Regulation of fatty acid oxidation in untrained vs. trained men during exercise

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Sidossis, Labros S., Robert R. Wolfe, and Andrew R. Coggan. Regulation of fatty acid oxidation in untrained vs. trained men during exercise. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E510–E515, 1998.—We have recently shown that increased carbohydrate flux decreases fat oxidation during exercise by inhibition of fatty acid entry into the mitochondria. Because endurance training reduces the rate of carbohydrate flux during exercise, we hypothesized that training increases fat oxidation by relieving this inhibition. To test this hypothesis, five sedentary and five endurance-trained men exercised on a cycle ergometer at an oxygen consumption (V˙O2) of ~2 l/min, representing 80 and 40% peak V˙O2, respectively. [1-13C]oleate and [1-14C]octanoate, long- and medium-chain fatty acids, respectively, were infused for the duration of the studies. Carbohydrate oxidation was significantly higher in the sedentary group (196 ± 9 vs. 102 ± 17 µmol·kg⁻¹·min⁻¹, P < 0.05). Oleate oxidation was higher in the trained group (3.8 ± 0.6 vs. 1.9 ± 0.3 µmol·kg⁻¹·min⁻¹, P < 0.05), whereas octanoate oxidation was not different between the two groups. The percentage of oleate that was taken up by tissues and oxidized was higher in the trained group (76 ± 7 vs. 58 ± 3%, P < 0.05). However, the percentage of octanoate taken up and oxidized was not different (62 ± 3 vs. 85 ± 4%, not significant). Because octanoate, unlike oleate, can freely diffuse across the mitochondrial membrane, the present results suggest that the difference in fatty acid oxidation between trained and untrained individuals may be due to enhanced fatty acid entry into the mitochondria.

METHODS

Volunteers. Five sedentary male volunteers and five endurance-trained cyclists participated in this study. Volunteer characteristics are presented in Table 1. All volunteers were healthy, as indicated by comprehensive history, physical examination, and standard blood and urine tests, and they consented to participate in this study, which was approved by the Institutional Review Board and the General Clinical Research Center of the University of Texas Medical Branch at Galveston.

Experimental design. All experiments were performed in the morning after the volunteers had fasted overnight (i.e., 12 h). Trained volunteers were instructed to limit the duration (to ~45 min) as well as the intensity of their final training bout, which was performed 24 h before the study. Teflon catheters were placed percutaneously into an antecubital vein for isotope infusion and into a contralateral dorsal hand vein, which was heated, for sampling of arterialized venous blood. After catheter placement, each volunteer sat quietly on a cycle ergometer for 15–30 min. Blood and breath samples were then obtained for the determination of background enrichments, after which the sedentary volunteer exercised for 30 min and the trained volunteer for 60 min at a V˙O2 of ~2.0 l/min. This represented ~80 and 40% of peak V˙O2 (V˙O2peak) for the sedentary and trained volunteers, respectively. At the beginning of exercise, infusions of [1-14C]octano-
FATTY ACID OXIDATION IN TRAINED AND UNTRAINED VOLUNTEERS

Table 1. Volunteer characteristics

<table>
<thead>
<tr>
<th></th>
<th>Sedentary</th>
<th>Trained</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>30 ± 2</td>
<td>24 ± 1*</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>76.8 ± 2.3</td>
<td>79.9 ± 3.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174 ± 4</td>
<td>170 ± 3</td>
</tr>
<tr>
<td>VO₂peak, l/min</td>
<td>25 ± 0.1</td>
<td>5.1 ± 0.2*</td>
</tr>
<tr>
<td>VO₂ during exercise, l/min</td>
<td>2.0 ± 0.1</td>
<td>1.9 ± 0.1</td>
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</table>

Values are means ± SE for 5 volunteers. *P < 0.05 vs. sedentary.

Values are means ± SE for 5 volunteers. *P < 0.05 vs. sedentary.

Materials. [1-13C]oleate, 99% enriched, was obtained from MSD Isotopes (Montreal, Canada), [1-13C]octanoate from Du Pont (Boston, MA), human albumin (5%) from Baxter Healthcare (Glendale, CA), the lipid emulsion [Intralipid, 20%, containing linoleic (50%), oleic (26%), palmitic (10%), linolenic (9%), and stearic (3.5%) acids] from Kabi (Clayton, NC), and heparin from Elkins Sinn, Cherry Hill, NJ.

