Fuel metabolism in men and women during and after long-duration exercise

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Horton, Tracy J., Michael J. Pagliassotti, Karen Hobbs, and James O. Hill. Fuel metabolism in men and women during and after long-duration exercise. J. Appl. Physiol. 85(5):1823–1832, 1998.—This study aimed to determine gender-based differences in fuel metabolism in response to long-duration exercise. Fuel oxidation and the metabolic response to exercise were compared in men (n = 14) and women (n = 13) during 2 h (40% of maximal \( \dot{V}O_2 \) uptake) of cycling and 2 h of postexercise recovery. In addition, subjects completed a separate control day on which no exercise was performed. Fuel oxidation was measured using indirect calorimetry, and blood samples were drawn for the determination of circulating substrate and hormone levels. During exercise, women derived proportionally more of the total energy expended from fat oxidation (50.9 ± 1.8 and 43.7 ± 2.1% for women and men, respectively, \( P < 0.02 \)), whereas men derived proportionally more energy from carbohydrate oxidation (53.1 ± 2.1 and 45.7 ± 1.8% for men and women, respectively, \( P < 0.01 \)). These gender-based differences were not observed before exercise, after exercise, or on the control day. Epinephrine (\( P < 0.007 \)) and norepinephrine (\( P < 0.0009 \)) levels were significantly greater during exercise in men than in women (peak epinephrine concentrations: 208 ± 36 and 121 ± 15 pg/ml in men and women, respectively; peak norepinephrine concentrations: 924 ± 125 and 659 ± 68 pg/ml in men and women, respectively). As circulating glycerol levels were not different between the two groups, this suggests that women may be more sensitive to the lipolytic action of the catecholamines. In conclusion, these data support the view that different priorities are placed on lipid and carbohydrate oxidation during exercise in men and women and that these gender-based differences extend to the catecholamine response to exercise.

EXERCISE FUEL METABOLISM has been well characterized in men but not in women. This is relevant, inasmuch as certain data suggest that men and women may place different priorities on lipid and carbohydrate utilization during exercise. For example, with the same relative intensity of submaximal exercise, women have been reported to oxidize relatively more lipid and less carbohydrate compared with men (1, 25, 31). Concurrently, a lesser decline in muscle glycogen was observed in response to endurance exercise in women (31, 32). Whether these gender-based differences in whole body fuel oxidation are related to differences in circulating substrate utilization is unclear (1, 9, 31). With respect to the hormonal response to exercise, there is some suggestion that this may differ between men and women (31) and, in particular, that there is a smaller increase in epinephrine during exercise in women (23, 31). However, it is still not clear how gender-based differences in the pattern of fuel oxidation during exercise are mediated metabolically.

One problem with the previous studies of gender-based differences in exercise metabolism is the lack of adequate control measurements. To establish an independent effect of exercise on fuel oxidation, it is important to establish that, under control conditions, i.e., resting and fasting for a duration similar to the exercise period, women do not oxidize significantly more lipid. In addition, fuel oxidation measurements continued into the postexercise period would establish whether women compensate for their greater exercise fat oxidation by utilizing less lipid than men during exercise recovery. Such measurements of fuel oxidation need to be made to appropriately interpret and assess the implications of the experimental data.

Studies of gender-based differences during exercise metabolism also require careful attention to issues of subject selection and study design. This may be the reason for the lack of gender-based differences in exercise metabolism observed in certain investigations (5, 26). For example, it is important to ascertain the menstrual status of female subjects, as the female gonadotrophic hormones are known to influence fuel metabolism (12, 16, 21, 27). Furthermore, when an attempt is made to match gender groups, it may not be practical or logical to do so on the basis of body composition or maximal \( \dot{V}O_2 \) uptake (\( V_{O_2\text{max}} \)), as these two parameters naturally differ between men and women. Thus it may be more appropriate to select and match subjects on the basis of training status and training quantity, as this is known to be a major determinant of exercise fuel utilization (3, 8, 13). Matching in this way, therefore, has implications for the exercise conditions selected. Choosing the same relative work intensity for the exercise test would represent a similar metabolic challenge for men and women rather than exercising individuals at the same absolute workload. Finally, it is important to control pretest dietary intake and exercise performance to ensure that subjects are in a similar state of energy balance and fuel repletion before testing. Only by consideration of these factors in the design of studies may it be possible to clearly establish any gender-based differences in exercise metabolism.

