Effects of exercise intensity and training on lipid metabolism in young women

ANNE L. FRIEDLANDER, GRETCHEN A. CASAZZA, MICHAEL A. HORNING, THOMAS F. BUDDINGER, AND GEORGE A. BROOKS
Exercise Physiology Laboratory, Department of Integrative Biology, University of California, Berkeley, California 94720-3140

Friedlander, Anne L., Gretchen A. Casazza, Michael A. Horning, Thomas F. Buddinger, and George A. Brooks. Effects of exercise intensity and training on lipid metabolism in young women. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E853–E863, 1998.—We examined the effects of exercise intensity and training (12 wk, 5 days/wk, 1 h, 75% peak oxygen consumption (\(\dot{V}O_{2peak}\)) on lipolysis and plasma free fatty acid (FFA) flux in women, \(n = 8\); 24.3 ± 1.6 yr). Two pretraining trials (45 and 65% of \(\dot{V}O_{2peak}\)) and two posttraining trials [same absolute workload (65% of old \(\dot{V}O_{2peak}\); ABT) and same relative workload (65% of new \(\dot{V}O_{2peak}\); RLT)] were performed using infusions of \([1,1,2,3,3-^2H]glycerol\) and \([1-13C]palmitate\). Pretraining rates of FFA appearance (Ra), disappearance (Rd), and oxidation (Roxp) were similar between the 45% \(\dot{V}O_{2peak}\) trials (6.2 ± 0.6, 6.2 ± 0.7, 3.1 ± 0.3 \(\mu\)mol·kg\(^{-1}\)·min\(^{-1}\), respectively) and the 45% of \(\dot{V}O_{2peak}\) trials. At ABT and RLT training increased FFA Ra to 8.4 ± 1.0 and 9.7 ± 1.1 \(\mu\)mol·kg\(^{-1}\)·min\(^{-1}\), Rd to 8.3 ± 1.0 and 9.5 ± 1.1 \(\mu\)mol·kg\(^{-1}\)·min\(^{-1}\), and Roxp to 4.8 ± 0.4 and 6.7 ± 0.7 \(\mu\)mol·kg\(^{-1}\)·min\(^{-1}\), respectively (P ≤ 0.05). Total FFA oxidation from respiratory exchange ratio was also elevated after training at ABT and RLT, with all of the increase attributed to plasma FFA sources. Pretraining, glycerol Ra was higher during exercise at 65% than 45% of \(\dot{V}O_{2peak}\) (6.9 ± 0.9 vs. 4.7 ± 0.6 \(\mu\)mol·kg\(^{-1}\)·min\(^{-1}\)) but was not changed by training. In young women 1) plasma FFA kinetics and oxidation are not linearly related to exercise intensity before training, 2) training increases FFA Ra, Rd, and Roxp, whether measured at given absolute or relative exercise intensities, 3) whole body lipolysis (glycerol Ra) during exercise is not significantly impacted by training, and 4) training-induced increases in plasma FFA oxidation are the major contributor to elevated total FFA oxidation during exercise after training.

stable isotopes; substrate utilization; fat metabolism; glycerol; reesterification; lipolysis; crossover concept; gender; exertion

At rest or during low to moderate intensity exercise, free fatty acids (FFA) can represent a major energy source sustaining whole body energy flux in men and women (26, 33, 36). Results of studies comparing substrate utilization in men and women during exercise have been interpreted to suggest that, for a given relative work intensity, FFA comprise a greater proportion of the source of energy in women than in men (8, 36, 37). It may be that ovarian hormones cause women to respond to physical stress (e.g., pregnancy, exercise, altitude exposure) in a way that favors greater lipid

utilization. Estrogen may impact lipid utilization directly by biasing metabolism toward FFA mobilization and oxidation or by altering the balance of substrate use by decreasing hepatic gluconeogenesis and insulin binding capacity (3). In contrast, progesterone may counteract the impact of estrogen directly by favoring FFA storage or may augment the effects of estrogen by decreasing glucose uptake and oxidation in adipose tissue, decreasing hepatic gluconeogenesis, increasing hepatic glycogen storage, or decreasing peripheral insulin sensitivity (20, 25, 36).

In men, there is strong evidence to suggest that endurance training enhances the capacity for lipid oxidation through increased mitochondrial content and enzymes of β-oxidation (13, 22, 38). Similar training adaptations have been observed in women (5). There is also evidence to suggest that, when measured at the same absolute workload after training, lipid oxidation is increased in men (23, 26). Although several studies have observed increased arteriovenous concentration ([a-v]) FFA differences across trained compared with untrained legs during exercise (1, 12, 22), the issue of whether the increase in lipid oxidation during exercise after training results from increased uptake of plasma FFA (11, 22, 38) or from mobilization of intramuscular sources (16, 23, 26) is still unresolved.

Few studies have measured the impact of endurance training on whole body lipid metabolism when measured at given relative workloads. Results of a previous study conducted in our laboratory (9) suggest that there is no increase in total lipid oxidation at the same relative workload after training in men. However, it has not yet been determined whether women adapt in a similar way as men to endurance training. Studies using biopsies of adipose tissue demonstrated that, after training, both men and women develop an increased lipolytic sensitivity to catecholamine stimulation in vitro. However, the impact of training on different body regions and the pathways of adaptations may differ between genders (6, 27, 32).

