Fat metabolism during low-intensity exercise in endurance-trained and untrained men

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Klein, Samuel, Edward F. Coyle, and Robert R. Wolfe. Fat metabolism during low-intensity exercise in endurance-trained and untrained men. Am. J. Physiol. 267 (Endocrinol. Metab. 30): E934–E940, 1994.—Whole body lipid kinetics were evaluated during basal resting conditions, 4 h of treadmill exercise eliciting an oxygen uptake of 20 ml·kg⁻¹·min⁻¹, and 1 h of recovery in five untrained and five endurance-trained men. Glycerol and free fatty acid (FFA) rate of appearance (Rₐ) values in plasma were determined by infusing [²H₅]glycerol and [¹-¹³C]palmitate, respectively, and lipid oxidation was determined by indirect calorimetry. The lipolytic response to 4 h of exercise, expressed as the average glycerol and FFA Rₐ values, was similar in both trained (0.85 ± 1.02 and 24.64 ± 3.76 μmol·kg⁻¹·min⁻¹, respectively) and untrained subjects (11.29 ± 0.99 and 24.13 ± 0.39 μmol·kg⁻¹·min⁻¹, respectively). However, mean triglyceride oxidation was greater during exercise in the trained than in the untrained group (7.51 ± 0.26 and 5.67 ± 0.51 μmol·kg⁻¹·min⁻¹, respectively). During recovery, glycerol and FFA Rₐ values decreased more rapidly in trained than in untrained subjects. We conclude that highly trained male endurance runners use more fat as a fuel during low-intensity exercise than do untrained healthy men despite similar rates of lipolysis and FFA uptake from plasma. Therefore, the increase in fat oxidation must be related to an increased percentage of FFA uptake oxidized, a greater contribution from intramuscular triglyceride stores, or both. Additionally, lipid kinetics return to baseline more rapidly in trained than in untrained subjects after completing an exercise bout of the same absolute intensity.

ENDOGENOUS TRIGLYCERIDE stores are a major source of fuel for low- and moderate-intensity exercise. Cross-sectional studies comparing endurance-trained athletes with sedentary subjects and longitudinal training studies demonstrate that endurance training increases the use of fat as a fuel during exercise. Several factors may be responsible for the stimulation of fat oxidation in endurance-trained subjects. First, an increase in oxidative enzymes and mitochondria content (12) in trained muscles enhances fat oxidation. Second, training has been shown to increase the oxidation of intramuscular triglycerides during moderate-intensity exercise (13). Third, training may increase muscle uptake of plasma free fatty acids (10, 15, 30).

Alterations in the mobilization of adipose tissue triglycerides could also affect the use of lipid as a fuel during exercise. However, how endurance training affects lipolysis during exercise is not clear because of conflicting results from different studies. Havel et al. (8, 9) found that free fatty acid (FFA) rate of appearance (Rₐ) in plasma was greater in trained athletes than in untrained persons performing different modes of exercise at similar absolute intensities. In contrast, Martin et al. (21) demonstrated that 12 wk of endurance training blunted the exercise-induced increase in FFA Rₐ during exercise performed at the same absolute intensity. Jansson and Kaijser (14) found that FFA Rₐ during exercise at the same relative intensity was the same in endurance-trained athletes and in untrained subjects. None of these studies evaluated glycerol Rₐ, which provides a better index of whole body lipolytic rates than does FFA Rₐ. Glycerol released during lipolysis of adipose tissue triglycerides must enter the bloodstream (20), whereas released fatty acids can be reesterified within the adipocyte, which would prevent fatty acid appearance in plasma. Therefore, differences in fatty acid reesterification could cause differences in measured fatty acid kinetics without real differences in whole body lipolytic rates. Recently, Kiens et al. (15) reported greater regional glycerol release during dynamic knee extension exercise in the trained than in untrained thigh muscle. We are unaware of any studies that have compared whole body glycerol Rₐ in trained and untrained subjects.

