Carbohydrate Loading and Female Endurance Athletes: Effect of Menstrual-Cycle Phase

Rebecca T. McLay, Christine D. Thomson, Sheila M. Williams, and Nancy J. Rehrer

This study compared 3 d of carbohydrate loading (CHOL; 8.4 g·kg⁻¹·d⁻¹ carbohydrate) in female eumenorrheic athletes with 3 d of an isoenergetic normal diet (NORM; 5.2 g·kg⁻¹·d⁻¹ carbohydrate) and examined the effect of menstrual-cycle phase on performance, muscle-glycogen concentration [glyc], and substrate utilization. Nine moderately trained eumenorrheic women cycled in an intermittent protocol varying in intensity from 45% to 75% VO₂max for 75 min, followed by a 16-km time trial at the midfollicular (MF) and midluteal (ML) phases of the menstrual cycle on NORM and CHOL. Time-trial performance was not affected by diet (CHOL 26.10 ± 1.04 min, NORM 26.16 ± 1.35 min; P = 0.494) or menstrual-cycle phase (MF 26.05 ± 1.10 min, ML 26.23 ± 1.33 min; P = 0.370). Resting [glyc] was lowest in the MF phase after NORM (575 ± 145 mmol·kg⁻¹·dw⁻¹), compared with the MF phase after CHOL (728 mmol·kg⁻¹·dw⁻¹) and the ML phase after CHOL and NORM (756 and 771 mmol·kg⁻¹·dw⁻¹, respectively). No effect of phase on substrate utilization during exercise was observed. These data support previous observations of greater resting [glyc] in the ML than the MF phase of the menstrual cycle and suggest that lower glycogen storage in the MF phase can be overcome by carbohydrate loading.

Key Words: midfollicular, midluteal, performance, muscle glycogen, substrate utilization

Carbohydrate loading has been shown to enhance muscle-glycogen stores and improve the endurance capacity (2, 29) and performance (23, 24) of men exercising longer than 90 min. Although these studies primarily examined male subjects, and most have not included a placebo-treatment group, the results have contributed to the recommendation for all endurance athletes (men and women) to carbohydrate load before important events. Limited data are slowly becoming available that examine the effect of carbohydrate loading in endurance-trained women at different stages during the menstrual cycle, but the benefits of carbohydrate loading in this population are far from substantiated.

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Menstrual-cycle phase might influence muscle-glycogen concentration [glyc] and, subsequently, exercise capacity or performance in eumenorrheic female athletes. Hackney (9) observed that resting muscle glycogen was significantly greater in the midluteal (ML) phase than in the midfollicular (MF) phase of the menstrual cycle. Nicklas et al. (20) found that although resting [glyc] was not different between the luteal and follicular phases, muscle-glycogen repletion was greater, and there was a tendency \( P < 0.07 \) for cycle time to exhaustion at 70% \( VO_{2\text{max}} \) to be greater, during the luteal phase, 3 d after an exhaustive exercise bout.

Tarnopolsky and colleagues (29) found that increasing dietary carbohydrate from 55% to 75% of total energy had no effect on [glyc] or endurance capacity in the MF phase of the menstrual cycle. More recently, Walker et al. (31) detected a significant increase in muscle-glycogen content and exercise capacity in the luteal phase of the menstrual cycle after a high-carbohydrate diet (~78% carbohydrate) compared with a mixed diet (~48% carbohydrate). These studies suggest that muscle-glycogen synthesis might be enhanced during the luteal phase of the menstrual cycle, when circulating levels of reproductive hormones are high (17).

Other researchers have investigated the effect of carbohydrate loading on [glyc] (13, 22, 30) and exercise performance (22) in the MF phase of the menstrual cycle. When fed a carbohydrate-loading diet containing high levels of carbohydrate (8.8–9.9 g·kg body weight\(^{-1}\)·d\(^{-1}\)), female athletes could significantly increase [glyc] during the MF phase of the menstrual cycle (13, 22, 30), although this had no effect on performance (22). Unfortunately, the results of these studies are confounded by the fact that some (22, 30) or all (13) of the participants were taking triphasic-type oral contraceptives.

To date, no investigation has studied the interaction of menstrual-cycle phase and a high-carbohydrate diet and exercise taper in the same eumenorrheic female subjects. Therefore, the purpose of this study was to test the efficacy of carbohydrate loading in eumenorrheic, female endurance athletes and to determine whether muscle-glycogen storage, exercise performance, and substrate metabolism at varying exercise intensities are influenced by menstrual-cycle phase.

**Methods**

**Subjects**

Nine moderately trained women volunteered to participate in the study and provided written informed consent after approval by the ethics committee of the Southern Regional Health Authority (Dunedin, New Zealand). All subjects (cyclists, triathletes, duathletes, or mountain bikers) were eumenorrheic with regular cycles (cycle length range = 24–34 d), and none had taken oral contraceptives for at least 6 mo before the study (Table 1).

