Substrate metabolism during different exercise intensities in endurance-trained women

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There is a common perception that women may be relatively more suited to endurance exercise than men because of a greater ability to use fatty acids as energy substrates during exercise. This notion is supported by several studies that have found some index of fat metabolism to be greater in women than in men at comparable exercise intensity (e.g., Refs. 7, 22). On the other hand, several other studies have failed to show a gender difference in substrate metabolism (3, 23). It has been suggested that the difference in substrate metabolism between men and women during exercise becomes less as training status increases (19), but all

data are not consistent with this explanation. For example, endurance-trained women were found to derive more energy from lipids than endurance-trained men did when exercising at 65% of maximal oxygen consumption (VO2max) (21). If women are in fact better able to rely on fat during exercise than men, it may be because women generally have a higher percentage of body fat than men. This could theoretically lead to greater availability of fatty acids during exercise and, in turn, to more fat oxidation at a given exercise intensity than in a man with less body fat. In this regard, changes in fatty acid availability have been shown to have an effect on fatty acid oxidation during high-intensity exercise (16). On the other hand, it may be erroneous to assume that women have an increased rate of lipolysis because of their greater amount of body fat. Our laboratory has previously shown that changes in the percent body fat result in altered lipolytic sensitivity that makes individuals with low body fat better able to mobilize fatty acids than might be anticipated otherwise. Thus lipolytic responsiveness is enhanced in subjects with low body fat (11) and decreased in obese subjects (29). Consistent with these observations, our laboratory found that highly trained athletes had higher rates of lipolysis at rest (18) than untrained controls did. However, it is not clear whether this reflects a response to training, a change in body composition, or some other factor. In trained men, it is difficult to distinguish a training effect on fat metabolism from a body-composition effect because body fat is low in all endurance-trained men. This is not necessarily the case in endurance-trained women, who may have a relatively large range in body composition. The purpose of the present study was therefore twofold: 1) to compare with the previously reported data from endurance-trained men (15) the response of glucose and lipid kinetics in endurance-trained women to three intensities of exercise and 2) to evaluate the role of body composition on the response of substrate kinetics to exercise in the trained women. To accomplish these goals, we have quantified glucose and fatty acid kinetics by using stable isotopically labeled tracers at three intensities of exercise in eight endurance-trained women with a range in body composition from 7.7 to 26.9% body fat.
METHODS

Subjects

We studied eight well-trained female cyclists with percent body fat ranging from 7.7 to 26.9%. Their physical characteristics are shown in Table 1. All subjects were healthy, as indicated by medical history and physical examination. They were consuming a weight-maintaining diet containing at least 300–400 g of carbohydrates daily. They were in energy balance, documented by their stable weight in the 2 mo preceding the study. They were studied in the postabsorptive state after a 10- to 12-h fast and did not exercise the day before the study. The subjects were eumenorrheic and studied through a variety of phases of the menstrual cycle. The men we used for comparison were described previously (15).

Body composition was determined by densitometry by using the hydrostatic weighing technique with correction for residual lung volume (25). Relative body fat was estimated from total body density (26). Assessment of body composition was performed within 1 wk of the isotope-infusion protocol.

V˙O2max had been determined several weeks before the present protocol while the subjects cycled on a stationary ergometer (model 819, Monark). V˙O2max was determined during an incremental cycling protocol lasting 7–10 min. The study was approved by the institutional review boards of the University of Texas, Galveston and Austin.

Exercise Protocol

The subjects were studied on 2 consecutive days in the postabsorptive state. On one occasion, the protocol consisted of at least 120 min of bed rest, 60 min of exercise at 25% of V˙O2max on a stationary ergometer (model 819, Monark), followed by 60 min of bed rest, and, finally, 30 min of exercise at 85% of V˙O2max. We have previously shown that the effects of very-low-intensity exercise (120 min at 25% of V˙O2max) on glucose and fat metabolism subside within 1 h of bed rest (15). On the other day, the protocol consisted of at least 120 min of bed rest followed by 60 min of exercise at 65% of V˙O2max. The order of the two protocols was determined by random allocation.

