No effect of menstrual cycle phase on lactate threshold

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1Section of Nutrition, Department of Pediatrics, and 2Division of Endocrinology, Metabolism, and Diabetes, Department of Medicine, University of Colorado Health Sciences Center, Denver 80262; and 3Department of Integrative Physiology, University of Colorado, Boulder, Colorado 80309

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Dean, Teresa M., Leigh Perreault, Robert S. Mazzeo, and Tracy J. Horton. No effect of menstrual cycle phase on lactate threshold. J Appl Physiol 95: 2537–2543, 2003; 10.1152/japplphysiol.00672.2003.—No previous exercise studies in women have assessed the effects of the normal menstrual cycle on the lactate threshold (LT) measured during a graded, maximal exercise test. This is relevant to our understanding of exercise training and metabolism in eumenorrheic women. The present study, therefore, examined the effect of menstrual cycle phase on the LT. Eight moderately active, eumenorrheic women performed three maximal exercise tests with simultaneous determination of LT. Tests were performed in the early follicular (low estrogen and progesterone), midfollicular (elevated estrogen and low progesterone), and midluteal (elevated estrogen and progesterone) phases of the menstrual cycle. No significant differences were observed in LT measured across phases of the menstrual cycle whether data were expressed in absolute terms (1,299 ± 70, 1,364 ± 80, and 1,382 ± 71 ml O2/min, respectively) or relative to maximal oxygen uptake (VO2max; 52.1 ± 1.7, 54.7 ± 1.7, and 55.7 ± 1.6%, respectively). In addition, there were no significant cycle phase differences in VO2max, maximal heart rate, heart rate at LT, or final lactate concentration. With data combined across all phases of the menstrual cycle, there was a significant correlation between the LT and the epinephrine breakpoint (r = 0.91, P < 0.0002) and norepinephrine breakpoint (r = 0.94, P < 0.0001). For epinephrine only, there was close correspondence between the epinephrine breakpoint (ml O2/min) and the LT. In conclusion, LT as well as VO2max and other measures of cardiorespiratory fitness are not significantly affected by the changing sex steroid levels observed across the normal menstrual cycle. Data suggest that the onset of the steep increase in epinephrine determines the LT during graded exercise.

catecholamines; female sex steroids; maximal exercise

The past few decades have seen an increased emphasis on health-related research in women. At the same time, there has been a significant rise in the number of physically active women, both at a recreational and a professional level. As exercise is a key factor in the prevention and treatment of many chronic diseases, understanding the metabolic effects of exercise in women is important in terms of maximizing health benefits. It is also relevant to the optimization of training practices in competitive athletes.

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When conducting studies in women, it is important to consider whether variations in the prevailing sex steroid environment can impact outcome measure(s). In adult women, circulating estrogen and progesterone levels vary normally throughout the menstrual cycle (45) and undergo a permanent change from the pre- to postmenopausal state (34). Secondary amenorrhea, common in athletic women, results in continuously decreased female sex steroid levels (27). Because estrogen and progesterone have many effects on energy and substrate metabolism, disregard for the sex steroid status in women when performing metabolic studies could confound the results and lead to erroneous conclusions. This may be particularly relevant during physical activity. Indeed, human studies have shown that administration of estrogen (5, 35), or use of combined oral contraceptives (OCPs) (39), decreases glucose utilization during submaximal exercise, whereas this effect is antagonized by coadministration of a high physiological dose of progesterone (9).

Despite this evidence for an effect of the female sex steroids on exercise substrate metabolism, data on the effects of the normal menstrual cycle on glucose kinetics and carbohydrate oxidation are conflicting. Some studies report that glucose rates of appearance and disappearance are significantly decreased in the luteal vs. follicular phase of the menstrual cycle (4, 46) coincident with a decrease in whole body carbohydrate oxidation. Nevertheless, equal data do not support such an effect (3, 19, 38). One factor that could contribute to these equivocal results is the mode of testing subjects. In these menstrual cycle studies, steady-state exercise was performed relative to either subject’s maximal oxygen uptake (VO2max) or lactate threshold (LT). It has repeatedly been shown that VO2max is not affected by menstrual cycle phase (1, 10, 11, 23, 37). In contrast, although many studies have measured certain lactate parameters during exercise throughout the menstrual cycle, no study has directly measured LT. It is quite possible for LT to vary without differences occurring in VO2max (29, 30). Because testing above or below the LT can, in of itself, significantly impact lipid and glucose metabolism (8, 24), this could confound results in relation to menstrual cycle effects on substrate metabolism. Understanding the potential im-
pact of menstrual cycle variation on LT is, therefore, important for appropriate interpretation of exercise studies in eumenorrheic women as well as appropriate study design.

