Substrate utilization during endurance exercise in men and women after endurance training

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Carter, S. L., C. Rennie, and M. A. Tarnopolsky. Substrate utilization during endurance exercise in men and women after endurance training. Am J Physiol Endocrinol Metab 280: E898–E907, 2001.—We investigated the effect of endurance training on whole body substrate, glucose, and glycerol utilization during 90 min of exercise at 60% peak O2 consumption (VO2peak) in males and females. Substrate oxidation was determined before and after 7 wk of endurance training on a cycle ergometer, with posttesting performed at the same absolute (ABS, W) and relative (REL, %VO2peak) intensities. [6,6-2H]glucose and [1,1,2,3,3-2H]glycerol tracers were used to calculate the respective substrate tracee flux. Endurance training resulted in an increase in VO2 peak for both males and females of 17 and 22%, respectively (P < 0.001). Females demonstrated a lower respiratory exchange ratio (RER) both pretraining and posttraining compared with males during exercise (P < 0.001). Glucose rate of appearance (Ra) and rate of disappearance (Rd) were not different between males and females. Glucose metabolic clearance rate (MCR) was lower at 75 and 90 min of exercise for females compared with males (P < 0.05). Glucose Ra and Rd were lower during exercise at both ABS and REL posttraining exercise intensities compared with pretraining (P < 0.001). Females had a higher exercise glycerol Ra and Rd compared with males both pre- and posttraining (P < 0.001). Glycerol Rd was not different at either the ABS or REL posttraining exercise intensities compared with pretraining. We concluded that females oxidize proportionately more lipid and less carbohydrate during exercise compared with males both pre- and posttraining, which was cotemporal with a higher glycerol Rd in females. Furthermore, endurance training resulted in a decrease in glucose flux at both ABS and REL exercise intensities after endurance training.

ENDURANCE EXERCISE TRAINING results in adaptive changes in muscle metabolic function characterized by a decrease in carbohydrate utilization and an increase in lipid oxidation when tested at the same absolute exercise intensity (21, 28, 34). The reduction in the reliance on carbohydrate oxidation during exercise includes a sparing of muscle glycogen (27, 33, 34) and a decrease in the rates of appearance and oxidation (13, 28, 34) of plasma glucose. There is consistency in the observed increase in mitochondrial potential and β-oxidation maximal enzyme capacity (21, 33), yet controversy exists regarding the source of the oxidized free fatty acids (FFA). For example, some studies have found an increase in intramuscular triglyceride utilization (21, 33, 34), whereas others have found an increase in plasma FFA uptake (1, 25) and peripheral adipocyte lipolytic sensitivity (6, 7). Although the metabolic adaptations to endurance exercise have been extensively reproduced (13, 27, 28, 32–34), almost all of these adaptations have been reported in studies involving exclusively or predominantly male participants. Consequently, less is known about the response of females to endurance exercise training.

Cross-sectional studies have found gender differences in the metabolic response to submaximal endurance exercise characterized by a lower respiratory exchange ratio (RER) (14, 20, 31, 41) and attenuated muscle glycogen depletion (10, 40) for females compared with males. Although the results from these studies are generally consistent, there remains a controversy regarding the most appropriate method of matching the genders on the basis of oxygen consumption (i.e., relative to total vs. fat-free mass) and training history. A longitudinal design eliminates the issue of potential gender differences in training background by exposing males and females to a similar training stimulus.

McKenzie et al. (27) found that leucine and total carbohydrate oxidation was higher in males compared with females during exercise both before and after endurance exercise training. Another study reported that, compared with males, females had a higher glycerol rate of appearance and lower carbohydrate oxidation during exercise than were present before and after endurance exercise training (14). These latter studies suggested that there may be gender differences in the metabolic response to exercise before and after training (14, 27); however, this remains an incompletely characterized area of investigation.

The purpose of the present study was to investigate the effect of endurance training on whole body substrate oxidation and glucose and glycerol turnover dur-
ing 90 min of exercise at 60% of peak oxygen uptake in both males and females. The longitudinal design allowed for the comparison of the metabolic response of males and females in the untrained as well as the trained state.

**METHODS**

**Subjects.** Sixteen healthy volunteers (8 males and 8 females) participated in the study (Table 1). Informed consent was obtained after a description of the study and advisement of the risks and benefits of participation, in accordance with and prior approval of the Research Ethics Committee.