Indirect Calorimetry

Indirect calorimetry was performed at rest for at least 15 min continuously during the first 30 min of exercise (all 3 levels of exercise) and for 5–10 min at 15-min intervals throughout the remainder of the exercise periods at 25 and 65% of V˙O2max. The values obtained from 20 to 30 min of exercise were used to calculate and compare substrate oxidation rates among the three levels of exercise. Our laboratory (14) has previously shown that indirect calorimetry is a valid method to measure substrate oxidation rates in trained subjects during cycling exercise at 80–85% of V˙O2max, because of the relative physiological state (i.e., constant lactate concentration, heart rate, and so forth) over this time interval. The resting values were obtained after the subjects had been lying on a bed for at least 1 h. Inspired volumes of air were measured with a dry-gas meter (model CD-4, Parkinson-Cowan). Aliquots of expired gas were sampled from a mixing chamber for O2 (model S3A, Applied Electrochemistry) and CO2 (model LB-2, Beckman). Analog outputs from the gas analyses and gas meter were directed to a laboratory computer for calculation of oxygen uptake and carbon dioxide production.

Isotope Infusion

Teflon catheters were placed percutaneously in an antecubital vein, and a sampling catheter was inserted in a dorsal hand vein of the contralateral side. The heated hand technique was used to obtain arterialized blood samples. The subjects lay on a bed for 1 h after catheter placement. Then, after a blood sample was drawn to determine background enrichment, primed, constant infusions of [6,6-2H2]glucose (99% enriched, Merck, Rahway, NJ); 0.22 μmol·kg⁻¹·min⁻¹; priming dose 17.6 μmol/kg) and [13C]palmitate (0.04 μmol·kg⁻¹·min⁻¹; no priming dose) were started by using calibrated syringe pumps (Harvard Apparatus, Natick, MA). The exact infusion rates in each experiment were determined by measuring the concentrations in the infusates. The palmitate tracer (99% enriched) was purchased from Tracer Technologies (Newton, MA). Palmitate was bound to albumin (Cutter Biological, Berkeley, CA) by following previously described procedures (27). After 2 h of infusion at rest, exercise was started and the rate of isotope administration was doubled for palmitate (25 and 65% of V˙O2max) and glucose (65% of V˙O2max) and tripled for glucose (85% of V˙O2max) to minimize changes in substrate isotopic enrichment. These changes in isotope infusion rates were determined from results of a previous study on metabolic effects of similar exercise intensities in men (15). During the interval between the experiments with exercise at 25 and 85%, the isotope infusions were continued at the basal rates.

Blood Sampling

The first blood samples were drawn before the isotope infusion was started to determine background enrichment. Blood was also taken 110, 115, and 120 min after the beginning of infusion to measure resting kinetics. During high-intensity exercise, blood was drawn after 5, 10, 15, 20, 25, and 30 min of exercise. In moderate- and low-intensity exercise, samples were drawn every 10 min for 60 min. All samples were collected in 10-ml vacuum containers containing lithium heparin and were placed on ice. Plasma was separated by centrifugation within 5 min of sampling and subsequently frozen until further processing.

Sample Analysis

Plasma glucose concentration was measured on a glucose analyzer (Beckman Instruments) by use of the glucose oxidase method. The enrichment of [6,6-2H2]glucose in plasma was determined as previously described (27). Briefly, plasma was deproteinized with barium hydroxide and zinc sulfate solutions. Glucose was extracted by mixed-bed anion-cation exchange chromatography (AG-1-X8 and AG 50W-X8, Bio-Rad Laboratories, Richmond, CA) and reacted with acetic anhydride and pyridine to form the pentaacetate derivative. Isotopic enrichment was determined by using gas chromato-

