Training-induced alterations of carbohydrate metabolism in women: women respond differently from men

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Although the use of stable isotopes has emerged as an important methodology for studying substrate utilization in humans, few studies have used tracers to investigate the effects of exercise intensity and endurance training on substrate flux rates in women. With use of labeled palmitate, Poelhman et al. (41) demonstrated that free fatty acid (FFA) and norepinephrine (NE) turnover increase at rest in response to training in postmenopausal women. In addition, Rudy et al. (43) used labeled glucose to demonstrate that glucose rate of appearance (Ra) was reduced in response to short-term estrogen treatment in amenorrheic females. However, to our knowledge, there are currently no published reports that used stable isotopes to measure the influence of training on glucose flux in women during exercise. Because metabolite concentration does not provide information about turnover rate and the respiratory exchange ratio (RER) alone cannot be used to distinguish between oxidation of blood borne and intracellular substrate sources, tracer studies can add valuable information on the use of specific substrates as energy sources.

Available data on gender differences in substrate utilization suggest that, at rest or for given relative submaximal exercise intensities, women oxidize a smaller proportion of carbohydrate (CHO) relative to lipid than do men, as indicated by lower RER values (24, 46, 47). Furthermore, decreased net glycogen utilization and lower blood lactate concentrations have been observed in women compared with men while both were exercising at similar relative intensities (39, 46). Whereas some studies have shown gender differences at submaximal exercise, others have shown that differences in RER between men and women disappear during higher intensity exercise (16) or when the individuals are highly trained (10, 15).

Some of the discrepancies in data comparing men and women may be attributed to the difficulties involved with accurately matching subjects or with the timing of measurements relative to the menstrual cycle. The ovarian hormones (estrogen and progesterone) are potential effectors of substrate utilization (7, 22, 25, 26), and lower circulating FFA, changed RER, and decreased ability to maintain blood glucose during exercise in women who are in the luteal rather than the follicular phase of the menstrual cycle have been reported (2, 20, 30). However, not all investigators have been able to demonstrate differences related to cycle phase, and there is still debate on whether there are menstrual variations in athletic performance (13, 31, 37).

Because of the limited information available on glucose kinetics in women, the purpose of the present study was to examine the effects of exercise intensity and training on glucose kinetics in female subjects to evaluate the hypothesis that, as was demonstrated in men (14), during hard exercise, blood glucose flux is not affected by training if relative exercise intensity is considered. In addition, to determine whether there are gender differences in the metabolic response to exercise and endurance training, the present investigation pro-
vides data for comparison with those from a similar study that used male subjects.

**METHODS**

Subjects. Eighteen healthy, nonsmoking, sedentary female subjects between the ages of 18 and 35 yr were recruited from the University of California campus community by flyers and mailings. Subjects were recruited in two groups of nine. One subject withdrew from the study before posttraining testing for reasons unrelated to the study protocol, leaving only eight subjects in the second group. Each group followed the same protocol except that different tracers were infused (see Tracer protocol). Subjects were considered sedentary if they had participated in <2 h of regular strenuous activity per week for at least the last year and if they had a peak oxygen consumption (VO\(_{2}\)peak) between 30 and 42 ml·kg\(^{-1}\)·min\(^{-1}\) as determined by a continuous-progressive maximal stress test on the cycle ergometer. To qualify for participation in the study, subjects were required to be diet and weight stable; to have a body fat percent of <30%; to have a regular (26- to 35-day) menstrual cycle; to not be pregnant, lactating, or taking oral contraceptives; and to be disease and injury free as determined by medical questionnaire and physical examination. All subjects provided informed consent, and the study protocol was approved by the University of California Committee for the Protection of Human Subjects (approval no. 93-12-45).

General experimental design. After an initial screening interview and screening tests, two stable isotope infusion trials were performed while the subjects pedaled on a cycle ergometer for 1 h at 45 and 65% of VO\(_{2}\)peak (45UT and 65UT, respectively). All isotope trials were performed on the women in the midfollicular phase of the menstrual cycle (between days 5 and 10 from the first day of menses) and after 36–48 h without exercise training. Isotope trials were performed ~1 mo apart (1 cycle length), and the order of the trials was randomized. Subjects began training 2 days after their second isotope trial and continued for 12 wk. The screening tests were repeated at 4, 8, and 12 wk of training. At ~8 and 12 wk of training, two more isotope trials were performed, one was at the same absolute workload that elicited 65% of pretraining VO\(_{2}\)peak (ABT), and the second was at a workload that elicited 65% of the new, postraining VO\(_{2}\)peak, or the same relative workload (RLT). The two postraining trials were also ~1 mo apart (again matched to the midfollicular phase of the menstrual cycle) and randomized, and training was continued between the two trials. In two cases, because of illness at 8 wk, the women performed both isotope trials during the same cycle (12 wk). In addition, two subjects performed both of their trials during second cycle because the approach of the holiday season and the end of the school term would have prevented completion of the final test at 12–15 wk (the timing problem was due to an increase in cycle duration for 1 subject and a delay in recruitment for the other). However, all subjects had at least 8 wk of training before the posttraining trials began. The trials completed within the same cycle were randomized and performed a minimum of 2 days apart but were still conducted within the 5- to 10-day postmenstrual window of testing. The exact duration of training varied slightly between subjects depending on the length of each woman's menstrual cycle. The timing of the isotope trials was determined by the menstrual cycle phase, not the duration of training. Throughout the training intervention, the subjects were weighed daily and instructed to increase their energy intake to compensate for increased energy expenditure and to ensure weight and body fat stability. Because of the extensive work by Schutz and associates (44), it was deemed necessary to prevent large changes in total body or fat mass because changes in tissue mass are likely to affect insulin action and the balance of substrate utilization, independent of training.

