INTERNATIONAL JOURNAL OF -EXERCISE SCIENCE-

Original Research

No Differences in Lactate Threshold Across the Menstrual Cycle in Untrained Females

Bianca A.R. Galletti*#, Grant A. Chesbro‡#, Rebecca D. Larson‡

Department of Health & Exercise Science, University of Oklahoma, Norman, OK, USA

*Denotes student investigator, ‡Denotes established investigator, # These authors share first authorship

Abstract

International Journal of Exercise Science 18(8): 193-205, 2025. https://doi.org/10.70252/JKXM8836 In addition to maximal oxygen consumption (VO2max), the Lactate Threshold (LT) is the other major determinant of aerobic exercise performance. This study examined the effects of the menstrual cycle (MC) phase on the LT in untrained females. Eight females and 8 males completed a LT test on a cycle ergometer. The tests were performed across three MC phases: early follicular (EF; menses), ovulatory (O), and mid-luteal (ML). The male participants (control group) were randomly assigned visits at similar time intervals as a regular 28-day cycle. Blood lactate was obtained during the last minute of each 3-minute stage of the LT test. The LT was determined by visual method (LT_{vis}), and fixed blood lactate concentrations of 2.0 mmol/L ($LT_{2.0mmol}$) and 4.0 mmol/L ($LT_{4.0mmol}$). There were no statistically significant differences in power at LT for any of the determination methods across the MC for the female participants ($p>0.05$) or across the time intervals for the male participants ($p>0.05$). The male participants tended to have higher power at LT for all three methods LT_{vis} (41.91W), $LT_{2.0mmol}$ (44.68W), and $LT_{4.0mmol}$ (55.94W). These findings support that the MC does not seem to influence power at LT in untrained naturally menstruating females.

Keywords: Graded exercise test, female physiology, pain, fatigue

Introduction

For many years, females have been underrepresented in exercise science research, often due to the fluctuations in hormone levels across the menstrual cycle (MC).[1] During the past decade, there has been a growing interest in understanding how ovarian hormones affect exercise performance.[2] This has led to an increased interest in advancing current knowledge about how the menstrual cycle (MC) and changes in estrogen and progesterone potentially affect physiological responses to performance.[3–6]

The rate of fatigue is often associated with the rate of blood lactate accumulation during exercise.[7] Lactate is a byproduct of carbohydrate metabolism, and its accumulation during exercise is due to an imbalance between lactate production and clearance in the blood, which increases as exercise intensity rises.[7] Although there have been several studies investigating the

effects of the MC on carbohydrate and fat metabolism during exercise, few studies have evaluated its effect on blood lactate concentrations. Several studies have found no difference in blood lactate concentrations across the menstrual cycle during incremental[8–11] or constant-intensity exercise.[8] However, several other studies have found lower blood lactate concentrations during the luteal phase compared to the follicular phase during incremental[3,12] and constant-intensity exercise.[11,13] During constant-intensity exercise, blood lactate differences tended to be noticed at higher intensities (above 75% of VO_{2max}).[11,13]

Exercise metabolism and performance have been shown to be impacted by the estrogen hormone complex (estradiol-β-17, estriol, and estrone) and progesterone.[14] Sex hormones may influence the metabolism of carbohydrates and fat utilization during exercise, which can affect performance since carbohydrate stores are more limited.[15] Some studies have observed that resting glycogen stores may be higher in the mid-luteal phase (ML, high estrogen, and progesterone) when compared to the early follicular phase (EF; low estrogen, and low progesterone) when carbohydrate intake is comparable.[16] Similarly, resting free fatty acid availability has been demonstrated to be enhanced at rest during ovulation (~day 14 of the cycle) and ML.[17] The increased reliance on fat oxidation during exercise is thought to be linked to the higher estrogen-to-progesterone ratio present in the luteal phase of the menstrual cycle.[14] Estrogen interacts with skeletal muscle and prioritizes fat metabolism and conserves carbohydrates.[18–20] Conserving carbohydrate resources during endurance exercise is considered a beneficial adaptation to delay the onset of fatigue, thus improving performance.[18–20]

While there have been a few studies which examine the effects of the menstrual cycle on exercise metabolism and lactate production, few have examined the effect of the menstrual cycle on the lactate threshold (LT).[11,21–23] In addition to maximal oxygen consumption (VO2max), the LT is the other major determinant of exercise capacity.[6] Most of the studies examining the effect of the menstrual cycle on LT have found no differences in LT across the follicular and luteal phases.[11,21,22] However, one study found that the LT occurred at a higher power output and that blood lactate concentrations were lower at LT during the luteal phase compared to the follicular phase.[23]

Nonetheless, most studies focus on subjects in high hormone states (MF and ML),[3,11–13,21,23] often ignoring the first few days of menstruation when estrogen and progesterone levels are low. Consequently, more research is needed to better understand how different MC phases affect LT and lactate accumulation during exercise. Thus, the purpose of this study is to investigate whether the MC phase affects the LT.