Some studies suggest lactate metabolism could be affected by the female sex steroids. Several studies have shown that during both steady-state, moderate- to high-intensity exercise and maximal exercise, lactate concentrations tend to be lower in the luteal vs. midfollicular (MF) phase of the menstrual cycle (3, 10, 11, 14, 23, 26, 40, 46). Furthermore, administration of medroxyprogesterone, a synthetic progesterone, to men lowers the lactate response to maximal exercise (11, 14, 23, 26, 40, 46). Therefore, menstrual cycle variation in LT is, therefore, important for appropriate interpretation of exercise studies in eumenorrheic women as well as appropriate study design.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Height, m</th>
<th>Body Weight, kg</th>
<th>BMI, kg/m²</th>
<th>Body Fat, %</th>
<th>Total, days</th>
<th>Ovulation, day</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 ± 5</td>
<td>1.66 ± 0.08</td>
<td>58.7 ± 6.5</td>
<td>21.3 ± 0.8</td>
<td>25.3 ± 4.3</td>
<td>27.2 ± 2.1</td>
<td>14.1 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SD. BMI, body mass index.

Determination of Menstrual Cycle Phase

Only women who had experienced a regular menstrual cycle over the preceding 6 mo were recruited. Details on previous menstrual history along with subject-monitored basal body temperature were used to identify phases of the menstrual cycle. Ovulation was indicated by a sustained increase in basal body temperature of at least 0.3°C after the luteinizing hormone surge and confirmed by sex hormone levels (estradiol and progesterone) measured on the study days. Menstrual cycle length was calculated from the first day of menses (day 1) to the day preceding the next menses. Average cycle length was 27 ± 2.1 days with ovulation occurring at day 14 ± 0.9. The goal was to test subjects at times characterized by very different estrogen-to-progesterone ratios: EF, low estrogen and progesterone; MF, elevated estrogen and low progesterone; and ML, elevated estrogen and progesterone. Study days were day 3 ± 1.6 for EF, day 10 ± 2.6 for MF, and day 21 ± 1.8 for ML and were associated with the expected levels of estrogen and progesterone (Table 2). Measurements were made in the same or consecutive cycles.

Preliminary Assessments

Each subject underwent preliminary assessments before any exercise testing. These included a health and physical examination, blood screening for anemia, determination of body composition via dual-energy X-ray absorptiometry (DPX-1Q, Lunar Radiation, Madison, WI) (32), meeting with a dietician, and resting metabolic rate measurement (RMR). RMR was measured by using indirect calorimetry (2900 metabolic cart, Sensormedics, Yorba Linda, CA) in the morning, after a 10- to 12-h fast. Metabolic rate was calculated from the flow rate of the exiting air, in conjunction with the oxygen and carbon dioxide concentrations (44). Results from this test were used to calculate energy intake for the controlled diet phase of each test period.

Diet and Exercise Control

Dietary intake was controlled the day before each test. All food was provided by the General Clinical Research Center (GCRC) at the University of Colorado Health Sciences Center, and energy intake was based on the subject’s RMR × 1.6 (18). Diet composition was fixed at 25% of the total energy intake from fat, 15% from protein, and 60% from carbohy-
drate. Subjects also refrained from planned exercise on this day. Diet and exercise control was used to minimize inter- and intra-subject variation in glycogen stores and state of energy balance before each test day, which might otherwise affect the LT or VO2max measures. Subjects fasted 12 h overnight, and abstained from caffeine, before arriving on the GCRC at 7:30 AM for the exercise test.