Screening tests. Body composition was determined both by skin-fold measurement and underwater weighing. VO\(_{2}\)peak was determined on an electronically braked cycle ergometer (Monark Ergometric 829E) during a continuous, progressive protocol that increased 25 or 50 W every 3 min until voluntary cessation. Respiratory gases were analyzed (Ametek S-3A1 O\(_2\) and Beckman LB-2 CO\(_2\) analyzers) and recorded by an online, real-time personal computer-based system every minute. Each subject underwent two VO\(_{2}\)peak tests before commencement of the study, and the tests were evaluated on maximal heart rate, RER values (>1.15) and O\(_2\) consumption (VO\(_{2}\)) uniformity to ensure a true maximum effort both before and after training. Three-day dietary records were kept at the beginning, 4 wk into training, and before each posttraining isotope trial to monitor the diet composition and quantity of intake for each subject. Dietary analysis of these records was performed by using the Nutritionist III program (N-Squared Computing, Salem, OR).

Tracer protocol. All subjects were studied in a postabsorptive state in the morning, and dietary intake was monitored for the 24 h immediately preceding each of the four isotope trials. Dinner the night before each trial (12 h) was selected by the individual subject and repeated before each trial. Each subject was given a standardized snack (535 kcal: 17% protein, 53% CHO, 30% fat) to consume before bed, 8–10 h before the trial, and a standardized breakfast (320 kcal: 18% protein, 82% CHO; skim milk and cereal) to consume 1–2 h before reporting to the laboratory. In the first group of nine subjects, on the morning of the trial, a catheter was placed in the radial artery for sampling, and an antecubital venous catheter was placed in the opposite arm for primed continuous infusion of the tracers for 90 min of rest and 1 h of exercise. In the second group of nine subjects, “arterialized” blood samples were obtained by using the “heated-hand vein” technique. Because there was no significant difference in the data obtained in the two groups of subjects, the glucose-flux data were pooled for analysis. The first group of subjects received [1-\(^{13}\)C]glucose, [6,6-\(^{2}\)H\(_2\)]glucose (D\(_2\)-glucose), and [1,1,2,3,3,-\(^{2}\)H\(_2\)]glycerol (D\(_5\)-glycerol) while the second group received [1-\(^{13}\)C]palmitate, [6,6-\(^{2}\)H\(_2\)]glucose, and [1,1,2,3,3,-\(^{2}\)H\(_2\)]glycerol. The glycerol and palmitate kinetics data will be reported separately. After the collection of background blood and expired-air samples, a priming bolus was given and the subjects rested semisupine for 90 min. For both glucose isotopes, the priming doses were 125 times the resting minute infusion rate. By using a Harvard Apparatus syringe pump (model 2400-01, Natick, MA), the resting infusion rate was set at 15 ml/h and the continuous infusion cocktail contained 6.4 mg/ml each of [1-\(^{13}\)C]glucose and [6,6-\(^{2}\)H\(_2\)]glucose. Thus, the resulting infusion rate was 1.6 mg/min for both isotopes. On initiation of exercise, the infusion rate was increased to 45 ml/h (4.8 mg/min) for the two pretraining isotope trials and for the 65% of the old VO\(_{2}\)peak posttraining trial (same ABT). Because of the increased metabolic flux anticipated for the 65% of the new VO\(_{2}\)peak, the exercise infusion rate was increased to 60 ml/h (6.4 mg/min). We chose our infusion rates based on the “steady-state” model that emphasizes constant concentrations and isotopic enrichments to facilitate the calculation of substrate kinetics. On the basis of previous experiments, we selected infusion rates to yield steady and comparable isotopic enrichments for all testing workloads. Because of the smaller mean body size in the female subjects relative to the previously used male subjects, the absolute infusion rates were reduced for the women, but...
the relative relationship of infusion rates between the trials was identical to that of the men (14). Arterial samples were taken at 0, 75, and 90 min of rest and at 5, 15, 30, 45, and 60 min of exercise. All isotopes were obtained from Cambridge Isotope Laboratories (Woburn, MA), diluted in 0.9% sterile saline, pharmaceutically tested for sterility and pyrogenicity (University of California San Francisco School of Pharmacy, San Francisco, CA), and, on the day of the experiment, passed through a 0.2-µm Millipore filter (Nalgen, Rochester, NY).

At each of the blood-sampling time points, respiratory gas exchange was determined by using the same system described above, and a sample of expired air was collected in a 10-ml vacuum container to determine $^{13}$CO$_2$ isotopic enrichment. The expired-air samples were stored at room temperature until they were analyzed by using isotope ratio mass spectrometry (IRMS) by Metabolic Solutions (Merrimack, NH). Heart rate was recorded throughout rest and exercise by using a Quinton Q750 electrocardiogram (Seattle, WA). Hematocrit was determined during the last 15 min of rest and exercise to ensure that the measurements of metabolite and hormone concentrations were not influenced by changes in plasma volume.