**Protocol.** A progressive exercise test on an electronically braked cycle ergometer was used to determine peak oxygen consumption (\(V\dot{O}_2\) peak), as previously described (42). The \(V\dot{O}_2\) peak test took place in the 2 wk before the initiation of the study. \(V\dot{O}_2\) peak was used to estimate the workload required to elicit 60% of the subject’s \(V\dot{O}_2\) peak for subsequent testing. Detailed metabolic, dietary, and anthropometric data were collected before (PRE) and after (POST) a 7-wk endurance exercise training program. Exercise testing was completed at 60% \(V\dot{O}_2\) peak for PRE training and at the same workload POST training (ABS; absolute trial). In addition, we completed a posttraining exercise testing session at 60% of the new POST training \(V\dot{O}_2\) peak (REL; relative trial). The order of the POST trials was randomized for each individual, and trials were completed within 3–5 days of each other to ensure that the females were tested in the early to midfollicular phase of the menstrual cycle. The exercise program consisted of 7 wk of cycle training with a 5:2 (exercise-rest day) protocol. Each session was 60 min in duration at an intensity of 60% \(V\dot{O}_2\) peak. After 3 wk of training, a second progressive exercise test was administered to reevaluate \(V\dot{O}_2\) peak, and adjustments were made to training intensities to ensure a progressive training stimulus for each subject.

Body composition (fat-free mass, fat mass, and percent body fat) was determined using dual-energy X-ray absorptiometry, or DEXA, as previously described (29). These measurements were determined 1 wk before the initiation and after the 7th wk of endurance exercise training. Subjects recorded their dietary intake for 4 days (one weekend and three weekdays) in the week before the initiation of training and during the 7th wk of training. Diets were analyzed using a computer-based nutrient analysis program (Nutritionist IV, N-Squared Computing, Silverton, OR). Participants were given an individual checklist diet to consume (and a checklist to record it) the day before each exercise trial (Table 2).

On the morning of the exercise trials, the participants arrived at the laboratory 3 h postabsorptive. Each participant was given a defined formula drink to consume 3 h before exercise testing. A blood sample was collected before the initiation of the exercise session [males 11%, and females 12%, of total daily energy intake: 60% carbohydrate (CHO); 30% fat; 10% protein]. Exercise trials were conducted at the same time of day for each participant and under identical environmental conditions (21 ± 2°C, 50–70% relative humidity).

Upon the subject’s arrival at the laboratory, a 20-gauge plastic catheter was inserted in a retrograde fashion into the antecubital vein for the infusion of the tracers with an infusion pump (model 74900, Cole-Palmer). A second catheter was inserted in an identical manner into the contralateral antecubital vein for subsequent blood collection. The sampling arm was placed in a heating pad (65 ± 5°C) to “arterialize” the blood for the entire duration of the experiment. [6,6-2H]glucose and [1,1,2,3,3,3-2H]glycerol (99% isotopic purity) were purchased from CDN Isotopes (Pointe Claire, QC, Canada). Glucose and glycerol were mixed with 0.9% saline and filtered through a 0.2-μm filter immediately before infusion. A blood sample was collected before the initiation of the infusion (~90 min) for the determination of the natural background isotopic enrichment of glucose and glycerol. A priming dose of glucose (17 μmol/kg) and glycerol (1.5 μmol/kg) tracers were given, followed by a constant infusion at the rates of ~0.22 μmol/kg/min or ~0.05 μmol/kg/min for glucose and glycerol tracers, respectively. Subjects

### Table 1. Descriptive characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Males (n = 8)</th>
<th>Females (n = 8)</th>
<th>Gender Difference (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>22 ± 1</td>
<td>22 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>Height, cm</td>
<td>176.6 ± 3.2</td>
<td>165.7 ± 1.2</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>78.1 ± 2.5</td>
<td>66.6 ± 3.0</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>FFM, kg</td>
<td>65.6 ± 2.5</td>
<td>48.9 ± 1.7</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>12.5 ± 1.0</td>
<td>17.8 ± 1.5</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>16.1 ± 1.4</td>
<td>26.4 ± 2.2</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>(V\dot{O}_2) peak, ml·kg·min(^{-1})</td>
<td>41.5 ± 2.4</td>
<td>32.3 ± 1.6</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>(V\dot{O}_2) peak, ml·kg·FFM·min(^{-1})</td>
<td>49.5 ± 2.1</td>
<td>45.7 ± 1.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. FFM, fat-free mass; \(V\dot{O}_2\) peak, peak oxygen consumption; NS, not significant. *Significantly different from pretraining (\(P < 0.01\)); †significantly different from pretraining (\(P < 0.001\)).

