Regulation of exercise carbohydrate metabolism by estrogen and progesterone in women

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Regulation of exercise carbohydrate metabolism by estrogen and progesterone in women. Am J Physiol Endocrinol Metab 283: E1046–E1055, 2002. First published July 30, 2002; 10.1152/ajpendo.00271.2002.—To assess the roles of endogenous estrogen (E2) and progesterone (P4) in regulating exercise carbohydrate use, we used pharmacological suppression and replacement to create three distinct hormonal environments: baseline (B), with E2 and P4 low; estrogen only (E), with E2 high and P4 low; and estrogen/progesterone (E + P), with E2 and P4 high. Blood glucose uptake (Rd), total carbohydrate oxidation (CHOox), and estimated muscle glycogen utilization (EMGU) were assessed during 60 min of submaximal exercise by use of stable isotope dilution and indirect calorimetry in eight eumenorrheic women. Compared with B (1.26 ± 0.04 g/min) and E + P (1.27 ± 0.04 g/min), CHOox was lower with E (1.05 ± 0.02 g/min). Glucose Rd tended to be lower with E and E + P relative to B. EMGU was 25% lower with E than with B or E + P. Plasma free fatty acids (FFA) were inversely related to EMGU (r² = 0.49). The data suggest that estrogen lowers CHOox by reducing EMGU and glucose Rd. Progesterone increases EMGU but not glucose Rd. The opposing actions of E2 and P4 on EMGU may be mediated by their impact on FFA availability or vice versa.

There is growing consensus that the ovarian hormones, estrogen and progesterone, have important roles in regulating substrate metabolism during exercise in women (5, 7, 10, 14, 39, 40, 43–46). In animal models, estrogen promotes lipolysis and increases fatty acid availability (3, 15, 21, 23, 31) while decreasing the rate of gluconeogenesis and sparing muscle and liver glycogen use (18, 23, 31, 34, 38). The addition of progesterone increases EMGU but not glucose availability (23, 29, 33). Conversely, the addition of progesterone appears to accentuate the carbohydrate-sparing actions of estrogen by decreasing hepatic glycogenolysis (23, 29, 37). Recently, Campbell and Febbraio (9) showed that estrogen upregulates mitochondrial enzymes favoring fat oxidation, whereas progesterone opposed these actions.

Metabolic regulation by endogenous estrogen (estradiol, E2) and progesterone (P4) has usually been studied in humans by comparison across different phases of the menstrual cycle: the menstrual phase (E2 and P4 both low), the midfollicular phase (E2 elevated, P4 low), and the midluteal phase (E2 and P4 both elevated). High inter- and intrasubject variability inherent to studying the “natural” hormonal environment complicates the ability to draw clear conclusions. In some cases (8, 20, 47), researchers observed a shift toward reduced blood glucose use and increased fat oxidation during submaximal exercise in the luteal phase of the cycle, but others have reported no significant differences (2, 4, 6, 25, 30). Even in well designed studies that minimize confounding variables with dietary control, careful timing of measurements, and the addition of stable isotope tracers to indirect calorimetry, the discrepancy remains. Furthermore, because both E2 and P4 concentrations vary in different phases of the natural menstrual cycle, it is not possible to attribute metabolic differences observed to independent effects of progesterone or estrogen.

Researchers have tried to solve the latter problem by “controlling” the ovarian hormone environment with estrogen administration. Ruby et al. (41) found that supplementing amenorrheic women (who have low E2) with transdermal estrogen reduced blood glucose flux during submaximal exercise. Carter et al. (12) supplemented men with estrogen and found comparable results. These data suggest that estradiol administration reduces the rate of blood glucose utilization. Neither research group reported a significant decrease in whole body carbohydrate oxidation, implying that there was no sparing of muscle glycogen utilization in the presence of estrogen.

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Table 1. Subject physical characteristics and demographic data

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>24.1 ± 7.2 (18–35)</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>55.8 ± 8.6 (45–67)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>158.7 ± 9.0 (146–175)</td>
</tr>
<tr>
<td>Percent fat</td>
<td>22.4 ± 4.1 (18–27)</td>
</tr>
<tr>
<td>(\dot{V}O_2) max, ml·kg(^{-1})·min(^{-1})</td>
<td>42.5 ± 8.0 (33–51)</td>
</tr>
</tbody>
</table>

Values are means ± SD (range) of 8 subjects. \(\dot{V}O_2\) max, maximal oxygen consumption.