Blood-sample collection and analysis. Blood samples for the analysis of glucose and lactate concentration and glucose isotopic enrichment were collected in 8% perchloric acid. Plasma glucose concentration was determined by using a hexokinase enzymatic kit (Sigma Chemical, St. Louis, MO), and plasma lactate concentration was determined by using the method of Gutmann and Wahlefeld (19). Glucose isotopic enrichment was measured by using gas chromatography-mass spectrometry (GCMS) (GC model 5890 series II and MS model 5989A, Hewlett-Packard) of the pentaacetate derivative. Plasma catecholamine concentrations were determined by using HPLC with electrochemical detection (35). The details of the GCMS and catecholamine analysis have been described extensively elsewhere (14).

Training protocol. Subjects were required to exercise with a personal trainer in our facility 5 days/wk for 1 h each day on the cycle ergometer. The personal trainers were current undergraduate students in, or recent graduates of, the Department of Human Biodynamics and, for the most part, were competitive or recreational athletes themselves. During the first 3 wk of training, exercise intensity was gradually increased from 50% of each participant's $V_\text{O}_2\text{peak}$ to 75% of their $V_\text{O}_2\text{peak}$. Subjects were asked to warm up for 5 min and stretch before their hour of exercise. The personal trainers used heart rate monitors and data from periodic $V_\text{O}_2\text{peak}$ tests to adjust workloads as the subjects improved. In addition to the supervised training, subjects were required to exercise an additional 1 h on the weekend in any manner they desired. Subjects were weighed daily to ensure that they remained weight stable and were asked to increase their caloric intake without altering their normal dietary composition to match their increased energy expenditure.

Calculations and statistics. The $R_\text{m}$, glucose rate of disappearance ($R_\text{dis}$), and glucose metabolic clearance rate (MCR) were calculated by using equations defined by Steele and modified for use with stable isotopes (48); data and calculations on men have been previously reported in detail (14). Glucose rate of oxidation was calculated by using the IRMS analysis of the expired air samples (14). Energy derived from glucose, total CHO, and lipid oxidation was calculated by using the following equations

$$\% \text{CHO in } V_\text{O}_2 = \left[ \frac{\text{RER} - 0.71}{0.29} \right] \times 100$$

(1)

$$\% \text{lipid in } V_\text{O}_2 = 100 - \% \text{CHO in } V_\text{O}_2$$

(2)

Total energy expenditure

$$= \left[ \% \text{CHO/100} \times V_\text{O}_2 \times 5.05 \text{ kcal/l} \right]$$

$$+ \left[ \% \text{lipid/100} \times V_\text{O}_2 \times 4.7 \text{ kcal/l} \right]$$

Energy from glucose oxidation

$$= (R_{\text{ox}} \times \text{BW/1,000}) \times 4.2 \text{ kcal/g}$$

(3)

(4)

where $V_\text{O}_2$ is in liters per minute, glucose oxidation rate ($R_{\text{ox}}$) is in milligrams per kilogram per minute, and BW represents body weight in kilograms. Numbers for kilocalories per liter oxygen and kilocalories per gram CHO are standard values used in the field (6).

Data are represented as means ± SE. Calculations of steady-state glucose kinetics were made by using the last two (75 and 90 min) and three (30, 45, and 60 min) isotopic enrichment measurements obtained during rest and exercise, respectively. Because the study was designed with two groups of subjects receiving slightly different isotopes, the number of subjects available for particular analyses differs throughout this report. Calculations done by using the $^{13}$C glucose isotope (e.g., $R_{\text{ox}}, \text{recycling rate}$) had nine subjects, whereas all other data such as $R_\text{dis}, R_\text{m}$, and metabolite concentrations were calculated with 17 subjects. To assess differences between the four isotope trials, an analysis of variance with repeated measures was used, and, where appropriate, the Fisher least significant difference test was used for post hoc analysis. Comparisons between genders were performed by using an unpaired two-tailed t-test. Statistical significance was set at $\alpha = 0.05$.

RESULTS

Subject characteristics. Pre- and posttraining characteristics of the 17 women who completed the study are listed in Table 1. Subjects were weight stable throughout the study period and did not lose a significant amount of body fat whether measured by skinfolds or underwater weighing. $V_\text{O}_2\text{peak}$ improved by 25.17 ± 2.43% over the training period. The workload characteristics for the four isotope trials are presented in Table 2. Because the training-induced increase in aerobic capacity, the posttraining trial at the same absolute workload was equivalent to 50% of the subject's new $V_\text{O}_2\text{peak}$. There was a significant exercise intensity and training effect on average exercising heart rate. Training re-

Table 1. Physical characteristics of young women before and after 12 wk of endurance training

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>%Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>23.8 ± 0.2</td>
<td>23.3 ± 0.3</td>
<td>0.0 ± 0.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>64.9 ± 0.8</td>
<td>64.2 ± 0.2</td>
<td>0.0 ± 0.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63.7 ± 2.1</td>
<td>64.2 ± 2.2</td>
<td>0.0 ± 0.5</td>
</tr>
<tr>
<td>Body fat, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UW weighing</td>
<td>24.2 ± 1.0</td>
<td>23.7 ± 1.1</td>
<td>-0.2 ± 0.5</td>
</tr>
<tr>
<td>Skinfolds</td>
<td>24.1 ± 0.9</td>
<td>23.7 ± 1.1</td>
<td>-0.4 ± 0.6</td>
</tr>
<tr>
<td>$V_\text{O}_2\text{peak}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ml·kg$^{-1}$·min$^{-1}$</td>
<td>33.5 ± 1.0</td>
<td>41.6 ± 1.3*</td>
<td>25.2 ± 2.4</td>
</tr>
<tr>
<td>l/min</td>
<td>2.1 ± 0.1</td>
<td>2.6 ± 0.1*</td>
<td>24.9 ± 2.2</td>
</tr>
<tr>
<td>ml·kg·LM$^{-1}$·min$^{-1}$</td>
<td>44.2 ± 0.9</td>
<td>54.5 ± 1.1*</td>
<td>23.3 ± 2.2</td>
</tr>
</tbody>
</table>