Study Days

A maximal exercise test with LT determination was performed on each study day. The order of testing was randomized.

Testing protocol. A sampling catheter was placed into an antecubital vein. Electrodes were placed for 12-lead ECG monitoring. Before beginning the exercise protocol, subjects lay quietly for 30 min before a baseline blood sample was drawn. Subjects then moved to an electronically braked, stationary bicycle ergometer (Lode Medical Technology, Groningen, The Netherlands) to begin the graded-exercise test. Subjects pedaled at a rate of 70–80 rpm beginning at a resistance of 20 W. Resistance was increased by 20 W every 2 min until volitional exhaustion was achieved. Heart rate was measured and recorded every minute, and blood pressure was monitored. Blood samples (3 ml) were drawn every 2 min during the maximal test for the analysis of blood lactate and plasma catecholamines (epinephrine and norepinephrine). Respiratory gas-exchange measurements were made throughout the test by using indirect calorimetry (2900 metabolic cart, Sensormedics). Subjects pedaled at a low resistance for a minimum 5-min cool-down period after completing the test.

Biochemical assays. At baseline, ~4 ml of whole blood were drawn and allowed to clot. Serum was separated after spinning and used to determine estradiol and progesterone levels. Another 3 ml of blood were taken at baseline and every 2 min during the graded-exercise test. This was immediately divided into two collection tubes. Approximately 0.5 ml of whole blood was added to a preweighed tube containing 1.5 ml of 8% perchloric acid, vortexed, and then stored on ice until completion of the test. All tubes were postweighed to determine blood lactate. For plasma catecholamines, 2.5 ml of whole blood were placed in iced collection tubes containing 40 ml of preservative [EGTA (3.6 mg) plus reduced glutathione (2.4 mg) in distilled water], and the tube was inverted then stored on ice until completion of the test and spun to separate the plasma. All samples were stored at −70°C until analysis.

Blood lactate was run in duplicate by spectrophotometric assay (Sigma Diagnostics, St. Louis, MO) with an intra-assay coefficient of variation (CV) of 4.2%. Catecholamines were determined in duplicate by high-performance liquid chromatography with electrochemical detection (7) with an intra-assay CV of 6.2% for epinephrine and 4.9% for norepinephrine. Radioimmunoassays were used to determine progesterone and estradiol (Kabi Pharmacia, Piscataway, NJ). For all assays, samples from all study days for a given subject were run simultaneously.

Calculations

VO2max was calculated from the plateau in oxygen uptake (VO2) achieved before exercise termination. VO2 was reported in 20-s intervals, with the last three intervals before exercise termination used to calculate the VO2max. To ensure that maximum effort was achieved, two of the following criteria had to be fulfilled: an increase in VO2 in the final vs. preceding minute of <2.0 ml·kg−1·min−1, a maximum heart rate within 5% of the subject’s age-predicted maximum, and/or whole body respiratory exchange ratio ≥1.1. LT was calculated by plotting the blood lactate concentration against VO2. Data were log transformed (43). A two-line regression model was used to describe the two phases of lactate accumulation. The point of intersection of these two lines was taken as the LT (43). The test data were entered onto a spreadsheet by one investigator, with all names and reference to cycle phase removed and replaced by a code. This ensured blinding of the investigator completing the LT calculation with respect to menstrual cycle phase. Two investigators (T. J. Horton and L. Perreault or T. Dean) provided independent verification of each LT plot. The breakpoint for the increase in epinephrine and norepinephrine was calculated from a two-line regression model as for identification of LT. The point of intersection of these two lines was taken as the epinephrine or norepinephrine breakpoint.

Statistical Analysis

A repeated-measures ANOVA, with cycle phase as the grouping factor, was used to determine whether a difference existed in the primary outcome variable of LT and other secondary outcome measures. Because there were a number of missing epinephrine and norepinephrine data points, catecholamine concentrations and breakpoint were compared across cycle phases by using a one-way ANOVA. Pearson product correlations were calculated for LT vs. epinephrine or norepinephrine breakpoint, and LT vs. estrogen or progesterone concentration. Statistical significance was set at a value of \( P = 0.05 \).