Taken together, results from animal and human studies present a complicated and contradictory story. Recently, Horton et al. (25), Campbell and Febbraio (10), and D’Eon and Braun (14) argued that examining the relative ratio of estrogen to progesterone might clarify some of the discrepancies. Therefore, the purpose of this study was to assess glucose kinetics and oxidation during submaximal exercise by use of pharmacological agents to create three tightly controlled hormonal environments: baseline (low estrogen and progesterone), estrogen only (high estrogen, low progesterone), and estrogen plus progesterone (high estrogen, high progesterone). Mainly on the basis of menstrual cycle phase studies in humans, we hypothesized that the addition of progesterone would potentiate the effects of estrogen alone to lower blood glucose uptake and total carbohydrate oxidation relative to the baseline condition.

METHODS

Subjects. Subjects for this study were healthy, physically active women who participated in regular aerobic activity ≥3 times/wk. All of the subjects were in excellent overall health, had no history of cardiovascular, metabolic, or hormonal disorders, used no medications other than occasional over-the-counter aspirin or ibuprofen, displayed no evidence of eating disorders and reported normal eating habits, and had not used oral contraceptives for ≥6 mo before the study. After study procedures were explained verbally, subjects signed a written informed consent document approved by Institutional Review Boards at both the University of Massachusetts and Baystate Medical Center. Initially, 12 women enrolled in the study. Two subjects withdrew after the first test due to discomfort with the blood-drawing procedures and/or hormonal treatments. In two other subjects who completed the study, either estrogen or progesterone concentrations were considerably elevated in a condition when they were expected to be low. Therefore, data from these two subjects were not included in the analysis, and all results are reported with \(n = 8\). Characteristics of these subjects are shown in Table 1.

Pretesting procedures. Body density was determined by hydrostatic weighing, with residual lung volume estimated as \((0.28 \times \text{maximal expired volume})\). Maximal oxygen consumption (\(\dot{V}O_2\) max) was measured on an electronically braked cycle ergometer with an incremental ramp protocol starting at 60 W and increasing by 30 W every 2 min until subjects could no longer maintain a cadence of 60 rpm. Oxygen consumption and carbon dioxide production were measured by indirect calorimetry with a TrueMax 2400 metabolic measurement system (Parvo Medics, Sandy, UT).

Overall study design. Subjects were tested in three different conditions: baseline, estrogen only, and estrogen plus progesterone (see Table 2). The treatments were originally administered in a double-blind manner, but one author inadvertently became aware of the code about halfway through the study and was therefore not blinded for the remainder of the study. The order in which the E and E+P treatments were administered was balanced across subjects.

Hormonal control. Subcutaneous injections of 0.25 mg Ganirelix (Organon, West Orange, NJ) were begun 2–5 days after the onset of menses to suppress endogenous production of gonadotropin-releasing hormone, or GnRH. The injections were given 30–34 and 6–10 h before baseline testing (B), which occurred on day 3. After B, subjects were given one of two treatments: estrogen-only (E), in which subjects wore three transdermal estradiol patches (Vivelle, 0.1 mg estradiol each) affixed to the skin of the upper pelvis and consumed an oral placebo for 3 days; and estrogen plus progesterone (E + P), which was exactly the same treatment but with oral progesterone (Prometrium, 200 mg/day) replacing the placebo. Posttreatment testing occurred on day 6, at the same time of day as baseline testing. The second round of treatment and testing was begun 2–5 days after the following onset of menses ~1 mo later.

Control of diet and activity. Subjects were regularly reminded to maintain a similar activity level and consistent dietary habits throughout the course of the study. They refrained from exercise for 24 h before testing in all conditions. Although diet was not rigidly controlled throughout the study, all subjects consumed the same preexercise meal 3 h before each test. The meal comprised 35% of estimated daily energy requirements and was composed of 55% carbohydrate, 15% protein, and 30% fat. The Harris and Benedict equation specific to women, \[655 + 9.5\times\text{(weight)} + 1.9\times\text{(height)} - 4.7\times\text{(age)},\] was used to calculate resting metabolic rate, and this value was multiplied by an activity factor of 1.7 (assuming women in this study were moderately active) to estimate daily energy requirements. Subjects were instructed to fast after this meal until after testing.