The advent of stable isotope technology facilitates the ability to evaluate the response of lipid metabolism to endurance training in women. One study by Poehlman et al. (31) demonstrated that, in postmenopausal women, lipid oxidation, but not FFA rate of appearance (Ra), was elevated at rest after 8 wk of endurance training. The current investigation was designed to evaluate the effects of exercise intensity and endurance training on lipid metabolism in young women during exercise. A longitudinal experimental design was selected to eliminate confounding influences of genetic differences between athletes and nonathletes. Because

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
it has been shown previously that women may have an increased propensity to conserve carbohydrate and oxidize lipid during periods of physical stress, we expected that women would respond to training by demonstrating enhanced abilities to oxidize FFA. Therefore, the purpose of this study was to test the hypothesis that young women would increase their reliance on FFA oxidation during exercise after 12 wk of endurance training when measured at both the same absolute and relative exercise intensities.

METHODS

Subjects. Nine healthy, nonsmoking, sedentary female subjects between the ages of 18–35 yr were recruited from the University of California campus community by flyers and mailings. One subject withdrew from the study before post-training testing for reasons unrelated to the study protocol, leaving data from only eight subjects available for analysis. Subjects were considered sedentary if they had participated in <2 h of regular strenuous activity per week for at least the last year and if they had a peak oxygen consumption (VO_{peak}) between 30 and 42 ml·kg⁻¹·min⁻¹ as determined by a continuous progressive maximal stress test on the 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%, to have a regular (28- to 35-day) menstrual cycle, to not be pregnant, lactating, or taking oral contraceptives, and to be disease/injury free as determined by medical questionnaire and physical examination. All subjects provided informed consent, and the study protocol was approved by the University of California Committee for the Protection of Human Subjects (approval no. 96–1–50).

General experimental design. After an initial interview and screening tests, two stable isotope infusion trials were performed on a cycle ergometer for 1 h at 45 and 65% of VO_{peak} (hereafter referred to as 45UT and 65UT, respectively). All isotope trials were performed on the women in the midfollicular phase of the menstrual cycle (between days 5 and 10 from the first day of menses) and after 36–48 h without exercise training. The two trials were randomized, performed a minimum of 2 days apart, but still conducted within the 5- to 10-day postmenstrual window of testing. Subjects did not train between the two trials. Subjects were required to be diet and weight stable, to have a body fat percentage of <30% and to have a regular (28- to 35-day) menstrual cycle, to not be pregnant, lactating, or taking oral contraceptives, and to be disease/injury free as determined by medical questionnaire and physical examination. All subjects provided informed consent, and the study protocol was approved by the University of California Committee for the Protection of Human Subjects (approval no. 96–1–50).

Isotopes. The glycerol infusion rate was increased three times for the posttraining isotope trial to monitor the subject’s dietary composition and quantity of intake. Dietary analysis of these records was performed using the Nutritionist III program (N-Squared Computing, Salem, OR).

Tracer protocol. All 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 (12 h) was selected by the individual subject and repeated before each trial. Each subject was given a standardized snack (505 kcal: 16% protein-52% carbohydrate-32% fat) to consume before bed, 8–10 h before the trial, 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 more applicable to a nonlaboratory environment. Typically, subjects ate 1–2 h before reporting to the laboratory; subject preparation took a minimum of 1 h, and rest ranged from 90 to 120 min. Thus we report data on resting subjects fed 3.5–5 h previously and exercising subjects fed 4.5–6 h before study. On the morning of the trial, a catheter was placed in a hand vein to obtain “arterialized” blood samples using the “heated hand vein” technique, 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. In parallel studies on men, radial arterial and heated hand vein samples were drawn simultaneously, and the two sampling sites were found to contain similar metabolite concentrations and isotopic enrichments (14). 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 prime) tracers were infused continuously (Baxter Travenol 6200 infusion pump). The resting infusion rate was set at 0.32 mg/min for [1,1,2,3,3-H]glycerol and 0.61 mg/min for palmitate during rest. Upon 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 VO_{peak} postraining trial (same ABT workload) at the start of exercise. Because glycerol tracer was prepared in the same infusion cocktail as [1,1,2,3,3-H]glucose (8) and because of the increased glucose metabolic flux anticipated for the 65% of the new VO_{peak} postraining, 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 [University of California at San Francisco (UCSF) School of Pharmacy, San Francisco, CA], 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 UCSF 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 sterilization testing.

At each of the blood sampling time points, respiratory gas exchange was determined using the calorimetry system described above, and a sample of expired air was collected in a 10-ml vacuum Exetainer tube to determine 13CO2 isotopic enrichment. The expired air samples were stored at room temperature until they were analyzed using isotope ratio mass spectrometry (IRMS) by Metabolic Solutions (Merrimack, NH). Heart rate was recorded throughout rest and exercise using a Quinton O750 electrocardiogram (Seattle, WA). Hematocrit was determined during the last 15 min of rest and exercise to ensure that the measurements of metabolite and hormone concentrations were not influenced by changes in plasma volume.