**Determination of Menstrual-Cycle Phase**

Subjects were given an oral digital thermometer and instructed how to accurately record basal body temperature every morning before rising from bed for 2–4 mo before the experimental testing. An increase of 0.3 °C in basal body temperature was used to identify the day of ovulation (10). The follicular phase of the menstrual cycle...
cycle was defined as the days from the onset of menses to ovulation, and the luteal phase, as the days from ovulation to the next menses. Confirmation of desired hormonal status was verified with blood estradiol and progesterone measurements from samples collected before the start of each experimental exercise session. Subjects took part in the experimental exercise sessions at Days 8–12 (MF) and Days 21–29 (ML) of their menstrual cycles, depending on individual menstrual-cycle length.

Preliminary Testing

All exercise testing took place on a King cycle ergometer (King Cycle Trainer/Tester, Bucks, England) using each subject’s own bicycle, which was adjusted and calibrated before each session. At the initial laboratory visit, $VO_{2\text{max}}$ was determined using a graded maximal cycling exercise test (15) during the ML phase of the menstrual cycle. Height and weight were recorded, and percentage body fat was estimated using the sum of 7 skinfolds (21). The $VO_{2\text{max}}$ test and anthropometric measures were repeated at the end of the study during the ML phase to determine changes caused by training.

Four days before each experimental exercise session subjects completed a 40-km cycle to deplete muscle-glycogen stores. Subjects cycled at 65% $VO_{2\text{max}}$ for 20 min followed by 5 intervals of 3 min at 80% $VO_{2\text{max}}$ and 2 min at 65% $VO_{2\text{max}}$. The remaining distance was completed by maintaining an exercise intensity $\geq$65% $VO_{2\text{max}}$. After completing the depletion ride, subjects were given an experimental diet to follow for the next 3 d and instructed to limit their physical activity to no more than 30 min/d at a low intensity. Subjects consumed either a normal diet (NORM) or a carbohydrate-loading diet (CHOL) once each during both the ML and MF phases of their menstrual cycles (a total of 4 trials over, maximally, 3 mo per subject). The order of the experimental exercise sessions (ML vs. MF) and the order of the diets (NORM vs. CHOL) were randomized, with some adjustment for individual menstrual-cycle phase and length and subject availability.

Dietary Treatments

Energy intake for each diet was calculated using each subject’s expected energy expenditure based on surface area and resting metabolic rate as a function of age and sex (18). The energy calculated to perform the experimental exercise session was added to give the total predicted energy intake for each diet. All diets were designed to be isoenergetic for each subject throughout the experimental period.
Any deviation from the experimental diet was recorded, and changes were made accordingly so that the subsequent experimental diet, in the alternate phase of the menstrual cycle, would remain as similar as possible. The nature of the diet was undisclosed to the subjects, with all the food necessary to complete the 3 d of each diet supplied to each subject. Food intake was recorded for each 3-d experimental-diet period to estimate compliance. Nutrient composition was determined using Diet Entry and Storage and Diet Cruncher software, which uses food-composition data from the New Zealand Institute for Crop and Food Research (3). The characteristics of the test diets are presented in Table 2.

**Experimental Protocol**

A standard breakfast (energy = 1672 kJ, carbohydrate = 87 g, protein = 14 g, fat = 2 g) was consumed by all subjects 1.5–2 h before each experimental exercise session to simulate precompetition practices. About 30 min before the start of each session, a tissue sample from the lateral portion of the vastus lateralis muscle was obtained by needle biopsy according to the modified technique of Evans et al. (8). The 4 muscle-biopsy samples were obtained from the same leg at sites located within 1 cm of each other. The samples (approximately 50–200 mg wet muscle) were immediately dissected of any connective tissue, cleaned of blood, and quenched in liquid nitrogen before being stored at −80 °C. Each session was conducted at room temperature (17 ± 2 °C, range 14–22 °C) and took place at the same time of day for each subject. Each session comprised a warm-up, a submaximal intermittent controlled-intensity (SICI) exercise period including 10-min stages at 45%, 60%, and 75% VO$_2$max, and a 16-km time trial (Figure 1). Including stages of varying exercise intensity and a time trial in this exercise protocol allows substrate utilization to be measured during steady-state exercise and to mimic the cycling

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Diet Analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary component</td>
<td>NORM, mean ± SD</td>
</tr>
<tr>
<td>Energy, kJ</td>
<td>12,135 ± 1168</td>
</tr>
<tr>
<td>CHO, g</td>
<td>359 ± 51</td>
</tr>
<tr>
<td>CHO, %TE</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>Protein, g</td>
<td>125 ± 11</td>
</tr>
<tr>
<td>Protein, %TE</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>Fat, g</td>
<td>116 ± 19</td>
</tr>
<tr>
<td>Fat, %TE</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>CHO, g·kg·BW$^{-1}$·d$^{-1}$</td>
<td>5.2 ± 0.9</td>
</tr>
<tr>
<td>Protein, g·kg·BW$^{-1}$·d$^{-1}$</td>
<td>1.8 ± 0.2</td>
</tr>
</tbody>
</table>

NORM indicates normal diet; CHOL, carbohydrate-loading diet; %TE, percentage of total energy intake; BW, body weight; and CHO, carbohydrate.
racing situation, where riders tend to take turns at the head of a bunch before the all-out effort at the end. During the time trial, subjects were self-motivated and not prompted by the researchers. The distance completed and current power-output display was visible to each subject, but the time taken was not.