Table 1. Physical characteristics of study subjects

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Weight, kg</th>
<th>Height, m</th>
<th>Weight, kg</th>
<th>Fat, % body weight</th>
<th>V˙O2max, l/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>48</td>
<td>1.65</td>
<td>44</td>
<td>7.7</td>
<td>7.7</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>49</td>
<td>1.63</td>
<td>42</td>
<td>13.4</td>
<td>7.5</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>55</td>
<td>1.60</td>
<td>47</td>
<td>14.8</td>
<td>3.0</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>63</td>
<td>1.66</td>
<td>52</td>
<td>17.8</td>
<td>3.6</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>64</td>
<td>1.65</td>
<td>53</td>
<td>18.4</td>
<td>3.8</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>69</td>
<td>1.78</td>
<td>55</td>
<td>20.5</td>
<td>3.9</td>
</tr>
<tr>
<td>7</td>
<td>29</td>
<td>65</td>
<td>1.65</td>
<td>51</td>
<td>21.6</td>
<td>4.1</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>72</td>
<td>1.77</td>
<td>53</td>
<td>26.9</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Mean ± SE 27 ± 1 61 ± 3 1.67 ± 0.02 50 ± 2 17.6 ± 2 3.49 ± 0.2

V˙O2max, maximal oxygen uptake.
graph-mass spectrometry (model 5985B, Hewlett-Packard, Fullerton, CA) with electronic-impact ionization, selectively monitoring ions at mass-to-charge ratio (m/z) 202, 201, and 200. Correction was made for the contribution of singly labeled molecules (m/z 201) to the apparent enrichment at m/z 202 (27).

FFA were extracted from plasma, isolated by thin-layer chromatography, and derivatized to their methyl esters. Palmitate and total free fatty acid (FFA) concentrations were determined by gas chromatography (model 5890, Hewlett-Packard) by using heptadecanoic acid as an internal standard (27). Isotopic enrichment of palmitate was measured by gas chromatograph-mass spectrometry analysis of the methyl ester derivatives (model 5992, Hewlett-Packard). Ions of m/z 270 and 272 were selectively monitored.

Calculations

Indirect calorimetry. Carbohydrate and fat oxidation rates were calculated using stoichiometric equations (4). Nitrogen excretion rate was assumed to be 135 µg·kg⁻¹·min⁻¹. This average value was taken from the measured values determined in another study performed in our laboratory (1). A 30% error in this assumed value (which exceeds the total range of values in the previous study) would have no significant effect on the calculated values of fat and carbohydrate oxidation in exercise in the present study. Fatty acid oxidation was determined by converting the rate of triglyceride oxidation (g·kg⁻¹·min⁻¹) to its molar equivalent, assuming the average molecular mass of triglyceride is 860 g/mol (4) and multiplying the molar rate of triglyceride oxidation by three because each molecule contains 3 mol of fatty acids.

Rates of appearance. Rate of appearance (Rₐ) and, when appropriate, rate of disappearance (Rₜ – tissue uptake) of glucose and palmitate at rest were calculated by using the equation of Steele (20), as modified for use with stable isotopes (27). During exercise and the first 30 min of recovery, the non-steady-state approximation of Steele was used in conjunction with a spline-fitting program to smooth the raw data (24). The effective volume of distribution was assumed to be 165 ml/kg for glucose and 40 ml/kg for palmitate. The value for palmitate was chosen because acute changes in palmitate concentration are essentially restricted to plasma (because FFA are bound to albumin). The FFA Rₐ was calculated by dividing the palmitate Rₐ by the fractional contribution of palmitate to the total FFA concentration, as determined by gas chromatography.

Statistical Analysis

The results obtained during 20–30 min of exercise were used for comparison of the subjects of the three levels of exercise. The effect of time on the response within each exercise level was analyzed by two-way analysis of variance for a randomized block, with the subjects as blocks and time as treatment. If necessary, the time effects were compared by Fisher’s least significant difference test. The results of the three exercise intensities were compared by two-way analysis of variance for randomized block design, in which the subjects are blocks and the three exercise levels are treatments. If necessary, the analysis of variance was followed by a multiple comparison to detect differences among groups.