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, immediately placed on ice, centrifuged for 10 min at 2,500 g, decanted, and frozen. Blood samples for the analysis of glucose and lactate concentrations were collected in 8% perchloric acid and vortex mixed before chilling and centrifugation. Plasma glucose concentration was determined using a hexokinase enzymatic kit (Sigma Chemical, St. Louis, MO). Plasma FFA and glycerol concentrations were collected in EDTA and measured using WAKO (Richmond, VA) and Sigma enzymatic colorimetric kits, respectively. Palmitate isotopic enrichments were measured by mixing a 1-ml aliquot of plasma with a solution of heptane, isopropanol, and H2SO4 that contained 100 nmol pentadecanoic acid as an internal standard. The solution was stored frozen for subsequent thin-layer chromatography (TLC) analysis. After the FFA samples had been separated for their lipid content by TLC, they were derivatized to the fatty acid methyl ester to permit analysis of substrate utilization, independent of training.

RESULTS

Subject characteristics. Pre- and posttraining characteristics of the eight women who completed the study are listed in Table 1. Subjects were weight stable throughout the study period and did not lose a significant amount of body fat whether measured by skin folds or underwater weighing. VO2peak improved by 20.0 ± 1.2% over the training period. The workload characteristics for the four isotope trials are presented in Table 2. Due to the training-induced increase in tissue mass are likely to affect insulin action and the balance of substrate utilization, independent of training.

Calculations and statistics. Palmitate and glycerol Rα, rate of disappearance (Rd), and metabolic clearance rate (MCR) were determined using equations defined by Steele and modified for use with stable isotopes (39). A detailed description of the equations has been reported previously (9). The volume of distribution for palmitate and glycerol were set at 40 and 270 ml/kg, respectively. Palmitate rate of oxidation was calculated using the IRMS analysis of the expired air samples. From a previous study in our laboratory (28), experimentally determined bicarbonate correction factors of 0.65 and 0.9 were used to account for labeled CO2 retained in the blood 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 FID. Rates of total FFA oxidation (Rα) were calculated using the RER and volume of expired CO2 (assuming 22.4 l/mol CO2 and an average of 18 carbons/FFA molecule). Percent of oxidative energy from FFA and lipid was calculated from RER (8). First, FFA including intramuscular triglyceride (IMTG) oxidation was calculated as the total lipid oxidation minus the rate of plasma FFA oxidation (Rα). The rate of whole body reesterification was estimated as the difference between the lipolytic rate (calculated as 3 times glycerol Rα) and Rα. Data are represented as means ± SE. Calculations of steady-state FFA and glycerol kinetics were made using the last two (75 and 90 min) and three (30, 45, and 60 min) isotopic enrichment measurements obtained during rest and exercise, respectively. 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’s least significant difference tests were used for post hoc analyses. Statistical significance was set at α = 0.05.

Table 1. Subject characteristics before and after 8–12 wk of endurance training in 8 young women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>Difference, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>24.3 ± 1.6</td>
<td>23.2 ± 1.1</td>
<td>0.0 ± 1.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165.3 ± 1.9</td>
<td>164.2 ± 1.4</td>
<td>0.0 ± 1.1</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65.9 ± 3.4</td>
<td>64.5 ± 3.0</td>
<td>0.0 ± 1.3</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>24.9 ± 1.9</td>
<td>23.8 ± 1.4</td>
<td>0.0 ± 1.1</td>
</tr>
<tr>
<td>UO2peak</td>
<td>24.2 ± 2.0</td>
<td>23.0 ± 1.5</td>
<td>0.0 ± 1.1</td>
</tr>
<tr>
<td>VO2peak</td>
<td>34.7 ± 1.7</td>
<td>42.1 ± 2.2</td>
<td>0.0 ± 1.2</td>
</tr>
<tr>
<td>ml·kg−1·min−1</td>
<td>2.3 ± 0.1</td>
<td>2.7 ± 0.2</td>
<td>0.0 ± 1.2</td>
</tr>
<tr>
<td>ml·kg·L−1·min−1</td>
<td>43.5 ± 1.2</td>
<td>54.2 ± 1.8</td>
<td>0.0 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. VO2peak, peak oxygen consumption; LM, lean mass; UW, underwater. *Significantly different from pretraining values, P < 0.05.
aerobic capacity, the posttraining trial at the same ABT was equivalent to 52% of the subject's new V\textsubscript{O2peak}.

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 same ABT but not RLT (Table 2). During all four exercise trials, significant hemoconcentration took place as indicated by elevated hematocrits in exercise compared with resting values (Table 2). However, there were no significant differences in hematocrit between any of the exercise intensities, and correction for hemoconcentration did not significantly impact the metabolite data reported below.