The aim of the present study, therefore, was to compare fuel oxidation during and after exercise and on a control day in women and men. This was done taking into consideration the above factors in the study design. Simultaneously, the circulating substrate and hormone environment was characterized to begin to elucidate possible mechanisms involved in any observed gender-based differences in exercise fuel oxidation. Finally, we
studied trained and untrained subjects to determine whether there was any interaction between gender and training status with respect to exercise fuel oxidation.

**METHODS**

**Subjects**

Trained and untrained men (n = 14) and women (n = 14) were recruited for the study (Table 1). Trained subjects were mainly competitive cyclists, with one triathlete in each male and female group. Subjects were matched on training status (volume and intensity of training as determined from self-reported activity records). Untrained subjects participated in <90 min of aerobic exercise per week. All subjects were of normal weight, were nondiabetic and nonsmokers, and were not suffering from any medical condition or taking any chronic medication known to interfere with intermediary metabolism. All women were eumenorrheic and not using steroidal contraceptives. The study protocol was approved by the University of Colorado Committee Institutional Review Board for the Protection of Human Subjects. All subjects read and signed a subject consent form before admission into the study.

**Preliminary Assessments**

\( \dot{V}O_2 \)max, \( \dot{V}O_2 \)max was determined using a graded exercise test on a cycle ergometer (Monark, Varberg, Sweden). Trained subjects pedaled at a rate of 90 rpm and untrained subjects at 70 rpm while the resistance was gradually increased (commencing at 1 kp and increasing by 0.5 kp every 1–2 min) until volitional exhaustion. Heart rate was continuously monitored via a 12-lead electrocardiogram, with blood pressure and perceived exertion measured in the final minute of each work level. To ensure that maximum effort was achieved, two of the following criteria had to be fulfilled: whole body respiratory exchange ratio >1.1, maximum heart rate within 5% of the predicted maximum, and an increase in \( \dot{V}O_2 \) consumption in response to the final workload of <2.0 ml·kg body wt \(^{-1}\)·min \(^{-1}\).

Body composition. Body composition was determined by measuring body density using underwater weighing, as previously described (14). Percent body fat was estimated from body density using the revised equation of Brozek et al. (4).

Resting metabolic rate. Resting metabolic rate (RMR) was measured using indirect calorimetry via a metabolic cart system (model 2900, Sensormedics, Yorba Linda, CA). RMR for untrained and trained women was determined using a graded exercise test on a cycle ergometer (Monark) for 2 h at 40% \( \dot{V}O_2 \)max after a 5-min warm-up. After the termination of the exercise bout, subjects rested in a semireclined position for 2 h.

**Study Days**

Subjects spent the evening before each study in the GCRC and were tested the next day after a 10-h fast. All subjects completed 2 test days: an exercise day and a control day. The order of testing was randomized within each group, and 3–6 wk separated the 1st from the 2nd study day. Women were studied in the follicular phase of their menstrual cycle (exercise study: days 7.3 ± 2.8 and 6.8 ± 3.5 in trained and untrained women, respectively, where day 1 is the 1st day of menses; control study: days 7.5 ± 3.9 and 6.5 ± 3.3, respectively). Previous menstrual history was used to estimate desired study day, and menstrual cycle phase and day were confirmed by report of most recent menses and determination of serum estradiol and progesterone on the study day. One trained woman was found to have been in her luteal phase on both study days but was not able to repeat either day. Analysis of the data with and without her results did not affect the substrate oxidation results but did impact some of the hormone data. Her results were therefore excluded from the final analysis. On the morning of the study, subjects were awakened at ~0545. An intravenous flexible catheter (18–20 gauge) was placed in a vein close to the antecubital space of one arm. A three-way stopcock was attached for sampling and infusion of normal saline. The subject was then transported by car (3-min drive) to the laboratory for testing.