RESULTS

LT and Lactate Concentrations

LT did not differ significantly across menstrual cycle phases whether data were expressed in absolute terms (ml O2/min) or as a percentage of the corresponding

Table 3. Lactate threshold

<table>
<thead>
<tr>
<th></th>
<th>EF</th>
<th>MF</th>
<th>ML</th>
</tr>
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<tbody>
<tr>
<td>LT</td>
<td>1.299 ± 0.07</td>
<td>1.364 ± 0.08</td>
<td>1.382 ± 0.07</td>
</tr>
<tr>
<td>VO2max</td>
<td>52.1 ± 1.7</td>
<td>54.7 ± 1.7</td>
<td>55.7 ± 1.6</td>
</tr>
<tr>
<td>Resting lactate concentration, mM</td>
<td>0.74 ± 0.1</td>
<td>0.70 ± 0.1</td>
<td>0.64 ± 0.1</td>
</tr>
<tr>
<td>Lactate concentration at LT, mM</td>
<td>1.27 ± 0.1</td>
<td>0.95 ± 0.1</td>
<td>1.33 ± 0.2</td>
</tr>
<tr>
<td>Final lactate concentration, mM</td>
<td>8.1 ± 0.7</td>
<td>7.2 ± 0.7</td>
<td>7.2 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. LT, lactate threshold; VO2max, maximal oxygen uptake.
VO_2max (Table 3). Because there appeared to be a tendency for LT to be higher in the ML vs. EF phase, data were directly compared by using a paired t-test. This comparison yielded a value of \( P = 0.09 \), suggesting borderline significance. Maximal lactate concentration tended to be lowest in the ML and MF phases and highest in the EF, but again the difference was not significant (Table 3). Lactate concentration at LT was highest in the EF, but again the difference was not significant across cycle phases nor did maximal heart rate. Although no significant difference in either the relative or absolute LT when measured in the EF, MF, or ML phases of the menstrual cycle. Similarly, there were no differences in maximal measures of VO_2, heart rate, workload, or lactate concentration, nor differences in lactate concentration at LT across the menstrual cycle. A significant correlation was observed between the epinephrine, or norepinephrine, breakpoint during the graded, maximal exercise test and the LT. This suggests a role for the catecholamines in determining the LT.

**DISCUSSION**

This study evaluated the effect of different phases of the menstrual cycle, characterized by different estrogen and progesterone levels, on LT. We observed no significant difference in either the relative or absolute LT when measured in the EF, MF, or ML phases of the menstrual cycle. Similarly, there were no differences in maximal measures of VO_2, heart rate, workload, or lactate concentration, nor differences in lactate concentration at LT across the menstrual cycle. A significant correlation was observed between the epinephrine, or norepinephrine, breakpoint during the graded, maximal exercise test and the LT. This suggests a role for the catecholamines in determining the LT.

**Maximal Exercise Performance**

The absolute and relative VO_2max did not vary significantly across cycle phases nor did maximal heart rate, final workload, time to exhaustion, or the respiratory exchange ratio at exhaustion (Table 4).

**Catecholamine Response**

At rest, epinephrine (24 ± 2, 23 ± 3, and 26 ± 5 pg/ml for EF, MF, and ML, respectively) and norepinephrine (242 ± 60, 229 ± 33, and 225 ± 16 pg/ml, respectively) concentrations were not different between cycle phases. Because of collection of inadequate blood volume, catecholamine measurements were not made on all subjects at all time points during each exercise test. Where enough data points were available, the epinephrine and norepinephrine breakpoint were calculated. Epinephrine breakpoint in the three phases of the menstrual cycle occurred at 58 ± 10 pg/ml \(( n = 6)\) for EF, 50 ± 7 pg/ml \(( n = 4)\) for MF, and 78 ± 22 pg/ml \(( n = 4)\) pg/ml for ML with the corresponding values for norepinephrine being 553 ± 91 \(( n = 6)\), 598 ± 97 \(( n = 4)\) and 757 ± 109 \(( n = 4)\) pg/ml, respectively. At exercise termination, epinephrine concentration was 418 ± 110 \(( n = 4)\), 336 ± 70 \(( n = 5)\) and 353 ± 51 \(( n = 6)\) pg/ml, respectively, and norepinephrine concentration 3,800 ± 1,109 \(( n = 5)\), 4,135 ± 1,076 \(( n = 5)\) and 4,034 ± 961 \(( n = 6)\) pg/ml, respectively. Although no significant differences were observed between cycle phases in terms of the catecholamine levels at LT, or at exercise termination, these comparisons are limited by the small number of observations in each phase.

