidized (~50 and 74%, respectively). In our study, only the pretraining plasma FFA oxidation rates are available. The values suggest that 50–60% of R_d was oxidized, which corresponds well to the data of Turcotte et al. (26) and Martin et al. (20). Therefore, the plasma FFA oxidation values that we obtained indicate that as much as 75% of FFA oxidation was from nonplasma sources during exercise at 65% of $\dot{V}O_{2peak}$ pretraining. This value is higher than the ~50% reported by others using subjects exercising at a similar relative power outputs as utilized in our investigation (23, 24). However, in those reports, the investigators assumed 100% oxidation of plasma FFA R_d. Recalculating our data, assuming 100% oxidation of FFA R_d, would change our estimates of nonplasma fatty

acid oxidation to account for between 40 and 50% of total lipid oxidation.

Whole body lipolytic rate. Whole body lipolytic rate, as represented by three times glycerol R_a, was higher during exercise relative to rest, but did not differ between any of the work intensities. This lack of training effect on whole body lipolysis has been observed in male subjects after 1 h of exercise when measured at the same absolute workload (18, 23). In addition, our previous parallel study using female subjects also demonstrated no training effect on lipolytic rate (7).

Our glycerol R_a values tended to be lower than those reported by others. Furthermore, the pattern of response in glycerol R_a to training and exercise is very different from that observed by Klein et al. (18). We observed resting glycerol R_a to decrease slightly but significantly after training, whereas they observed a threefold greater glycerol R_a in endurance of trained, compared with untrained, subjects at rest. Our explanation for the differences in magnitude and pattern of glycerol R_a in comparison with those observed by others is attributable to the study design and dietary controls employed. Our subjects rested the day before study and were fed within 4 h before data acquisition, whereas subjects in the study by Klein et al. (18) were fed a standard meal 12 h before commencement of experimental protocols. Therefore, the trained subjects studied by Klein et al. (18), who exercised the day before testing, may have been in negative energy balance, possibly susceptible to liver and muscle glycogen depletion and hypoglycemia during subsequent exercise. Because glycerol turnover has been shown to increase as the time past feeding increases (22), it is likely that our inability to demonstrate a training effect raising glycerol flux in either men (Fig. 3) or women (7) relates to the dietary conditions imposed. In the aggregate, results of the two sets of investigations are of value, as they illustrate the interactive effects of preexercise nutrition and training on substrate utilization.

Gender differences in lipid flux and oxidation. We have recently published data using eight healthy female subjects and an identical protocol to the one described in the present investigation (7). For the most part, the patterns of intensity and training effects on lipid oxidation in the men and women were similar. Men and women demonstrated similar patterns and magnitude of FFA R_a and R_d, but because the women were working at lower absolute workloads than the men, plasma FFA flux rates represented a greater percentage of the energy contribution than in the men (Table 3). In addition, total fat oxidation tended to be higher in the women pretraining at the 65UT workload and was significantly higher after training at both workloads because of the lower RER values in the women. Neither the women nor the men demonstrated a training effect on glycerol flux, although the women did show elevated glycerol R_a at the higher workloads pre- and posttraining (Table 3). In general, the women demonstrated a more pronounced shift toward lipid metabolism in response to training, especially when measured at a given relative exercise intensity. One confounding effect on the comparisons between the men's and women's data is that the women increased their $\dot{V}O_{2peak}$ in response to training by ~20%, whereas the men in this study only increased their $\dot{V}O_{2peak}$ by one-half that amount. However, in both studies, we also measured glucose flux rates, and the men demon-

Table 3. *Lipid kinetic parameters for men and women following similar exercise and training protocols*