In the present study lipid kinetics were evaluated during basal resting conditions, 4 h of low- to moderate-intensity endurance exercise, and 1 h of recovery in endurance-trained male athletes and in untrained healthy young adult men. The rates of glycerol and palmitate appearance in plasma and the rate of triglyceride oxidation were measured using stable isotope tracers and indirect calorimetry. The absolute intensity of the exercise bout was the same in both groups, and therefore the relative intensity was lower in the trained than in the untrained persons.

METHODS

Subjects. Five untrained and five endurance-trained healthy men participated in the study, which was approved by the Institutional Review Board of The University of Texas Medical Branch at Galveston. The characteristics of the study subjects are shown in Table 1. Maximal oxygen consumption (VO₂max) determined during exhausting exercise on a treadmill was 50% greater in trained than in untrained subjects. All subjects were considered to be healthy, did not smoke or take medications, and had no evidence of cardiovascular or metabolic disease. The untrained subjects were physically active but did not participate in any regular exercise program, whereas the endurance-trained subjects were competitive distance runners who were participating in a strenuous training program involving 1–3 h of running a day, 5–7 days/wk for 3–8 yr.

Measurement of VO₂max. VO₂max was determined during 8–12 min of continuous multistage exercise on a treadmill. At least
ENDURANCE TRAINING AND LIPID KINETICS

Table 1. Characteristics of study subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Untrained</th>
<th>Trained</th>
</tr>
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<tbody>
<tr>
<td>Age, yr</td>
<td>28 ± 2</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>177 ± 3</td>
<td>181 ± 5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69 ± 4</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>VO2max, ml·kg⁻¹·min⁻¹</td>
<td>46 ± 2</td>
<td>72 ± 4</td>
</tr>
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Values are means ± SE; n = 5 subjects/group. VO2max, maximal oxygen consumption. *Significantly different from untrained subjects, P < 0.05.

two of the following three criteria were met to establish that VO2max was attained: 1) respiratory exchange ratio > 1.15, 2) a leveling off of VO2 and heart rate despite increases in work load, and 3) attainment of predicted maximal heart rate. Exercise testing was started with a "warm up" by having the subjects walk and/or jog for 10 min on the treadmill. The speed and incline were then increased every minute until volitional exhaustion was reached. VO2 and carbon dioxide production were measured continuously by open-circuit respirometry, using a Horizon metabolic measurement cart (Sensormedics, Anaheim, CA).

Experimental protocol. All subjects were admitted to the Clinical Research Center of The University of Texas Medical Branch. In the afternoon of the day of admission, VO2 was determined in the untrained subjects, whereas the trained subjects participated in their customary 7- to 10-mile training run. VO2max had been measured previously in the trained subjects. The subjects ingested at least 300 g of carbohydrate the day before the study. After the subjects fasted overnight (12 h), Teflon catheters were placed percutaneously into the antecubital vein of one arm for the infusion of stable isotopes (MSD Isotopes, Montreal, Canada) dissolved in normal saline (12 h), Teflon catheters were placed percutaneously into the antecubital vein of one arm for the infusion of stable isotopes and into the contralateral dorsal hand vein, which was heated, for arterialized venous sampling (22). A primed (0.9 pmol/kg), constant (0.06 μmol·kg⁻¹·min⁻¹) infusion of [2H₅]glycerol (MSD Isotopes, Montreal, Canada) dissolved in normal saline and a constant (0.03 μmol·kg⁻¹·min⁻¹) infusion of [1-¹³C]palmitate (MSD Isotopes) bound to human albumin were started and infused with calibrated syringe pumps (Harvard Apparatus, Natick, MA) for 60 min while the subjects rested comfortably in a chair. After 60 min of infusion the subjects walked continuously on a treadmill for 4 h. The speed and incline of the treadmill were adjusted during the early portion of exercise so that VO2 was maintained at 20 ml·kg⁻¹·min⁻¹. At the end of the 4-h exercise period the subjects rested by sitting in a chair for 1 h. The rates of isotope infusion were altered during exercise and recovery to minimize changes in plasma substrate isotopic enrichment as described previously (33). At the onset of exercise the infusion rate of [2H₅]glycerol was increased four- and threefold and the infusion rate of [1-¹³C]palmitate was increased 2.5- and 2-fold in the trained and untrained subjects, respectively. The isotope infusion rates were increased more during exercise in the trained subjects to ensure adequate substrate isotopic enrichment in the event that exercise caused a greater stimulation of lipolytic activity in trained than in untrained subjects. At the onset of recovery the rates of isotope infusion were decreased to basal values. Blood samples were taken before beginning the isotope infusion to measure baseline glycerol and palmitate enrichment. Blood samples were then taken to measure lipid kinetics at rest (45, 50, 55, and 60 min), during exercise (10, 20, 30, 60, 90, 120, 150, 180, 210, and 240 min of exercise), and during recovery from exercise (10, 20, 30, and 60 min of recovery). All blood samples were collected in heparinized tubes and placed in a 4°C ice bath. Plasma was promptly separated by centrifugation and stored at −20°C until analysis. VO2 and carbon dioxide production were measured by indirect calorimetry using a metabolic cart (Sensormedics) at the end of the resting period and at the time of each blood sample during exercise and recovery.