### Table 2. Dietary composition PRE and POST endurance training

<table>
<thead>
<tr>
<th></th>
<th>Total kcal</th>
<th>PRO, g</th>
<th>CHO, g</th>
<th>FAT, g</th>
<th>PRO, %</th>
<th>CHO, %</th>
<th>FAT, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>3,005 ± 322</td>
<td>110.2 ± 11.1</td>
<td>395.0 ± 39.7</td>
<td>113.0 ± 15.2</td>
<td>15 ± 1</td>
<td>52 ± 2</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>POST</td>
<td>3,402 ± 256</td>
<td>132.8 ± 11.2*</td>
<td>479.0 ± 41.0</td>
<td>111.3 ± 11.3</td>
<td>16 ± 1*</td>
<td>56 ± 2</td>
<td>29 ± 2*</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>1,861 ± 110†</td>
<td>66.3 ± 8.4†</td>
<td>271.0 ± 21.6†</td>
<td>61.0 ± 7.6†</td>
<td>14 ± 1</td>
<td>57 ± 3</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>POST</td>
<td>2,155 ± 277‡</td>
<td>89.8 ± 10.7†‡</td>
<td>320.4 ± 34.1†‡</td>
<td>60.5 ± 13.6†‡</td>
<td>17 ± 1*</td>
<td>59 ± 3</td>
<td>24 ± 2‡</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 8). kcal, Kilocalories; PRO, protein; CHO, carbohydrate. PRE, pretraining; POST, posttraining. *Significantly different from PRE (\(P < 0.05\)); †significantly different from males (\(P < 0.01\)); ‡significantly different from males (\(P < 0.001\)).
were infused for 90 min at rest, before the onset of exercise. At the onset of exercise, the infusion rates were increased in a stepwise fashion to −0.55 and −0.125 μmol·kg⁻¹·min⁻¹, respectively, for glucose and glycerol tracers, as previously described (4). The infusion rate remained at −0.55 and −0.125 μmol·kg⁻¹·min⁻¹ for glucose and glycerol tracers for the remainder of the 90 min of exercise. Blood samples were drawn at 75 min after the initiation of the constant infusion (−15 min), at rest (0 min), and at 30, 60, 75, and 90 min during exercise. (Thus the first blood sample for tracer kinetic determination was taken at 4 h and 15 min after the consumption of the high-CHO snack drink described above.) Blood samples were collected into heparinized tubes and centrifuged immediately, and the plasma was stored at −50°C for subsequent analysis. For catecholamine determination, 5 ml of whole blood were added to a tube containing 100 μl of EDTA and reduced glutathione. The tube was centrifuged at 2,000 for 10 min, and the plasma was stored at −80°C for subsequent analysis. Blood samples collected for hormone analyses were allowed to stand for 10 min in unheparinized tubes and then were centrifuged, and the serum was stored at −50°C for subsequent analysis. 

Respiratory measurements were made using a computerized open-circuit gas collection system as described previously (42). Respiratory gases were collected at rest and at 30, 60, 75, and 90 min of exercise. Heart rate was also monitored continuously throughout the 90 min of exercise and was recorded at the same time points as respiratory gases. The proportions of CHO and lipid utilized were calculated using the RER corrected for protein oxidation (11). Protein oxidation rates were estimated from leucine oxidation rates measured in a previous study in our laboratory (27), with the assumption that tissue protein contains 590 μmol of leucine/g.

Analysis. Plasma lactate and glucose concentrations were analyzed with a blood glucose and lactate analyzer (YSI model 2300 STAT Plus, Yellow Springs Instrument, Yellow Springs, OH). Plasma glycerol concentration was determined by using an enzymatic colorimetric assay (Triglyceride-GPO-Trinder), Sigma Diagnostics, St. Louis, MO.

Resting serum samples were analyzed for 17β-estradiol, progesterone, testosterone, and insulin. Insulin was also analyzed during exercise. 17β-Estradiol, progesterone, testosterone, and insulin were analyzed using a single incubation radioimmunassay (Coat-a-Count: kit nos. TKE22, TKEP1, TKTE1, and TKIN5; Diagnostics Products, Los Angeles, CA). Serum FFA concentrations were determined at rest and during exercise using an enzymatic colorimetric assay (NEFAC-ACS, Wako Chemicals, Richmond, VA). Catecholamines (epinephrine and noradrenaline) were analyzed using high-performance liquid chromatography (injector WISP 710B and pump model 570, Waters, Milford, MA) with electrochemical detection (Coulochem II, ESA, Chelmsford, MA), as previously described (4).

Isotopic enrichment of glucose and glycerol was determined using gas chromatography-mass spectrometry (GC model 6890 and MS model 5973, Hewlett-Packard, Fullerton, CA) of the pentacacetate and tris-trimethylsilyl derivatives, respectively, as previously described (4). Mass analysis was performed in the electron impact ionization (EI⁺) mode to monitor selected ions with a mass-to-charge ratio (m/z) of 200 and 202 atomic mass units (amu) for glucose enrichment and an m/z of 205 and 208 amu for glycerol enrichment.