Metabolite concentration and isotopic enrichment data. Blood glucose concentrations fell significantly (~10%) during the first 15 min of exercise; however, there were no significant differences in blood glucose concentrations among the four trials during steady-state exercise, and the concentration remained steady at ~4.6 mM (Table 2). Plasma glycerol concentration demonstrated an 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. In addition, plasma glycerol concentration was significantly reduced during exercise after training at the same ABT but not RLT (Fig. 1A). Plasma FFA concentrations were stable during the last 15 min of rest and increased steadily throughout the 1-h exercise period. However, there were no significant differences between any of the four exercise trials in FFA concentration (Fig. 1B). Glycerol, palmitate, and \textsuperscript{13}CO\textsubscript{2} isotopic enrichments for the four isotope trials are presented in Fig. 2, A–C, respectively. Palmitate as a percentage of total FFA concentration was significantly reduced after training from ~34 to 28% during both rest and exercise (Table 2). The mean values obtained for palmitate as a percentage of total FFA concentration pre- and posttraining were used to calculate the FFA kinetic data.

Glycerol kinetics. Glycerol R\textsubscript{a} did not differ at rest pre- and posttraining; however, during all four exercise trials, glycerol R\textsubscript{a} was significantly elevated above resting values (Fig. 3). There was an intensity effect pre- and posttraining, with higher intensity exercise eliciting significantly elevated values for R\textsubscript{a}. Training
did not alter glycerol Ra at either ABT or RLT, although Ra tended to be lower at the same ABT after training (Fig. 3). The pattern of glycerol Rd was similar to that of Ra during rest and exercise for all workloads (data not shown).

FFA kinetics. FFA Ra was significantly elevated in exercise compared with rest during all four trials. There was no significant intensity effect on FFA Ra pretraining, but Ra was elevated after training at both the same ABT and RLT (Fig. 4A). Responses of FFA Rd to exercise and training were similar to those of appearance and are presented in Fig. 4B. Compared with 65UT, FFA Rd increased by 33% at the same ABT and 52% at the same RLT after training. The MCR tended to be higher at rest after training, but the difference did not reach significance ($P = 0.065$). MCR did not increase during exercise relative to rest, and, pretraining, there was only a trend toward lower clearance at 65UT compared with 45UT ($P = 0.058$). However, MCR was significantly higher after training at the same ABT and RLT (Fig. 4C).

The palmitate Ra values in micromoles per kilogram per minute were as follows: 1.22 ± 0.18 (pretraining rest), 1.02 ± 0.18 (posttraining rest), 2.45 ± 0.32 (45UT), 2.19 ± 0.20 (65UT), 2.39 ± 0.31 (ABT), and 2.73 ± 0.31 (RLT). The Rd values were essentially identical to Ra. Exercise flux rates for palmitate were significantly elevated during exercise compared with rest and significantly higher during the RLT trial than the 65UT trial. Thus use of the measured difference in palmitate percentage (34% pretraining vs. 28% posttraining) did impact the calculated flux rates in that significance was established in FFA Ra and Rd at the same ABT after training, where no significant difference in palmitate Ra and Rd was observed at that workload before conversion.

**Fig. 2.** A: isotopic enrichments (IE) of [1,1,2,3,3-2H]glycerol over time for the four isotope trials. Values are means ± SE; n = 8. B: isotopic enrichments of [1-13C]palmitate over time for the four isotope trials. Values are means ± SE; n = 8. C: isotopic enrichments of 13CO2 over time for the four isotope trials. Values are means ± SE; n = 8.

**Fig. 3.** Effect of exercise intensity and training on the plasma glycerol rate of appearance (Ra). Values are means ± SE of the last 15 and 30 min for rest and exercise, respectively; n = 8. # Significantly different from rest; * significantly different from 45UT; # significantly different from ABT ($P < 0.05$).
FFA oxidation. The rate of \( R_{\text{oxp}} \) was significantly higher during exercise than rest. \( R_{\text{oxp}} \) was not affected by intensity pretraining but was significantly higher after training at the same ABT (58%) and RLT (117%) (Fig. 4D). Part of the increase in \( R_{\text{oxp}} \) after training can be attributed to the increase in plasma FFA \( R_d \), but there was also a significant increase in the percentage of \( R_d \) that was oxidized after training during exercise (Table 3). \( R_{\text{oxp}} \) as determined from the indirect calorimetry presented in Table 2, also tended to be elevated.