Testing procedures. Subjects reported to the laboratory 3 h after the meal, and a catheter was inserted into an antecu-

Table 2. Summary of study design

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
</tr>
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<tr>
<td>Cycle 1</td>
<td>Ganirelix</td>
<td>AM: Test B PM: estradiol patch + oral treatment</td>
<td>estradiol patch + oral treatment</td>
<td>estradiol patch + oral treatment</td>
<td>AM: Test E or E + P</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>Ganirelix</td>
<td>PM: estradiol patch + oral treatment</td>
<td>estradiol patch + oral treatment</td>
<td>estradiol patch + oral treatment</td>
<td>AM: Test E or E + P</td>
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</tbody>
</table>
bital vein for infusion of stable isotope. A second catheter was placed in a forearm or wrist vein of the contralateral arm for blood sampling. A venous blood sample was collected before infusion for determination of background isotopic enrichment, and a priming bolus of 200 mg [6,6-2H]glucose in 0.9% sterile saline was then rapidly infused into the venous catheter. To reach and maintain isotopic equilibrium, [6,6-2H]glucose was then continuously infused at 2.5 mg/min with a peristaltic infusion pump (Harvard Apparatus, South Natick MA). Venous blood samples and 5-min collections of expired oxygen and carbon dioxide were taken at rest 75 and 90 min after the start of the infusion.

Immediately after the last resting measurement, the subject began submaximal exercise on a bicycle ergometer (LifeFitness888). To maintain a steady isotopic enrichment of blood glucose, the [6,6-2H]glucose infusion rate was increased to 6.0 mg/min. During the first 15 min of exercise in the B condition, the intensity was adjusted by manipulating the pedaling resistance until oxygen consumption reached a steady state at ~60% of the previously measured VO2max. Blood and breath samples (5 min) were collected at 15, 30, 45, and 60 min of exercise (see Fig. 1).

Biochemical assays. Samples of venous blood for analysis of glucose, lactate, insulin, and glucose isotopic enrichment were collected in heparinized syringes and then transferred to vacutainers containing sodium fluoride (to inhibit glycolysis). Samples for analysis of free fatty acids (FFA) were collected in heparinized syringes and then transferred to vacutainers containing EDTA. Samples for analysis of estradiol, progesterone, epinephrine, and norepinephrine were collected in nonheparinized syringes, transferred to vacutainers specific for serum analysis (glutathione added for epinephrine and norepinephrine), and allowed to clot. All samples were immediately centrifuged, and the plasma was transferred to cryogenic vials and frozen at −70°C until analysis. Glucose and lactate concentrations were determined using a glucose/lactate analyzer (GL5 Analyzer, Analox Instruments, Lunenburg, MA). Estradiol and progesterone levels were determined using enzyme immunoassays (Diagnostic Systems Laboratories, Webster, TX). Insulin was measured using radioimmunoassay (Linco Research, St. Charles, MO). FFA concentrations were measured using a standard colorimetric assay (Wako Chemicals, Richmond, VA). Epinephrine and norepinephrine concentrations were determined by high performance liquid chromatography with electrochemical detection.

Glucose isotopic enrichment was measured by GC-MS. Plasma was first neutralized by back titration with 2 N KOH, passed through anion and cation exchange resins, lyophilized, reconstituted with acetic anhydride-pyridine (2:1), dried under a stream of nitrogen, and reconstructed in 100 μl of ethyl acetate. A 25-μl sample was injected and separated on a gas chromatograph, with spectra recorded on a mass spectrometer (Hewlett-Packard 6890, Palo Alto, CA). Selected ion monitoring was used to compare the abundance of the unlabeled fragment with that of the enriched isotopeomer (Chemstation Software). After correction for background enrichment, the abundance of the deuterated isotopeomer [mass-to-charge ratio (m/z) = 333] was expressed as a percentage of total glucose species (m/z = 331 + 32 + 333).

Calculations. To test the rate at which glucose is taken up from the blood (rate of disappearance, Rd) and replaced by the liver (rate of appearance, Ra), equations specifically designed for use with stable isotopes in biological systems were used

\[
R_g = \frac{F - V(C_1 + C_2)/2}{[IE_2 + IE_1]/2}
\]

where Rg represents the isotopic infusion rate, IE1 and IE2 are the concentrations of plasma glucose with deuterated glucose at time points t1 and t2, respectively; C1 and C2 are the concentrations of plasma glucose at t1 and t2; and V is the estimated volume of distribution for glucose (180 ml/kg). percent energy from carbohydrate was determined from total RER as [(RER − 0.71)/0.29] × 100 for total carbohydrate oxidation = (blood glucose Rg)

This estimate is based on the assumption that 100% of blood glucose taken up from the blood is oxidized, which is unlikely to be true; i.e., the percentage of Rg oxidized is probably 70–90% (16, 28) but may vary across the conditions used in this study. Thus the calculation underestimates glucose use and is best described as minimal muscle glycogen utilization.