*Values are means ± SE for 17 women. UW, underwater weighing; $V_\text{O}_2\text{peak}$, peak $O_2$ consumption; LM, lean mass. *Significantly different from pretraining values, $P \leq 0.05$. 
also demonstrated an intensity effect pretraining four exercise intensities compared with resting values. Related as a percent of total energy expenditure (R dE), the values pre- and posttraining. The same relative intensity, there was no difference in lower (17%) after training. However, when measured at the same ABT and lower at 65%UT than at 45%UT. After training, RdE values were significantly lower during exercise vs. rest to those of R a and are presented as the average of the MCR and exercise intensity among the four trials in Fig. 2. Furthermore, glucose R a increased significantly higher in RLT than ABT. Also, when measured at the same ABT pre- and postraining, R a was significantly lower (17%) after training. However, when measured at the same relative intensity, there was no difference in the values pre- and postraining.

Responses of R d to exercise and training were similar to those of R a and are presented as the average of the last 15 min of rest and 30 min of exercise for the four trials in Fig. 3A. There was a significant intensity effect pre- and postraining, as well as a training effect at the same ABT but not the same RLT. In addition, because blood glucose concentration was not significantly different between exercise trials, the relationship between MCR and exercise intensity among the four trials tracked responses in R d (Fig. 3B). When R d was calculated as a percent of total energy expenditure (R dE), values were significantly lower during exercise vs. rest and lower at 65%UT than at 45%UT. After training, R dE tended to decrease at both the same ABT and RLT, but the decrease did not reach significance (Fig. 3C).

R ox and RER. R ox was significantly higher during all four exercise intensities compared with resting values. Also, R ox demonstrated an intensity effect pretraining and a significant training effect at the same ABT. R ox tended to be lower at the same RLT after training, but the difference was not significant (Fig. 3D).

RER values increased significantly in the transition between rest and exercise. Values for RER during steady-state exercise were significantly higher for the 65%UT trial compared with the 45%UT trial. After training, RER was significantly lower during rest. RER was also lower during steady-state exercise at both the same ABT and RLT compared with the 65%UT (Table 2). However, there were no differences between the two postraining workloads (ABT vs. RLT).

![Image](http://jap.physiology.org)
Glucose recycling rate and lactate concentrations. Glucose recycling rate, calculated as the difference between the $R_a$ values as determined from $[1-13C]$ and $[6,6-^{2}H]$-labeled tracers, estimates the carbon recycling through gluconeogenesis from three carbon precursors, predominantly lactate. The recycling rate in the women tended to follow a pattern similar to $R_a$; however, because of the high SEs and low calculated values for recycling, none of the values were significantly different between trials (Fig. 3E). Blood lactate concentrations were significantly elevated at higher intensities both pre- and posttraining and were significantly lower after training at both the same ABT and RLT (Fig. 3F).

Ovarian hormone responses. All of the isotopic measurements were performed between days 5 and 10 of the menstrual cycle, with a mean day of menses of ~6 for each trial (Table 3). During the early to midfollicular phase of the menstrual cycle, both estrogen (E$_2$) and progesterone (P$_4$) concentrations and variability are lowest (17). We did not measure hormonal variations throughout the cycle, but the resting values of P$_4$ and E$_2$ in our subjects were in the normal range for the midfollicular phase (17). There was no training effect at rest on either P$_4$ or E$_2$ levels. P$_4$ increased significantly between rest and exercise for all but the 45UT trial, and, during exercise, a pattern similar to that seen in the glucose flux data was observed between trials; e.g., concentrations were higher during steady-state exercise at 65UT vs. 45UT, and values for ABT, but not RLT, were lower after training (Table 3). During exercise, E$_2$ increased significantly for all exercise trials. In the first group of nine women, E$_2$ demonstrated a significant intensity and training effect (Table 3). However, the second group of eight women did not show the same effect. The reason for this apparent discrepancy between groups in E$_2$, but not P$_4$, is unclear at this time.

Catecholamine responses. Concentrations of epinephrine (Epi) were significantly higher during all four exercise trials than at rest, but they were not different between any of the exercise intensities (Fig. 4A). NE also was higher during exercise than rest, but, unlike Epi, NE demonstrated both an increase with intensity pretraining, and a decrease during both workloads after training (Fig. 4B).