**Table 3. FFA oxidation parameters in women during rest and exercise**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prerest</th>
<th>Postrest</th>
<th>45UT</th>
<th>65UT</th>
<th>ABT</th>
<th>RLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA oxidation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, µmol·kg(^{-1})·min(^{-1})</td>
<td>4.21 ± 0.38</td>
<td>4.27 ± 0.37</td>
<td>11.79 ± 0.99(^a)</td>
<td>15.69 ± 1.60(^a)</td>
<td>17.49 ± 1.84(^a)</td>
<td>19.48 ± 2.12(^a)</td>
</tr>
<tr>
<td>Plasma, µmol·kg(^{-1})·min(^{-1})</td>
<td>0.45 ± 0.12</td>
<td>0.38 ± 0.06</td>
<td>3.08 ± 0.42(^a)</td>
<td>3.06 ± 0.30(^a)</td>
<td>4.84 ± 0.43(^a)</td>
<td>6.65 ± 0.66(^a)</td>
</tr>
<tr>
<td>Other FFA, µmol·kg(^{-1})·min(^{-1})</td>
<td>3.62 ± 0.47</td>
<td>3.31 ± 0.51</td>
<td>8.11 ± 1.36(^a)</td>
<td>12.64 ± 1.94(^a)</td>
<td>12.41 ± 1.81(^a)</td>
<td>12.79 ± 2.70(^a)</td>
</tr>
<tr>
<td>FFA from other, %</td>
<td>91.36 ± 1.26</td>
<td>89.52 ± 2.56</td>
<td>67.61 ± 6.03(^a)</td>
<td>80.50 ± 2.05(^a)</td>
<td>65.61 ± 6.19(^a)</td>
<td>51.76 ± 3.91(^a)</td>
</tr>
<tr>
<td>( R_d ) oxidized, %</td>
<td>10.62 ± 1.03</td>
<td>10.93 ± 0.76</td>
<td>49.58 ± 5.54(^a)</td>
<td>51.33 ± 4.43(^a)</td>
<td>61.22 ± 5.51(^a)</td>
<td>71.76 ± 3.91(^a)</td>
</tr>
<tr>
<td>Other if 100% ( R_d ) oxidized, %</td>
<td>19.5</td>
<td>16.2</td>
<td>38.3</td>
<td>61.0</td>
<td>53.8</td>
<td>51.1</td>
</tr>
<tr>
<td>Reesterification rate, µmol·kg(^{-1})·min(^{-1})</td>
<td>1.40 ± 0.50</td>
<td>1.30 ± 0.40</td>
<td>3.20 ± 1.12(^a)</td>
<td>5.58 ± 2.05(^a)</td>
<td>2.82 ± 1.06(^a)</td>
<td>4.80 ± 1.36(^a)</td>
</tr>
<tr>
<td>( R_s ) as % lipolysis</td>
<td>21.74 ± 6.63</td>
<td>19.66 ± 6.16</td>
<td>18.06 ± 6.18</td>
<td>21.25 ± 6.77</td>
<td>14.15 ± 5.00</td>
<td>19.68 ± 4.84</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 8 \) subjects. \( R_d \), rate of disappearance; \( R_s \), reesterification rate. \(^a\)Significantly different from rest, \( P < 0.05; \(^b\)significantly different from 45UT, \( P < 0.05; \(^c\)significantly different from 65UT, \( P < 0.05; \(^d\)significantly different from ABT, \( P < 0.05; \(^e\)% total oxidation coming from nonplasma sources if assumption is made that \( R_d = \) rate of plasma FFA oxidation (see Discussion).
after training, although the differences did not reach significance when 65UT was compared with either posttraining workload (Table 3). Subtracting $R_{\text{oxo}}$ from $R_{\text{tot}}$ gives an estimate of other FFA oxidation ($R_{\text{oxo}}$), including IMTGs. For rest and all exercise conditions, the calculated $R_{\text{oxo}}$ was >50%, suggesting that the majority of whole body FFA oxidation was derived from nonplasma sources (Table 3).

**FFA reesterification rate.** Rate of total body reesterification ($R_s$) did not differ at rest pre- and posttraining but was significantly higher during all four exercise trials. There was also a reduction in $R_s$ after training when measured at the same ABT but not RLT exercise intensity (Table 3). However, $R_s$ approximated 20% of the total lipolytic rate at rest and did not change significantly during exercise. In addition, $R_s$ as a percentage of total lipolysis did not differ significantly between exercise trials (Table 3).

Total lipid metabolism. Figure 5A represents the impact of exercise intensity and training on the contributions of different lipid components to overall lipid metabolism during rest and exercise in women. Most of the increase in lipid oxidation during the 65UT trial compared with the 45UT trial was derived from nonplasma sources. When the same absolute workloads are compared pre- and posttraining, total lipid metabolism did not change, but oxidation of plasma FFA increased while there was a reduction in the reesterification rate. During exercise at the same RLT posttraining, oxidation of plasma FFA was elevated, and reesterification did not change compared with 65UT. In Fig. 5B, the relative contributions of plasma FFA, other FFA, and carbohydrates are presented normalized to total energy expenditure.

**DISCUSSION**

The results of the current investigation suggest that women increase their reliance on lipid after endurance training whether exercise is normalized to either absolute or relative power outputs. The increase in FFA oxidation in response to training was derived predominantly from plasma sources, based on a combination of both increased FFA $R_d$ and a greater percent of $R_d$ oxidized. There was no training-induced increase in other (e.g., intramuscular) FFA oxidation. In contrast, the elevated FFA oxidation observed pretraining at 65 vs. 45% of $V\dot{O}_2\text{peak}$ resulted entirely from nonplasma sources. There were no differences between FFA $R_d$ or percent of $R_d$ oxidized measured in the two pretraining intensities.

