Luteal and follicular glucose fluxes during rest and exercise in 3-h postabsorptive women

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The metabolic effects of the ovarian hormones have been addressed by investigating the response to acute exercise during the follicular (FP) and luteal (LP) menstrual phase (25, 29, 43, 44) or by comparing men with women (11, 14, 22, 39). Those studies showed discrepancies in glucose flux [rate of appearance and rate of disappearance (Ra and Rd, respectively)], in muscle glycogen utilization rates, and in circulating levels of glucose and lactate during rest and exercise. For example, two recent studies (8, 44) observed that, when women were allowed to fast for 10–12 h, significant reductions occurred in glucose flux rates during exercise in LP compared with FP. However, the menstrual phase difference disappeared when CHO was supplied in a fluid-energy-electrolyte replacement beverage during exercise (8). In addition to differences in dietary controls imposed, some of the discrepancies found in data that compared men with women and that examined the role of ovarian hormones may be attributed to the difficulties involved with matching subjects and with timing of the measurements relative to the menstrual cycle, respectively. Collectively, these previous studies have shown a trend toward lower levels of blood lactate and small differences in the levels of blood glucose during rest and exercise in the LP. In addition, muscle glycogen appears to be utilized less in LP compared with FP (18).

Because limited data are available on blood glucose flux rates in response to menstrual cycle phase changes and because of the potential effects of dietary and activity histories, we examined the effects of exercise intensity and menstrual cycle phase on blood glucose and exercise of moderate (14) and high (30) intensities. Given similar glucose flux and lower CHO oxidation, the results are consistent with reports of greater lipid, lesser muscle glycogen and relatively higher blood glucose use in women. The potential mechanism for lower CHO oxidation in women compared with men may be the decreased rate of glycogenolysis, which may be partially attributable to lower circulating epinephrine concentrations (38) and lesser muscle glycogen levels (20).

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flux during rest and exercise. We hypothesized that 1) blood glucose flux would scale to exercise intensity and that menstrual cycle effects, if any, would be small by comparison; 2) dietary status would have a greater effect than menstrual cycle phase on glucose flux and overall CHO and lipid oxidation rates in exercising women; and 3) blood glucose flux would be similar during high-intensity exercise [at 65% peak oxygen consumption (VO2 peak)] regardless of menstrual cycle and dietary status because of the overriding effects of exercise stimulating muscle glycogenolysis and glycolysis (7).

METHODS

Subjects. Eight healthy, moderately active women between the ages of 22 and 30 yr with normal menstrual cycles (24–32 days) were recruited from the University of California, Berkeley campus, community by posted notices and E-mail. Subjects were nulliparous, reported having normal menstrual cycles (for at least 6 mo), had not taken oral contraceptives, and had not experienced any large weight, exercise, or diet changes within the last 6 mo. Subjects had a percent body fat between 19 and 25%, a VO2 peak between 38 and 54 ml·kg−1·min−1, and a normal lung function (forced expiratory volume in 1 s of 70% or more) and were injury and disease free as determined by a health history questionnaire and physical examination. Subjects provided informed consent, and the study protocol was approved by the University of California Committee for the Protection of Human Subjects (no. 2000-8-30).

Experimental design. After the initial screening interview and physical examination were completed, to determine the VO2 peak, subjects performed continuous graded exercise tests in randomized order in each phase of the menstrual cycle. Subjects were subsequently tested in a random order during early FF (days 3–9) and LP (days 18–24 or 4–9 days past the luteinizing hormone surge). Urinary luteinizing hormone levels were measured with ovulation kits (First Response, Carter Products) starting at day 10 after the start of menses until a positive test was achieved. A positive test result indicated the surge in luteinizing hormone that occurred within 48 h. Cycle phases were later confirmed by plasma estradiol and progesterone concentrations (FP: estradiol < 50 pg/ml and progesterone < 1 ng/ml; LP: estradiol > 50 pg/ml and progesterone > 3 ng/ml) (6, 8, 32). Four stable isotope tracer infusion trials were conducted within two sequential menstrual cycles, with each trial consisting of a 90-min rest period followed by a 60-min exercise protocol. Exercise tasks involved leg ergometer cycling at 45 and 65% VO2 peak.