Contributions from different fuel sources. On the basis of the RER and average Vo$_2$ during rest and exercise for the four isotope trials, the percent contributions of total CHO and lipid were calculated. At rest, >50% of the energy was derived from lipid sources. During exercise in all conditions, there was a shift to a greater reliance on CHO with >50% of the energy coming from CHO sources. A maximum of 73% of energy from CHO sources was obtained in the 65UT trial and a minimum of 53% during the ABT trial (Table 2). The caloric equivalents from the oxidation of blood glucose, other CHO, and lipid fuel sources are presented for rest and the four isotope trials in Fig. 5. Total energy expenditure during exercise was 4.67, 6.63, 6.53, and 8.47 kcal/min for 45UT, 65UT, ABT, and RLT, respectively.
applicable to active adults as well as athletes in a nonlaboratory setting. As athletes and others train and improve, they are capable of performing at higher absolute as well as relative power outputs. Several studies have indicated that highly trained men work at high intensity levels and, therefore, rely predominantly on CHO regardless of their muscle oxidative capacity (40). In one study by Kjær et al. (27), who used male subjects, the glucose kinetics of trained and untrained subjects were compared at maximal workloads. No differences were observed in Rd between groups, although elevated glucose concentrations in the trained group resulted in lower MCRs. Another study by Cogan et al. (9) compared male endurance athletes and untrained individuals for 30 min of exercise at 80% $\dot{V}O_2\text{peak}$ and showed no difference in Rd but a lower Rd in the trained subjects. The present data, obtained from a longitudinal study of women, demonstrated that glucose flux was similar when measured at the same relative intensity (65% of $\dot{V}O_2\text{peak}$) before and after endurance training.

Importance of menstrual cycle phase. We tested all of our female subjects in the midfollicular phase of their menstrual cycles to ensure lower and more consistent levels of E2 and P4 and thus to reduce the variability of ovarian hormone impact on substrate utilization. Estrogen can alter glucose metabolism directly by decreasing gluconeogenesis and insulin-binding capacity (7), whereas P4 has been shown to decrease glucose uptake and oxidation in adipose tissue, decrease hepatic glucose...
neogenesis, increase hepatic glycogen storage, decrease peripheral insulin sensitivity, and increase insulin production by directly affecting the pancreas (25).

Alternatively, the ovarian hormones may impact CHO utilization indirectly by biasing lipid metabolism toward FFA mobilization and oxidation by E2 or FFA storage by P4 (7, 25). Hackney et al. (20) demonstrated elevated levels of lipid oxidation in women who were in the midluteal phase of their menstrual cycles. The differences were observed at rest and 35 and 65% of V\textsubscript{O2}peak, but at 75% of V\textsubscript{O2}peak the differences disappeared. Similarly, Graham et al. (18) showed differences in FFA and lactate levels between amenorrheic and eumenorrheic women during rest and exercise at 30% of maximum capacity but not at 60 and 90% of maximum capacity (18). At higher intensities, it is likely that glycolytic flux governs substrate selection and the impact of mitigating factors such as menses, training, and nutritional status is reduced. Several investigators have shown that women in the follicular phase of their menstrual cycle are more capable of maintaining blood glucose levels during prolonged exercise (15, 46) or after CHO-deficient diet (30) than are men. Ovarian hormones (and androgens) may have a suppressive effect on hepatic gluconeogenesis (30, 46); therefore, in terms of glucose metabolism, women may be more similar to men during the luteal phase of the menstrual cycle. Because we only measured our female subjects during the follicular phase of the menstrual cycle, gender differences observed in this investigation may not be as apparent in women studied throughout all phases of the menstrual cycle.

Gender differences in substrate utilization. Because there are inherent problems in matching subjects of different genders, especially when using a cross-sectional design (e.g., problems accounting for body composition, training status, maximal V\textsubscript{O2}, and relative vs. absolute exercise intensity), it is difficult to obtain directly comparable data on men and women. However, the longitudinal study design, training intervention, and methodologies used in the present investigation on women were identical to an earlier published training study on glucose flux in men (14) and thus provide parallel information for comparison. Although the glucose-flux data were similar (both in magnitude and direction) to the data we obtained on men (Table 4), the magnitudes of total CHO and lipid oxidation differed between genders. During rest and each of the parallel workloads matched as percentage of V\textsubscript{O2}peak, female subjects tended to have lower RER values than did the men and the values were significantly lower in all cases after training (Table 4). Lower RER values in women compared with men during exercise at similar relative intensities have been reported previously in cross-sectional studies that used well-matched subjects (46, 47). Additionally, when women and men are compared,

![Fig. 3—Continued.](image)

Table 3. Menstrual status and hormone concentrations in female subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rest</th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>Pretraining</th>
<th>Posttraining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days past start of menses</td>
<td></td>
<td>Rest: Pretraining</td>
<td>Posttraining</td>
<td>Pretraining (45UT)</td>
<td>Posttraining (65UT)</td>
</tr>
<tr>
<td>Progesterone, ng/ml</td>
<td>0.35 ± 0.03</td>
<td>0.34 ± 0.04</td>
<td></td>
<td>0.54 ± 0.08</td>
<td>0.60 ± 0.05</td>
</tr>
<tr>
<td>Estrogen-group 1, pg/ml</td>
<td>31.7 ± 3.2</td>
<td>27.9 ± 2.5</td>
<td></td>
<td>36.2 ± 6.5</td>
<td>39.5 ± 3.0</td>
</tr>
<tr>
<td>Estrogen-group 2, pg/ml</td>
<td>30.4 ± 3.1</td>
<td>29.0 ± 3.9</td>
<td></td>
<td>41.01 ± 3.6</td>
<td>43.4 ± 5.0</td>
</tr>
</tbody>
</table>

Values are means ± SE for 17 women. All exercise values are significantly different than rest (except progesterone at 45UT). P < 0.05.