Body weight was recorded before and after each session, and ad libitum water ingestion throughout each session was documented. Each exercise testing session required subjects to cycle for at least 100 min.

Blood samples were collected via venipuncture before the start of the SICI exercise period and in the 1–2 min before and immediately after the termination of the performance time trial. Finger-prick blood samples were collected for lactate analysis before the start of the SICI exercise period, in the last minute of each sub-maximal block of increasing exercise intensity, and before and after the time trial (Figure 1). Expired air was sampled continuously to determine minute ventilation ($V_e$), oxygen consumption ($VO_2$), and carbon-dioxide production ($VCO_2$; Sensor-medics, Model 2900Z BXB, Yorba Linda, CA). Heart rate (HR) was assessed every minute throughout the experimental exercise sessions (Polar HR monitor, Model PE 4000, Kemplele, Finland).

**Analyses**

Finger-prick blood samples were immediately analyzed for blood lactate (YSI 1500 Sport, Yellow Springs, OH). Serum progesterone and estradiol concentrations were determined using radioimmunoassay ($^{125}$I-labelled hormone) Coat-a-Count kits (Diagnostic Products Corp, Los Angeles, CA). The intra-assay coefficients of variation were 6.6% and 9.8% for the progesterone and estradiol assays, respectively. Concentrations of serum free fatty acids were determined using an optimized enzymatic colorimetric assay (Boehringer Mannheim, Germany, Cat No 1383 175) with an intra-assay coefficient of variation of 5.5%. Free glycerol concentration
was determined using a quantitative enzymatic assay (Sigma Diagnostics, St Louis, MO) with an intra-assay coefficient of variation between 10% and 13%.

Muscle samples were freeze-dried using a Free Zone 6-L freeze-dry system (Model 77530, Labconco, Kansas City, MO) for 24 h and then weighed to obtain a dry weight. The samples were digested chemically and enzymatically with sodium hydroxide and amyloglucosidase (19). The resulting glucose was analyzed on the Cobas Mira Plus (Roche, Nutley, NJ) using a Roche Diagnostic Systems Ultimate 5 Gluc HK glucose test kit (Art 0736724). The intra-assay coefficient of variation was 11.6%, and the calculated recovery was 104.4% ± 4.3%, using a glycogen standard (Sigma rabbit-liver glycogen type III).

Calculations

Gas samples were collected and analyzed every 20 s during the SICI exercise period and time trial. Means were calculated during Minutes 7–9 of each 10-min block at 45%, 60%, and 75% \( \text{VO}_{2\text{max}} \) by averaging all the data points collected during the time trial. The \( \text{VO}_2 \) and \( \text{VCO}_2 \) measures from the SICI exercise period were used to calculate the nonprotein respiratory-exchange ratio (RER), and carbohydrate and fat utilization and oxidation rates were estimated using standardized metabolic calorimetry formulas (18). Because of a significant anaerobic contribution to fuel utilization, RER and estimated carbohydrate and fat utilization were not calculated during the time trial. For the purposes of the calculation of estimated carbohydrate and fat utilization during the SICI exercise period, any RER value greater than 1.00 was assigned the value 1.00 (5 individual data points were treated in this manner). Data were also analyzed with these values removed, and this did not influence the conclusions or significance.

Hematocrit and hemoglobin (Hexiometer, OSM 3, Copenhagen, Denmark) results were used to calculate plasma-volume changes according to the method of Dill and Costill (7).

Statistical Analyses

A mixed model with a random effect for each woman was used to analyze the data. The model included terms for the effects of diet and menstrual-cycle phase and accounts for the underlying covariance structure. An interaction term between these factors was considered but not included if it was not statistically significant. The physical characteristics of the subjects at the beginning and the end of the study were compared using paired, 2-tailed \( t \)-tests. The data were analyzed with STATA statistical software package, Release 8.0 (STATA, College Station, TX). Statistical significance was accepted as \( P \leq 0.05 \). Data are presented as means and standard deviations or differences and 95% confidence intervals.

Results

Hormone Analyses

Serum estradiol and progesterone results are summarized in Table 3. Resting serum progesterone levels indicated that 5 individual experimental exercise sessions
Table 3  Hormone Analyses, Mean ± SD

<table>
<thead>
<tr>
<th>Condition</th>
<th>Serum estradiol (pmol/L)</th>
<th>Serum progesterone (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOL</td>
<td>309 ± 207</td>
<td>15.3 ± 20.0</td>
</tr>
<tr>
<td>NORM</td>
<td>286 ± 126</td>
<td>19.1 ± 23.2</td>
</tr>
<tr>
<td>MF</td>
<td>251 ± 191</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>ML</td>
<td>354 ± 115</td>
<td>36.6 ± 18.3</td>
</tr>
<tr>
<td>MF/CHOL</td>
<td>275 ± 252</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>ML/CHOL</td>
<td>355 ± 132</td>
<td>33.9 ± 18.4</td>
</tr>
<tr>
<td>MF/NORM</td>
<td>228 ± 114</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>ML/NORM</td>
<td>353 ± 110</td>
<td>39.0 ± 19.4</td>
</tr>
</tbody>
</table>