Sample analysis. Plasma glycerol concentration was determined enzymatically with an automated analyzer using a glycerol oxidase method (Technicon, Tarrytown, NY). Plasma FFA concentrations were quantified by gas chromatography. Hepatodecanolic acid was added to each sample as an internal standard.

Isotopic enrichment of palmitate and glycerol in plasma was determined by gas chromatography-mass spectrometry (GC-MS) using an MSD 5971 system (Hewlett-Packard, Palo Alto, CA) with an HP-1 12 m × 0.2-mm fused silica capillary column (Hewlett-Packard). Plasma samples were analyzed for [1-¹³C]palmitate enrichment as described previously (17). FFAs were isolated from plasma and converted to their methyl esters. The derivatized sample was injected into the GC-MS where ions were formed by electron impact ionization. Ions at mass-to-charge ratio (m/e) 270.2 and 271.2, representing the molecular ions of unlabeled and labeled methyl esters, respectively, were selectively monitored to determine the tracer-to-tracer ratio. Plasma samples were prepared for analysis of glycerol isotopic enrichment as described previously (17). Plasma proteins were precipitated with barium hydroxide and zinc sulfate. After centrifugation the supernatant was passed through a mixed cation and anion exchange column. A trimethylsilyl derivative of glycerol was formed and injected into the gas chromatograph mass spectrometer. Ions were produced by electron impact ionization, and glycerol enrichment was determined by selectively monitoring ions at m/e 205.1 and 208.1.

Calculations. A physiological and isotopic steady state was present during the last 15 min of the resting (preexercise) period, so Steele's equation (29) for steady-state conditions was used to calculate substrate (palmitate and glycerol) R, rate of disappearance (R₀) from plasma. During exercise and recovery non-steady-state conditions were present, and Steele's equation (29) for non-steady state conditions was used to calculate R, and R₀. Fatty acid R, was calculated by dividing palmitate R₀ by the percentage of contribution of palmitate to total FFA concentration. The effective volume of distribution was assumed to be 270 ml/kg for glycerol and 50 ml/kg for palmitate. Samples obtained at 10 min of exercise and recovery were not used to estimate glycerol and palmitate kinetics because of the potential confounding effect of a sudden change in isotope infusion rate on calculated R₀ values.

Triglyceride oxidation rates were calculated from measurement of carbon dioxide production, VO₂, and estimated urinary nitrogen excretion (6). Nitrogen excretion was assumed to be 80 μg·kg⁻¹·min⁻¹ based on the results of an earlier study in subjects completing a similar exercise protocol (3). The rate of triglyceride oxidation (expressed as g·kg⁻¹·min⁻¹) was converted to its molar equivalent (expressed as μmol·kg⁻¹·min⁻¹) by assuming that palmitoyl oleoyl triglyceride (860 g/mol) was a typical triglyceride. Potential errors in estimating nitrogen excretion would not have had a significant effect on the accuracy of calculating fat oxidation. In the present study, altering the value for urinary nitrogen by as much as 300% would cause less than a 5% change in the calculated rate of fat oxidation.