*Significantly different between resting conditions, P < 0.05. †Significantly different from pretraining 45% (45UT), P < 0.05. ‡Significantly different from pretraining 65% (65UT), P < 0.05. §Significantly different from posttraining (ABT), P < 0.05.
the lower RER values for a given relative workload in women, despite the similar magnitude of glucose flux values between genders in our data, suggest that a higher percentage of total CHO oxidation could be obtained from blood glucose in women. Also, because the women were working at substantially lower ABTs than were the men, the women had higher glucose-flux values per unit energy expenditure than did the men (Table 4). Observations of decreased RER with similar or relatively greater glucose flux in women are consistent with findings of decreased glycogen utilization and decreased lactate levels in women exercising at the same relative workloads as men (39, 46). We too observed lactate levels that were lower in our female subjects than in the male subjects for a given relative exercise intensity (Table 4).

The mechanism for lower total CHO oxidation in women relative to men has not yet been determined. Some investigators who have analyzed biopsies of the vastus lateralis of training-matched men and women have demonstrated that the women have a higher percentage of type I (slow-oxidative) fibers and a higher ratio of oxidative to glycolytic enzymes than do the men (29, 32, 38). These results may suggest that women have greater capacity for muscle lipid oxidation than do men. However, a more likely explanation comes from previous data showing men to have higher circulating levels of Epi than do women at similar relative intensities (46). Higher circulating Epi in men could increase the rate of muscle glycogenolysis without increasing glucose uptake (3). In addition, higher initial muscle glycogen concentration in men could promote increased glycogenolysis and lactate production during exercise (21). Our data demonstrated no gender difference in catecholamines at rest or before training, but our male subjects did have significantly higher concentrations of both Epi and NE during exercise after training (see Gender differences in training response). Thus the observed differences in RER may have been influenced by differences in lactate production and oxidation. Gender differences in receptor availability and affinity may also be important in determining substrate utilization in response to circulating hormone levels. For example, insulin-receptor binding was shown to be greater and more similar to observations of binding in men when women were in their follicular rather than luteal phase of the menstrual cycle (1, 12). It is likely that even low levels of ovarian hormones may interact with other circulating hormones to modulate the substrate response in a way that reduces glycogen utilization in women.

The rate of glucose recycling was significantly lower in women than in men at rest and during exercise at all four workloads. A combination of lower circulating lactate and catecholamine levels likely impacted the differences in glucose recycling.
Gender differences in training response. The major difference in training response between our male and female subject was that, at the same RLT after training, women demonstrated a significant reduction in RER, whereas the men did not. Because glucose flux values were unchanged in both men and women at the same relative intensity, the implication is that, in women, training had a greater glycogen-sparing effect than in men for a given RLT. The reason for such a gender difference is unclear, although it could result from differences in the training-induced response of the sympathetic nervous system. The endurance-training protocol that we used resulted in a trend of elevated Epi levels in the men at both the same ABT and RLT after training. In contrast, our female subjects showed no difference in Epi levels and significantly reduced NE levels during exercise at both workloads after training (Fig. 5). Others have shown training-induced hypertrophy of the adrenal medulla in rats (45) and increased capacity for Epi secretion in highly trained male athletes (28). Why the sympathetic nervous system adapts differently to training in women and men has yet to be determined.

Our female subjects improved their $\dot{V}O_{2\text{peak}}$ by 25.2% overall, which is substantially greater than the increase of 9.4% observed in our male subjects. It is possible that the larger CHO-sparing effect after training in women (vs. men) was a result of the greater training effect rather than differences in type of adaptations between genders. The capacity to increase $\dot{V}O_{2\text{peak}}$ is dependent on both the initial starting value and genetic disposition (4). The women that were recruited for our study might have been more untrained relative to the male subjects, who tended to be generally more active, and thus the women may have been able to increase their maximum capacity to a larger extent than their male counterparts. However, $\dot{V}O_{2\text{peak}}$ is more closely related to central cardiovascular capacity and is not necessarily the best indicator of endurance-training adaptations. Changes that impact endurance capacity usually relate more to peripheral adaptations such as increases in mitochondrial content (11). Because substrate utilization is also closely related to peripheral adaptations, one could argue that the endurance-training component was actually more effective in the male subjects, because our male subjects exhibited a larger decrease in glucose flux at a defined power output in response to training (21%) than did our female subjects (17%). In addition, short-term training studies that recorded a reduction in $R_d$ and an increase in whole body fat oxidation with no concomitant changes in $\dot{V}O_{2\text{peak}}$ provide evidence that large changes in maximum aerobic capacity are not critical to alter substrate selection (36).

Methodological considerations. When measured at the same relative workload, both men and women demonstrated a decline in $R_{ox}$ (significant in men, trend in women), despite the similar flux values pre- and posttraining. The discrepancy between $R_d$ and $R_{ox}$ at the same relative workload in both men and women suggests increased nonoxidative disposal of glucose after training that may be a result of increased cycling of glycogen in nonworking muscles (J. Azevedo, J. K. Linderman, S. L. Lehman, and G. A. Brooks; unpublished observations). However, an alternative explanation might be found in the equation used to calculate $R_{ox}$. A correction factor is used to account for retention of labeled CO2 in the bicarbonate buffering system. On the basis of previous research in our laboratory, a correction factor (k) of 0.65 at rest and 0.9 during exercise was used for the retention of CO2 in body pools. Differences in acid-base status related to gender or training-induced changes in lactate concentration or the capacity of the bicarbonate system are not accounted for in our calculations. Only a small reduction in k after training (from 0.9 to 0.8 in women or from 0.9 to 0.75 in men) would have resulted in the same values for pre- and posttraining $R_{ox}$ at the same RLT (Table 5). An increased retention of label after training is consistent with the physiology of
training (5), but further research is needed to determine the magnitude of the adjustment.