Statistical analysis. All data in Tables 1–4 and in Figs. 2–7 are group means ± SE (SD for demographic information). Summary measures of the exercise time points for each subject were calculated using the trapezium rule for area under the curve (AUC). The summary data were analyzed as raw data by ANOVA with repeated measures by use of the PROC-MIXED univariate analysis for all variables (SAS Institute, Cary, NC). Statistical significance was defined as α < 0.05. Post hoc analysis with planned comparisons was made using Fischer’s protected least significant difference test. Nonlinear regression analysis was performed using SPSS 10.0.7 (SPSS, Chicago, IL).

RESULTS

Hormonal environment. The treatments resulted in three very distinct hormonal environments (Table 3). Compared with the baseline condition (B), the serum concentration of estradiol was considerably elevated in the estrogen-only condition (E), with no difference in serum progesterone. With the addition of progesterone (E + P), there was a substantial elevation in serum progesterone, but the serum estrogen concentration was comparable to E. Therefore, E and B differed only in the levels of serum estradiol, whereas E and E + P differed only in the levels of progesterone.
Table 3. Estrogen and progesterone levels for each treatment

<table>
<thead>
<tr>
<th>Condition</th>
<th>Estrogen, pg/ml</th>
<th>Progesterone, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>49 ± 19*</td>
<td>1.36 ± 0.58</td>
</tr>
<tr>
<td>E</td>
<td>256 ± 57</td>
<td>1.55 ± 0.72</td>
</tr>
<tr>
<td>E + P</td>
<td>232 ± 101</td>
<td>47.6 ± 29.0*</td>
</tr>
</tbody>
</table>

Values are means ± SD averaged over 3 time points: rest, 30 min of exercise, and 60 min of exercise. B, baseline testing; E, estrogen only; E + P, estrogen + progesterone. *P < 0.05, significantly different from the other 2 conditions.

Exercise oxygen consumption and heart rate. Oxygen consumption, percent \( V_{O_2 \text{max}} \), and heart rates were almost identical in B, E, and E + P (Table 4), implying that the exercise intensity did not vary among the three conditions.

Gas exchange. The RER was significantly lower both at rest and during exercise in E compared with B (Table 4). Compared with E, the RER in E + P was elevated and almost indistinguishable from B. As shown in Table 4, total carbohydrate oxidation was reduced with E relative to B, and the addition of progesterone did not potentiate the estrogen effects as expected. Instead, total carbohydrate oxidation was significantly higher with E + P compared with E, and very similar to B. Total fat oxidation is the reciprocal of carbohydrate oxidation and so followed the same pattern, in which fat oxidation was elevated with E relative to either B or E + P (Table 4).

Blood glucose kinetics and EMGU. The isotopic enrichment of plasma glucose at rest and during exercise is shown in Fig. 2. The increase in isotope infusion rate at the onset of exercise appears to have resulted in relatively constant isotope enrichment over time. The rate of glucose appearance into (glucose \( R_d \)) and uptake from the blood (glucose \( R_a \)) were not significantly different among conditions (Fig. 3, A and B) but tended to be lower with E and E + P relative to B (0.05 < P < 0.10). When \( R_d \) was scaled to the ambient plasma glucose concentration to assess the metabolic clearance rate (MCR) of glucose, there were no differences among conditions (Fig. 3C). Muscle glycogen use, estimated as the difference between total carbohydrate oxidation and glucose \( R_o \) (EMGU), was significantly lower in E compared with B (Table 4). Contrary to the stated hypothesis, the addition of progesterone increased EMGU to values that were very similar to those observed in B. Therefore, the decrease in total carbohydrate oxidation with E relative to B resulted from reductions in both blood glucose and EMGU. Compared with E, total carbohydrate oxidation was restored by E + P to a level indistinguishable from B and was attributable solely to increased EMGU. A summary of the relative proportions of nonprotein energy expenditure derived from blood glucose, other carbohydrate (i.e., glycogen), and fat oxidation is shown in Fig. 4.

Plasma glucose and lactate. Plasma concentrations of glucose did not change from rest to exercise and were not different among conditions (Fig. 5A). Plasma lactate concentrations rose steeply in the first 15 min of exercise in all conditions and then declined toward baseline at each subsequent time point (Fig. 5B). At 60 min, plasma lactate concentrations were ~2.0–2.5 mM in B and E. Although lactate concentrations were low-

Table 4. Work intensity, oxygen consumption, substrate use, and catecholamine concentrations during steady-state exercise in the 3 conditions