Statistical analysis. The significance of differences between the trained and untrained subjects was evaluated by using the Student's t-test for independent samples and analysis of variance with repeated measures. A P value of < 0.05 was considered to be statistically significant. All data are expressed as means ± SE.
RESULTS

Fatty acid concentrations. Basal plasma FFA concentrations were similar in the trained and untrained subjects. However, the increase in plasma FFA concentration throughout the 4 h of exercise was greater in the untrained subjects, with differences becoming statistically significant by 120 min of exercise (Fig. 1). At the end of exercise, values approached 2 mM in the untrained compared with only 1 mM in the trained subjects. Plasma glycerol concentration also increased more during exercise in untrained than in trained subjects (data not shown).

Indirect calorimetry and triglyceride oxidation. Adjustments in treadmill speed and incline were made during early exercise to achieve a VO2 of 20 ml·kg⁻¹·min⁻¹. The target rate was reached within 15 min of exercise in each subject and was maintained throughout the exercise period. VO2 during the last 30 min of exercise was 20.8 ± 1.12 and 21.2 ± 1.1 ml·kg⁻¹·min⁻¹ in the trained and untrained subjects, respectively. Mean respiratory exchange ratio during the last 30 min of exercise was 0.79 ± 0.02 in the trained and 0.83 ± 0.02 in the untrained subjects (P < 0.05).

The rates of triglyceride oxidation during rest, exercise, and recovery are shown in Fig. 2. Basal fat (triglyceride) oxidation rates were higher in trained than in untrained subjects (1.39 ± 0.19 and 0.82 ± 0.15 μmol·kg⁻¹·min⁻¹, respectively, P < 0.01). During exercise fat oxidation increased fivefold and threefold within 10 min in the trained and untrained subjects, respectively, and increased progressively thereafter. The average rate of fat oxidation during 4 h of exercise was greater in trained than in untrained subjects (7.51 ± 0.26 and 5.67 ± 0.51 μmol·kg⁻¹·min⁻¹, respectively; P < 0.001). During recovery fat oxidation decreased rapidly in both trained and untrained subjects. The average rate of fat oxidation during recovery was higher in trained than in the untrained subjects (2.05 ± 0.13 and 1.11 ± 0.07 μmol·kg⁻¹·min⁻¹, respectively; P < 0.05).

Glycerol and fatty acid kinetics. Glycerol Rₜ and FFA Rₜ during rest and exercise are shown in Fig. 3. Glycerol Rₜ and fatty acid Rₜ at rest before exercise were ~50% higher in the trained than in the untrained subjects (P < 0.05; Table 2). During exercise, glycerol Rₜ and FFA Rₜ increased to similar values in both trained and untrained subjects. The lipolytic response to exercise, expressed as the average glycerol or FFA Rₜ during 4 h of exercise, was similar in trained and untrained subjects (Table 2). During recovery, glycerol Rₜ and fatty acid Rₜ returned rapidly to resting preexercise values in the trained subjects but remained two- to threefold higher than resting values in the untrained group. Average glycerol Rₜ and fatty acid Rₜ during recovery were closer to preexercise values in trained than in untrained subjects (Table 2).