In addition to changes with training, our overall values for blood Rox were lower than anticipated during rest (∼0.56 mg·kg⁻¹·min⁻¹), given the glucose-flux values in the range of 2.8–3.0 mg·kg⁻¹·min⁻¹. Therefore, at rest only ∼20% of Rg was oxidized. Although we primed the glucose pool before commencing the resting infusion, we did not specifically prime the bicarbonate pool. Such a low oxidation rate could be explained by additional metabolically generated labeled CO₂ not accounted for by the correction factor, being trapped as bicarbonate. During exercise the percentage of Rg oxidized ranged from 56 to 80% for the women and from 72 to 93% for the men. Although there is less label retention during exercise, the values could still be impacted by overall label retention or by gender-based differences in retention.

Whole body vs. working muscle substrate use. The present study presents some of the first isotopic data available on glucose flux in women. However, the data can only provide insights into whole body adaptations to training. It is possible that the reduction in glucose flux at the same absolute workload after training is not a response that is localized in the working muscle. Data that are currently emerging from our laboratory (by means of measurements of arteriovenous differences for O₂, CO₂, and metabolites across the working limbs of male subjects) suggest that, in men, the respiratory quotient of working muscle is close to 1.0 and does not change with training, despite a significant reduction in whole body RER (B. C. Bergman, G. E. Butterfield, E. E. Wolfel, G. A. Casaza, G. D. Lopaschuk, and G. A. Brooks, unpublished observations). The implication is that the working muscle oxidizes CHO regardless of intensity or training and that whole body tracer studies measure adaptations from active as well as nonactive tissues. Such adaptations are important in that they assist with the ability of the body to provide working muscle with a readily available CHO supply, but caution should be used when attributing changes in whole body measurements to adaptations in working muscle.

Summary and conclusions. The results of this study support the hypothesis that glucose flux in women is related to relative exercise intensity both before and after training. After training, we observed a significant decrease in glucose flux and oxidation (Rox, Rg, MCR, Rα) at the same ABT but not at the same RLT. Although the results of the glucose-flux data were consistent with those we observed in men (14), the

Table 4. Glucose kinetic parameters for men and women following similar exercise and training protocols