Assays. Expired air was collected in 3-liter anesthesia bags, and 14CO₂ carbon SA was determined using a liquid scintillation counter, as previously described (21). For determination of breath CO₂ carbon enrichment, 10 ml of expired air were injected into evacuated tubes, and the 13CO₂-to-12CO₂ ratio (tracer-to-trace ratio; enrichment) was determined using isotope ratio mass spectrometry (SIRA VG Isotech, Cheshire, UK) as previously described (21).

Blood samples (6 ml) were collected into prechilled tubes containing 120 µl of 0.2 M ethylene glycol-bis-beta-aminoethyl ether-N,N',N'-tetraacetic acid to inhibit in vitro lipolysis as well as clotting, and plasma was immediately separated by centrifugation and frozen until further processing. Plasma oleate carbon enrichment was determined by following previously described procedures (26). Briefly, FFA were extracted from plasma, isolated by thin-layer chromatography, and converted to their methyl esters. The isotopic enrichment of oleate carbon was determined by gas chromatography-mass spectrometry (GC-MS; Hewlett-Packard 5890 Series II, Palo Alto, CA) by selectively monitoring the mass-to-charge ratio of ions 296 and 297 (26).

Plasma glucose and lactate concentrations were determined on a 2300 STAT analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma oleate and total FFA concentrations were determined by GC (Hewlett-Packard 5890) with heptadecanoic acid as internal standard.

Calculations. Plasma oleate and breath CO₂ carbon enrichment (13C-to-12C ratio) and SA (13C-to-12C ratio) reached plateau over at least the last 15 min of exercise (Figs. 1 and 2, respectively). The average enrichment values during the last 10 min of exercise were used for the calculation of substrate kinetics and oxidation. Substrate oxidation calculated at 20–30 min was not different from that calculated at 50–60 min of exercise at ~40% VO₂peak in the trained group. Therefore, we compared substrate oxidation values calculated for the last 10 min of exercise in the sedentary and trained groups.

Carbohydrate and total fatty acid oxidation rates were calculated from indirect calorimetry by use of stoichiometric equations (5). VO₂ and VCO₂ values were the average over the last 10 min of each study. Fatty acid oxidation was determined by converting the rate of triacylglycerol (TG) oxidation (g·kg⁻¹·min⁻¹) to its molar equivalent, with the assumption of the average molecular weight of TG to be 860 g/mol (5), and multiplying the molar rate of TG oxidation by three because each molecule contains three moles of fatty acids.

The rate of appearance (Rₐ) of oleate in plasma, which under steady-state conditions is equal to the rate of disappear-
Plasma fatty acid oxidation was determined by dividing the rate of oxidation of oleate by the fractional contribution of oleate to the total FFA concentration, as determined by GC. The rate of oxidation of fatty acids oxidized directly without first passing through the plasma pool (so-called “intramuscular” or “nonplasma” fatty acids) was calculated as

\[ \text{Nonplasma fatty acid oxidation} = \frac{\text{total fatty acid oxidation (indirect calorimetry)}}{\text{plasma fatty acid oxidation (tracer methodology)}} \]

The percentage of oleate and octanoate tracer that was taken up by tissues (i.e., cleared from the circulation) and oxidized was calculated as

\[ \text{Percent uptake oxidized} = \left( \frac{\text{substrate oxidation}}{R_\text{a}} \right) \times 100 \]

Statistical analysis. Differences between the two groups were identified using an unpaired t-test. Statistical significance was set at \( P < 0.05 \).

RESULTS

Plasma substrate concentrations are presented in Table 2 as the average of the last 10 min of exercise. By infusing lipids and heparin in the sedentary volunteers, we were able to maintain similar plasma oleate and total FFA concentrations in the two groups. Plasma glucose concentration was also similar in the two groups; however, plasma lactate concentration was significantly higher in the sedentary volunteers (\( P < 0.05 \)). Similarly, carbohydrate oxidation was significantly higher in the sedentary volunteers (196 ± 9 vs. 102 ± 17 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \), \( P < 0.05 \)).