Tissue uptake of plasma fatty acids (FFA Rᵦ) was similar in both trained and untrained subjects. Mean total FFA Rᵦ was greater than FFA oxidation during exercise in untrained subjects, but FFA Rᵦ and oxidation rates were similar during exercise in the trained group (Fig. 4). Fatty acid uptake was greater than FFA Rᵦ in all untrained subjects, whereas FFA Rᵦ was less than the rate of FFA oxidation in three of five trained subjects.
In the present study we found that endurance-trained athletes oxidize more fat during low-intensity exercise than do untrained subjects exercising at the same absolute intensity. Additionally, plasma FFA concentration during exercise was significantly lower in trained than in untrained subjects. These results agree with data from earlier studies demonstrating that training increases the contribution of fat as a fuel during exercise (12, 13, 21). The increase in total fat oxidation in our trained subjects occurred despite having the same rate of whole body lipolysis (glycerol $R_d$), FFA released into plasma (FFA $R_d$), and FFA taken up from plasma (FFA $R_u$) as the untrained group. Therefore, during exercise trained muscles oxidize a greater percentage of fatty acids delivered from plasma or they rely more on fatty acids derived from intramuscular triglycerides than do untrained muscles. Our data cannot definitively distinguish between these two possibilities. However, the relationship between FFA $R_u$ and FFA oxidation suggests that the trained subjects oxidized more intramuscular triglycerides than the untrained subjects. Plasma FFA availability could account for total FFA oxidation during exercise in all untrained subjects because FFA $R_d$ was always greater than FFA oxidation. In contrast, FFA $R_d$ was lower than FFA oxidation in three of the five trained subjects, and mean FFA $R_d$ was closer to mean FFA oxidation rates in trained than in untrained subjects. Furthermore, it is likely that a smaller percentage of total FFA uptake (FFA $R_d$) was directed toward skeletal muscle in trained than untrained subjects because of differences in splanchnic blood flow between groups. Splanchnic blood flow, and presumably splanchnic FFA uptake, should have decreased more during exercise in untrained subjects because they exercised at a higher intensity (~40% $V_{O2\max}$) than the trained subjects (~25% $V_{O2\max}$) (28). Therefore, a greater proportion of plasma FFA uptake was available for muscle oxidation in untrained than in trained subjects.

Several factors may have facilitated fat oxidation during exercise in the endurance-trained subjects. Training in humans may increase triglyceride content in skeletal muscle (25). An increase in mitochondrial density in exercising muscle of our trained subjects, probably double that of the untrained subjects, would provide them with a greater ability to oxidize fat (12, 14). Furthermore, the greater number of mitochondria in trained muscle decreases the rate of respiration per mitochondrion needed to maintain a given rate of $V_{O2}$ during exercise (i.e., 20 ml·kg$^{-1}$·min$^{-1}$), thereby decreasing carbohydrate oxidation in favor of fatty acids (11, 12, 24).

Our data appear to be inconsistent with the results reported by Martin et al. (21). In that study endurance training blunted the increase in FFA $R_u$ during exercise. However, there are several significant differences between studies that may be responsible for the contrasting results. First, the absolute and relative intensities of exercise in our subjects, particularly in the athletes,

**Table 2. Plasma FFA and glycerol kinetics in untrained and trained subjects**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Untrained</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol $R_a$ Basal</td>
<td>2.13 ± 0.33</td>
<td>3.20 ± 0.67*</td>
</tr>
<tr>
<td>Exercise†</td>
<td>11.29 ± 0.99</td>
<td>9.85 ± 1.02</td>
</tr>
<tr>
<td>Recovery‡</td>
<td>8.18 ± 0.77</td>
<td>5.04 ± 0.52*</td>
</tr>
<tr>
<td>FFA $R_d$ Basal</td>
<td>5.30 ± 0.84</td>
<td>8.55 ± 1.07*</td>
</tr>
<tr>
<td>Exercise†</td>
<td>24.13 ± 0.39</td>
<td>24.64 ± 3.76</td>
</tr>
<tr>
<td>Recovery‡</td>
<td>19.22 ± 1.04</td>
<td>15.19 ± 2.60*</td>
</tr>
</tbody>
</table>

Values are means ± SE in μmol·kg$^{-1}$·min$^{-1}$; n = 5 subjects/group. FFA, free fatty acid; $R_a$, rate of appearance. *Significantly different from corresponding value in untrained subjects, $P < 0.05$. †Values represent average values from 30 to 240 min of treadmill exercise. ‡Values represent average values during 1 h of recovery from exercise.
β-Adrenergic stimulation of adipose tissue has been proposed to be the most important mechanism for stimulating lipolysis during exercise (2, 7). Endurance exercise training (4, 5) has been shown to increase the lipolytic response to catecholamines in adipocytes isolated from abdominal adipose tissue. Our results support the idea that adipose tissue sensitivity to β-adrenergic stimulation in vivo was enhanced in the trained subjects. In the present study, lipolytic rates, expressed per kilogram body weight, during exercise were similar in both trained and untrained subjects despite presumably lower circulating catecholamines in the trained group (19, 32). Lipolytic rates expressed per kilogram body fat mass may provide a better index of adipose tissue sensitivity to a stimulus. This can be calculated by estimating body composition in our subjects based on measurements we have made previously in a similar group of trained and untrained subjects (27). In that study the percent body weight as fat in our untrained subjects (11% body weight as fat) was 50% greater than in our trained subjects (17% body weight as fat). Correcting for differences in body composition increases the differences between study groups; lipolytic rates per kilogram fat mass during exercise was 35% greater in trained than in untrained subjects (P < 0.05).