To analyze the effects of body composition and gender on different metabolic responses (e.g., glucose production) we used the repeated-measures analysis of covariance. Letting Yₛₑ,eff denote the measured metabolic response from subject(s) [person(s)] exercising at intensity level e, with body composition parameter f, the model is $Y_{s,e}^{eff} = S_s + E_e + f(FE)_e + e_{s,e}$, in which the error terms are assumed to be independent, identically distributed, normal random variables with mean value 0 and common SDₑ. The body composition factor was either fat mass (FM), fat free mass (FFM), or the ratio FM/FFM. Thus the repeated-measures aspect is included via the subject effect $S_s$, and the effect of body composition is accounted for by the f(FE) term. To ensure unique estimates, the baseline constraints $E_1 = 0$ and $(FE)_e = 0$ were imposed. In this case, $E_s$ and $(FE)_e$ represent the rise over baseline levels of intercept and slope, respectively. The effect of body composition at baseline, along with any other baseline variability associated with body composition or other fixed characteristics of subjects, is embodied in the $S_s$. If the body composition were the only such factor, the values of the $S_s$ would specify the average effect of body composition on the baseline metabolic response. Simultaneously, 0.95 level confidence intervals for the parameters $E_s$ and $(FE)_e$ were generated in association with a repeated-measures analysis of covariance F-test of the hypothesis $H_0: E_s = 0, (FE)_e = 0, e = 2, 3, 4$. Data from a previous paper (15) from our laboratory, in which the exact same experimental procedures were used in trained male subjects, were used for evaluation of gender effect.

RESULTS

Resting State

At rest, there were no differences in concentrations, $R_a$, or oxidation rates of FFA or glucose before the three levels of exercise. The mean values are shown in Table 2.

Exercise

Lactate concentrations. Lactate concentration did not change during low-intensity exercise and increased from 0.84 ± 0.03 to 1.68 ± 0.18 mmol/l ($P < 0.05$) during moderate exercise intensity. During high-intensity exercise, lactate concentrations were considerably higher, but, nonetheless, physiological steady state was maintained. This was reflected in plasma lactate concentrations over the last 15 min of exercise (0 min: 0.84 ± 0.03, 15 min: 7.92 ± 0.17, 30 min: 7.30 ± 0.71 mmol/l).

Glucose metabolism. Tracer-to-tracee ratios and substrate kinetics are shown in Figs. 1 and 2, respectively. During low-intensity exercise, plasma glucose concentration and glucose $R_a$ did not change from the resting values. In contrast, during moderate- and high-intensity exercise, plasma glucose concentration and glucose $R_a$ increased significantly in relation to exercise intensity (Tables 2 and 3).

FFA metabolism. FFA concentrations increased gradually during low-intensity exercise, whereas they transiently decreased and subsequently increased during moderate exercise intensity (not significant (NS) vs. low-intensity exercise). During high-intensity exercise, FFA concentrations were considerably decreased compared with the values obtained at rest and during low-intensity exercise ($P < 0.05$; Table 3). Palmitate $R_a$, FFA $R_a$, and FFA uptake were significantly increased to the same extent in low- and moderate-intensity exercise (Fig. 2, Tables 2 and 3). During high-intensity exercise, palmitate $R_a$, FFA $R_a$, and FFA uptake were significantly
Table 2. Comparison of anthropometric values and substrate metabolism at rest and after 20–30 min of exercise at different exercise intensities in endurance-trained men and women