The data for all measures of epinephrine or norepinephrine breakpoint (ml O_2/min) were combined across cycle phases and correlated to the corresponding LT (ml O_2/min) measured during the same test. There was a significant correlation between the epinephrine \(( P < 0.0002)\) or norepinephrine \(( P < 0.0001)\) breakpoint and the LT (Fig. 1). Only for the epinephrine breakpoint vs. LT, however, was the regression line not different from the line of identity showing equivalence between the two measures.

**Fig. 1.** A: relationship between the breakpoint in epinephrine concentration and the lactate threshold measured during graded, maximal exercise. \( P < 0.0002, r = 0.91 \). B: relationship between the breakpoint in norepinephrine concentration and the lactate threshold measured during graded, maximal exercise. \( P < 0.0001, r = 0.94 \).
Certain studies have reported that lactate concentrations tend to be greater for a given work rate in the EF than the MF or ML phases of the menstrual cycle (11, 23, 40). In this context, the reduced lactate levels in the luteal phase of the menstrual cycle appear to be due to a lower rate of lactate production rather than a lower rate of muscle efflux or increased lactate clearance (11, 23). Indeed, there is evidence for decreased production of lactate in association with a reduced carbohydrate utilization (4, 10, 11, 23, 40, 46) and/or a decline in catecholamine response (28, 35, 40, 41) in the presence of elevated female sex steroids, as characterized the luteal phase. Lower catecholamine levels during exercise in the luteal phase of the menstrual cycle could contribute to a lower rate of lactate production (36, 42). If the rate of appearance of lactate were lower in the luteal vs. the follicular phase, yet the rate of disappearance remained the same, then the upward deflection of lactate concentration defining LT would be expected to occur at a higher \( V_o_2 \) and thus at a higher percent \( V_o_2_{\text{max}} \). Our present observations, however, do not support any significant effect of menstrual cycle phase on LT measured during maximal incremental exercise. Observations of lower lactate levels during submaximal exercise in the luteal phase of the menstrual cycle could, therefore, be due to increased lactate clearance, or decreased muscle lactate efflux, rather than decreased lactate production. It cannot be overlooked, however, that a subtle effect of the sex steroids on lactate production between cycle phases is masked by the greater stress imposed during a graded-exercise test. As exercise intensity increases during maximal exercise, there will be a greater catecholamine stimulation of muscle glycolysis and enhanced calcium-mediated glycolysis (16). Thus a direct effect of the sex steroids on lactate metabolism in the menstrual cycle may be difficult to discern if they are overridden by other factors that activate lactate production.

An indirect effect of the sex steroids on LT could be mediated through an effect on catecholamine levels. Indeed, it has been reported that epinephrine and norepinephrine, or muscle sympathetic activation, are reduced in the luteal phase of the menstrual cycle in response to stress (12, 28, 35, 40). In the present study, however, we observed no cycle phase differences in catecholamine levels. Therefore, the lack of menstrual cycle phase differences in LT may be due to a lack of an effect on the catecholamine response. Indeed, our results agree with previous studies that show a significant correlation between the breakpoint in epinephrine or norepinephrine during graded exercise and the LT (20, 29, 30, 33). In contrast, there was no correlation between estrogen (\( r = 0.35 \)) or progesterone (\( r = 0.26 \)) concentrations and the LT measured on the same day. This suggests that, within the range of female sex hormones observed in this study, there was no significant effect, either direct (for example via cell membrane receptors) or indirect (via effects on catecholamine levels) on the LT. For epinephrine, but not norepinephrine, the regression line relating the breakpoint in epinephrine to LT was not significantly different from the line of identity, suggesting that epinephrine plays a causative role in determining the LT. Epinephrine is important in the stimulation of muscle glycolysis during exercise (13, 22), and as glycolysis increases, so does glycolytic flux and lactate production (13, 22). Indeed, epinephrine infusion alone increases muscle lactate production at rest or during exercise (36, 42). Furthermore, \( \beta \)-adrenergic blockade decreases lactate production during exercise (21). This supports the contention that the onset of the sharp increase in epinephrine level during graded exercise initiates the rapid increase in plasma lactate concentrations, thus determining the LT. Because epinephrine is a more potent agonist of the \( \beta_2 \)-adrenergic receptors than norepinephrine, the regression line relating the break-point in epinephrine to LT would be recommended in this population.