FFA metabolism (effects of training). Our data on the impact of endurance training on lipid metabolism in women are similar to those showing increased total body lipid oxidation after training at a given absolute workload in men (9, 26, 30). However, the finding that women also displayed increased lipid oxidation at the same relative workload after training differs from our previous findings in men showing no increased total lipid use at the same relative exercise intensity (9). In addition, the increased FFA flux rates that we observed in female subjects after training differed from previous reports of others on men demonstrating reduced FFA $R_d$ after training at the same absolute workload (26, 30) or no difference in values at the same relative workload (18). It may be that adipose tissue of women responds differently to training from that of men. Several studies investigating lipolysis in adipose tissue samples obtained from trained and untrained subjects have shown increased catecholamine sensitivity in both trained men and women in vitro (6, 27, 32). However, results of studies comparing men and women suggest that training may lead to better subcutaneous abdominal lipid mobilization in women (6) and that in women the increased mobilization results from both an upregulation of the $\beta$-adrenergic stimulation pathway of FFA lipolysis and a downregulation of the inhibitory $\alpha_2$-adrenergic pathway (6, 27, 32). In addition, estrogen

---

Fig. 5. A: contributions of different lipid sources to total lipid metabolism during rest and exercise before and after training. SE and statistical symbols are for total lipid metabolism only; $n = 8$. △Significantly different from rest; *significantly different from 45UT ($P < 0.05$). B: contributions of energy from different substrate sources during rest and exercise normalized to percent energy expenditure; $n = 8$. CHO, carbohydrate.
has been shown to enhance lipid oxidation in rats (10, 21) and mobilize peripheral adipose triglycerides, perhaps with an estrogen-growth hormone interaction in humans (4). Thus women could have enhanced plasma FFA availability for a given submaximal workload or after training than men. However, testing our subjects in the midfollicular phase of the menstrual cycle when estrogen is low should have minimized the ovarian interactive effects.

Our finding of increased FFA $R_d$ in women after training fits well with data from $[\text{a-v}]$ difference studies comparing trained vs. untrained men (38) or using one-leg training protocols comparing trained vs. untrained legs (12, 22). Kiens et al. (22) demonstrated that, during 2 h of dynamic leg extension exercise performed at the same absolute workload, FFA concentration continued to increase throughout exercise in the circulation to both legs, but only the trained leg continued to increase its net uptake of FFA. After the first hour of exercise, the untrained leg exhibited a plateau in net uptake despite similar FFA availability in both legs, suggesting that FFA uptake could be a saturable process. Similarly, Turcotte et al. (38) demonstrated that, during 3 h of knee extension exercise at 60% of maximum capacity, leg FFA uptake increased linearly over time in trained but not untrained subjects, reaching a significant difference after 2 h. Because FFA delivery increased similarly in both groups, the trained subjects had higher fractional extraction by the end of exercise than the untrained subjects. Thus, on the whole body level, our findings of increased FFA $R_d$ and increased MCR after training in our female subjects are consistent with data obtained across working limbs. In addition to an increased uptake of FFA from plasma in trained subjects, the results of rat and human biopsy studies suggest that training-induced increases in FFA binding proteins, mitochondrial density, $\beta$-oxidation, and tricarboxylic acid cycle enzymes could enhance the ability of FFA in the cytosol to be taken up by the mitochondria and utilized after training (12, 13, 22, 29, 38). Because of the complex regulation of the balance of substrate utilization, increased capacity does not necessarily imply increased oxidation. However, our female subjects did demonstrate an increase in the percent of FFA oxidized after training (Table 3).

Our values for percent of FFA oxidized compare well with those measured in the training studies presented by Martin et al. (50%; see Ref. 26) and Turcotte et al. (74–76%; see Ref. 36). However, in those studies, male trained subjects did not exhibit a higher percent oxidation than the untrained subjects. Whether the increase in percent oxidation that we observed in response to training can be attributed to gender differences or methodological differences is unclear at this time.

There was a significant difference in the composition of individual FFA between the pre- and postraining tests. Pretraining, palmitate comprised ~34% of total FFA, whereas, after training, palmitate made up only 28% of the total FFA (Table 3). These findings were not only significant in and of themselves but also impacted the calculation of FFA kinetics (e.g., whereas the increase in FFA $R_d$ after training would not have been significant at the same absolute workload using a constant value of 34% for palmitate, the reduced value of 28% after training yielded a significant increase for FFA $R_d$ after training at ABT). However, the increases in FFA flux that we observed at the RLT workload, as well as the increases in plasma FFA oxidation after training at both workloads, would have remained significantly elevated (although smaller in magnitude) if a constant palmitate percentage had been utilized pre- and postraining. Contrary to our expectations, the 3-day dietary records obtained from the subjects indicated that there was an increase, rather than a decrease, in the consumption of saturated fat by our subjects throughout the training intervention. Thus the shift away from palmitate as a constituent of plasma FFA does not appear to correspond to a change in dietary composition. More research is needed to determine whether the observed change in palmitate reflects a reduction in saturated fat storage and/or utilization induced by training. However, regardless of the cause, our results are interpreted to mean that care should be taken to measure the palmitate-to-total FFA ratio in future studies as differences can impact the calculated kinetic values.