Screening tests. VO2 peak during leg cycling was determined during a continuous graded exercise test on a bicycle ergometer (Monark Ergometric 889E) beginning at 75 W and increasing 25 W every 3 min until voluntary cessation. Respiratory gases were continuously monitored via an open-circuit system (Ametek S-3A1 O2 and Ametek CD-3A CO2 analyzers) recorded every minute by an on-line, real-time PC-based mixing chamber system that our laboratory has used previously (4, 14, 41). This system was calibrated against two standard gases before, during, and after trials; runs at 100 Hz; and calculates and reports respiratory parameters over 10-s and 1-min intervals. In each trial, the open-circuit system was calibrated twice before rest and exercise with room air and a certified calibration gas (16% O2 and 4% CO2). VO2 peak tests were accepted as maximal if heart rate was within 10% of predicted and RER values exceeded 1.1. Immediately before the second exercise screening test, a catheter was placed in a forearm vein for withdrawal of blood for the determination of lactate threshold. The second screening test was done to ensure reliability of the measures, evaluate the possibility of a menstrual cycle phase effect on VO2 peak, and determine lactate threshold; lactate threshold was determined as the intensity of exercise at which blood lactate concentration is 1 mM above baseline (10). Body composition was determined by skinfold measurement (six-site skinfold with a Harpenden skinfold caliper) (24). Three-day diet records were collected four times to assess dietary habits and to monitor the subject’s caloric intake and macronutrient composition. Analysis of dietary records was performed with the Nutritionist III program (N-squared Computing, Salem, OR).

Tracer protocol. Subjects were studied in a postabsorptive state in the morning, and dietary intake was controlled for the 24 h immediately preceding each of the four isotope trials. Subjects rested the day before tracer trials and were given a standardized daily diet [2,183 kcal: 65% CHO (5.5 g·kg−1·day−1), 15% protein, and 20% fat (cereal, milk, and apple juice)] in the laboratory 3 h before exercise. We chose to test our subjects in a rested and recently fed, postabsorptive state to control for effects of meal size, composition, and timing as well as to mimic conditions in a nonlaboratory environment. On the morning of the trial, a catheter was placed in a hand or wrist vein to obtain “arterialized” blood samples by using the “heated hand vein” technique, and a forearm venous catheter was placed in the contralateral arm for continuous infusion of tracers. After collection of background blood and expired gas samples, a priming bolus of [6,6-2H2]glucose (D2-glucose), at 125 × the resting minute infusion rate, was given, and the subjects rested supine or semisupine for 90 min while the D2-glucose was continuously infused (Baxter Travenol 6300 infusion pump). The glucose tracer was infused at 1.6 mg·min−1. Infusion rates were increased to 4.8 and 6.4 mg·min−1 during exercise at 45% and 65% VO2 peak, respectively.

The isotope tracer infusion rates employed have been previously demonstrated by our laboratory to maintain stable plasma isotopic enrichment for the measurement of substrate kinetics throughout rest and the two exercise intensities (13, 14). Isotope tracers were obtained from Cambridge Isotope Laboratories (Woburn, MA), diluted in 0.9% sterile saline, and pharmacologically tested for sterility and pyrogenicity (School of Pharmacy, University of California, San Francisco, CA); on the day of the experiment, tracers were passed through a 0.2-µm Millipore filter (Nalgene, Rochester, NY) before infusion.