Exercise day. Subjects emptied their bladder and completed a urine collection over the subsequent 24 h. They then lay down and rested for 30 min before a 15- to 20-min measurement of RMR. Immediately thereafter, baseline blood samples were taken. Subjects then rode on a stationary bicycle ergometer (Monark) for 2 h at 40% \( \dot{V}O_2 \)max after a 5-min warm-up. After the termination of the exercise bout, subjects rested in a semireclined position for 2 h.

Control day. The control day followed exactly the same format as the exercise day, except the subjects did not exercise but rested for the same study duration in a semireclined position (4 h total).

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**Table 1. Subject characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Trained Subjects</th>
<th>Untrained Subjects</th>
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<tbody>
<tr>
<td></td>
<td>Men (n = 8)</td>
<td>Women (n = 7)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>25 ± 4</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>176 ± 6†</td>
<td>166 ± 6</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69.1 ± 7.0†</td>
<td>57.8 ± 6.5</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>16.1 ± 3.4†</td>
<td>19.1 ± 3.0†</td>
</tr>
<tr>
<td>( \dot{V}O_2 )max, ml·kg (^{-1})·min (^{-1})</td>
<td>64.4 ± 3.7†</td>
<td>53.3 ± 6.6†</td>
</tr>
<tr>
<td>( \dot{V}O_2 )max, ml·min (^{-1})</td>
<td>4,450</td>
<td>3,196</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>57.9 ± 5.6†</td>
<td>46.7 ± 4.4†</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>11.2 ± 3.0†</td>
<td>11.1 ± 2.9†</td>
</tr>
</tbody>
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Values are means ± SD. Trained women (n = 7) excludes one trained woman found to be in luteal phase of her menstrual cycle. \( \dot{V}O_2 \)max, maximal \( V_2 \) uptake. *P < 0.05 vs. untrained women. †P < 0.05 vs. trained women. ‡P < 0.05 vs. untrained men.
Metabolic Determinations

Respiratory gas exchange measurements. Respiratory gas exchange during each 4-h test period was measured via indirect calorimetry using the metabolic cart and Hans Rudolph mask. Measurements were made continuously for periods of ~30 min, with recalibration of the metabolic cart between measurements. Carbohydrate and fat oxidation were calculated from the volume of O2 consumed and the volume of CO2 expired after correction for protein oxidation (17). Protein oxidation was estimated by dividing 24-h urinary nitrogen excretion into 2-h portions. This time frame was used rather than the study period itself, as 1) some subjects could not urinate immediately after the exercise and 2) we observed a time delay in the excretion of nitrogen resulting from exercise protein oxidation. We estimated a difference of less than ±0.5% in the estimation of carbohydrate or fat grams oxidized when using the study period urine collection vs. 2-h nitrogen excretion values estimated from the 24-h urine collection.

Determination of circulating hormone and substrate levels. Blood was sampled at baseline, every 30 min on the exercise day, and at baseline and every hour on the control day. Blood samples were drawn from a forearm vein; therefore, the circulating measurements represent arterial concentrations as well as venous drainage from the local muscle bed. Approximately 1 ml of whole blood was added to a preweighed tube containing 3 ml of iced perchloric acid (8%). After it was vortexed, the tube was weighed and spun to separate the supernatant. Whole blood (2.5 ml) was added to 40 µl of preservative (3.6 mg EGTA + 2.4 mg glutathione in distilled water) for plasma catecholamine determinations. Samples were immediately placed on ice and spun. Approximately 7 ml of whole blood were allowed to clot, and the serum was separated off after spinning. All plasma, serum, and supernatant samples were stored at −70°C until analysis. Catecholamines were determined in duplicate by radioenzymatic assay (intra-assay coefficients of variation (CV) = 16 and 8% for norepinephrine and epinephrine, respectively) (24). RIAs were used to determine serum insulin (Kabi Pharmacia, Piscataway, NJ), cortisol, progesterone, estradiol, and testosterone (Diagnostic Products, Los Angeles, CA) and sex-hormone binding globulin (SHBG; Wien Laboratories, Succasunna, NJ). Samples were run in duplicate, with intra-assay CVs of 10, 6.7, 8.8, 7.5, 9.1, and 7.0%, respectively. Serum glucose was measured enzymatically on an automated Roche COBAS Mira Plus glucose analyzer (intra-assay CV = 0.7%). Enzymatic assays were used to determine serum glycerol (Boehringer Mannheim Diagnostics, Indianapolis, IN) and free fatty acids (FFA; Wako Chemical, Richmond, VA) one time only (intra-assay CVs = 7.8 and 1.2%, respectively). β-Hydroxybutyric acid (β-HBA) and blood lactate (Sigma Diagnostics, St. Louis, MO) were run in duplicate with intra-assay CVs of 10.8 and 4.2%, respectively. For each subject, samples from both study days were run simultaneously for all assays.