Variable	Rest		Exercise			
	Pretraining	Posttraining	45UT	65UT	ABT	RLT
FFA R_a, μmol·kg^{-1}·min^{-1}						
M	4.47 ± 0.20	4.51 ± 0.32	8.14 ± 1.28	6.64 ± 0.46[b]	8.84 ± 1.05[c]	8.44 ± 1.10[c]
W	3.79 ± 0.56	3.64 ± 0.63	7.66 ± 0.99	6.81 ± 0.63	8.40 ± 1.00[c]	9.73 ± 1.12[c]
FFA R_d, μmol·kg^{-1}·min^{-1}						
M	4.36 ± 0.20	4.48 ± 0.32	8.03 ± 1.28	6.42 ± 0.41[b]	8.82 ± 1.06[c]	8.35 ± 1.11[c]
W	3.39 ± 0.29	3.58 ± 0.63	7.28 ± 1.02	6.20 ± 0.69	8.30 ± 0.97[c]	9.53 ± 1.10[c]
Total fat R_{ox}, μmol·kg^{-1}·min^{-1}						
M	3.32 ± 0.55	3.74 ± 0.74	13.30 ± 1.72	12.27 ± 2.78	13.01 ± 2.11	11.41 ± 2.08
W	4.21 ± 0.38	4.27 ± 0.37	11.79 ± 0.99	15.69 ± 1.60[b]	17.99 ± 1.04[b,c] e	19.48 ± 2.12[b,c] e
Respiratory exchange ratio						
M	0.86 ± 0.02	0.85 ± 0.01	0.89 ± .01	0.94 ± 0.02[b]	0.92 ± 0.01	0.95 ± 0.02[b,d]
W	0.84 ± 0.01	0.81 ± 0.01[a] e	0.89 ± 0.01	0.92 ± 0.01[b]	0.86 ± 0.01[c] e	0.87 ± 0.02[c] e
FFA R_d as %energy expenditure						
M	54.2 ± 3.5	53.0 ± 3.9	18.9 ± 2.8	10.8 ± 1.4[b]	14.8 ± 2.1[c]	12.5 ± 2.1[b]
W	43.8 ± 4.2	47.4 ± 5.3	22.9 ± 1.8	13.2 ± 1.8[b]	18.6 ± 1.9[c]	16.5 ± 2.4[b]
Glycerol R_a, μmol·kg^{-1}·min^{-1}						
M	1.8 ± 0.2	1.3 ± 0.2[a]	4.7 ± 0.3 e	4.7 ± 0.3 e	4.2 ± 0.4 e	4.6 ± 0.4 e
W	2.0 ± 0.2	1.6 ± 0.1	5.7 ± 0.5	6.9 ± 0.8[b,d]	5.6 ± 0.5	7.2 ± 0.5[b,d]

Values are means ± SE; $n = 9$ for men (M), and 8 for women (W), except glycerol rate of appearance (R_a) were $n = 19$ and 17 for men and women, respectively. R_d, rate of disappearance. All exercise values were significantly different from resting. [a] Significantly different between resting conditions at $P < 0.05$; [b] significantly different from 45UT at $P < 0.05$; [c] significantly different from 65UT at $P < 0.05$; [d] significantly different from posttraining (ABT) at $P < 0.05$; [e] significantly different between genders ($P < 0.05$).

strated a more exaggerated training-induced decrease in glucose flux at a given absolute workload. These results suggest that, in terms of the peripheral adaptations that govern substrate selection, the men may actually have been more responsive than the women to the identical training regimens. An increase in $\dot{V}O_{2peak}$ [which is thought to relate more to central adaptations (4, 5)] without a concomitant change in peripheral adaptations would likely result in decreased lipid use at the same relative workload (e.g., it would be performed at a higher absolute workload). Thus we do not believe that the differences that we observed in the men and women were solely a result of the magnitude of the training effect.

In the present investigation in men, palmitate comprised 28% of circulating FFA both before and after training. In contrast, we observed plasma palmitate as a percentage of circulating FFA composition to decline from 34 to 28% in women in response to training (7). That change exaggerated the training effect of an increased FFA flux in women. Absence of a training effect on percentage plasma palmitate composition in men makes the calculated increase in FFA flux in trained men during exercise smaller but more robust than in women. With men, our conclusion that training increases FFA flux during exercise is based only on changes in isotopic dilution, not on the combination of isotopic dilution and altered fatty acid fractional composition.

Given that training has a relatively greater effect on lipid oxidation in women compared with men, it is unclear why active women maintain greater lipid stores than men. The difference is especially puzzling as we tested our subjects in the midfollicular phase of the menstrual cycle, when estrogen is low, which should have minimized the ovarian effects on lipid metabolism (7, 8). However, Jensen et al. (14) have demonstrated that epinephrine and estrogen may interact in such a way as to cause elevated FFA availability during estrogen deficiency. We cannot offer an explanation with our data, but we suspect the apparent discrepancy between our measurements of lipid oxidation and body composition in men and women relates to the timing of our observations. We determined FFA flux and oxidation rates during exercise, not recovery. Therefore, for the present, we speculate that there are gender differences that favor preservation of lipid depots in women during rest or recovery from exercise that relate, perhaps, more to lack of androgens than to the presence of the ovarian hormones.

Conclusion. In conclusion, this investigation demonstrated that, after 10 wk of endurance training, men increased FFA R_a and R_d, whether measured at the same absolute or relative exercise intensities. However, total fat oxidation and whole body lipolysis were not significantly impacted by endurance training, and carbohydrate energy sources remained the major contributor to energy production during exercises requiring power outputs eliciting moderate and greater exercise intensities (i.e., 45% or more of $\dot{V}O_{2peak}$). Taken in combination with our previous investigations in men and women, when identical protocols were used (1, 7–9), it appears that after endurance training women may have a more pronounced shift toward lipid utilization during exercise than do men. In men, substrate use is more closely related to relative exercise intensity after training than in women.

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