Blood sampling and analysis. Blood samples were taken at 0, 60, 75, 90 min of rest and at 15, 30, 45, 60 min of exercise and were immediately chilled on ice before centrifugation at 2,800 g for 13 min. Supernatants were stored at either −20°C or −80°C until analysis. Blood samples for glucose isotopic enrichment and glucose and lactate concentrations were collected in 8% perchloric acid and thoroughly mixed before centrifugation. Blood samples for determination of hormones were collected in heparinized syringes and transferred to sterile vacutainers containing EDTA and mixed before centrifugation. Aprotinin (4 mg/ml of blood) was added to prevent cross-reaction of glucagon fragments arising from proteolytic degradation. Blood glucose concentrations were determined with hexokinase kits (Sigma Chemical, St. Louis, MO), whereas blood lactate concentrations were determined by using the methods of Gutmann and Wahlefeld (17).
Plasma hormone concentrations were determined by 125I radioimmunoassay (Coat-A-Count kits; Diagnostic Products, Los Angeles, CA). Samples for each subject trial were analyzed together. The intra-assay coefficient of variations ranged from 2–5%, and the sensitivities of the assays were 2.9 pmol/l for estradiol, 0.06 nmol/l for progesterone, 1.2 μIU/ml for insulin, and 13 pg/ml for glucagon. Hematocrit was measured at each sampling point with the use of circular microcapillary tube readers (no. 2201, International Equipment) to ensure metabolite concentrations and isotopic enrichments were not affected by changes in plasma volume. Subjects drank tap water during each trial to maintain hydration status.

**Isotopic enrichment analysis.** Glucose isotopic enrichments were determined by using gas chromatography-mass spectrometry (GCMS) (GC model 5890 series II and MS model 5898A, Hewlett-Packard) of the penta-acetate derivative. In preparation for GCMS analysis, samples were neutralized with 2 N KOH and transferred to cation (AG 50W-X8, 50–100 mesh H+ resin) and anion (AG 1-X8, 100–200 mesh formate resin) exchange columns, and the glucose was eluted with distilled deionized water. Samples were then lyophilized, resuspended in methanol, and transferred to a 1-ml GCMS vial. One hundred microliters of 2:1 acetic anhydride-pyridine solution were added to each vial, and each vial was heated at 60°C for 10 min. Samples were subsequently dried under nitrogen, resuspended in 100 μl of ethyl acetate, and transferred to GCMS vials for analysis. For GCMS analysis, the injector temperature was set at 200°C and initial oven temperature was set at 110°C. Oven temperature was gradually increased by 35°C/min until it reached a final temperature of 255°C. Helium was used as the carrier gas for all analyses with a 35-to-1 ml/min splitless injection ratio. The transfer line temperature was set at 250°C, the source temperature was set at 200°C, and the quadrupole temperature was set at 116°C. Chemical ionization was performed with methane gas, and selected ion monitoring was used to monitor ion mass-to-charge ratios (331.20 and 333.20 for [12C]glucose and [6,6-2H]glucose, respectively).

**Calculations.** Glucose Ra, glucose Rd, and metabolic clearance rate (MCR) were calculated by using equations defined by Steele (37) and modified for use with stable isotopes

\[ R_a (\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \frac{F - V[(C_1 + C_2)/2][(IE_2 - IE_1)(t_2 - t_1)]}{[IE_1 + IE_2]V^2} \]

\[ R_d (\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = R_a - V[(C_2 - C_1)/(t_2 - t_1)] \]

\[ \text{MCR (ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \frac{R_d}{[(C_1 + C_2)/2]} \]

where F represents the isotopic infusion rate, V is the estimated volume distribution of glucose (180 ml/kg), C1 and C2 are concentrations at sampling times t1 and t2, respectively, and IE1 and IE2 are the glucose isotopic enrichments of [2H]glucose at sampling times t1 and t2, respectively. Values for isotopic enrichment were corrected for baseline enrichments from background blood samples taken before infusion of the isotopes. Energy derived from total CHO and lipid oxidation was calculated (12)

\[ \% \text{Energy from CHO} = \left[ (\text{RER} - 0.707)/0.293 \right] \times 100 \]

\[ \% \text{Energy from lipid} = 100 - \left[ (\text{RER} - 0.707)/0.293 \right] \times 100 \]