Rates of appearance (Ra) and disappearance (Rd) of glucose and glycerol were calculated according to the Steele equation (38), which was modified for use with stable isotopes according to Romijn et al. (35), because the amount of tracer infused is no longer considered negligible. Enrichment and concentration data were fitted to curves by use of spline fitting (35); then the kinetics were calculated as described in the previous paragraph. The volume of distribution was assumed to be 100 ml/kg for glucose and 230 ml/kg for glycerol (35). We attempted to minimize the changes in enrichment by increasing the infusion rate in step increments when exercise was initiated, as previously described (4).

Statistical analysis. The physical characteristics of the participants and the hormone concentrations were analyzed using a two-way analysis of variance (ANOVA). All other data were analyzed using a three-way ANOVA, with gender being the between-factor variable, condition (PRE, ABS, and REL) being the first within-factor variable, and time (t = 0, 30, 60, 75, 90 min) being the second within-factor variable. When significance was obtained, the location of the difference was determined using Newman-Keuls post hoc analysis. The level of significance was set at P ≤ 0.05. Values are presented as means ±SE.

RESULTS

Physical characteristics. Males had a significantly higher absolute VO₂peak than females; however, there was no significant difference between the genders in relative VO₂peak when expressed per kilogram of fat-free mass (FFM). Training resulted in a significant increase (P < 0.001) in VO₂peak for both males and females (Table 1). Male participants were heavier, taller, and leaner than the female participants. Body mass and FFM were unchanged, whereas fat mass and percent body fat decreased (P < 0.01) after training for both males and females (Table 1).

Diet. Total energy intake was not altered by training (Table 2). The proportion of energy derived from CHO was not changed, whereas that derived from fat was decreased and that from protein was increased, after training. Males had a higher total energy intake compared with females, yet the proportion of energy derived from fat, CHO, and protein was not different between males and females (Table 2).

Basal hormone levels. Seven weeks of endurance training had no effect on resting serum testosterone concentration in males (19.3 ± 1.8 → 21.5 ± 2.8 nmol/l) or females (1.6 ± 0.3 → 1.3 ± 0.3 nmol/l), and males had a significantly higher testosterone concentration compared with females (P < 0.001). Endurance training did not alter resting serum 17β-estradiol concentration in males = 96.3 ± 13.9 → 161.2 ± 20.9 pmol/l; females = 119.3 ± 31.6 → 139.5 ± 40.4 pmol/l). Because the females were tested during the early to mid follicular phase of their menstrual cycle, resting serum 17β-estradiol concentrations were not significantly different between the genders. Serum progesterone concentrations were not different for females before and after training (PRE = 3.5 ± 0.5 nmol/l, POST = 2.1 ± 0.4 nmol/l), which ensured that they were tested in the early to mid follicular phase of their menstrual cycle.

For clarity of Figs. 1–3, we have collapsed the data across gender (see A, C, and E) to show the training effect(s) and have demonstrated the gender effects by collapsing across exercise trials (B, D, and F).
Exercise trial (90-min). By design, the percentage of VO₂ peak elicited in the REL exercise trial was not different from that elicited in the PRE-training exercise trial, yet the absolute power output (in W) was higher. Oxygen consumption (VO₂) was greater in the REL exercise trial compared with both the PRE and ABS trials (Fig. 1A). At 75 and 90 min, VO₂ was lower for the ABS compared with PRE trial (P < 0.001) despite the workloads being the same (Fig. 1A). Females had a lower absolute VO₂ during the exercise trial compared with males (Fig. 1B). Endurance training resulted in a lower RER in the ABS trial, but not in the REL trial, compared with PRE (P < 0.001). RER increased from rest at the onset of exercise in all exercise trials (Fig. 1C), and females had a lower exercise RER compared with males (P < 0.01; Fig. 1D). Heart rate was lower during exercise in the ABS trial compared with both the PRE and REL trials (P < 0.001; data not shown). Resting heart rate was significantly lower after endurance training in both the ABS and REL compared with the PRE trial (P < 0.001; data not shown). Males had a lower resting heart rate compared with females (P < 0.05; data not shown).

Endurance training resulted in an increase in the proportion of fat oxidized during exercise at the same ABS workload (P < 0.01); however, the proportion of fat oxidized at the same REL intensity was not affected by training. Consequently, training resulted in a decrease in the proportion of CHO oxidized during exercise at the same ABS (P < 0.01), but not REL, intensity (Fig. 1E). Females oxidized a greater percentage of energy from fat during exercise compared with males (P < 0.001; Fig. 1F); conversely, males oxidized a higher percentage of CHO compared with females during exercise in all trials (P < 0.01; Fig. 1F).