The distance runners in our study had higher resting, preexercise lipolytic rates and lipid oxidation rates than did the untrained subjects. These results are similar to those we have previously observed in highly trained cyclists (27). The increase in basal lipid oxidation is probably related to the increase in whole body lipolytic rates. The mechanism responsible for the increase in lipolysis is not known but may be related to differences in carbohydrate homeostasis between groups. Although all subjects who participated in this study ingested >300 g of carbohydrate daily, the trained group undoubtedly had hepatic and muscle glycogen reduction because of their rigorous training regimen. Inadequate carbohydrate ingestion alone can increase lipolysis and fat oxidation even when calorie intake is sufficient to meet resting energy requirements (18). It is also possible that alterations in hormonal regulation of lipid metabolism contributed to the increase in basal lipolytic rates. Training may increase basal plasma catecholamine levels (16) and lipolytic sensitivity to β-adrenergic stimulation (4, 5) but decrease basal plasma insulin concentration (23).

Recovery from exercise with respect to lipid kinetics occurred more rapidly in our trained than in our untrained subjects. After subjects completed the exercise bout, the $R_0$ values of glycerol and palmitate in plasma returned to baseline levels within 30 min in the endurance-trained subjects but remained two- to threefold higher than baseline in the untrained subjects even after 60 min of recovery. These results suggest that although the absolute intensity of exercise was the same in both trained and untrained subjects, the exercise bout was less stressful in the trained subjects presumably because of their daily training program and the lower relative intensity of the exercise. Weber et al. (31) also found a rapid return of glycerol $R_0$ to preexercise values.
after 2 h of treadmill exercise in trained African pygmy goats, suggesting that training can affect postexercise lipid metabolism in other species.

Steele's equation during non-steady-state conditions (29) was used to calculate \( R_A \) and \( R_D \) during exercise and recovery. This equation assumes a uniformly, well-mixed, single-pool model that may not accurately describe the true physiological parameters of fatty acid or glycerol kinetics. Therefore, the value chosen for the effective volume of distribution (\( V_d \)) can have dramatic effects on the calculated value for \( R_A \) when there are large changes in isotopic enrichment (1). We altered the infusion rate of tracer during exercise and recovery to minimize changes in isotopic enrichment (tracer-to-tracer ratio). This manipulation reduces the impact of \( V_d \) on \( R_A \) so that when there is no change in isotopic enrichment (e.g., steady-state conditions), \( V_d \) does not affect \( R_A \). In the present study, doubling the \( V_d \) for palmitate (from 50 to 100 ml/kg) or decreasing by one-half the \( V_d \) for glycerol (from 270 to 135 ml/kg) would have caused less than a 5% change in palmitate \( R_A \) or glycerol \( R_A \) values during exercise or recovery. Therefore, our data are not influenced by potential errors in estimated \( V_d \).

In summary, the data from the present study demonstrate that the increased rate of fat oxidation during low-intensity exercise (i.e., 20 ml·kg⁻¹·min⁻¹) in trained subjects, the hallmark of endurance training, is not accompanied by differences in the rates of whole body lipolysis (i.e., glycerol \( R_A \)), the appearance of FFA in plasma, or FFA uptake from plasma. The relationship between FFA \( R_A \) and FFA oxidation during exercise suggests that trained subjects oxidize more fatty acids from a nonplasma source, presumably intramuscular triglycerides. Additionally, lipid kinetics return to baseline more rapidly in trained than in untrained subjects after they complete an exercise bout of the same absolute intensity.

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REFERENCES