Data Analysis

On each study day, data were compared by sex and by training status, with any interaction between the two grouping factors also determined. Inasmuch as the only significant effects on exercise fuel oxidation were observed between the genders, all data are presented using the gender-based separation. For fuel oxidation and energy expenditure (EE), each study day was divided into two parts comprising the first 2 h of measurement (cycling period for the exercise day) and the second 2 h of measurement (recovery period for the exercise day). Fuel oxidation was expressed as absolute oxidation per 2-h period and as relative oxidation (percent): (kJ fuel oxidized/total kJ oxidized) × 100. The latter mode of expression allows for the different EE between individuals and groups, which would automatically result in different absolute fuel oxidation rates. Univariate ANOVA was initially used to determine group differences in EE and fuel oxidation variables for comparable time periods on each study day. Where significant differences were observed, these were confirmed by multivariate analysis that included percent body fat and VO2max as covariates. These two variables were included in the statistical model, as they may be considered confounding factors for the univariate results. All data are, however, presented as the unadjusted means with statistics from the univariate analysis. For substrate and hormone measures, a repeated-measures ANOVA was used with gender as the grouping factor and each time point of sampling as repeated measures. Any interaction between gender and time was determined to establish whether the pattern of change in each parameter differed between the genders. Where significant gender-by-time interactions were observed, an unpaired t-test was used to determine whether there were also specific time points where gender-based differences occurred. Group characteristics were compared using ANOVA, and where appropriate, post hoc analysis was performed using the Fisher least significant difference test. Within-group differences in parameters (between periods of measurements and between days) were compared using a paired t-test. Significant differences were established where P < 0.05.

RESULTS

Physical Characteristics

There were no gender differences in age and fat mass. However, men were significantly heavier and taller and had a greater fat-free mass than women (P < 0.05). VO2max was marginally higher in men than in women (P < 0.06), whereas percent body fat was significantly greater in women (P < 0.05). Levels of activity were similar in trained women and men (cycling: 135 ± 41 and 132 ± 54 miles/wk; other aerobic activity: 3 and 2.5 h/wk; strength training: 2 and 2.5 h/wk, respectively). Dietary intake and body weight change over the 3 days before each study day are shown in Table 2. Energy and nutrient intakes were similar for the 3 days preceding the control or exercise day, and weight

<table>
<thead>
<tr>
<th>Table 2. Dietary intake and body weight change before each study day</th>
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<tr>
<td><strong>Cycling day</strong></td>
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<tr>
<td>Men</td>
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<tr>
<td>Women</td>
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<tr>
<td><strong>Control day</strong></td>
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<tr>
<td>Men</td>
</tr>
<tr>
<td>Women</td>
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</tbody>
</table>

Values are means ± SD. CHO, carbohydrate. Weight change was estimated from fasting body weight measured on the morning of the 1st day of diet control — fasting body weight measured the morning of the study day.
stability was maintained in men and women before each study day.

Energy Expenditure

RMR was similar in men and women relative to fat-free mass (Fig. 1). There was no difference between RMR measured on the cycling day and that measured on the control day (Table 3: 5.38 ± 0.21 and 5.33 ± 0.13 kJ/min, respectively, for men; and 4.37 ± 0.08 and 4.41 ± 0.12 kJ/min, respectively, for women). EE during the 120-min cycle ride was significantly greater in men than in women (Table 4). This was related to the greater absolute work rate performed by the men (338 ± 16 and 219 ± 14 kpm/min for men and women, respectively, P < 0.05). However, the relative intensity of the exercise was similar between the two groups (average heart rate: 121 ± 4 and 125 ± 4 beats/min for men and women, respectively; intensity = 42.1 ± 0.9 and 42.3 ± 0.8% VO2max for men and women, respectively). During exercise recovery, EE was significantly elevated above the value on the control day in men (P < 0.001) but not in women.