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gender</th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>45UT</th>
<th>65UT</th>
<th>ABT</th>
<th>RLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rₐ, mg·kg⁻¹·min⁻¹</td>
<td>W</td>
<td>2.81±0.21</td>
<td>2.96±0.26</td>
<td>4.52±0.25</td>
<td>5.53±0.33</td>
<td>4.70±0.20</td>
<td>6.0±0.34</td>
</tr>
<tr>
<td>M</td>
<td>2.69±0.17</td>
<td>2.74±0.27</td>
<td>4.73±0.19</td>
<td>5.84±0.22</td>
<td>4.63±0.25</td>
<td>5.81±0.27</td>
<td></td>
</tr>
<tr>
<td>R₉, mg·kg⁻¹·min⁻¹</td>
<td>W</td>
<td>2.80±0.21</td>
<td>3.02±0.26</td>
<td>4.46±0.25</td>
<td>5.54±0.33</td>
<td>4.69±0.20</td>
<td>6.04±0.35</td>
</tr>
<tr>
<td>M</td>
<td>2.70±0.18</td>
<td>2.75±0.23</td>
<td>4.73±0.19</td>
<td>5.78±0.23</td>
<td>4.65±0.24</td>
<td>5.82±0.27</td>
<td></td>
</tr>
<tr>
<td>Rₐ as %total energy</td>
<td>W</td>
<td>67.85±7.88</td>
<td>72.87±7.82</td>
<td>25.03±1.58</td>
<td>21.72±1.33</td>
<td>20.15±1.46</td>
<td>18.79±0.90</td>
</tr>
<tr>
<td>M</td>
<td>54.41±3.67</td>
<td>48.72±3.19</td>
<td>17.13±0.94</td>
<td>15.30±0.40</td>
<td>12.85±0.81</td>
<td>13.87±1.21</td>
<td></td>
</tr>
<tr>
<td>MCR, ml·kg⁻¹·min⁻¹</td>
<td>W</td>
<td>3.07±0.24</td>
<td>3.10±0.22</td>
<td>5.44±0.37</td>
<td>6.67±0.44</td>
<td>5.41±0.29</td>
<td>7.1±0.46</td>
</tr>
<tr>
<td>M</td>
<td>2.88±0.28</td>
<td>2.99±0.31</td>
<td>5.20±0.28</td>
<td>7.03±0.56</td>
<td>5.39±0.35</td>
<td>6.45±0.32</td>
<td></td>
</tr>
<tr>
<td>Recycling rate, mg·kg⁻¹·min⁻¹</td>
<td>W</td>
<td>0.69±0.04</td>
<td>0.13±0.07</td>
<td>0.29±0.14</td>
<td>0.43±0.19</td>
<td>0.32±0.10</td>
<td>0.42±0.18</td>
</tr>
<tr>
<td>M</td>
<td>0.45±0.07</td>
<td>0.28±0.05</td>
<td>0.78±0.13</td>
<td>1.40±0.11</td>
<td>0.53±0.05</td>
<td>0.70±0.10</td>
<td></td>
</tr>
<tr>
<td>Rox, mg·kg⁻¹·min⁻¹</td>
<td>W</td>
<td>0.56±0.07</td>
<td>0.57±0.08</td>
<td>2.45±0.16</td>
<td>4.35±0.26</td>
<td>3.54±0.50</td>
<td>3.88±0.22</td>
</tr>
<tr>
<td>M</td>
<td>0.68±0.07</td>
<td>0.68±0.09</td>
<td>3.41±0.23</td>
<td>5.36±0.15</td>
<td>3.77±0.43</td>
<td>4.41±0.42</td>
<td></td>
</tr>
<tr>
<td>%E energy from CHO</td>
<td>W</td>
<td>45.0±4.12</td>
<td>34.1±4.52</td>
<td>63.1±3.51</td>
<td>72.6±7.46</td>
<td>52.7±3.52</td>
<td>56.7±3.46</td>
</tr>
<tr>
<td>M</td>
<td>55.6±8.83</td>
<td>50.0±7.3</td>
<td>68.9±3.88</td>
<td>78.2±5.39</td>
<td>73±4.72</td>
<td>78.4±5.85</td>
<td></td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>W</td>
<td>0.84±0.01</td>
<td>0.81±0.01</td>
<td>0.89±0.01</td>
<td>0.92±0.01</td>
<td>0.86±0.01</td>
<td>0.87±0.02</td>
</tr>
<tr>
<td>M</td>
<td>0.86±0.02</td>
<td>0.86±0.01</td>
<td>0.91±0.01</td>
<td>0.94±0.01</td>
<td>0.92±0.01</td>
<td>0.94±0.02</td>
<td></td>
</tr>
<tr>
<td>Plasma lactate, mM</td>
<td>W</td>
<td>0.91±0.06</td>
<td>0.85±0.05</td>
<td>1.24±0.07</td>
<td>2.86±0.28</td>
<td>1.14±0.07</td>
<td>1.22±0.13</td>
</tr>
<tr>
<td>M</td>
<td>0.78±0.04</td>
<td>0.85±0.03</td>
<td>1.69±0.30</td>
<td>3.25±0.33</td>
<td>1.77±0.30</td>
<td>1.66±0.30</td>
<td></td>
</tr>
<tr>
<td>Arterial glucose, mM</td>
<td>W</td>
<td>5.14±0.07</td>
<td>4.95±0.11</td>
<td>4.66±0.08</td>
<td>4.53±0.13</td>
<td>4.65±0.11</td>
<td>4.68±0.10</td>
</tr>
<tr>
<td>M</td>
<td>4.99±0.11</td>
<td>4.81±0.14</td>
<td>4.62±0.10</td>
<td>4.50±0.13</td>
<td>4.60±0.13</td>
<td>4.65±0.08</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Rₙ, rate of glucose appearance; R₉, rate of glucose disappearance; MCR, metabolic clearance rate; Rox, rate of glucose oxidation; M, men; W, women. All exercise values were significantly different from resting values (P < 0.05) except as marked (a).

(a)Significantly different between resting conditions at P < 0.05. Significantly different from pretraining 45% (45UT) at P < 0.05. Significantly different from posttraining (ABT) at P < 0.05. Significantly different between genders, P < 0.05.

Table 5. Glucose oxidation rates in men and women pre- and posttraining at same relative workload calculated using different correction factors

<table>
<thead>
<tr>
<th>k</th>
<th>65UT</th>
<th>RLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td>4.35±0.26</td>
<td>3.88±0.22</td>
</tr>
<tr>
<td>0.8</td>
<td>4.36±0.24</td>
<td>4.65±0.26</td>
</tr>
<tr>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td>5.36±0.15</td>
<td>4.41±0.42</td>
</tr>
<tr>
<td>0.8</td>
<td>4.96±0.47</td>
<td>5.31±0.50</td>
</tr>
<tr>
<td>0.75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Correction factor for retention of CO₂ in body pools (k) is from following equation: Rox (mg·kg⁻¹·min⁻¹) = Rg ([IE CO₂] / [VCO₂] / 100) (F × k) where IE is isotopic enrichment of expired 13CO₂; VCO₂ is volume of CO₂ expired per minute; and F is infusion rate.
response of total CHO oxidation to endurance training differed between men and women when measured at the same relative intensity. Women had a more exaggerated substrate shift toward lipid use than did the men in response to similar training protocols. Therefore, caution should be used when combining substrate utilization data obtained from both genders. Furthermore, because $R_b$ is as high but pulmonary $RER$ is lower in women than men during exercise at similar relative intensities, it appears that women rely less on muscle glycogen and lactate during exercise than do men. The glucose flux data in this investigation are interpreted to mean the following in women: 1) glucose use is directly related to exercise intensity; 2) training decreases glucose flux for a given power output; and 3) when expressed as relative exercise intensity, training does not affect blood glucose flux. Our results are consistent with those of others showing decreased CHO oxidation in exercising women compared with men.

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