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>E</th>
<th>E + P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Work, W</td>
<td>96 ± 3</td>
<td>96 ± 3</td>
<td>96 ± 3</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>148 ± 5</td>
<td>146 ± 4</td>
<td>147 ± 5</td>
</tr>
<tr>
<td>( V_{O_2 \text{max}} ), ml·kg(^{-1})·min(^{-1} )</td>
<td>22.9 ± 1.2</td>
<td>22.9 ± 1.1</td>
<td>23.0 ± 1.1</td>
</tr>
<tr>
<td>%( V_{O_2 \text{max}} )</td>
<td>53.8 ± 2.7</td>
<td>53.8 ± 2.6</td>
<td>53.9 ± 2.6</td>
</tr>
<tr>
<td>Energy expenditure, kcal/min</td>
<td>7.1 ± 0.2</td>
<td>7.1 ± 0.2</td>
<td>7.1 ± 0.3</td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>0.918 ± 0.018</td>
<td>0.882 ± 0.015*</td>
<td>0.913 ± 0.009</td>
</tr>
<tr>
<td>Total CHO use, g/min</td>
<td>1.26 ± 0.10</td>
<td>1.04 ± 0.08*</td>
<td>1.27 ± 0.07</td>
</tr>
<tr>
<td>Estimated glycogen use, g/min</td>
<td>0.79 ± 0.11</td>
<td>0.60 ± 0.08*</td>
<td>0.83 ± 0.09</td>
</tr>
<tr>
<td>Total lipid use, g/min</td>
<td>0.20 ± 0.04</td>
<td>0.30 ± 0.04*</td>
<td>0.20 ± 0.3</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>185 ± 33</td>
<td>176 ± 39</td>
<td>175 ± 34</td>
</tr>
<tr>
<td>Norepinephrine, ng/ml</td>
<td>1.45 ± 0.17</td>
<td>1.66 ± 0.15</td>
<td>1.69 ± 0.21</td>
</tr>
</tbody>
</table>

Values are means ± SE of exercise period from 30 to 60 min. \( V_{O_2} \), oxygen consumption; CHO, carbohydrate. *P < 0.05, significantly different from the other 2 conditions.
At every exercise time point with E/H11001 (final concentration ~1.5 mM), there were no significant differences among conditions.

**Glucoregulatory hormones.** Plasma insulin concentrations fell during exercise in all conditions (Fig. 5C). Insulin levels plateaued at ~25 pM in E and E/H11001. In B, plasma insulin concentrations were significantly higher at rest and for the first 30 min of exercise relative to E and E/H11001, but there were no differences among conditions at 45 and 60 min. Plasma concentrations of epinephrine and norepinephrine (Table 4) rose considerably during exercise compared with rest, and the responses were very similar in all three conditions.

**Plasma FFA.** Relative to E/H11001, the FFA concentration was significantly elevated with E (Fig. 6). As shown in Fig. 7, there was an inverse nonlinear (power function) relationship between the concentration of fatty acids in plasma and the EMGU rate.

**DISCUSSION**

The main findings in this study were that 1) high circulating levels of estrogen reduced total carbohydrate oxidation during exercise compared with a low-estrogen condition, with decreases in both estimated muscle glycogen use and (a tendency toward) blood glucose uptake, and 2) when high levels of progesterone were added to the high-estrogen environment, there was no measurable impact on blood glucose uptake but a complete reversal of the muscle glycogen sparing induced by estrogen alone, which restored total carbohydrate oxidation to baseline values. The first
finding supports the stated hypothesis and is generally consistent with the majority of the animal and human literature. The second finding is contrary to the stated hypothesis and is inconsistent with human studies across phases of the menstrual cycle, but it is concordant with the bulk of the animal literature.

Control of confounding variables. There are several limitations to this study that could potentially confound the interpretation of the data. Systematic changes in energy balance or carbohydrate intake could influence the results obtained. Although it would have been desirable, dietary energy intake and composition were not controlled throughout the study. However, to minimize the contribution of dietary variation to the observed results, the final meal before each test was of a standardized energy content and nutrient composition, and it was consumed by the subjects at the same time before the infusion began. Similarly, changes in physical activity patterns over the course of the study could alter the relative intensity of the submaximal exercise and affect substrate utilization. The observations that submaximal heart rate and oxygen consumption were almost identical in all three conditions imply that the exercise intensity did not vary and that the fitness level of the subjects did not change between tests. In addition, the use of a crossover design, with the order of E and E + P balanced across

Fig. 5. Glucose (A), insulin (B), and lactate (C) concentrations at rest and during exercise. Insulin concentrations fell during the course of exercise and were significantly higher in the baseline testing condition at rest and at 15 and 30 min of exercise.