CHOL indicates carbohydrate-loading diet; NORM, normal diet; MF, midfollicular; ML, midluteal.

were conducted in the wrong phase of the menstrual cycle. Expected values were 0.48–4.5 nmol/mL in the follicular phase and 5.1–67 nmol/mL in the luteal phase (6). Results from these mistimed exercise sessions were excluded from all data analyses. Data were therefore available on the following number of subjects for each experimental condition: MF/CHOL, n = 8; ML/CHOL, n = 6; MF/NORM, n = 8; ML/NORM, n = 7.

Muscle-Glycogen Measurements

A small but nonsignificant difference in resting [glyc] after CHOL compared with NORM was detected when the data were pooled for menstrual-cycle phase: NORM, 666 ± 170, vs. CHOL, 739 ± 126 mmol/kg dry weight (–75 mmol/kg dry weight, 95% CI = –167, 17 mmol/kg dry weight, \( P = 0.111 \)). There was a significant effect of menstrual-cycle phase on resting [glyc], and a significant dietary-treatment and menstrual-cycle-phase interaction was detected (\( P = 0.016 \); Figure 2). The lowest levels of resting [glyc] were observed in the MF phase of the menstrual cycle after NORM (575 mmol/kg dry weight), compared with the MF phase after CHOL (728 mmol/kg dry weight) and the ML phase after CHOL and NORM (756 and 771 mmol/kg dry weight, respectively; Figure 2).

Physiological Responses

Heart rate, \( \text{VO}_2 \), and \( \text{VCO}_2 \) increased steadily with increasing exercise intensity. Comparison of mean values of pooled menstrual-cycle and dietary data for these measures at each exercise intensity revealed no significant differences. These data and the data for each separate condition are presented in Table 4.

Performance Variables

Performance during the 16-km time trial was not significantly different on NORM (26.16 ± 1.35 min) versus CHOL (26.10 ± 1.04 min; +0.23 min, 95% CI = –0.35, 0.81 min, \( P = 0.434 \)). Performance was also not affected by menstrual-cycle phase (ML 26.23 ± 1.33 vs. MF 26.05 ± 1.10 min; +0.26 min, 95% CI = –0.31, 0.83 min,
Figure 2 — Resting muscle-glycogen concentration at the midfollicular versus midluteal phase of the menstrual cycle after carbohydrate-loading (CHOL) or normal (NORM) diet. *Significant difference between midfollicular/NORM compared with the other experimental treatments ($P = 0.016$).

$P = 0.370$). The treatment order also had no effect on performance. Time-trial results for each experimental condition were as follows: MF/CHOL, 25.91 ± 0.78 min; ML/CHOL, 26.35 ± 1.35 min; MF/NORM, 26.18 ± 1.39 min; and ML/NORM, 26.13 ± 1.42 min.

**Estimated Carbohydrate and Fat Utilization**

Menstrual-cycle phase had no effect on RER during exercise at 45%, 60%, or 75% $V_O^{2max}$ (Table 5). There was, however, a significantly greater RER after CHOL than after NORM at 45% $V_O^{2max}$ (NORM 0.90 ± 0.03 vs. CHOL 0.92 ± 0.02; $-0.03$; 95% CI = $-0.05$, $-0.01$; $P < 0.001$), 60% $V_O^{2max}$ (NORM 0.89 ± 0.03 vs. CHOL 0.92 ± 0.02; $-0.04$; 95% CI = $-0.05$, $-0.02$; $P < 0.001$), and 75% $V_O^{2max}$ (NORM 0.96 ± 0.03 vs. CHOL 0.99 ± 0.04; $-0.02$; 95% CI = $-0.04$, 0.00; $P = 0.033$). The estimated amounts of carbohydrate and fat utilized during exercise at 45%, 60%, and 75% $V_O^{2max}$ were not affected by menstrual-cycle phase (Table 5). Carbohydrate utilization was significantly greater after CHOL at 45% ($P = 0.001$) and 60% $V_O^{2max}$ ($P < 0.001$), but dietary treatment had no effect on carbohydrate utilization at 75% $V_O^{2max}$ (Figure 3). The amount of fat utilized during exercise at 45% ($P < 0.001$), 60% ($P < 0.001$), and 75% $V_O^{2max}$ ($P = 0.049$), was significantly greater on NORM than on CHOL (Figure 3).

**Blood Measurements**

Blood lactate, free-fatty-acid, and free-glycerol concentrations at baseline and throughout the experimental exercise period are shown in Table 6. Neither dietary treatment nor menstrual-cycle phase had an effect on blood lactate concentration.
Carbohydrate Loading and Female Athletes

Free glycerol concentration at baseline and before the time trial was significantly greater after NORM than after CHOL. No differences were observed after the time trial. Free glycerol concentration was not affected by menstrual-cycle phase. Free-fatty-acid concentration was greater after NORM than after CHOL at baseline, before the time trial, and after the time trial. There was no effect of menstrual-cycle phase on free-fatty-acid concentration.