The \( R_\text{a} \) of oleate in plasma was similar in the two groups, as was the \( R_\text{a} \) of total FFA (Table 3). Oleate oxidation (Fig. 3) and plasma fatty acid oxidation (tracer methodology; Table 3) were significantly higher in the trained group, whereas octanoate oxidation rate was not different in the two groups (1.2 ± 0.4 vs. 1.3 ± 0.1 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) in the sedentary and trained volunteers, respectively). Total fatty acid oxidation, calculated using indirect calorimetry and representing the sum of plasma derived and nonplasma fatty acids (intramuscular, fatty acids that are oxidized without first entering the plasma), was 16.7 ± 4.4 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) in the trained and 0 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) in the untrained. Given that there was significant plasma fatty acid oxidation even in the untrained volunteers, as measured using the labeled oleate (Fig. 3), indirect calorimetry significantly underestimated fat oxidation in this group of untrained volunteers.

Table 2. Plasma substrate concentrations during exercise

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Sedentary (( \mu \text{mol} \cdot \text{l}^{-1} ))</th>
<th>Trained (( \mu \text{mol} \cdot \text{l}^{-1} ))</th>
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</thead>
<tbody>
<tr>
<td>Oleate</td>
<td>0.13 ± 0.02</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td>FFA</td>
<td>0.35 ± 0.04</td>
<td>0.24 ± 0.07</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.1 ± 0.4</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>Lactate</td>
<td>6.2 ± 0.4</td>
<td>1.3 ± 0.1*</td>
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</table>

Values are means ± SE for 5 volunteers expressed in mmol/l. FFA, free fatty acid. * \( P < 0.05 \) vs. sedentary.
The percent [1-13C]oleate uptake that was oxidized was higher in the trained (P, 0.05; Fig. 4) group, whereas the percent [1-14C]octanoate uptake that was oxidized was similar in the two groups (Fig. 4). The ratio of percent [1-13C]oleate to percent [1-14C]octanoate oxidized was also higher in the trained group (Fig. 5), suggesting that fatty acid oxidation is higher in the trained partly because of accelerated entry of fatty acids into the mitochondria. Interestingly, unlike the sedentary group, in the trained group the ratio of percent [1-13C]oleate to percent [1-14C]octanoate oxidized was not significantly different from one, suggesting that mitochondrial fatty acid uptake is not the rate-limiting step in endurance-trained cyclists exercising at a V̇O₂ of ~2 l/min.

DISCUSSION

Using isotopically labeled LCFA and MCFA, we have provided evidence that mitochondrial fatty acid uptake is rate limiting for fatty acid oxidation during high-intensity exercise in sedentary individuals. In contrast, during exercise of the same absolute intensity in trained subjects, fatty acid entry into the mitochondria was enhanced and was apparently no longer limiting for fatty acid oxidation. These findings suggest that, during exercise of the same intensity, fat oxidation is higher in endurance-trained vs. sedentary individuals in part because of accelerated fatty acid entry into the mitochondria.

The comparison of oleate to octanoate oxidation requires the assumption that the main difference between the fate of the two tracers is the need for oleate to utilize CPT for transport across the mitochondrial membrane. McGarry and Foster (15) tested this assumption by comparing relative rates of oxidation of octanoate, oleate, and (-)-octanoylcarnitine in perfused rat livers. (-)-Octanoylcarnitine, in contrast to octanoate, is transported across the mitochondrial membrane utilizing CPT but enters the same β-oxidation pathway as octanoate once inside the mitochondria. It was found that the relative rate of (-)-octanoylcarnitine oxidation followed that observed for oleate rather than octanoate in livers from fed and fasted rats, suggesting that the main difference in the oxidation of oleate and octanoate is the need for oleate to utilize CPT 1 for transport across the mitochondrial membrane. However, there is still the possibility that LCFA and MCFA may also

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Table 3. Plasma and total fatty acid kinetics and oxidation

<table>
<thead>
<tr>
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<th>Sedentary</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleate Ra (µmol·kg⁻¹·min⁻¹)</td>
<td>4.0 ± 1.0</td>
<td>5.1 ± 0.9</td>
</tr>
<tr>
<td>FFA Ra (µmol·kg⁻¹·min⁻¹)</td>
<td>11.1 ± 2.8</td>
<td>11.6 ± 2.3</td>
</tr>
<tr>
<td>Plasma fatty acid oxidation (µmol·kg⁻¹·min⁻¹)</td>
<td>5.3 ± 1.0</td>
<td>8.4 ± 1.4*</td>
</tr>
<tr>
<td>Nonplasma fatty acid oxidation (µmol·kg⁻¹·min⁻¹)</td>
<td>NA</td>
<td>8.3 ± 2.4</td>
</tr>
<tr>
<td>Total fatty acid oxidation (µmol·kg⁻¹·min⁻¹)</td>
<td>0</td>
<td>16.7 ± 4.4*</td>
</tr>
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</table>