Fuel Oxidation

Resting respiratory exchange ratio did not differ between the two groups on either study day (cycling day: 0.80 ± 0.01 and 0.79 ± 0.01 for men and women, respectively; control day: 0.80 ± 0.01 and 0.79 ± 0.01 for men and women, respectively). During the cycle exercise, absolute total carbohydrate oxidation and total protein oxidation were significantly greater in men than in women (Table 4; P < 0.001 and P < 0.01, respectively), but there were no gender-based differences in the absolute total oxidation of fat. When the oxidation data were expressed as a percentage of the total EE, the contribution of fat oxidation to exercise EE was significantly greater in women than in men (P < 0.02), whereas the contribution of carbohydrate oxidation to total exercise EE was significantly greater in men (P < 0.01; Table 4). No gender-based differences in the relative contribution of fat and carbohydrate oxidation to total EE were observed during the postexercise recovery period or on the control day.

As expected, absolute fuel oxidation was greater during period 1 of the cycling day (exercise) than during period 2 (recovery) and period 1 of the control day (Table 4). During the postexercise period the contribution of fat to total EE significantly increased relative to the exercise period in men (P < 0.001) and women (P < 0.01), whereas the contribution from carbohydrate significantly decreased in both groups (P < 0.01). On the control day, there was also an increase in the contribution of fat to total EE from period 1 to period 2, but this was significant only for women (P < 0.01). Consequently, the increased relative fat oxidation in men during recovery on the exercise day was greater than the relative fat oxidation during the same time period on the control day (P < 0.01).

Circulating Substrate Levels

Circulating substrate levels on the exercise and control days are shown in Fig. 2. For both groups on the cycling day, glucose, FFA, glycerol, and lactate increased with exercise and decreased after exercise (P < 0.0001), whereas β-HBA increased mainly in the postex-

Table 3. RMR, exercise EE, and nonprotein RER

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>RMR, kJ/min</th>
<th>RMR RER</th>
<th>EE Period 1, kJ/min</th>
<th>RER</th>
<th>EE Period 2, kJ/min</th>
<th>RER</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Cyclling day</td>
<td></td>
<td>Control day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td>14</td>
<td>5.38±0.21*</td>
<td>0.80±0.01</td>
<td>34.5±2.1‡</td>
<td>0.86±0.01‡</td>
<td>6.17±0.17‡</td>
<td>0.79±0.01‡</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>4.37±0.08</td>
<td>0.79±0.01</td>
<td>23.5±1.9‡</td>
<td>0.84±0.01‡</td>
<td>4.43±0.20</td>
<td>0.79±0.01‡</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>14</td>
<td>5.32±0.13*</td>
<td>0.80±0.01</td>
<td>5.32±0.20*</td>
<td>0.82±0.01</td>
<td>5.38±0.21*</td>
<td>0.81±0.01</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>4.41±0.12</td>
<td>0.79±0.01</td>
<td>4.29±0.16</td>
<td>0.82±0.01</td>
<td>4.33±0.15</td>
<td>0.80±0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. RMR, resting metabolic rate; RER, respiratory exchange ratio; EE, energy expenditure. Period 1, rate of EE during first 2 h of measurement (encompasses entire cycling period on cycling day); period 2, rate of EE during second 2 h of measurement (encompasses postexercise recovery period on cycling day). Significantly different from control: *P < 0.001; †P < 0.05. ‡Significantly different from period 1.
exercise period (P < 0.0001). Although lactate levels increased with exercise, the rise was relatively small (maximum change of ~0.2 mmol/l for both groups) and was unlikely to have had any impact on nonmetabolic CO₂ production. During exercise the increase in FFA was significantly greater in women than in men (P < 0.004). Conversely, glucose and ß-HBA were higher in men during the postexercise period (P < 0.005 and P < 0.003, respectively). On the control day the small increases in FFA, glycerol, lactate, and ß-HBA were also significant with time (P < 0.001). On this day, substrates increased similarly in both groups. Lactate level was significantly higher in men than in women on the control day (P < 0.02), although the differences were small in magnitude (~0.2 mmol/l).