Energy from CHO oxidation (kJ/min)

\[ = \left[ (%\text{CHO}/100) \times (\text{VO}_2) \right] \times (21.1 \text{ kJ/liter O}_2) \]

Energy from lipid oxidation (kJ/min)

\[ = \left[ (1 - %\text{CHO}/100) \times (\text{VO}_2) \right] \times (19.7 \text{ kJ/liter O}_2) \]

**Statistics.** Representative values for hormone concentrations and glucose kinetics were obtained by averaging results from the final 15 min (75, 90 min) of rest and 30 min (30, 45, 60 min) of exercise. Despite concerted efforts to control prior activity and diet and to standardize time of day and menstrual cycle phase, cell sizes in ANOVA varied because endocrine status criteria were not always met, mainly due to inconsistencies in progesterone rise after luteinizing hormone surge. Hence, data are presented as means ± SE of parameters for which paired (luteal-follicular) data are available. Because there were no significant differences between resting values for the two trials in each phase of the menstrual cycle, the resting values were pooled to obtain one follicular (n = 7) and one luteal (n = 5) value. Significance of differences between mean values for physical characteristics was determined by paired t-tests. Significance of differences among mean values representing metabolic concentrations and flux rates for the four conditions were determined by using two-way ANOVA with repeated measures followed by multiple comparisons (S-Plus 2000, Professional Release 2). Significance of differences over time during exercise was determined by using one-way ANOVA with post hoc Scheffe’s test. Statistical significance (α) was set at 0.05.

**RESULTS**

**Subject characteristics.** Physical characteristics of subjects are listed in Table 1. Subjects were weight stable throughout the study period, with no changes in percent body fat or VO2peak between menstrual phases.

**RER, CHO, and lipid oxidation.** There was an increase in RER in the transition between rest and exercise at both intensities in both menstrual phases (Table 2), but this increase was not significant, except for the 65% trial in FP (P < 0.05). Values for RER during exercise were higher for the 65% trials compared with the 45% trials in both menstrual phases, but these values were not significantly different between phases.

At rest, >50% of the energy was derived from CHO sources for both menstrual phases, but no significant phase difference was observed. During exercise in all isotope trials, there was a shift to a greater reliance on CHO sources, which was significant (P < 0.05, Table 2). Total energy coming from CHO during exercise at

<table>
<thead>
<tr>
<th>Variable</th>
<th>FP</th>
<th>LP</th>
</tr>
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<tbody>
<tr>
<td>Age, yr</td>
<td>24.9 ± 1.4</td>
<td>26.0 ± 1.8</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165.6 ± 1.6</td>
<td>165.9 ± 2.2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>61.4 ± 1.1</td>
<td>60.2 ± 1.4</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>23.3 ± 1.6</td>
<td>21.4 ± 1.5</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>14.4 ± 0.8</td>
<td>12.9 ± 0.7</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>47.0 ± 0.8</td>
<td>47.3 ± 1.1</td>
</tr>
<tr>
<td>VO2peak, ml · kg⁻¹ · min⁻¹</td>
<td>44.2 ± 1.8</td>
<td>42.9 ± 2.1</td>
</tr>
<tr>
<td>l/min</td>
<td>2.7 ± 0.1</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Lactate threshold, %VO2peak</td>
<td>NM</td>
<td>67.0 ± 3.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of women. FP, follicular phase; LP, luteal phase; VO2peak, peak oxygen consumption; NM, not measured.

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In all four isotope trials, there was a significant contribution of lipid to 75% of the energy used to do work. The difference was not significant during exercise compared with resting values ($P < 0.05$, Table 2), but no significant phase or intensity effect was observed.