FFA metabolism (effects of exercise intensity). Our data on the effects of exercise intensity on lipid metabolism in women before training are similar in some respects to those of Romijn et al. (33) obtained in men. Romijn et al. demonstrated that the majority of the increase in fat oxidation observed in response to increasing intensity [65 vs. 25% of maximal aerobic capacity ($V_{\text{O2max}}$)] could be attributed to nonplasma sources and proposed that heavy exercise limits lipolysis in adipose tissue thus reducing plasma FFA availability. In an earlier study, Jones et al. (19) suggested a similar hypothesis to explain their observations of decreased FFA $R_d$ with increased glycerol concentration at 70 vs. 36% of $V_{\text{O2max}}$. Pretraining, our women also demonstrated a significant increase in lipid oxidation derived from nonplasma sources at the higher intensity workload. In contrast, in our female subjects postraining, the increase in lipid oxidation at relative workload (65% of $V_{\text{O2peak}}$ vs. absolute workload (52% of $V_{\text{O2peak}}$)) was comprised of an increase in plasma FFA oxidation (a trend toward higher FFA $R_d$ and a significant increase in percent $R_d$ oxidized). Because FFA kinetics have been described previously as relating to exercise intensity in a manner similar to an inverted parabola with a peak somewhere between 50 and 65% of maximum capacity (2, 15), it is possible that a training-induced shift upward or to the right of the curve could alter the response of FFA kinetics to the exercise intensities in differing ways pre- and postraining. Although the current investigation does not include a sufficient number of exercise intensities postraining to define such a shift, the available data are consistent with a shift to a higher relative power output for peak FFA flux after training (Fig. 6).
FFA metabolism (sources of FFA). An additional difference in our results compared with those obtained in previous research on men is that we observed a larger magnitude of nonplasma sources relative to plasma sources of energy coming from lipid. For example, our data suggest that as much as 80% of lipid oxidation came from nonplasma sources during the 65% of pretraining trial, unlike the ~50% reported by Romijn et al. (33) for trained men exercising at a similar relative power output. It could be that women rely more heavily on nonplasma FFA than do men. However, Romijn et al. (33) assumed 100% oxidation of plasma FFA in their male subjects. According to our data, such an assumption could result in an overestimation from between 30 and 50% depending on training state. Recalculating our values making the same assumption of 100% oxidation of FFA Rd (Table 3) illustrates that our data in women would be similar to those presented in papers making the assumption Rd = R_{awp} in males (30, 33).

Whether IMTGs provide a significant source of energy during exercise remains an issue of debate, and results are influenced by the methodology used. Reports that have attempted to measure IMTG content of muscle biopsy samples before and after exercise are inconsistent (16, 22). Those using [a-v] differences of FFA and glycerol report either a significant contribution (38) or minimal contribution (1) from IMTG sources. Results of studies on whole body metabolism that calculate IMTG use as the difference between \( R_{ext} \) (as determined by RER) and plasma FFA oxidation (using the rate of oxidation or \( R_d \)) generally find a large proportion of lipid oxidation coming from nonplasma sources (50–80%; see Refs. 26, 30, 33). Often the nonplasma sources calculated in the whole body studies are attributed to working muscle IMTG oxidation. However, there is no way to determine from such methodologies where the lipid is being oxidized, and results from our laboratory indicate the leg [v-a] for glycerol approximates zero during exercise in men even after training (1). In contrast, we believe that it is likely that the working muscle is utilizing more carbohydrate than nonworking muscle or other tissues during exercise, thus the majority of putative “IMTG” oxidation could occur in nonworking tissues.

Lipolysis and reesterification. Whole body lipolytic rate, as represented by three times glycerol \( R_g \), was elevated at the higher of the two intensities pretraining and postraining but did not demonstrate a significant training effect during exercise at either workload after training. This lack of training effect on whole body lipolysis has been observed by others after 1 h of exercise using male subjects when measured at the same absolute workload pre- and postraining (23, 30). Our values in women are also comparable in magnitude to those obtained in men, despite the fact that our female subjects were fed within 4 h before data acquisition, whereas the men were fed 6 h before (30) and 12 h before (23) commencement of experimental protocols.

Reesterification rates in our subjects were substantially lower at rest and during exercise than rates of others (40). Furthermore, we did not show a training-induced increase in resting recycling rate as others have shown (34). Such discrepancies could be attributed to gender differences, nutritional status, or level of training as highly trained male endurance athletes who were exercised the day before and fed a standard evening meal were used by Romijn et al. (34) and Klein et al. (23).