Circulating Hormones

Figures 3 and 4 show the hormone changes during both study days. On the cycling day, all circulating hormone levels changed significantly with time (P < 0.0001), generally increasing with exercise and decreasing after exercise. Most notably, there was a significantly greater elevation in epinephrine (P < 0.007) and norepinephrine (P < 0.0009) during exercise in men than in women. Postexercise insulin levels were significantly greater in men than in women (P < 0.0009), but the absolute magnitude of this difference was small (1 µU/ml). Although cortisol levels were greater in men than in women on the cycling day, this did not achieve statistical significance (P = 0.08).

With respect to the gonadotrophic hormones, on the control and the exercise day, SHBG levels were significantly greater in women than in men (P < 0.01 and P < 0.009, respectively), whereas testosterone levels were significantly greater in men (P < 0.0001). The increase in estradiol during exercise was greater in women than in men (P < 0.04). Testosterone increased with exercise, more in men than in women (P < 0.0001), but levels were not significantly different from the values on the control day in either group.

DISCUSSION

This study demonstrated significant gender-based differences in fuel oxidation and the hormonal response to exercise. With the same relative intensity of mild-to-moderate exercise, fat oxidation contributed more to the total EE in women than in men. Consequently, the contribution of carbohydrate oxidation to exercise EE was lower in women than in men. The most striking observation, however, was our finding of significantly greater epinephrine and norepinephrine levels in men than in women during exercise. Whether these hormones played a role in the observed gender-based differences in exercise fuel oxidation could not be directly established.

The present data confirm previous observations of a greater relative fat oxidation and lower relative and/or absolute carbohydrate oxidation in women than in men (1, 25, 31, 32). It also extends previous observations by establishing that these gender-based differences are limited to the exercise condition itself. We observed no difference between men and women in the contribution of lipid and carbohydrate fuels to EE before exercise, after exercise, or on a control day. Data were analyzed by taking into account the differences in VO₂max and percent body fat between men and women. Therefore, it appears that there is a gender-specific difference in the prioritization of fat and carbohydrate fuels for oxidation during exercise. Furthermore, this was independent of training status in both men and women.

Although the magnitude of the differences observed in the present study was small, others have observed greater gender-based differences between the contribution of fat and the contribution of carbohydrate oxidation to exercise EE (25, 31, 32). These studies used a
somewhat higher exercise intensity (65–85% \( \dot{V}O_{2\text{max}} \)) than the present study (40% \( \dot{V}O_{2\text{max}} \)). Therefore, the magnitude of the gender difference between fat and carbohydrate oxidation may be related to the intensity of exercise performed. It is possible that at mild exercise intensities the gender-based differences in fat oxidation may be smaller, as both groups can readily utilize lipid as an energy source. Conversely, it seems probable that at very high exercise intensities, where carbohydrate utilization predominates and anaerobic metabolism is increased, gender-based differences in fuel utilization may not occur.

In the present cross-sectional study, trained subjects had a nonsignificantly greater lipid oxidation than untrained subjects. This contrasts with longitudinal training studies, in which significantly higher rates of lipid oxidation are observed after training (15). This discrepancy may be explained by the differences in study design (cross-sectional vs. longitudinal) and the use of different exercise regimens (relative vs. absolute exercise intensity). In addition, the exercise intensity used in the present investigation was relatively mild to moderate for all subjects and would favor lipid utilization in untrained as well as trained subjects alike.

The implication of gender differences in fuel oxidation during aerobic exercise relates to the control of fuel stores, both lipid and carbohydrate (glycogen). With respect to total body fat, it may be counterintuitive that women oxidize more lipid than men during exercise, given the fact that women normally maintain a higher body fat level than men. However, the magnitude of the gender-based difference in the relative fat oxidation observed during exercise was small and could readily be offset by postexercise differences in EE and/or food intake. In regard to carbohydrate stores, the lower carbohydrate oxidation during exercise in women has been shown to be related to less glycogen depletion (31, 32). Therefore, increasing glycogen stores to enhance performance may be less effective in women than in men (32).