Hydration Status

Hematocrit and hemoglobin concentrations were significantly different at baseline (hematocrit = 0.39 ± 0.02, hemoglobin = 138 ± 6 g/L) from those observed after the time trial (hematocrit = 0.43 ± 0.02, hemoglobin = 149 ± 6 g/L; P = 0.0001) for all treatments. There were no significant differences observed in hematocrit or hemoglobin concentration resulting from dietary treatment or menstrual-cycle phase. Plasma-volume changes (−13.3% ± 5.3%) and body-weight changes (−0.61%...
Table 5  Nonprotein Respiratory-Exchange Ratio (RER) and Estimated Carbohydrate and Fat Utilization During Different Phases of the Menstrual Cycle

<table>
<thead>
<tr>
<th>RER</th>
<th>ML, mean ± SD</th>
<th>MF, mean ± SD</th>
<th>Difference between ML and MF</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>45% VO₂max</td>
<td>0.91 ± 0.03</td>
<td>0.91 ± 0.03</td>
<td>0.00</td>
<td>–0.01, 0.02</td>
<td>0.755</td>
</tr>
<tr>
<td>60% VO₂max</td>
<td>0.90 ± 0.03</td>
<td>0.91 ± 0.03</td>
<td>0.00</td>
<td>–0.02, 0.01</td>
<td>0.955</td>
</tr>
<tr>
<td>75% VO₂max</td>
<td>0.97 ± 0.03</td>
<td>0.98 ± 0.03</td>
<td>–0.01</td>
<td>–0.03, 0.01</td>
<td>0.431</td>
</tr>
</tbody>
</table>

Estimated carbohydrate utilization (g/min)

<table>
<thead>
<tr>
<th>RER</th>
<th>ML, mean ± SD</th>
<th>MF, mean ± SD</th>
<th>Difference between ML and MF</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>45% VO₂max</td>
<td>1.29 ± 0.23</td>
<td>1.27 ± 0.25</td>
<td>0.04</td>
<td>–0.06, 0.14</td>
<td>0.389</td>
</tr>
<tr>
<td>60% VO₂max</td>
<td>1.71 ± 0.38</td>
<td>1.67 ± 0.27</td>
<td>0.07</td>
<td>–0.08, 0.22</td>
<td>0.377</td>
</tr>
<tr>
<td>75% VO₂max</td>
<td>2.80 ± 0.48</td>
<td>2.83 ± 0.47</td>
<td>0.00</td>
<td>–0.19, 0.19</td>
<td>0.993</td>
</tr>
</tbody>
</table>

Estimated fat utilization (g/min)

<table>
<thead>
<tr>
<th>RER</th>
<th>ML, mean ± SD</th>
<th>MF, mean ± SD</th>
<th>Difference between ML and MF</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>45% VO₂max</td>
<td>0.26 ± 0.11</td>
<td>0.24 ± 0.09</td>
<td>0.00</td>
<td>–0.04, 0.05</td>
<td>0.957</td>
</tr>
<tr>
<td>60% VO₂max</td>
<td>0.36 ± 0.13</td>
<td>0.35 ± 0.12</td>
<td>–0.00</td>
<td>–0.07, 0.06</td>
<td>0.896</td>
</tr>
<tr>
<td>75% VO₂max</td>
<td>0.16 ± 0.13</td>
<td>0.12 ± 0.14</td>
<td>0.03</td>
<td>–0.06, 0.11</td>
<td>0.565</td>
</tr>
</tbody>
</table>

ML indicates midluteal, and MF, midfollicular.

Figure 3  — Carbohydrate and fat utilization during the experimental exercise session after carbohydrate-loading (CHOL) versus normal (NORM) diet (N = 9). CHO indicates carbohydrate. *Significant difference between dietary treatments (P ≤ 0.05).
Table 6  Blood Measurements