Plasma lactate concentration was lower during exercise (P < 0.001) in the ABS trial compared with both the PRE and REL trials (Fig. 2F). Plasma lactate

Fig. 1. Oxygen consumption (VO₂), respiratory exchange ratio (RER), and substrate oxidation. A: VO₂ during 90 min of exercise before (PRE) and after (absolute (ABS) and relative (REL)) training. *Significantly different from PRE and ABS (P < 0.001); †significantly different from PRE (P < 0.001). B: gender difference in VO₂ during 90 min of exercise. *Significantly different from males (P < 0.001). C: RER during 90 min of exercise before (ABS) and after (ABS and REL) training. Main effect for condition: ‡P < 0.01 (ABS < PRE and REL). D: gender difference in RER during 90 min of exercise. Main effect for gender: *P < 0.01 (females < males). E: effect of training on the proportion of substrate utilized during 90 min of exercise. Main effect of condition: *P < 0.01 (ABS < PRE and REL). F: gender differences in the proportion of substrate utilized during 90 min of exercise. Main effect for gender: *P < 0.01 (females < males); ‡P < 0.001 (males < females).
concentration was significantly lower in the REL trial compared with PRE at 30 min of exercise only (Fig. 2F). Plasma lactate concentration increased from rest at the onset of exercise in all three conditions (Fig. 2F). There was no difference in plasma lactate concentration between males and females at any time point.

After training, glucose $R_a$ and $R_d$ were significantly lower for both the ABS and REL trials at all exercise time points compared with PRE ($P < 0.001$), with no effect on resting glucose $R_a$ or $R_d$. Glucose $R_a$ and $R_d$ were significantly lower in the REL trial at 60, 75, and 90 min of exercise than in PRE, but they were greater than in ABS ($P < 0.001$; Fig. 2A). There were no differences in glucose $R_a$ or $R_d$ between males and females before or after training (Fig. 2B). Metabolic clearance rate (MCR) was lower ($P < 0.001$) during exercise in both the ABS and REL trials compared with PRE (Fig. 2C). The MCR of glucose was lower ($P < 0.05$) for females at 75 and 90 min of exercise compared with males (Fig. 2D). Endurance training resulted in a higher ($P < 0.05$) plasma glucose concentration during exercise in the REL compared with both the PRE and
ABS trials, which were not different from each other (Fig. 2E).

Plasma glucose concentration increased from rest to 60 min of exercise ($P < 0.05$) and then decreased to resting levels by 75 min of exercise (Fig. 2E). Plasma glucose concentration was not significantly different between the genders at rest or during exercise; however, there was a trend toward females having a higher plasma glucose concentration throughout exercise compared with males ($P = 0.056$; Table 3). The kinetics of plasma glucose are summarized in Table 3.

Plasma epinephrine concentration increased with exercise in both males and females ($P < 0.05$).

Endurance training did not alter glycerol $R_a$ and $R_d$ at rest or during exercise. Acute exercise increased glycerol $R_a$ and $R_d$ before and after training ($P < 0.001$; Fig. 3A). Glycerol $R_a$ and $R_d$ were significantly higher for females compared with males at all time points ($P < 0.01$; Fig. 3B). Plasma glycerol concentration increased at the onset of exercise, but at 75 and 90 min, values were lower ($P < 0.05$) during the ABS trial compared with both the PRE and REL trials (Fig. 3C). Plasma glycerol concentration was not different between males and females (Fig. 3D). There was an increase in FFA concentration with exercise, with FFA concentration being higher in the PRE trial at 90 min of exercise compared with the REL trial. There was no difference in FFA concentration between the ABS and REL trials at 90 min of exercise (Fig. 3E). Females had a significantly higher serum FFA concentration compared with males ($P < 0.05$; Fig. 3F).

Serum insulin concentrations at rest and during exercise were not affected by exercise training. Serum insulin concentrations decreased during exercise and