Fig. 2. Circulating substrate levels in men (open symbols) and women (filled symbols) on exercise (circles) and control (triangles) days. A: for lactate, there was a significant effect of time (\( P < 0.0001 \)) on cycling and control days and a significant effect of gender (\( P < 0.02 \)) on control day. B: for glucose, there was a significant effect of time (\( P < 0.0001 \)) and a significant gender-by-time interaction (\( P < 0.005 \)); for the cycling day only, *\( P < 0.05 \) (unpaired t-test). C: for free fatty acids, there was a significant effect of time (\( P < 0.0001 \)) on cycling and control days and a significant gender-by-time interaction (\( P < 0.004 \)) on cycling day; *\( P < 0.05 \) (unpaired t-test). D: for glycerol, there was a significant effect of time on cycling and control days (\( P < 0.0001 \) and \( P < 0.001 \), respectively). E: for \( \beta \)-hydroxybutyric acid, there was a significant effect of time (\( P < 0.0001 \)) on cycling and control days and a significant gender-by-time interaction (\( P < 0.003 \)) on cycling day.
The significantly greater catecholamine response that was observed during exercise in men than in women suggests a role for these hormones in the gender-based differences in fuel oxidation. Previous observations have suggested a greater epinephrine (23, 31), but not norepinephrine (9, 31), response to exercise in men than in women. One of the major effects of the catecholamines is the stimulation of lipolysis (10, 20). However, in the present study we observed no gender-based difference in circulating glycerol levels, an estimate of whole body lipolysis. This has also been observed previously (9, 31), although reports are inconsistent (1, 31). The authors recognize that changes in circulating glycerol levels do not solely reflect the release of glycerol from triglyceride stores; however, these two parameters do appear to be highly correlated (2, 29). Thus, on the basis of the assumption that a change in glycerol level is a reasonable estimate of lipolysis, the glycerol data from this study suggest a greater sensitivity to the lipolytic action of one or both of the catecholamines in women relative to men. Epinephrine can also stimulate glycogenolysis, particularly in muscle (7). Therefore, the greater catecholamine level during exercise in men could have resulted in a greater mobilization and, potentially, utilization of muscle glycogen. Indeed, a greater decline in muscle glycogen stores after exercise has been previously observed in men than in women (31). However, in the present study we had no measure of glycogenolysis. It could be postulated that a greater sensitivity in women than in men, to the lipolytic action of the catecholamines, in conjunction with a similar glycogenolytic effect, would favor lipid over carbohydrate mobilization at any given catecholamine level.

The main gender-based difference observed in circulating substrate concentrations during exercise was the greater FFA levels in women than in men. This has also been reported by other authors using mild exercise (1), whereas with more moderate exercise no difference in circulating FFA has been observed (9, 31). However, circulating substrate levels do not reflect fuel utilization but represent a balance between production and utilization. This is relevant, as we observed gender-based differences in circulating FFA concentrations but not circulating glycerol levels. Therefore, if lipolysis
were stimulated to the same extent in both groups, the lower FFA levels in men could be due to a greater oxidation of adipose tissue-derived FFA than in women. This could only be confirmed by measurements of substrate turnover. Adipose tissue-derived FFA may not, therefore, be contributing to the increased relative fat oxidation in women during exercise. Other sources of lipid that could contribute to the greater relative fat oxidation in women include intramuscular triglyceride-derived FFA (31) and, to a lesser extent, circulating very-low-density-lipoprotein triglycerides (33).

In the present investigation, circulating glucose levels were consistently lower in women than in men, either in response to the same duration of fasting on the control day or during the exercise and the postexercise recovery. Others have reported that, with more moderate exercise (65% \(V_{\text{O2max}}\)), exercise blood glucose is lower in men than in women (31). This again implies that exercise intensity is an important determinant of gender-based differences in exercise fuel metabolism. In the present study it appeared that women were better able than men to maintain blood glucose levels (albeit at an overall lower level) during exercise. In the only report to measure glucose kinetics in men and women (1 h of cycling, 50% \(V_{\text{O2max}}\)), exercise glucose utilization was not different between the genders (23). However, it is difficult to make inferences from this tracer study relative to our present investigation, as the former study observed no gender-based differences in whole body fuel oxidation, likely because of the small number of subjects studied (\(n = 4/\text{gender}\)). In the present study, whether the greater catecholamine levels during exercise in men than in women resulted in differences in glucose production cannot be determined. Therefore, potential gender-based differences in the regulation of blood glucose production and utilization during exercise require further investigation.