<table>
<thead>
<tr>
<th></th>
<th>Men (n=5)</th>
<th>Women (n=8)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>75.2 ± 3.6</td>
<td>60.6 ± 3.2</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Height</td>
<td>1.78 ± 0.03</td>
<td>1.67 ± 0.02</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Lean body mass</td>
<td>68.2 ± 3.3</td>
<td>49.5 ± 1.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fat mass</td>
<td>8.1 ± 0.2</td>
<td>11.1 ± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td>VO2max l/min</td>
<td>5.01 ± 0.3</td>
<td>3.5 ± 0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>VO2max ml/kg lean body mass</td>
<td>73.6 ± 3.5</td>
<td>70.1 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Rd glucose, μmol·kg lean body mass⁻¹·min⁻¹</td>
<td>Rest</td>
<td>17.0 ± 1.4</td>
<td>15.9 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>65%</td>
<td>29.0 ± 2.9</td>
<td>26.6 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>85%</td>
<td>25.2 ± 2.5</td>
<td>29.4 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>Rest</td>
<td>19.0 ± 3.7</td>
<td>15.6 ± 1.5</td>
</tr>
<tr>
<td>Fatty acid oxidation, μmol·kg lean body mass⁻¹·min⁻¹</td>
<td>Rest</td>
<td>6.7 ± 0.5</td>
<td>5.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>65%</td>
<td>26.1 ± 1.6</td>
<td>30.1 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>85%</td>
<td>47.0 ± 4.6</td>
<td>53.3 ± 3.5</td>
</tr>
<tr>
<td>Rd glucose, μmol·kg lean body mass⁻¹·min⁻¹</td>
<td>Rest</td>
<td>33.4 ± 5.0</td>
<td>36.8 ± 3.9</td>
</tr>
<tr>
<td>Carbohydrate oxidation, μmol·kg lean body mass⁻¹·min⁻¹</td>
<td>Rest</td>
<td>6.4 ± 0.9</td>
<td>6.2 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>65%</td>
<td>11.5 ± 0.4</td>
<td>11.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>85%</td>
<td>14.3 ± 0.4</td>
<td>12.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Rest</td>
<td>24.5 ± 1.2</td>
<td>28.5 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>65%</td>
<td>56.9 ± 2.1</td>
<td>51.7 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>85%</td>
<td>331.1 ± 17.5</td>
<td>285 ± 15.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. RER, respiratory exchange ratio; FFA, free fatty acid; Rd, rate of disappearance; NS, not significant.

lower than the values during the two lower exercise intensities (P < 0.05; Fig. 2, Tables 2 and 3).

Substrate oxidation rates. Substrate oxidation rates are given in Tables 2 and 3. During moderate-intensity exercise, carbohydrate oxidation rates increased above the value during low-intensity exercise, and carbohydrate oxidation was the highest during high-intensity exercise. The highest rate of fat oxidation was during exercise at 68% of VO2max (Table 3), whereas there was no significant difference between low- and high-intensity exercise.

During exercise at 25% of VO2max, FFA uptake and FFA oxidation rates, expressed in fatty acid equivalents, were similar (Table 2), indicating that plasma could transport adequate amounts of FFA for oxidation from adipose tissue. However, during moderate and high exercise intensity, fat oxidation rates exceeded the maximal amounts that could be obtained from plasma, the difference being the minimal contribution of muscle triglycerides to energy requirements (Fig. 3). From Fig. 3, it is also evident that the maximal combined contribution of plasma glucose and FFA to oxidation rates hardly differs between the three levels of exercise. Apparently, the decrease in FFA Rd during high-intensity exercise more or less balances the increase in glucose Rd.

Relationship of Results to Gender

Table 2 shows the comparison between the women and the previously described men. VO2max, expressed per kilogram of lean body mass, was similar in both groups. In addition, respiratory exchange ratio (RER) values obtained during different exercise intensities were not different. Despite decreased glucose Rd and increased rate of carbohydrate oxidation during low-intensity exercise in the women, there were no differences in glucose and fat metabolism between men and women after correction for differences in lean body mass. The minimal contribution of muscle triglycerides to energy requirements were 0 vs. 9% (men vs. women) at 25% of VO2max (P < 0.05), 23 vs. 25% at 65% of VO2max (NS) and 11 vs. 17% at 85% of VO2max (NS). Muscle glycogen contributed minimally, 0 vs. 9% (men vs. women) at 25% of VO2max (P < 0.05), 41 vs. 34% at 65% of VO2max (NS), and 63 vs. 58% at 85% of VO2max (NS).