### Table 3. Summary of glucose kinetics during endurance exercise

<table>
<thead>
<tr>
<th>Gender</th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>PRE</th>
<th>ABS</th>
<th>REL</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
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</tr>
<tr>
<td>M</td>
<td>10.44 ± 0.31</td>
<td>10.69 ± 0.99</td>
<td>25.92 ± 2.73</td>
<td>16.65 ± 1.65*</td>
<td>19.18 ± 2.00</td>
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<tr>
<td>F</td>
<td>10.06 ± 0.95</td>
<td>11.23 ± 0.59</td>
<td>21.58 ± 2.32</td>
<td>15.52 ± 1.59*</td>
<td>18.76 ± 1.55</td>
</tr>
<tr>
<td>M</td>
<td>10.43 ± 0.31</td>
<td>10.69 ± 0.46</td>
<td>26.44 ± 2.94</td>
<td>17.22 ± 1.61*</td>
<td>19.27 ± 2.02</td>
</tr>
<tr>
<td>F</td>
<td>10.06 ± 0.73</td>
<td>11.23 ± 0.59</td>
<td>21.75 ± 2.21</td>
<td>15.59 ± 1.61*</td>
<td>18.62 ± 1.59</td>
</tr>
<tr>
<td>M</td>
<td>19 ± 2</td>
<td>14 ± 1</td>
<td>11 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>27 ± 4</td>
<td>23 ± 5</td>
<td>14 ± 1</td>
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</tr>
<tr>
<td>M</td>
<td>2.23 ± 0.08</td>
<td>2.29 ± 0.11</td>
<td>5.69 ± 0.66</td>
<td>3.56 ± 0.35*</td>
<td>3.94 ± 0.39*</td>
</tr>
<tr>
<td>F</td>
<td>2.05 ± 0.19</td>
<td>2.34 ± 0.11</td>
<td>4.33 ± 0.47</td>
<td>3.17 ± 0.29*</td>
<td>3.51 ± 0.36*</td>
</tr>
<tr>
<td>M</td>
<td>4.69 ± 0.07</td>
<td>4.59 ± 0.07</td>
<td>4.69 ± 0.11</td>
<td>4.87 ± 0.10</td>
<td>4.90 ± 0.00 17‡</td>
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<tr>
<td>F</td>
<td>4.91 ± 0.05</td>
<td>4.84 ± 0.27</td>
<td>5.06 ± 0.12</td>
<td>4.91 ± 0.22</td>
<td>5.37 ± 0.18 13‡</td>
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</tbody>
</table>

Values means ± SE ($n = 8$). PRE, pretraining; ABS, absolute exercise trial; REL, relative exercise trial; $R_a$, glucose rate of appearance; $R_d$, glucose rate of disappearance; MCR, metabolic clearance rate; CHO, carbohydrate. *Significantly different from PRE ($P < 0.001$); †significantly different from ABS ($P < 0.001$); ‡significantly different from PRE and ABS ($P < 0.005$); §significantly different from PRE ($P < 0.05$). For detailed results see RESULTS.

### Table 4. Catecholamine responses during prolonged exercise

<table>
<thead>
<tr>
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<th>Time, min</th>
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</tr>
<tr>
<td>Norepinephrine, nmol/l</td>
<td>PRE</td>
</tr>
<tr>
<td>Males (n = 8)</td>
<td>ABS</td>
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<tr>
<td>Females (n = 8)</td>
<td>REL</td>
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<tr>
<td>Epinephrine, pmol/l</td>
<td>PRE</td>
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<tr>
<td>Males (n = 8)</td>
<td>ABS</td>
</tr>
<tr>
<td>Females (n = 8)</td>
<td>REL</td>
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</tr>
<tr>
<td>ABS</td>
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<tr>
<td>REL</td>
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<tr>
<td>PRE</td>
<td>300 ± 77</td>
</tr>
<tr>
<td>ABS</td>
<td>186 ± 33</td>
</tr>
<tr>
<td>REL</td>
<td>202 ± 27</td>
</tr>
</tbody>
</table>

Values are means ± SE. For norepinephrine, *significantly different from 0 min ($P < 0.001$); †significantly different from ABS during exercise ($P < 0.001$). For epinephrine, *significantly different from 0 min ($P < 0.05$); †significantly different from PRE males at 90 min ($P < 0.05$); ‡significantly different from PRE females at 90 min ($P < 0.05$).
were significantly lower than at rest at 60 and 90 min of exercise for both genders \( P < 0.01; \) data not shown.

**DISCUSSION**

We found that females oxidized proportionately more fat than males during all exercise trials. There was no gender difference in glucose \( R_a; \) however, females had a lower MCR at later exercise time points compared with males. Endurance training resulted in an increase in \( \dot{V}O_2 \) peak of similar magnitude for both males and females. CHO utilization was lower after endurance training during exercise at the same \( \dot{V}O_2 \) peak; however, glucose \( R_a \) and MCR were lower after endurance training at both the \( \dot{V}O_2 \) peak and REL exercise intensities.