**Ovarian hormone responses.** Individual estradiol and progesterone concentrations at rest and during exercise are shown in Table 3. Values that did not meet the endocrine status criteria were excluded from mean value representation and statistical analyses. Extra-

<table>
<thead>
<tr>
<th>Table 2. Ergonomic and physiological parameters of subjects during rest and exercise in follicular and luteal menstrual phases</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Workload, W</td>
</tr>
<tr>
<td>VO$_2$, ml·kg$^{-1}$·min$^{-1}$</td>
</tr>
<tr>
<td>RER</td>
</tr>
<tr>
<td>EE kcal/min</td>
</tr>
<tr>
<td>Energy from CHO kcal/min</td>
</tr>
<tr>
<td>Energy from lipid kcal/min</td>
</tr>
<tr>
<td>Minute ventilation, l/min</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
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<tr>
<td>Blood pressure, mmHg</td>
</tr>
<tr>
<td>diastolic</td>
</tr>
<tr>
<td>systolic</td>
</tr>
<tr>
<td>Hematocrit, %</td>
</tr>
</tbody>
</table>

### Table 3. Ovarian hormone concentrations during rest and exercise for each subject

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Rest</th>
<th></th>
<th></th>
<th>Exercise</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>FP45</td>
<td>LP45</td>
<td>FP65</td>
<td>LP65</td>
<td></td>
</tr>
<tr>
<td>Estradiol, ng/ml</td>
<td>238.7§</td>
<td>82.7</td>
<td>88.7§</td>
<td>47.9§</td>
<td>269.3§</td>
</tr>
<tr>
<td>Progesterone, ng/ml</td>
<td>0.04§</td>
<td>4.9</td>
<td>0.3§</td>
<td>2.3§</td>
<td>0.8§</td>
</tr>
</tbody>
</table>

Values are mean ± SE; $n = no.$ of women. 45 and 65, Exercise intensities in % of VO$_2$peak; CHO, carbohydrate; EE, energy expenditure; RER, respiratory exchange ratio; VO$_2$, oxygen consumption; CO$_2$, carbon dioxide production. *Significantly different from resting conditions, $P < 0.05$. †Significantly different from 45% trials, $P < 0.05$. §Values excluded because endocrine status criteria were not met.

45% VO$_2$peak was 9.7% lower in LP compared with FP, but the difference was not significant. During exercise at 65% VO$_2$peak in both menstrual phases, as much as 6.7 kcal/min (>75%) of the energy used to do work was derived from CHO sources. The contribution of lipid to energy expenditure was similar to that of CHO at rest. In all four isotope trials, there was a significant increase in energy derived from lipid in response to exercise compared with resting values ($P < 0.05$, Table 2), but no significant phase or intensity effect was observed.
diol and progesterone concentrations before the commencement of exercise were significantly higher in LP compared with FP \( (P < 0.05, \text{Table 3}) \). Increases in estradiol and progesterone occurred during exercise in both menstrual phases, with estradiol concentration being significantly greater at 65% trials \( (P < 0.05, \text{Table 3}) \). Estradiol and progesterone levels remained significantly elevated during exercise at both intensities in LP compared with FP \( (P < 0.05, \text{Table 3}) \). The overall pattern of response was similar in the two phases of the menstrual cycle.

**Blood glucose and lactate concentrations.** Blood glucose concentrations tended to fall in response to exercise. However, the change was not significant for any of the four trials, and concentrations remained relatively constant at 4.2–4.7 mM throughout exercise. Furthermore, there were no significant differences in blood glucose concentration between any of the four trials during rest or exercise at each time point (Fig. 1A).

Blood lactate concentrations increased in the transition between rest and exercise in both menstrual phases in an intensity-dependent manner (Fig. 1B). The increase in blood lactate concentrations was significant during exercise at 65% \( \dot{V}O_2\text{peak} \) in both menstrual phases. Lactate concentrations during exercise were significantly higher for the 65% compared with the 45% trials in both menstrual phases \( (P < 0.05) \), but there was no significant phase effect on blood lactate response during exercise at either intensity.