DISCUSSION

The results of this study indicate that substrate metabolism in endurance-trained women responds similarly to moderate- and high-intensity exercise, as our laboratory has previously reported in endurance-trained men (15). A greater relative amount of body fat in women is a potential basis for expecting a difference in substrate metabolism between trained men and women. In this study, we were able to assess the effect of body composition on substrate metabolism by comparing men and women with comparable training status but with different body composition. Because we found no metabolic effect of gender, substrate metabolism during exercise is determined by energy requirements of lean body mass, rather than by gender or body composition.

There was a slight difference in study design between the present study and the previous study in men (15). In the present study in women, exercise at 85% of VO2max was performed on the same day as the exercise study at 25% VO2max. The two exercise studies were separated by an interval of 1 h. In the study in men, we performed the three exercise protocols on 3 separate days. One might argue that this difference in design affects our conclusion as to the comparison of the results at 85% of VO2max. However, there was no gender effect after correction for differences in lean body mass. Moreover, in the study in men, we documented that all changes induced by exercise at 25% of VO2max subsided within the first hour of recovery. Finally, exercise at 25% of VO2max in trained subjects only involves cycling without any resistance. This very low exercise intensity is not reflected in any change in glucose Rd. Therefore,
it seems unlikely that the slight differences in study design between the two studies affect our conclusion to a considerable extent.

Several other studies have evaluated the changes in substrate metabolism in relation to exercise intensity. Our data extend the observations of Friedlander et al. (5), which involved the effects of exercise at 52 and 65% of \( V\text{O}_2\text{max} \) in women after 8–12 wk of endurance training. They observed that there was no significant difference in FFA Ra between these exercise intensities, in line with our observation that FFA Ra is not different even between 25 and 65% of \( V\text{O}_2\text{max} \) in trained women.
Conversely, glucose Rd was higher in women after the study of Friedlander et al. (5) than in our study. Fat oxidation rates were considerably lower in women in the study. For instance, FFA Ra and whole body fat oxidation were much lower in women in the study of Friedlander et al. (6) than in our study. These discrepancies are at least in part explained by a difference in training status. Moreover, our data show for the first time that, in women, FFA Ra at 85% of \( \dot{V}_{\text{O}_2}\text{max} \) is much lower and not different from the values obtained at rest, as occurs in men. In a second study with a similar design, Friedlander et al. (6) demonstrated that glucose Rd was directly related to exercise intensity in trained and untrained women. Our data are in accordance with this conclusion over a wider range of exercise intensities. However, there were also quantitative differences between the studies of Friedlander et al. and the present study. For instance, FFA Ra and whole body fat oxidation rates were considerably lower in women in the study of Friedlander et al. (5) than in our study. Conversely, glucose Rd was higher in women after training in the study of Friedlander et al. (6) than in our study. These discrepancies are at least in part related to differences in subject characteristics. For instance, the women in our study had a lower amount of body fat than those in the studies of Friedlander et al. (18 vs. 24%) and a higher \( \dot{V}_{\text{O}_2}\text{max} \) (70 vs. 54 ml·kg\(^{-1}\)·min\(^{-1}\)). These anthropometric values suggest that the differences in the absolute values between the studies of Friedlander et al. and our study are at least in part explained by a difference in training status.