It is presently unknown whether differences in fat and carbohydrate utilization in men and women can be explained by differences in substrate delivery, hormone action, or the capacity for substrate utilization. It could be hypothesized that the capacity for carbohydrate oxidation is greater in men than in women; therefore, by default, women must use more lipid. This hypothesis is supported by data that demonstrate a higher ratio of phosphofructokinase to 3-hydroxacyl-CoA dehydrogenase in men than in women, suggesting a greater enzymatic potential for glycolysis in men (11). Future work needs to establish how circulating factors (hormones and substrates) and muscle metabolic characteristics dictate the gender-based preferences for carbohydrate vs. lipid fuels.
Similar to previous observations, plasma β-HBA levels were significantly increased in men and women after exercise (18). In addition, β-HBA levels were significantly greater in men than in women after exercise. The elevated FFA after exercise provide a source for the production of ketones (22). However, the greater β-HBA level in the men was not associated with a greater FFA concentration in the postexercise period. The accumulation of ketones may lead to errors in the estimation of fuel oxidation (30) after exercise. However, the magnitude of these errors is likely small (30). The contribution of the greater ketone levels in men than in women to postexercise fuel oxidation cannot be estimated, inasmuch as it is not known whether the greater β-HBA accumulation in men was due to more production or less removal. Nor did we measure acetacetate, the other major ketone. Nevertheless, it is unlikely that the magnitude of these postexercise gender-based differences in ketone levels would significantly affect the estimations of fuel oxidation, such that the conclusion of no gender difference in postexercise fuel oxidation would be changed.

Other hormones measured in the present study showed less of a dramatic difference between men and women than the catecholamines. We observed a nonsignificantly greater cortisol level in men on the exercise day during cycling and recovery. No such difference was apparent during the control day. This suggests that cortisol could have some role, although not primary, in determining the gender differences in exercise fuel oxidation. All the gonadotrophic hormones were observed to increase during exercise. This has previously been reported by others (19, 36). This elevation in estradiol, progesterone, and testosterone during exercise appeared to be due to a rise in the free hormone, inasmuch as SHBG levels did not change. It has been suggested that a decreased hepatic clearance of these steroid hormones as a result of a decrease in hepatic blood flow is responsible for the exercise-induced rise in their circulating levels (28). Estradiol levels were very variable in women on the cycling day. This was because subjects were studied at different points of the follicular phase, and estradiol is known to increase from the early through the late part of this phase. Nevertheless, it is possible that the greater increase in estradiol during exercise in women had an effect on fuel metabolism. For example, estrogen has been reported to decrease glucose utilization but may increase lipid availability by stimulating lipolysis (12, 27). Further studies are needed to confirm a role, direct or indirect, for the gonadotropic hormones and especially estradiol in exercise fuel metabolism.

In summary, we observed a significant gender-based difference in the pattern of exercise fuel oxidation in both trained and untrained men and women matched for level of physical activity. Women derived more energy during exercise from fat oxidation and less from carbohydrate oxidation than did men. There were no gender-based differences in relative fuel oxidation under resting conditions before exercise, after exercise, or on a control day. The exercise differences in fuel oxidation between men and women were associated with a significant gender-based difference in the catecholamine response to exercise. Men had a greater elevation in both epinephrine and norepinephrine levels during exercise. It is hoped that future studies will provide insight into the sources of the different fat and carbohydrate fuels that contribute to the observed gender-based differences in exercise fuel oxidation and also the hormonal and/or metabolic mechanisms involved in determining the gender-specific pattern of fuel oxidation. Finally, it could be speculated that, because of their reproductive role, women have a greater priority for carbohydrate conservation than men under conditions of increased energy demand.

The authors thank all the subjects who volunteered for the study and greatly appreciate their time and cooperation. The authors also thank the GCRC Core Laboratory and especially Teddi Wiest Kent for assistance with analytic aspects of the study.

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