<table>
<thead>
<tr>
<th></th>
<th>NORM, mean ± SD</th>
<th>CHOL, mean ± SD</th>
<th>Difference between NORM and CHOL</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood lactate (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>1.53 ± 0.56</td>
<td>1.85 ± 0.53</td>
<td>−0.28</td>
<td>−0.70, 0.13</td>
<td>0.182</td>
</tr>
<tr>
<td>45% VO_{2max}</td>
<td>1.50 ± 0.58</td>
<td>1.73 ± 0.35</td>
<td>−0.19</td>
<td>−0.57, 0.19</td>
<td>0.318</td>
</tr>
<tr>
<td>60% VO_{2max}</td>
<td>2.05 ± 1.01</td>
<td>2.22 ± 0.88</td>
<td>−0.12</td>
<td>−0.81, 0.58</td>
<td>0.746</td>
</tr>
<tr>
<td>75% VO_{2max}</td>
<td>3.79 ± 1.57</td>
<td>4.15 ± 1.16</td>
<td>−0.01</td>
<td>−1.21, 1.19</td>
<td>0.984</td>
</tr>
<tr>
<td>pretrial</td>
<td>1.63 ± 0.68</td>
<td>1.70 ± 0.58</td>
<td>−0.03</td>
<td>−0.52, 0.45</td>
<td>0.888</td>
</tr>
<tr>
<td>posttrial</td>
<td>5.96 ± 1.69</td>
<td>6.50 ± 1.44</td>
<td>−0.34</td>
<td>−1.45, 0.77</td>
<td>0.553</td>
</tr>
<tr>
<td>Free glycerol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>0.05 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>0.02</td>
<td>0.00, 0.03</td>
<td>0.020</td>
</tr>
<tr>
<td>pretrial</td>
<td>0.16 ± 0.06</td>
<td>0.11 ± 0.06</td>
<td>0.05</td>
<td>0.00, 0.09</td>
<td>0.029</td>
</tr>
<tr>
<td>posttrial</td>
<td>0.29 ± 0.08</td>
<td>0.24 ± 0.07</td>
<td>0.04</td>
<td>−0.02, 0.10</td>
<td>0.163</td>
</tr>
<tr>
<td>Free fatty acids (µmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>161 ± 51</td>
<td>76 ± 46</td>
<td>85</td>
<td>53, 118</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pretrial</td>
<td>330 ± 138</td>
<td>187 ± 96</td>
<td>141</td>
<td>53, 230</td>
<td>0.002</td>
</tr>
<tr>
<td>posttrial</td>
<td>485 ± 217</td>
<td>248 ± 138</td>
<td>206</td>
<td>65, 347</td>
<td>0.004</td>
</tr>
</tbody>
</table>

NORM indicates normal diet, and CHOL, carbohydrate-loading diet.

± 0.38%) during the experimental exercise session were not affected by dietary treatment or menstrual-cycle phase. Body weight, percentage body fat, and VO_{2max} were not significantly different at the end than at the beginning of the study.

Discussion

The purpose of this study was to investigate the muscle-glycogen changes, exercise performance, and substrate responses of endurance-trained women to carbohydrate loading during the luteal and follicular phases of the menstrual cycle. To our knowledge, this is the first study to examine the ability to supercompensate muscle glycogen in both phases of the menstrual cycle in the same eumenorrheic women.

Menstrual-Cycle Phase and Resting Muscle Glycogen

When data were pooled for menstrual-cycle phase, the mean resting [glyc] was 11% greater after CHOL than after NORM. It is possible that this is a real difference, but one that was not significant given the present sample size. A limitation
of the present study is that food consumption and activity levels were not fully standardized before the glycogen-depleting exercise bout performed before NORM or CHOL. This raises the possibility that preloading glycogen levels might have varied among participants, accounting for some of the findings. Unfortunately, additional muscle biopsies could not be included in the study protocol to address this possibility because of the increased burden on subjects. Another explanation is that the differences in carbohydrate intake between the 2 dietary treatments (5.2 vs. 8.4 g·kg body weight$^{-1}$·d$^{-1}$) might not have been enough to produce a concomitant difference in [glyc].

Resting [glyc] was, however, significantly higher during the ML than during the MF phase of the menstrual cycle (765 vs. 651 mmol·kg$^{-1}$·dry weight$^{-1}$). This is similar to the observations of Hackney (9), who measured a resting [glyc] of 90.75 µmol/g wet muscle during the MF versus 102.05 µmol/g wet muscle during the ML phase. This finding might result from enhanced repletion of muscle glycogen during the ML phase. Nicklas et al. (20) found greater repletion of [glyc] during the ML phase than during the MF phase (88.2 vs. 72.8 µmol/g wet muscle) after depletion exercise and 3 d on a diet with a carbohydrate content (~56%) lower than what is considered optimal for maximal glycogen resynthesis.

We observed resting [glyc] to be lowest in the MF phase on NORM. Carbohydrate loading during the MF phase appeared to overcome this suboptimal storage of glycogen, in that resting [glyc] observed in the MF phase after the CHOL was comparable to that observed after CHOL and NORM in the ML phase. Two studies have reported similar findings. Tarnopolsky et al. (30) and Paul et al. (22) reported significant increases in resting [glyc] in female endurance athletes after carbohydrate loading in the MF phase of the menstrual cycle.

Other investigators have reported results that are in contrast to the present study. Walker and colleagues (31) observed a small but significant increase (13%) in resting [glyc] in the ML phase of the menstrual cycle after carbohydrate loading. James and coworkers (13) attempted to examine carbohydrate loading in the same women in both phases of the menstrual cycle. They observed no difference in resting [glyc] in the MF and ML phases of the menstrual cycle and found a similar increase in resting [glyc] in response to a carbohydrate-loading diet in both phases of the menstrual cycle.