**Blood glucose kinetics.** The \([6,6-^2H]\)glucose isotopic enrichments are shown in Fig. 2A. The isotopic enrichments for all four trials were stable during rest and the last 30 min of exercise. Glucose \( R_a \) increased significantly during exercise compared with at rest \( (P < 0.05) \) for all four of the exercise conditions, and values are presented as the average of the last 15 min of rest and 30 min of exercise (Fig. 2B). Glucose \( R_a \) was 30% higher during the 65% trials compared with the 45% trials \( (P < 0.05) \), demonstrating a significant intensity effect in both menstrual phases \( (P < 0.05) \). However, there was no significant effect of menstrual cycle phase on glucose \( R_a \) during rest and exercise \( (P > 0.05) \).

Responses of glucose \( R_d \) to rest and exercise in both menstrual phases were similar to those of \( R_a \) (Fig. 2C). The similarity between our glucose \( R_a \) and \( R_d \) is consistent with the observed stable glucose concentrations and isotopic enrichments during exercise. The MCR of glucose (Fig. 2D) was similar to glucose \( R_d \) among the four trials because there was no significant difference in blood glucose concentrations between exercise trials.

**Insulin and glucagon responses.** In all exercise-isotope trials, insulin concentrations decreased significantly in response to exercise compared with resting values \( (P < 0.05, \text{Fig. 3A}) \). There was a significant intensity effect in FP \( (P < 0.05, \text{rest} > 45\% \geq 65\%) \), but no significant phase effect was observed during rest and exercise in both menstrual phases.

Glucagon concentrations increased significantly during exercise at 65% \( \dot{V}O_2\text{peak} \) in both menstrual phases \( (P < 0.05, \text{Fig. 3B}) \). However, no significant phase or intensity effect was observed. There was one significant menstrual cycle effect on the insulin-to-glucagon ratio during exercise at 45% \( \dot{V}O_2\text{peak} \) \( (\text{insulin-to-glucagon ratio in LP < FP; P < 0.05}) \).

**DISCUSSION**

Results of the present investigation corroborate those of previous studies that demonstrated a direct relationship between exercise intensity and blood glucose flux \( (13, 14, 35) \). Furthermore, this study demonstrates that in a 3- to 4-h postabsorptive state there were no significant effects of menstrual cycle phase on blood glucose \( R_a, R_d, \text{MCR}, \) or whole body CHO and lipid oxidation rates during moderate-intensity exercise. In the aggregate, our results and those of others reveal overriding effects of exercise and CHO nutrition on glucose flux, CHO, and lipid oxidation rates in women exercising during various menstrual cycle phases.

Our results present similarities as well as differences compared with results of others \( (8, 13, 14, 44) \). Similar to a study of men \( (13) \) and a study of women tested in the FP \( (14) \), in our study, glucose flux rose during exercise and as exercise intensity increased in

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**Fig. 1.** Blood glucose (A) and lactate (B) concentrations over time for the 4 isotope trials. Values are means ± SE at each time point for 7 women (FP45, FP65), 6 women (LP45), and 5 women (LP65). FP, follicular phase; LP, luteal phase; 45 and 65, exercise intensities in percent of peak oxygen consumption. *Significantly different from resting conditions, \( P < 0.05 \). +Significantly different from 45% trials, \( P < 0.05 \).
When women were studied fasted as opposed to postabsorptive, the glucose flux rates between menstrual cycle phase were small, ranging from 14% (44) to 26% (8). Hence, small differences in glucose flux due to menstrual cycle phase are easily overridden by other factors, such as exercise and recent CHO intake.