**Gender differences.** Before and after training, females had a lower RER than males. These findings support the observations that females oxidized a greater proportion of fat compared with males during acute exercise at the same REL intensity \( (20, 31, 41) \). Females were also found to have a higher glycerol \( R_a \) compared with males. The higher glycerol \( R_a \) in females suggested that females have an increased rate of lipolysis compared with males, although the source of the lipid remains unclear. Similar findings have been reported in one training study in which males \( (15) \) were compared with data previously reported by the same group in female subjects \( (12) \). Studies investigating gender differences in the metabolic response to a single bout of prolonged submaximal exercise have found that females have a lower RER \( (20, 31, 41) \), attenuated muscle glycogen utilization \( (10, 40) \), and lower leucine oxidation \( (27) \) compared with males. The overall conclusion is that females oxidize a greater proportion of fat and less CHO and amino acids compared with males.
Although there was no gender difference in glucose Ra before or after training at either exercise intensity, glucose MCR was lower in females compared with males at later time points during endurance exercise. Females were also found to have a strong trend toward a higher plasma glucose concentration \((P = 0.056)\) compared with males. These findings demonstrated that females have a lower skeletal muscle glucose uptake compared with males for a given glucose concentration. Two studies have found that 17β-estradiol administration can decrease glucose Ra during submaximal exercise in humans \((4, 37)\). We have previously shown a better maintenance of plasma glucose and a lower glucose MCR and Ra during endurance exercise in males given 17β-estradiol \((4)\).

Another example of the effect of 17β-estradiol on plasma glucose maintenance comes from experiments using peroxisome proliferator-activated receptor-α double-knockout (PPARα \(-/-\)) mutant mice \((8)\). This group found that all male, but only 25% of female, PPARα \(-/-\) mice given etomoxir (an inhibitor of carnitine palmitoyl transferase I) developed fatal hypoglycemia \((8)\). The hypoglycemic effect of the PPARα \(-/-\) genotype + etomoxir was prevented entirely when the male mice were pretreated with 17β-estradiol \((8)\). These findings indicate that 17β-estradiol can mediate glucose Ra and the maintenance of plasma glucose during metabolic stress situations. Given that the plasma 17β-estradiol concentration was identical in the current study between males and females at the time of testing, a potential role for 17β-estradiol would not be occurring acutely.

Previously, we also found that transdermal 17β-estradiol administration had no effect on skeletal muscle glycogen utilization in males \((43)\). The marked hepatic glycogen sparing \((23, 24)\) and the lack of an effect on GLUT-4 content \((39)\) suggest that 17β-estradiol has a more significant effect on hepatic glycogen production than on skeletal muscle glucose uptake. These observations are similar to animal data demonstrating muscle and hepatic glycogen sparing \((23, 24)\) and increased lipid oxidation \((18)\) in 17β-estradiol-supplemented male or oophorectomized female rats.

In the present study, the lower epinephrine concentration in females was cotemporal with a lower glucose MCR at 90 min compared with males. This observation is similar to the results of Ruby et al. \((37)\), who demonstrated that the provision of 17β-estradiol to amenorrheic females reduced plasma epinephrine concentration during endurance exercise. It is also similar to previous findings from our laboratory, in which the 17β-estradiol administered to males resulted in a significantly lower glucose Ra and MCR and a trend \((P = 0.09)\) toward a decrease in plasma epinephrine concentration \((4)\).

**Training.** There was a decrease in total CHO utilization during exercise after endurance training when tested at the same ABS workload compared with pretraining. However, there was no change in the proportion of CHO utilized during exercise at the same REL intensity posttraining. Our results are similar to those of others \((21, 34)\) who have found a decrease in CHO utilization \((2, 28, 34)\) and an increase in fat utilization \((21, 27, 34)\) during prolonged exercise at the same ABS workload after endurance exercise training. Friedlander and colleagues \((13, 14)\) also did not find a decrease in CHO oxidation for either men or women during exercise at the same REL intensity after 10–12 wk of endurance training. Together, these data demonstrate that, despite an increase in fat utilization after endurance exercise training when subjects were tested at the same ABS exercise intensity (which is a lower percentage of the V o2 peak posttraining), there is no increase in the proportion of fat utilized at the same REL intensity. It is possible that the duration of training was not sufficient for seeing adaptations in lipid oxidation with a longitudinal study design, for cross-sectional studies have shown that well trained endurance athletes have a lower RER compared with untrained persons at the same REL-intensity exercise \((5, 22)\).