Several studies have compared substrate metabolism during exercise between men and women. Friedlander et al. (6) compared the training-induced alterations of carbohydrate metabolism in men and women. They found no gender difference in glucose Ra during exercise in trained men and women, in accordance with our data. Moreover, we found no differences in FFA Ra between endurance-trained men and women after correction for differences in lean body mass. In contrast to our results, however, Friedlander et al. found a gender difference in substrate oxidation, in that women showed a reduction in RER after training, in contrast to men. In accordance, other authors observed that women derived more of the total energy expended from fat oxidation than men did during exercise at 40 or 65% \( \dot{V}_{\text{O}_2}\text{max} \). The reason for this discrepancy in the results derived from indirect calorimetry between different studies is unclear. Most studies evaluate exercising women in the midfollicular phase (e.g., Refs. 6, 22), whereas our study was not controlled for the effects of the menstrual phase. There are indications that the menstrual cycle affects the metabolic response to exercise. For instance, lipid oxidation during moderate exercise intensities is higher in the midluteal phase (8). Gender differences may, therefore, be less clear in women studied throughout all phases of the menstrual cycle. During submaximal exercise intensity, this gender difference in lipid oxidation disappeared (8). Therefore, at higher intensities, it is likely that substrate selection is governed by the exercise itself rather than by mitigating factors like menstrual cycle.

In this study, we compared five men with eight women. In this respect, it is appropriate to evaluate the statistical power of the comparisons between both groups. We calculated the detectable difference between the means as a function of the \( \alpha \)-value of 0.05, a \( \beta \)-value of 0.80, the number of subjects, and the SD of the observations. Although the detectable difference varied somewhat between the different variables, the mean difference between both groups that could be detected was 15%. Therefore, we cannot exclude the possibility that there may have been smaller differences between both groups, which could have been detected in a study with a larger number of subjects. However, it is unlikely that this explains the absence of a difference in RER values between men and women.

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Table 3. Substrate concentrations, kinetics, and oxidation in women after 30 min of exercise

<table>
<thead>
<tr>
<th></th>
<th>25% ( \dot{V}_{\text{O}_2}\text{max} )</th>
<th>65% ( \dot{V}_{\text{O}_2}\text{max} )</th>
<th>85% ( \dot{V}_{\text{O}_2}\text{max} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA concentration, ( \mu \text{mol/l} )</td>
<td>698 ± 103*</td>
<td>771 ± 102*</td>
<td>359 ± 68</td>
</tr>
<tr>
<td>FFA uptake, ( \mu \text{mol·kg}^{-1}·\text{min}^{-1} )</td>
<td>21.6 ± 1.7*</td>
<td>25.3 ± 3.1*</td>
<td>12.6 ± 1.2</td>
</tr>
<tr>
<td>Fat oxidation, ( \mu \text{mol·kg}^{-1}·\text{min}^{-1} )</td>
<td>21.5 ± 1.6*</td>
<td>43.1 ± 3.5*</td>
<td>30.1 ± 3.6</td>
</tr>
<tr>
<td>Glucose concentration, mg/dl</td>
<td>83 ± 2*†</td>
<td>95 ± 6*</td>
<td>141 ± 17</td>
</tr>
<tr>
<td>( R_d ) glucose, ( \mu \text{mol·kg}^{-1}·\text{min}^{-1} )</td>
<td>9.1 ± 0.5†</td>
<td>23.2 ± 2.0*</td>
<td>41.7 ± 9.8</td>
</tr>
<tr>
<td>Carbohydrate oxidation, ( \mu \text{mol·kg}^{-1}·\text{min}^{-1} )</td>
<td>18.0 ± 3.2†</td>
<td>106.5 ± 11.5*</td>
<td>232.1 ± 15.1</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8 subjects. * \( P < 0.05 \) vs. 85% \( \dot{V}_{\text{O}_2}\text{max} \). † \( P < 0.05 \) vs. 65% \( \dot{V}_{\text{O}_2}\text{max} \).
because these values were almost identical in both groups.