Fig. 6. Plasma free fatty acid (FFA) concentrations at rest and during exercise. FFA were significantly higher (P < 0.05) in E compared with E + P at rest and during exercise.

Fig. 7. Relationship between plasma FFA concentrations and the estimated rate of muscle glycogen use. The power function shown above the figure describes the relationship between the 2 parameters, and the correlation coefficient (R2) indicates that about one-half of the total variance is explained by that relationship.
subjects, minimizes the possibility that variations in diet or training across conditions could explain the results.

In eumenorhic women, both estrogen and progesterone levels vary across phases of the menstrual cycle, making it difficult to tease apart their independent and combined actions. Using a suppression/replacement model in the present study, we were able to create hormonal environments in which the only differences between conditions were the estrogen concentration (E relative to B) or the progesterone concentration (E + P relative to E).

Other hormone supplementation studies in humans. The tendency toward a lower blood glucose $R_{d}$ with estrogen observed in the present study is concordant with results reported in previous estrogen supplementation studies (12, 41). Ruby et al. (41) and Carter et al. (12) both found that glucose $R_{d}$ was lower after several days of estrogen supplementation in amenorrheic women (41) or men (12) compared with the baseline condition. The sample size in the present study was equal to or greater than those prior studies, but we may still have lacked sufficient statistical power to detect a significant difference in glucose $R_{d}$. In addition, the reduction in blood glucose might have been accentuated if the exercise protocol had extended beyond 60 min.

Total carbohydrate oxidation was not different from baseline in those studies, however. In the present study, we report that carbohydrate oxidation was reduced by estrogen. In addition, we observed a reduction in estimated muscle glycogen use with estrogen that was not seen by Ruby et al. (41) or Carter et al. (12). The differences between the studies are unlikely to be explained by simple differences in circulating estrogen concentrations. Blood estrogen concentrations were raised 2- to 3-fold after supplementation in our subjects, which is within the range of >2-fold (41) and >10-fold (12) spanned by the two prior studies. Subjects in the other studies were fasted overnight before exercise testing, whereas in the current study, subjects were given a preexercise meal 4 h before exercise, which may have influenced reliance on blood glucose vs. muscle glycogen. The differences between the studies are likely more quantitative, in terms of the magnitude of the differences observed, than qualitative discrepancies. There are consistent data from animal studies that estrogen spares endogenous carbohydrate use in both liver and muscle (23, 31, 32, 37, 38). Because Ruby et al. and Carter et al. supplemented subjects with estrogen only, there are no human data to which our estrogen + progesterone condition can be compared.

Studies in animal models. Results from the present study generally parallel results from experiments in which circulating estradiol concentrations were suppressed and reintroduced in animals. In two separate studies, Kendrick and colleagues (31, 32) showed that muscle glycogen utilization during exercise was lower after estradiol administration in male rats. These results were confirmed by Rooney et al. (38), who also found that resting intramuscular lipid levels were higher in estradiol-treated rats, suggesting that carbohydrate conservation was secondary to increased fat availability. Hatta et al. (23) found that estradiol administration increased fat oxidation and subsequently reduced glucose oxidation during exercise. When they gave estrogen and progesterone concurrently, substrate oxidation was "restored" to control values. Recently, Campbell and Febbraio (9) used exogenous supplementation with estrogen and/or progesterone to study their independent and combined actions on key enzymes regulating the transport [carnitine palmitoyltransferase I (CPT I)] and oxidation [β-hydroxyacyl dehydrogenase (β-HAD)] of fatty acids in muscle from control and ovariectomized rats. In that study, Campbell and Febbraio reported that physiological levels of estrogen increased the maximal activity of CPT I and β-HAD by ~15% relative to control. Ovariectomy and progesterone, alone or in combination with estrogen (physiological levels), reduced the activity of those enzymes by ~20%. However, when the physiological dose of progesterone was combined with a pharmacological dose of estrogen to raise circulating estrogen concentrations to very high levels, the activities of both enzymes were similar to the estrogen-only condition. Our data are consistent with this work by Campbell and Febbraio, suggesting that up- or downregulation of key enzymes in fat and/or carbohydrate oxidation pathways may play an important role in mediating the changes observed at the whole body level.

It appears that progesterone and estrogen act in opposition to each other with respect to setting the mixture of oxidized substrates during exercise. Estrogen alone reduces carbohydrate oxidation by decreasing muscle glycogenolysis and blood glucose uptake (ultimately sparing liver glycogen). The addition of progesterone reverses the carbohydrate-sparing effect on muscle glycogenolysis but does not seem to oppose the reduction in blood glucose uptake (and may even potentiate it). The results suggest that the modulation of exercise substrate utilization by ovarian hormones is dependent on the relative concentrations of estrogen and progesterone. These data may help to explain the discrepant results obtained from studies in which the natural variations in estrogen and progesterone across the menstrual cycle have been used to study their regulatory roles in substrate metabolism.