The discrepancy between the resting [glyc] results obtained in the present study and those of James et al. (13) and Walker et al. (31) can be explained. First, in contrast to the present study, all 6 female subjects in the study by James et al. (13) were taking triphasic oral contraceptives (ethinyl estradiol/levonorgestrel). In a normal natural menstrual cycle, resting levels of both estradiol and progesterone are higher during the ML phase than during the MF phase (17). No differences in resting estradiol or progesterone in the MF or ML phases were reported by James et al. (13). In fact, the authors considered this lack of difference to signify compliance, showing that all subjects were indeed taking the oral contraceptives.

In contrast, there were large increases in the levels of these hormones measured in the present study in the ML phase when compared with the MF phase. To illustrate, in the present study the estradiol levels were 3 times greater while the progesterone levels were 28 times greater in the ML phase compared with the hormone-analysis results of James et al. (13). Because of the lack of difference in hormone profiles between the MF and ML phases, the ML results of James et
al. (13) could actually be interpreted in the same way as the MF results. That is, carbohydrate loading in the MF phase increased resting \([\text{glyc}]\), as shown by the present study and those of others (22, 30).

In the study by Walker and colleagues (31), ingestion of 464 g/d of carbohydrate for 3 d in the ML phase of the menstrual cycle resulted in a small but significant increase in resting \([\text{glyc}]\) compared with 266 g/d for 3 d. This intake of 266 g/d is considerably lower than the 361 g/d reported as the habitual carbohydrate intake of the subjects in the Walker et al. (31) study. Other studies investigating carbohydrate loading in female athletes, including our study, have used comparative diets containing 304–360 g/d carbohydrate (13, 22, 30), which are considerably higher in carbohydrate than the diet employed by Walker and colleagues (31). This low carbohydrate intake (especially compared with the habitual intake) might be responsible for the contrasting results reported by Walker et al. (31) compared with the present study.

The mechanisms responsible for changes in resting muscle glycogen during the menstrual cycle are unknown. In animal models, fluctuations in ovarian hormones have been shown to affect specific aspects of muscle metabolism, such as the insulin-to-glucagon molar ratio (16) and skeletal-muscle glycogen-synthase activity (1). The mechanisms at work in humans, however, require further elucidation and are beyond the scope of this article. What is clear is the importance of controlling for menstrual-cycle phase in all exercise-metabolism studies involving female subjects, because of the potential influence that fluctuations in endogenous sex steroids can have on muscle \([\text{glyc}]\).

**Carbohydrate Loading and Exercise Performance**

Consistent with the finding of no difference in resting \([\text{glyc}]\) between the dietary treatments, in this group of moderately trained female athletes there was also no difference in performance. This is in contrast to previous data from men, in whom carbohydrate loading exerted a positive effect on endurance capacity and performance (2, 23, 24, 29). This lack of improvement in performance in women after a carbohydrate-loading diet and exercise taper is consistent with the results of Paul et al. (22) and Tarnopolsky et al. (29) but contradicts those of Walker et al. (31).

Tarnopolsky and colleagues (29) were unable to detect an improvement in endurance capacity or resting \([\text{glyc}]\). On closer examination of the results, however, they found that a suboptimal amount of carbohydrate (~6.7 g·kg body weight\(^{-1}\)·d\(^{-1}\)) was ingested on the high-carbohydrate diet because of a low energy intake, as they outlined in a subsequent investigation (30). Paul and coworkers (22) were also unable to detect a statistically significant improvement in mean performance time of a 1200-pedal-revolution time trial. Walker et al. (31), who used time to fatigue rather than a time trial as their performance criterion, found an improvement in endurance capacity and a small increase (13%) in resting \([\text{glyc}]\) after carbohydrate loading. As outlined previously, the low carbohydrate intake compared with habitual intake in the Walker et al. (31) study might be responsible for the enhancement in resting \([\text{glyc}]\) after carbohydrate loading and, consequently, the observed longer cycle time to exhaustion at 80% \(\text{VO}_{2\text{max}}\).

Exercise performance can be influenced by the preexercise fed or fasted state of the participants. Many of the investigations in which carbohydrate loading was
shown to positively affect the performance of male athletes were conducted after an overnight fast (2) or 3–6 h postprandially (23, 24, 29). In the present study a standardized breakfast containing approximately 87 g of carbohydrate was consumed by all subjects 1.5–2 h before each experimental exercise session to simulate precompetition practices. According to Sherman (27), lowered liver glycogen after an overnight fast might affect the maintenance of blood glucose concentrations during exercise, particularly when muscle glycogen becomes depleted, which might in turn influence endurance time to fatigue. Schabort and colleagues (26) found an increase in exercise-cycle time to fatigue at 70% VO$_{2\text{max}}$ when male subjects were fed a breakfast containing 100 g carbohydrate 3 h before exercise, compared with an overnight fast. This possibility has yet to be investigated in female athletes.

Carbohydrate loading is typically not effective in enhancing endurance exercise lasting less than 90 min (11). The exercise protocol used in the current investigation required subjects to cycle for approximately 100 min at a variety of exercise intensities, including the 16-km performance time trial. Over half of the exercise protocol (55 min), however, was spent exercising at ≤50% VO$_{2\text{max}}$. The moderately low intensity, combined with the relatively short exercise duration, might have meant that glycogen reserves were not limiting performance. This combined with the provision of carbohydrate in the standardized breakfast might well have masked any potential differences in performance.