Values are means ± SE for 5 volunteers expressed in µmol·kg⁻¹·min⁻¹. Rₐ, rate of appearance. NA, not applicable. Nonplasma fatty acid oxidation is from "intramuscular" triacylglycerols. The fact that total fatty acid oxidation (indirect calorimetry) was lower than plasma fatty acid oxidation (tracer methodology) in the sedentary group suggests that indirect calorimetry underestimated fatty acid oxidation during exercise in this group. The difference between total and plasma fatty acid oxidation in the trained group (nonplasma or intramuscular fatty acids) represents fatty acids that are oxidized directly without first passing through the plasma pool. *P < 0.05 vs. sedentary.

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Fig. 3. Plasma oleate oxidation during cycle ergometer exercise at a V̇O₂ of ~2.0 l/min in sedentary and endurance-trained volunteers. Lipids and heparin were infused in the sedentary group to maintain plasma free fatty acid concentration constant in the 2 groups. Values are means ± SE for 5 volunteers. *P < 0.05 vs. sedentary.

Fig. 4. Percentage of oleate and octanoate tracer taken up by tissues and oxidized during cycle ergometer exercise at a V̇O₂ of ~2.0 l/min in sedentary and endurance-trained volunteers. Values are means ± SE for 5 volunteers. *P < 0.05 vs. sedentary.

Fig. 5. Ratio of percent oleate uptake oxidized to percent octanoate taken up and oxidized during cycle ergometer exercise at a V̇O₂ of ~2.0 l/min in sedentary and endurance-trained volunteers. A ratio significantly different from unity suggests that mitochondrial fatty acid uptake is rate limiting for fatty acid oxidation. Values are means ± SE for 5 volunteers. *P < 0.05 vs. unity.
differ in their mode of transport across the sarcolemmal membrane. Unlike MCFA, which are thought to be able to diffuse across the membrane, LCFA may require a transport system to gain access into cells (1, 10). Therefore, the increase in the capacity of the trained volunteers to oxidize LCFA could also be interpreted as reflecting a training-induced increase of the capacity of muscle for uptake of fatty acids. Whereas this is certainly a possibility that cannot be excluded from our data, it could not explain the increased oxidation of fatty acids derived from triacylglycerols stored inside the cell (intramuscular triacylglycerols) that was observed in the trained volunteers (Table 3).

The relative concentration of various fatty acids in plasma may affect their kinetics and oxidation. To prevent the effect of different concentrations of oleate on oleate uptake and oxidation, we infused lipids and heparin during exercise in the sedentary group to increase oleate availability to the same level as in the trained group. This was necessary because peripheral lipolytic rate decreases significantly during high-intensity exercise (20). By matching oleate availability in the sedentary and trained groups, we ensured a valid comparison for oleate oxidation between them. On the other hand, oleate and octanoate concentrations were significantly different, which raises the possibility that the observed differences in their kinetics and oxidation may have been due to differences in their relative concentrations. We have previously tested this possibility (23). We increased the R₃ of octanoate in plasma during exercise at 40 and 80% VO₂peak by exogenous infusion of unlabeled octanoate to match the R₃ of oleate. In these studies we did not observe any significant changes in the percent octanoate tracer oxidized between the “low” and “high” octanoate availability experiments, suggesting that the observed differences between octanoate and oleate tracer oxidation are not due to differences in their relative concentrations (23).

The fact that the sedentary volunteers oxidized a greater percentage of octanoate than oleate during high-intensity exercise supports the concept that flux through CPT I may be rate limiting for fat oxidation during exercise when the rate of carbohydrate utilization is very high (4, 23). However, this did not appear to be true in trained volunteers exercising at the same absolute intensity. In these subjects, the percentage of oleate uptake that was oxidized was similar to that of octanoate, such that the ratio of their relative rates of oxidation did not differ significantly from unity. Thus some factor other than mitochondrial fatty acid uptake was apparently rate limiting for fat oxidation under these conditions in the trained volunteers. Possibly, during the early stages of low-intensity exercise, fat oxidation is limited by the rate of lipolysis and thus fatty acid availability.