Studies of menstrual cycle phase. As previously described, data from well controlled studies of women in different phases of the menstrual cycle suggest that blood glucose uptake and/or whole body carbohydrate oxidation is similar in both phases (4, 6, 25) or lower in the luteal phase (8, 20, 47). Methodological issues, such as whether subjects were exercising below the lactate threshold and whether they were overnight fasted or received a preexercise meal, might explain some of the discordance. However, even when exercise is below the lactate threshold and at comparable intensity, investigators have reported no cycle phase differences in either fasted (4, 25) or fed (6, 8) subjects.

When the relative changes in estrogen and progesterone between the follicular and luteal phases are
compared across studies, however, a potentially useful pattern emerges. In four studies, stable isotope tracers were used to quantify glucose kinetics, exercise intensity was below the lactate threshold, and timing of the preexercise meal was controlled for. When no differences across cycle phase were observed (6, 25), estrogen concentrations were ~1.5-fold greater in the luteal phase. In the two studies in which carbohydrate oxidation was lower in the luteal phase, estrogen levels were 2.3- to 2.8-fold elevated relative to the follicular phase (8, 47). Campbell and colleagues (8, 10) suggest that antagonistic actions by estrogen and progesterone could underlie the differences noted across menstrual cycle phase, and they argue that progesterone may counter the actions of estrogen to facilitate glucose uptake during exercise. Hansen et al. (22) reported that glucose uptake during muscle contractions was impaired in estrogen-deficient rats, and recent data from the Febbraio laboratory (see Campbell and Febbraio, Ref. 11) show that estrogen, but not progesterone, replacement restored glucose uptake to control conditions in ovariectomized rats. Thus the specific actions of estrogen on blood glucose kinetics appear to be complex and influenced by the presence/absence of other factors. In contrast, progesterone, even when paired with physiological concentrations of estrogen, reduces blood glucose uptake relative to the baseline condition (11 and present study) and, at least under the conditions we used, raised total carbohydrate oxidation by increasing other carbohydrate (i.e., glycogen) use. A more accurate representation of the metabolic events will require understanding what is happening in specific tissues. In the luteal phase, the presence of progesterone may constrain blood glucose uptake by peripheral tissues and thereby spare liver glycogen use, at the expense of increased muscle glycogen use. The net effect would be no change in whole body substrate oxidation. But if the concurrent rise in estrogen is high enough to overcome the progesterone effect on muscle glycogenolysis, the dual constraints on both blood glucose uptake and muscle glycogen utilization would clearly have the net effect of reducing whole body carbohydrate use. The magnitude of the estrogen rise in the luteal phase may therefore determine the balance between changes in blood glucose uptake, liver glucose output, and intramuscular glycogenolysis.

Interactions between carbohydrate and lipid metabolism. Whether the effects of ovarian hormones on carbohydrate utilization are primary or secondary to their influence on the availability/oxidation of lipid has been the topic of much discussion (5, 7, 10, 14, 39, 43, 45). Estrogen may reduce glucose/glycogen availability such that maintaining a given rate of energy expenditure requires increased fat oxidation (a "carbohydrate constraint pull," Ref. 5). Alternatively, enhanced lipid availability and oxidation could displace carbohydrate utilization (i.e., a "lipolytic push," Ref. 5). As we have mentioned, data from some studies in animal models suggest that estrogen increases fat availability and oxidation by raising levels of intramuscular triglyceride and/or by elevating the rate of lipolysis (15, 23, 38).

Data in humans are consistent with the idea that estrogen increases lipolysis and, thereby, fatty acid availability. Conversely, progesterone is antilipolytic in animals (21, 33), and we found that blood levels of FFA were lower with the addition of progesterone relative to estrogen alone.