The calculation for total body reesterification assumes that there are only two ultimate fates for FFA broken down from triglyceride, oxidation or reesterification. However, if there is accumulation of FFA in plasma or other tissues such as adipose, the assumption does not hold true. Our data showed an accumulation of ~0.35–0.45 \( \mu \text{mol·kg}^{-1}·\text{min}^{-1} \) of FFA in the blood (assuming 5 liters of blood/subject) throughout exercise for each of the exercise intensities. Such a change in plasma FFA concentration would account for somewhere between 8 and 15% of our calculated reesterification rate. Perhaps more important are those FFA that accumulate in peripheral adipose tissue. Many studies have shown a rapid increase in plasma FFA concentration at the start of recovery immediately after exercise of 40, 65, and 85% of maximum capacity (33, 40). The rapid rise in FFA has been attributed to inadequate blood flow to adipose tissue during exercise while blood is being shunted to working muscles operating at moderate to high intensities. After exercise, blood returning to adipose tissue may flush the accumulated FFA from the tissues into the circulatory system. Unlike the hydrophobic FFA, which are carrier dependent, glycerol, which is water soluble, is not dependent on blood flow and therefore can diffuse into the system. We did not measure FFA concentration during recovery, but any FFA that were trapped in peripheral, nonexchanging tissues would cause an overestimation in our calculations of reesterification. In addition, the amount of FFA accumulation may differ depending on intensity and training in a way that parallels changes in blood flow. Thus our values along with those from previous studies using similar calculations must be considered estimates of the maximal rate of reesterification in each of the exercise testing conditions.
Methodological considerations. The use of glycerol $R_a$ as an estimate of whole body lipolytic rate is based on the assumption that all glycerol liberated from the breakdown of triglycerides in adipose or muscle tissue must enter the blood since it cannot be reused in peripheral tissues without significant amounts of the enzyme glycerol kinase. Reesterification in adipose and muscle tissue is thought to take place using $\alpha$-glycerol phosphate, a glycolytic intermediate, rather than glycerol. Nontracer measurements of glycerol release across tissue beds to estimate MTG lipolysis and use rely on a similar assumption that peripheral tissues do not take up glycerol. However, in a recent paper, Landau et al. (24) suggest that, in 60 h fasted subjects, only approximately one-half of glycerol $R_a$ is taken up by splanchic or kidney beds, thus leaving the periphery to take up the rest. In addition, preliminary data by Elia et al. (7) suggest that muscle may metabolize a significant amount of glycerol thus limiting the validity of glycerol $R_a$ as a measure of lipolysis. Such findings need to be corroborated but could impact many of the assumptions and calculations used in the study of lipid metabolism.

The magnitude and pattern of responses at rest of FFA reesterification and glycerol $R_a$ to training that we observed in women differ greatly from Romijn et al. (34) and Klein et al. (23). Rather than gender differences, we believe the data reflect variations in dietary status of the subjects used. Our subjects rested the day before their isotope trials and were fed to be in energy balance. In contrast, subjects studied by Romijn et al. and Klein et al. were athletes who trained the day before the trials and nonathletes who did not train and who were then each fed an isocaloric standardized meal 12 h or more before commencement of tracer studies. Thus we believe that the large differences in glycerol $R_a$ observed by Klein et al. and Romijn et al. between trained and untrained men relate more to differences in dietary status than training status. Furthermore, because of the differences in dietary control employed, it is premature to ascribe differences in magnitude and pattern of glycerol kinetics and FFA reesterification in our respective studies to the effects of gender.

Our plasma FFA oxidation rates at rest were low relative to plasma FFA $R_d$ and the total FFA rates of oxidation. As a result, the percent of $R_d$ oxidized was lower than anticipated, and the percent of total oxidation coming from nonplasma sources was high (Table 3). It is possible that, without a priming dose of $[1-^{13}C]$palmitate and without directly priming the bicarbonate pool, 90–120 min of rest were not sufficient to bring the bicarbonate pool to equilibrium. Therefore, during rest and perhaps during the early stage of exercise, metabolically generated labeled $^{13}CO_2$ could have been trapped in the blood. This may have caused an underestimation of plasma FFA oxidation with a resulting overestimation of "other" FFA oxidation. However, Fig. 2C demonstrates that, during the end stages of exercise, the isotopic enrichments of $^{13}CO_2$ were not increasing in any of the isotope trials, thus indicating that the tracer had attained equilibrium by that time. Therefore, we believe that our calculations of plasma FFA oxidation are valid during exercise in each of the four isotope trials.

In conclusion, the women in this investigation increased their reliance on lipid oxidation after endurance training when measured at the same absolute workload. In addition, the shift in total lipid oxidation as determined by RER values was greater in the women than in our previous study using male subjects (9). Unlike the males we studied, females demonstrated a significant shift toward increased lipid utilization after training at the same relative workload (65% of $V_{O_2peak}$) as well. The increase in $R_{ext}$ at both postraining workloads in women after training indicate that, in terms of lipid metabolism, women respond more dramatically to endurance training than do males performing a similar training regimen.

The results of this investigation suggest that in young women 1) FFA kinetics and oxidation are not linearly related to exercise intensity before training, 2) training increases FFA $R_a$, $R_d$, and MCR whether measured at the same absolute or relative workload, 3) whole body lipolysis (glycerol $R_a$) is not significantly impacted by training during rest or exercise, and 4) training-induced increases in plasma FFA oxidation are the main contributor to elevated $R_{ext}$ in the trained state.

We thank Gail Butterfield for consultation on all aspects of the study. We also thank Katie Milano, Robin Rynbrandt, Tam Ho, Tani Brown, Matt Inlay, Catherine Chen, and Chung Lu who provided much of the laboratory support throughout the study. We are also grateful to all of the student trainers who assisted with the subject training and the Tang Health Center physicians (especially Elizabeth Hedelman and Cheryl Tanouye) who provided medical coverage for the procedures.

This work was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR-42906.

Current address for A. L. Friedlander: GRECC, 1828, Palo Alto VA Health Care System, 3801 Miranda Ave., Palo Alto, CA 94304, e-mail: friedlan@eland.stanford.edu.

Address for reprint requests: G. A. Brooks, Dept. of Integrative Biology, 3060 Valley Life Science Bldg., Univ. of California, Berkeley, CA 94720-4480.

Received 2 April 1998; accepted in final form 17 July 1998.

REFERENCES


15. Henriksson, J.


18. Hultman, E.