The exact mechanism explaining the higher fatty acid oxidation in the trained volunteers cannot be determined from the present study. However, several possibilities can be suggested. It is well established that activated LCFA must bind to carnitine, a reaction catalyzed by the enzyme CPT I, to gain access into the mitochondrial matrix (6). The product of this reaction, fatty acyl-carnitine, is transported across the inner mitochondrial membrane via the carnitine-acylcarnitine translocase mechanism (17). Studies in vitro (16) and in vivo (23, 24) have suggested that accelerated carbohydrate flux inhibits CPT I, possibly via malonyl-CoA (16), and, as a consequence, fatty acid uptake into the mitochondria and fatty acid oxidation decrease. Our data suggest that the increase in fat oxidation after training is at least in part due to accelerated entry of fatty acids into the mitochondria. This is perhaps mediated via increased CPT I activity as a consequence of decreased carbohydrate flux. Alternatively, it is well established that endurance training increases the number of mitochondria in trained muscles (see Ref. 8). Thus training may enhance fatty acid entry into the mitochondria by increasing the number of mitochondria without any change in CPT I activity per mitochondrion. Furthermore, chronic increased fatty acid availability to the mitochondria, as is probably the case in endurance training, may also increase CPT I [rat hepatocytes (14)] and β-hydroxyl-CoA-dehydrogenase [human vastus lateralis; (7)] activities, explaining the ability of the trained volunteers to oxidize more fatty acids.

In the present study we found that 58 ± 3% and 76 ± 7% (P < 0.05) of oleate tracer were oxidized in the sedentary and trained volunteers, respectively. Martin et al. (13) did not see any difference in the percentage of LCFA tracer oxidized before and after 12 wk of endurance training. Similarly, Kanaley et al. (12) did not observe any difference between marathon runners and moderately trained runners in the percentage of fatty acid uptake oxidized. In the above mentioned studies (12, 13), the differences in oxidative capacity between the two groups [i.e., before and after training (13) and marathoners vs. moderately trained runners (12)] may not have been as large as in the present study. It is possible that a difference in the percentage fatty acid uptake oxidized between trained and untrained persons may only become obvious, or measurable, when the difference in oxidative capacity is very large, as was probably the case in our two groups of volunteers.

There was a clear discrepancy between fatty acid oxidation calculated using indirect calorimetry and tracer methodology during exercise in the untrained group (Table 3). Indirect calorimetry suggested that there was virtually no fatty acid oxidation during exercise, whereas tracer estimates indicated that fatty acid oxidation contributed significantly to energy production. High-intensity exercise is often associated with a progressive increase in plasma lactate concentration, progressive hyperventilation, and a decrease in the bicarbonate pool as reflected in plasma bicarbonate concentrations (25). Under such conditions indirect calorimetry may not be valid in estimating substrate oxidation, because changes in the bicarbonate pool preclude reliable measurements of tissue VCO₂ by breath CO₂ analyses. On the other hand, if lactate concentration increases in the initial phase of the exercise but remains stable subsequently, as is the case with highly
trained cyclists exercising at ~80% maximal aerobic capacity (19), indirect calorimetry may still be valid in estimating whole body fatty acid oxidation (19).

Because muscle glycogen concentrations were not measured in this study, it is possible that the two groups differ in their preexercise glycogen stores (lower in trained), which could potentially affect the interpretation of the findings of the present study. However, it seems unlikely that potential differences in preexercise glycogen stores have significantly influenced the pattern of glycogen use in these two groups, because on the basis of the literature, the trained volunteers exercising at 40% $V_{\text{O}_2}\text{peak}$ used significantly less muscle glycogen than the sedentary volunteers exercising at 80% $V_{\text{O}_2}\text{peak}$. However, it is still possible that the observed differences in fatty acid oxidation between the two groups reflect genetic variation in physiological characteristics of the subjects, such as fiber type, percent body fat, or hormonal responses. As a cross-sectional design, the present study shares all the limitations inherent in this type of research design.

In summary, the results of the present study suggest that endurance training may increase fat oxidation during exercise in part by enhancing fatty acid entry into the mitochondria. This may be due to decreased carbohydrate flux and thus increased CPT I activity per mitochondrion, or simply to increased mitochondrial number and thus increased total CPT I. Furthermore, our data suggest that in untrained human volunteers exercising at a $V_{\text{O}_2}$ of 2 l/min, mitochondrial fatty acid uptake is one factor limiting the rate of fat oxidation. However, when trained volunteers exercise at the same absolute intensity, fatty acid uptake by the mitochondria does not appear to be limiting fat oxidation.

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