Resting glucose Ra and Rd were not altered by endurance training. However, glucose Ra and Rd and MCR were decreased during exercise at both the ABS and REL exercise intensities after endurance training. These findings are in agreement with previous longitudinal training studies \((1, 13, 14, 28, 34)\) showing that there is a decrease in CHO utilization and glucose Ra at the same absolute workload after as few as 10 days of training \((28, 32)\). These findings are also in agreement with previous cross-sectional research \((5, 22)\) illustrating that trained individuals have a reduction in plasma glucose uptake compared with untrained individuals. Decreased glucose Ra during exercise at the same REL exercise intensity has been observed with cross-sectional studies comparing trained and untrained individuals \((5, 22)\); however, it has not yet been observed in longitudinal training studies \((13, 14)\). Friedlander and colleagues \((13, 14)\) did not find an attenuation in glucose Ra or MCR at the same REL intensity after training in either males or females. The differing results regarding glucose Ra and MCR between our study and previous studies by Friedlander and coworkers \((13, 14)\) may be associated with the intensity of the exercise trials. The exercise trials in the present study were conducted at \(~60\%\) of V o2 peak, whereas those in the previous exercise trials \((13, 14)\) were performed at a higher intensity \((~65 \text{ V o2 peak})\). Furthermore, at higher exercise intensities the proportion of CHO oxidation increases \((3, 35, 36)\), which may supersede any training-induced attenuation of glucose Ra.

In the present study, the decrease in glucose Ra and MCR, in conjunction with a similar RER during exercise at the same REL intensity after training, illustrated that there was not a net decrease in CHO utilization but rather a shift in the source of CHO utilized. If there is a decrease in plasma glucose uptake and no change in RER, then there must be a compensatory increase in skeletal muscle glycogen utilization. With endurance exercise training there is an increase in resting skeletal muscle glycogen concentration \((22, 27, 33, 34)\) and an increased rate of glycogen resynthe-
sis after exercise (16, 19). Although many studies have found an attenuation of skeletal muscle glycogen utilization at the same ABS exercise intensity after endurance training, there is limited research on the glycogen utilization before and after endurance exercise training at the same REL intensity. McKenzie et al. (27) found that the absolute change in glycogen concentration was similar during exercise at the same REL intensity before and after training. Another important consideration in the evaluation of the data is that a significant decrease in glucose Ra may amount to an increase in glycogen use that is not measurable with the muscle biopsy technique. It is also possible that whole body RER does not reflect tissue-specific respiratory quotient (RQ). For example, Bergman et al. (2) reported that working muscle RQ and whole body RER can be disparate during endurance exercise (i.e., a reduction in whole body RER with no change in muscle RQ); however, this discrepancy has not been consistently found (30).

Glycerol Ra and Rd were not affected by training at rest or during exercise at either the same ABS or REL intensity. Although an increase in glycerol Ra is sometimes found at rest after training (34), this is not always the case (14). However, when glycerol Ra was compared between trained and untrained individuals, trained individuals were found to have a greater glycerol Ra compared with untrained individuals during exercise (5). Although plasma glycerol concentration is considered an indicator of changes in whole body lipolysis, this is based upon the concept that glycerol can only be taken up by the liver and kidney. A recent study (26) has illustrated that only ~50% of circulating glycerol is taken up by the liver and kidney, suggesting that there is glycerol kinase present in extraliver and extrakidney tissues. Together with the data by Elia et al. (9), which indicated that glycerol may not always be released by skeletal muscle and that glycerol taken up by skeletal muscle may be used for the synthesis of intramuscular triglycerides (17), small changes in whole body lipolysis after 7 wk of endurance training may not be detected with glycerol Rd measurements. As a consequence, although our data demonstrate a higher glycerol Ra in females than in males, both before and after training, it is difficult to speculate on the mechanism(s) behind this observation.

Endurance training resulted in a lower plasma norepinephrine concentration at the same ABS exercise intensity; however, there was no effect of endurance training on plasma norepinephrine concentration at the same REL exercise intensity. Plasma epinephrine was lower at 90 min after subjects trained at the same ABS exercise intensity. The plasma epinephrine concentration at 90 min of exercise paralleled the lower glucose MCR that was observed in females at the later time points during endurance exercise. Our data are similar to those of previous training studies with male participants (21, 34) demonstrating a decrease in plasma norepinephrine and epinephrine concentrations after endurance exercise training when the participants were tested at the same ABS exercise intensity. However, the data are not in complete agreement with those of other investigators (14), who found change in plasma norepinephrine concentration in males, yet females had lower concentrations at both the ABS and REL exercise intensities after training. They also found no change in plasma epinephrine concentration in women; however, males had a higher plasma epinephrine concentration after training at both the ABS and REL exercise intensities (14). As previously addressed, the differences between our data and those of Friedlander et al. (14) may be due to the lower exercise testing intensity in the present study.

In conclusion, we found that there is a shift toward an increase in fat utilization and a decrease in CHO utilization after training at the same ABS exercise intensity. However, there was not an increase in fat utilization after training at the same REL exercise intensity. There is a decrease in plasma glucose uptake during exercise after training during exercise at both ABS and REL exercise intensities. Finally, females have an increased rate of lipolysis and utilize proportionately more fat during endurance exercise compared with males, regardless of training state.

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REFERENCES