In the present study, the group mean for aerobic capacity was 50.0 mL·min$^{-1}$·kg$^{-1}$, with a range of 44.0–57.5 mL·min$^{-1}$·kg$^{-1}$. This wide range suggests that some subjects were in a moderate training status and others were better trained. Thus, some of the true effects on performance and performance reliability might have been masked by variability introduced by sample-group heterogeneity.

Possibly the most important factor that might have influenced the performance results of the present study was a combination of the composition of the dietary treatments and the exercise protocol. The amount of carbohydrate consumed was 359 g/d (5.2 g·kg body weight$^{-1}$·d$^{-1}$) on the NORM and approximately 551 g/d (8.4 g·kg body weight$^{-1}$·d$^{-1}$) on the CHOL. It is possible that 359 g/d of carbohydrate was sufficient to provide carbohydrate energy to support the demands of the exercise protocol that these moderately trained female athletes undertook, especially when combined with the consumption of the high-carbohydrate standardized breakfast. These carbohydrate intakes correspond with those reported by Paul et al. (22), who also failed to observe an improvement in a 1200-pedal-revolution time trial in female athletes during the MF phase of the menstrual cycle after carbohydrate loading.

**Carbohydrate Loading, Menstrual-Cycle Phase, and Substrate Utilization**

Diet significantly affected substrate oxidation and plasma substrate concentrations, as would be expected based on earlier work (22, 25, 31). It is interesting that carbohydrate oxidation is greater after CHOL than after NORM, despite there being no significant dietary effect on muscle [glyc]. Again, this might indicate that glycogen levels were actually greater after CHOL but below our resolution to detect a difference. This greater availability of carbohydrate might have caused the difference in substrate oxidation between trials. Another possibility is that liver-glycogen concentration was greater after CHOL than after NORM. This difference
in liver-glycogen concentration might have contributed to a higher carbohydrate-oxidation rate during submaximal exercise after CHOL. Blood glucose values might have been able to support this possible explanation, but, unfortunately, samples were not collected during the present study.

Carbohydrate oxidation was greater after CHOL than after NORM at all submaximal exercise intensities except 75% VO$_{2\max}$. This observation might be related to the assignment to any RER values greater than 1.00 the value 1.00 for calculating CHO oxidation; therefore, carbohydrate oxidation might have been underestimated in some of these high-intensity exercise periods. Another possibility is that at 75% VO$_{2\max}$, the workload has reached a sufficiently high intensity to cross over into carbohydrate dependence (4), and thus differences between trials because of diet are minimized.

Menstrual-cycle phase had no effect on the amount of carbohydrate and fat utilized during exercise at any intensity in the present study. Some data suggest that there might be lower fat oxidation and greater carbohydrate oxidation during submaximal exercise in the follicular than in the luteal phase of the menstrual cycle (5, 10, 20, 32), although this has not always been observed (12, 14, 28). In the present study substrate-utilization data were collected in the last minute of each 10-min block of submaximal exercise. It is possible that 10 min was not long enough to achieve steady-state conditions. Although this is speculative, longer exercise periods might have allowed small differences in substrate oxidation to be detected.

Zderic et al. (32) and Campbell et al. (5) studied fasted women performing submaximal exercise in the follicular and luteal phases and found small differences in glucose flux rates between menstrual-cycle phases, ranging from 14% (32) to 26% (5). Suh and colleagues (28) observed that when women were studied in a postabsorptive state, there were no significant effects of menstrual-cycle phase on glucose flux or whole-body carbohydrate- and fat-oxidation rates during submaximal exercise. Based on this finding, the authors suggest that small differences in glucose flux resulting from the subtle effects of endogenous ovarian hormones are easily overridden by other factors such as exercise and recent carbohydrate intake. In the present study, subtle menstrual-cycle effects on substrate utilization might have been overridden by a plentiful dietary intake and the inclusion of a standardized breakfast before exercise.

**Summary**

The observation of greater resting [glyc] in the midluteal than in the midfollicular phase of the menstrual cycle supports previous findings. The lower level of glycogen storage in the midfollicular phase appears to be overcome by consuming a carbohydrate-loading diet consisting of at least 8 g·kg body weight$^{-1}$·d$^{-1}$. The effects of carbohydrate-loading diets on exercise performance in eumenorrheic women remain inconclusive. Despite achieving an improvement in glycogen storage in the midfollicular phase, carbohydrate loading did not enhance 16-km time-trial cycling performance after 75 min of low- to moderate-intensity cycling in this group of moderately trained eumenorrheic female athletes when they consumed breakfast beforehand. Menstrual-cycle phase had no effect on substrate utilization at various submaximal intensities, which seems to suggest that the subtle effects of the endogenous female sex hormones were overridden by the dietary-control aspects
of this study. In light of these results, and those of others, it seems prudent to recommend that menstrual-cycle phase be controlled for in any exercise-metabolism study using women as subjects.

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