Our results are in agreement with others who have also found no effect of menstrual cycle phase on \( V_o_2_{\text{max}} \), maximal heart rate, and maximal workload (1, 10, 11, 23, 37). The present observation of no menstrual cycle differences in lactate concentration at exercise termination is similar to what has been observed in some studies (1, 25) but not others (23). We also observed no significant difference in the lactate concentration at LT between cycle phases. Thus normal cyclic variations in estrogen and progesterone are without significant effect on any measure of maximal exercise performance. Nevertheless, the potential of larger and/or more chronic changes in the female sex steroid environment to affect exercise performance cannot be disregarded. Indeed, recent data (6) have shown that use of OCPs for 4 mo significantly decreases \( V_o_2_{\text{max}} \). This appeared to be due to a chronic rather than acute effect of OCPs as differences were observed during both the active and inactive pill phases. Although it was not clear whether these changes in cardiorespiratory fitness were due to changes in activity patterns coincident with OCP use, it would be informative to know whether LT is affected by OCP use. Furthermore, we did observe a tendency for LT to be higher in the MF vs. EF phase, but this was not statistically significant. Thus chronic and/or greater changes in the female sex steroid environment, including amenorrhea, menopause, and use of hormone replacement therapy, could significantly affect measures of exercise performance. Furthermore, even the small differences observed across cycle phases may be relevant for professional female athletes in whom fitness testing is a measure of training progress. Therefore, standardizing LT determinations to the same sex steroid hormone background would be recommended in this population.

It is worth considering whether our lack of ability to detect a significant effect of menstrual cycle phase on LT was due to confounding factors. Confounding factors include issues related to pretest control and/or statistical power. In terms of prestudy control, we attempted to control as many factors as possible that could potentially impact exercise performance. Subjects were fed the same diet the day before each test.
day and did not exercise to minimize within-subject variation in muscle glycogen stores. All tests were performed in subjects first thing in the morning, after an overnight fast (including abstinence from caffeinecontaining beverages). In addition, subjects were instructed to drink fluids the day and evening before each test as well as the morning of each study to avoid any effect of dehydration on LT measurement (30). By instigating these controls, the impact of factors, other than menstrual cycle phase, on exercise performance were avoided. From a statistical view point, test order was randomized with respect to cycle phase to prevent any effect of order on the outcome measures. Subject number was based on our previous observations (unpublished) of infrasubject variability in LT, which were very similar to those reported in the literature (infrasubject CV of 1.3%, range within subjects of 0.1–2.7% for 3 repeat tests) (31) and should have given enough power to detect a significant difference between cycle phases. Hence, it is felt that statistical issues did not limit the ability to detect a true effect of menstrual cycle on the LT.

In conclusion, we observed that the cyclic fluctuations in estrogen and progesterone normally observed in the menstrual cycle are without significant effect on any parameter of maximal exercise performance, including LT. Thus, when performing maximal exercise testing in eumenorrheic women who do not use OCPS, cycle phase does not need to be controlled for. Nevertheless, an effect on exercise performance of more chronic changes in the female sex steroid environment, and/or larger changes in sex steroid levels, cannot be dismissed. Our data also confirm previous studies that suggest the onset of the steep increase in catecholamine levels during graded exercise, in particular epinephrine, may be the overriding determinant of LT.

We thank all the subjects for their generous cooperation while participating in the study and all the GCRC staff who worked on various aspects of the study.

DISCLOSURES

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