Although not previously recognized, the overriding effect of recent CHO nutrition shifting the balance of substrate use to CHO oxidation in resting exercising humans has been reported (3). Several studies have found similar RER during rest and exercise between menstrual phases when the subjects were studied in a 3-h postabsorptive state (2, 5, 6). In our investigation, women were studied after 1 day of rest and with controlled energy and CHO intake. Our subjects consumed a standardized supper that we provided and consumed a prescribed breakfast in the laboratory; hence, we report data on fed and rested 3- to 4-h postabsorptive subjects. Furthermore, the two exercise tasks we used raised metabolic rate five to seven times above resting and resulted in 5- to 11-fold increments in CHO oxidation. More energy was derived from CHO sources during exercise (in an intensity-dependent manner) in both menstrual phases. At higher intensities, it is likely that glycolytic flux governs substrate selection (7). Thus the presence of relatively full muscle and liver glycogen stores as well as the exercise-induced crossover to dependence on CHO oxidation overcame the relatively smaller, if any, effects of ovarian hormones in promoting lipid oxidation.

Consistent with our interpretation of the influence of CHO intake on energy substrate partitioning in women are results of two recent reports in which glucose flux in fasted subjects was measured (8, 44). Zderic et al. (44) reported a significant (14%) decrease in glucose Ra in women exercising in LP compared with FP. Similarly, in their studies of glucose kinetics in women exercising at 70% V02peak, Campbell et al. (8) observed that the consumption of a CHO-containing “sports drink” abolished differences in glucose Ra between

Fig. 2. A: isotopic enrichments (IE) of [6,6-2H]glucose (D2-glucose) over time. Also shown is effect of menstrual cycle phase and exercise intensity on [6,6-2H]glucose rate of appearance (Ra; B), rate of disappearance (Rd; C), and metabolic clearance rate (MCR; D). Values for IE are means ± SE at each time point, and values for Ra, Rd, and MCR are means ± SE of last 15 and 30 min of rest and exercise, respectively, for 7 women (FP45, FP65), 6 women (LP45), and 5 women (LP65). *Significantly different from resting conditions, P < 0.05. +Significantly different from 45% trials, P < 0.05.
Because maternal glucose is the main fuel for fetal development and growth, there may be an evolutionary adaptation in women that acts to preserve blood glucose under situations of physiological stress. These adaptations may be unmasked only under extreme conditions, such as when fasting and vigorous exercise combine.

Several lines of evidence indicate that estradiol and progesterone participate in the regulation of substrate utilization during rest and prolonged exercise, but studies examining the relationship between ovarian hormones and metabolic responses in humans (6, 36, 43, 44) and rodents (1, 19, 21, 26) have produced equivocal results. This is not surprising given the difficulty in trying to assess metabolic actions of a single hormone in vivo. For example, as in the present investigation, no between-phase differences in blood glucose (2, 5, 25, 32) concentrations or glucose flux (44) or lactate (5, 25, 32, 34) concentrations in resting women have been reported (44). Similarly, Ruby and colleagues (36) reported no differences in resting levels of glucose or lactate or resting glucose flux in response to transdermal estradiol administration in amenorrheic women. In addition, Minson et al. (31) found no differences in resting plasma epinephrine concentrations between menstrual phases, which could partially explain the absence of differences in resting blood glucose flux.

Blood glucose and lactate concentrations in our study were not affected by menstrual phases during prolonged exercise. In this respect, our results are consistent with those of some previous studies showing no effect of menstrual cycle phases on blood glucose (2, 5, 6, 25) or lactate (5, 6, 25) concentration responses to prolonged exercise. However, others observed greater blood glucose concentrations in women during prolonged exercise in LP (9, 44). This result may be because blood glucose concentration was elevated before exercise in LP compared with FP. In contrast, Lavoie et al. (29) observed lower glucose concentrations during prolonged exercise in LP compared with FP in overnight-fasted women previously fed a CHO-poor diet. The investigators speculated that ovarian hormones impaired hepatic gluconeogenesis, contributing to lower glucose concentrations observed in LP (29). Hence, differences between our findings and those of Lavoie et al. may be due to differences in nutritional status of subjects studied. To summarize, our results and those in the literature can reasonably be interpreted to indicate that blood glucose and lactate responses to prolonged exercise are similar in the FP and LP when subjects are several hours postabsorptive. It may require a stronger stress (>12 h of fasting, >60 min of prolonged exercise) to elicit a change in glucose homeostasis. Long-term adaptation to changes in ovarian hormones due to the menstrual cycle may have adapted the body to respond well to exercise to maintain glucose homeostasis.