In light of the lack of a relationship between relative fat mass and the lipolytic response, as reflected by FFA $R_a$, to exercise, it is appealing to consider the energy requirements of performing the exercise as the major determinant of the rate of FFA $R_a$, because it is the rate of energy utilization that determines the requirement for substrate oxidation. Nonetheless, several aspects of our data argue against this interpretation. Most importantly, fatty acid uptake did not increase as the energy requirement increased. In the absence of a change in uptake, plasma fatty acid concentrations will not decrease, and thus any given peripheral fat cell would have no direct feedback signal. In any case, there is no evidence that FFA concentrations influence FFA $R_a$ in vivo.

For example, in short-term fasting the concentration of FFA increases two- to threefold, and FFA $R_a$ also increases (28). Thus there is no direct feedback mechanism whereby FFA concentration affects FFA $R_a$.

The absence of a direct link between the energy utilization of the lean body mass and FFA $R_a$ requires the identification of an alternative mechanism to explain how the change in energy utilization during exercise could control lipolysis. Insulin, epinephrine, and adenosine are the most important short-term regulators of lipolysis (2), but none provides a link between energy utilization of lipolysis or FFA $R_a$ during exercise at different intensities. Thus catecholamines are only elevated to a great extent during high-intensity exercise (15), yet in this study [as in previous studies (10, 15)] fatty acid release was less during high-intensity exercise than during low intensities. Insulin concentration is generally suppressed during exercise (16), and differences in insulin concentration at different exercise intensities cannot explain the corresponding rates of lipolysis. Adenosine is a potent inhibitor of lipolysis in some circumstances (12). However, because an increase in adenosine will generally accompany increased ATP turnover, changes in adenosine concentration would not be expected to cause lipolysis to be positively correlated with energy expenditure. In fact, our laboratory (13) has previously shown that changes in adenosine activity cannot explain the lipolytic response to exercise. Therefore, it is impossible to provide a physiological basis for the notion that energy utilization during exercise controls lipolysis, either directly or indirectly, through any of the control mechanisms known to be important in vivo regulations of lipolysis.

An implication of the preceding argument is that the regulation of lipolysis is not necessarily related to the rate of energy expenditure. This notion is supported by empirical data from a variety of circumstances. In the present study, the maximal lipolytic response was elicited at 25% of $V_{O_2}$max (Fig. 2), yet energy expenditure increased several-fold at higher intensities. In contrast, the response to fasting elicits a doubling or more in the rate of lipolysis, yet the rate of energy utilization does not increase (28). Furthermore, the extra fatty acids supplied by increased lipolysis in fasting far exceed the decrease in caloric equivalents due to decreased hepatic glucose production (17). Thus, in a wide variety of physiological circumstances, the rate of lipolysis is not directly related to the rate of energy expenditure. The major hormones regulating lipolysis, insulin and catecholamines, are primarily controlled by factors other than availability of fatty acids. Therefore, direct feedback control of lipolysis via hormonal control is unlikely.

Because our data indicate that changes in lipolysis are not primarily responsible for changes in fat oxidation during exercise, it is not surprising that we found no effect of body composition on any metabolic parameter. Differences in fat mass per se should be expected to affect substrate metabolism by means of differences in the availability of fatty acids. Because differences in body composition would be expected to be one of the major reasons for differences in substrate metabolism in exercise between men and women, the lack of an effect of body composition is consistent with the similarity in responses between men and women. On the other hand, there is evidence that sex steroids may play a role in substrate metabolism in exercise (19). The present study was not designed to assess the role of sex steroids because the phase of the menstrual cycle during which our subjects were studied was not controlled. It is unlikely, however, that this affected our conclusion because the coefficient of variation of the data in the female subjects in the present study was generally less than in the male athletes who were studied previously (15). Consequently, whereas variations in the concentrations of sex steroids may have contributed to variability of the results, that variability cannot explain the inability to find significant differences between men and women. Rather, it is likely that any possible effects of sex steroids were overshadowed by the effects of strenuous endurance training.

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