Whether changes in the availability of fatty acids alter the rate of fat oxidation and hence spare carbohydrate use has not been conclusively demonstrated. In the present study, we found a reciprocal relationship between fatty acid availability and glycogen utilization. Others have shown this same relationship in other contexts (24, 26, 36, 42); for example, when exogenous fatty acids or lipolytic agents are infused, the rate of fat oxidation goes up and muscle glycogen/blood glucose use decreases (36). In addition, the study by Campbell et al. (8), showing that estrogen and progesterone cause opposing changes in the key enzymes related to fatty acid transport and oxidation, lends weight to the hypothesis that the carbohydrate-sparing effect of estrogen is driven by a lipolytic push and that the opposing effects of progesterone are consequent to a reduction in lipid availability. Although these data suggest that alterations in fatty acid utilization precede changes in carbohydrate oxidation, several investigators have shown that changes in carbohydrate utilization (e.g., in response to raising or lowering exercise intensity) can be primary and can drive subsequent alterations in fat oxidation (42). The design of the current study does not allow us to definitively answer this question; our data could be explained by either the lipolytic push or the carbohydrate constraint pull models (or a combination of both). In addition, whether the impact of the sex hormones is on adipose tissue lipolysis only or also impacts other sources of oxidizable lipids (e.g., intramyocellular or blood triglycerides) cannot be determined on the basis of data presented here.

Direct and indirect actions of sex hormones. The ovarian hormones may act directly on metabolic pathways, and/or their actions could be mediated via other hormones. In the present study, the plasma insulin concentration fell during exercise in all three conditions but tended to be lower (especially at rest and early in exercise) with E or E + P compared with B. Differences in plasma insulin could potentially impact blood glucose uptake and total carbohydrate oxidation, but close examination of the data suggests otherwise. Despite higher insulin concentrations in the baseline condition (which, in the absence of any change in glucose concentrations, indicates some fasting insulin resistance, as noted recently by Campbell and Febbraio (11) after ovariectomy in rats), total carbohydrate oxidation was the same as with E + P, when insulin concentrations were lowest. Also, the differences in glucose Ra are greatest near the end of exercise, when plasma insulin concentrations are very similar among conditions. The actual plasma insulin concentrations in the last 30 min of exercise are very low in all three conditions, varying from 23 pM (E + P) to 35 pM (B), which, in clinical terms, is a difference of only 2 μU/ml.

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There is likely to be little physiological relevance to such small differences at the extremely low end of the plasma insulin range.

Several investigators have theorized that the major influence of the sex hormones on metabolic regulation is mediated via the catecholamines epinephrine and/or norepinephrine. Epinephrine concentrations were lower after estradiol supplementation in amenorrheic women in one study (41), but not significantly different across menstrual cycle phases (6, 25) or with estrogen supplementation in men (12). Studies in both humans (27) and rats (1, 3) suggest that high concentrations of estrogen alter tissue responses to favor the lipolytic compared with the glycogenolytic actions of epinephrine. We observed an increased fatty acid availability and oxidation in the presence of estrogen alone relative to baseline, despite no difference in the concentrations of circulating epinephrine or norepinephrine. The addition of progesterone also had no impact on catecholamine levels but caused a dramatic shift toward lower fatty acid availability and oxidation. Our data therefore support the idea that lipolytic sensitivity to catecholamines is accentuated by estrogen and imply that the shift is reversed with the addition of high levels of progesterone.

The potential importance of glucoregulatory hormones not measured in the current study, e.g., glucagon, growth hormone, and cortisol, cannot be evaluated. The general consensus, based on available data, is that glucocorticoids play relatively subtle roles in mediating substrate utilization during submaximal exercise (5, 17, 30, 39, 44).

Conclusions. Results from this study suggest that estrogen alone reduces total carbohydrate oxidation during exercise by decreasing both blood glucose uptake and other carbohydrate (i.e., glycogen) use. The addition of progesterone may further reduce blood glucose uptake but, conversely, increases glycogen use such that total carbohydrate utilization is indistinguishable from an estrogen-absent condition. There is tantalizing evidence that the changes observed are mediated via opposing effects of the ovarian hormones on fatty acid availability and oxidation, but further studies at the tissue-specific level will be required to address that question. The present data suggest that alterations in substrate use across the menstrual cycle are dependent on the relative changes in both estrogen and progesterone. From a practical perspective, the balance between use of carbohydrate and fat for energy could shift in response to hormone replacement therapy in postmenopausal or amenorrheic women. The specific formulation (estrogen only or combined with progesterone) might alter the pattern of substrate use and could potentially impact exercise performance and macronutrient requirements in active individuals.

We thank the research subjects for their exceptional commitment of time and effort. We also acknowledge exceptional assistance from Cindy Baecher and Kathy Dudzinski. Donna Storton was extremely helpful with the GC-MS work, and Dr. Juli Jones assisted with the insulin radioimmunoassays. Catecholamine analyses were done at the University of Colorado Health Sciences Center core laboratory.

Dr. George Wade provided helpful scientific insight and perspective. Thanks also to Organon USA for donating Ganirelix.

Funding for this study was provided by the University of Massachusetts/Baystate Biomedical Research Program.

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