Blood glucose concentrations in the present study remained relatively constant among the four trials during steady-state exercise. Constancy of blood glucose concentrations over time in exercising women (Fig. 1A) indicates good matching of glucose production and disposal rates when liver and muscle glycogen levels are abundant. Although we did not measure rates of gluconeogenesis or liver glycogenolysis in the present investigation, in other recent studies, our laboratory used dual-label (2H and 13C) glucose tracers and mass isotopomer distribution analysis to assess carbon recycling and gluconeogenic rates in exercising men (40–42). These studies indicated that the menstrual cycle phase differences in glucose Ra observed by others (8, 44), but not by us (Fig. 2B), may be attributable to the dietary controls we imposed and the overriding (in comparison to menstrual cycle) effects of CHO nutrition and liver glycogen storage.

The exercise intensity-dependent changes in glucose Ra that we observed in both LP and FP were coordinated with changes in insulin and glucagon concentrations. In the women that we studied, insulin fell during exercise at both intensities and glucagon rose during exercise at 65% VO2peak, but we did not observe consistent menstrual phase effects on glucagon or insulin concentrations in exercising women (Fig. 3). However, we did observe one significant phase difference in the
insulin-to-glucagon ratio in the 45% trials (LP < FP, 
P < 0.05). However, because neither insulin nor glucagon concentrations changed significantly between menstrual cycle phases, at this time we cannot attribute a physiological significance to the one change in insulin-to-glucagon ratio that we observed.

The current body of knowledge of the roles of ovarian hormones in the regulation of glucose metabolism is also derived from studies of insulin action after administering of exogenous estradiol, progesterone, or both in ovarietomized rats (28, 33). In ovarietomized rodents, insulin-stimulated glucose uptake decreases (28, 33). This insulin resistance of ovarietomy was mainly an effect of estradiol deficiency because estradiol replacement without progesterone was followed by full restoration of insulin action. In contrast to estradiol, progesterone appeared to suppress the effects of estradiol on insulin-stimulated glucose uptake (28, 33). The suppression by progesterone of the augmentation of insulin-stimulated glucose uptake by estradiol may provide a physiological counterbalance during pregnancy conditions, when concentrations of both ovarian hormones are high. In addition, Hansen and colleagues (19) also reported a significant decrease in contraction-stimulated glucose uptake but no significant changes in skeletal muscle glycogen concentration or glucose transporter-4 content in ovarietomized rats. Although it has been shown that elevations in circulating levels of estradiol and progesterone in rodents can influence insulin action and, therefore, glucose metabolism in hyperinsulinemic condition (plasma insulin of ~5.1 nM), altered insulin-stimulated glucose disposal does not appear to change in a major way in response to menstrual cycle variations in concentrations of endogenous ovarian hormones in humans.

Summary and conclusions. Results of this study indicate that 1) as seen in previous studies, glucose flux is directly related to exercise intensity regardless of menstrual cycle phase; and 2) menstrual cycle phase does not alter glucose flux in rested, 3-h postabsorptive women during rest, moderate-intensity exercise (45% VO₂_peak), or high-intensity exercise (65% VO₂_peak). In combination with the results of others (8, 44), we conclude that the effects of endogenous ovarian hormones on glucose flux and overall CHO oxidation are small compared with the much larger effects of exercise and recent CHO nutrition.

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