Methods

Participants

A sample size calculation was performed prior to the beginning of the study, indicating a minimum of 6 participants per group (males v. females). The analysis was based on 3 time points (menstrual cycle phases) and a within-between repeated measures ANOVA with an α of 0.05, effect size of 0.30, and a power of 0.80.[5] Twenty-five individuals were enrolled in the study, but

only 16 were included in the data analysis. Seven individuals (6F) did not complete data collection, and 2 individuals (1F) were removed from data analysis. One individual had an abnormally high lactate reading during the first stage, possibly indicating an error with the lactate analyzer and one individual had outlying VAS pain scores (more than 3 SD from the mean). Sixteen individuals were included in the data analysis, of which 8 were males (Age: 24.8 yrs ± 5.5; Height: 175.5 cm ± 7.7) and 8 were females (20.9 yrs ± 3.9; 162.7 cm ± 2.1). The control group was composed of males because they lack fluctuations in progesterone and estradiol throughout the month.[24]

All participants met the inclusion criteria, being free from cardiovascular, respiratory, metabolic, neurological, and musculoskeletal diseases and debilitating musculoskeletal injuries. The subjects were also required to be free from any external hormonal influences (e.g. hormonal contraceptives, antidepressants, or other medications for at least 6 months). The female participants reported that they were naturally menstruating and had a cycle lasting at least 21 days and no more than 35 days (length: 27.5 days ± 1.93) and a luteal phase lasting at least 10 days based on ovulation detected from luteinizing hormone levels.[25] All female participants were required to have reported a regular menstrual cycle with a similar length for at least six months prior to the study. Height and body weight were measured at the beginning of each visit to establish the correct load during the protocol.

This study was approved by the University of Oklahoma Institutional Review Board and complied with the Declaration of Helsinki. All participants provided written informed consent before participating in the study and were familiarized with all protocols. This research was carried out fully in accordance with the ethical standards of the *International Journal of Exercise Science*.[26]

Protocol

The female participants had their testing visits at the early follicular phase (EF; within 3 days of the onset of menstruation), ovulation (O; within 24 hours of a positive ovulation test), and mid-luteal (ML; 7-10 days following the positive ovulation test). Ovulation was assessed using a urine test to track a surge in luteinizing hormone (LH50 Urine Test, Shenzhen Mommy Medical, Shenzhen, China). The participants were instructed to start testing for ovulation on day 10 of the cycle (counting from the first day of bleeding), until a positive test, when the ovulation visit was scheduled. If the test failed to be positive within days 10 and 17, they were instructed to start testing on day 8 of the next cycle, until a positive test appeared.[27] This happened to two of the female participants.

After the familiarization visit, the first exercise test was scheduled according to the phase of the menstrual cycle that was occurring next, as determined by the menstrual history questionnaire. This approach ensured that the visit order was randomized among the female participants, reducing the potential impact of the learning effect on study outcomes. Four participants had their first visit during the early follicular phase (EF), two during the ovulatory phase (O), and two during the mid-luteal phase (ML). The time between visits varied for each participant due to differences in menstrual cycle length and availability.[24]

For the control group, visits were randomly assigned using a comparable time frame to the female 28-day cycle. For example, if a male's first visit was allocated to be during EF, his O visit was scheduled to occur 14 days later, and his visit ML would be 7-10 days after that (depending on availability). Five participants had their first visit scheduled as "EF", 1 participant had his first visit scheduled as "O", and 2 had their first visit scheduled as "ML".

A cycle ergometer (LODE Excalibur Sport, Groningen, The Netherlands) was used to perform all LT tests. Before exercise, a heart rate monitor (Model H1, Polar Inc., Bethpage, NY, USA) was placed on the participant's chest. The protocol began with a 5-minute self-selected warm-up, starting at an intensity equal to 0.5 Watts per kilogram of body weight (W/kg BW). The LT test consisted of an incremental protocol starting at 1.0 W/kg BW and increasing by 0.5 W/kg BW every 3 minutes[28]. The participants maintained a self-selected cycling cadence (RPM). The protocol was terminated if the participant's cadence fell more than 10 RPM, or the subject voluntarily stopped exercising. A cool down at 25 W for a minimum of 3 minutes was conducted at the end of the protocol.

A metabolic cart (TrueOne 2400, ParvoMedics, Sandy, UT, USA) was used to analyze oxygen consumption (VO_2), which was collected breath-by-breath continuously during the LT protocol and was analyzed using a 30-second average. At the end of the test, VO_{2max}, HRmax, end-test respiratory exchange ratio (RER), peak power, and rating of perceived exertion (RPE) were recorded.

A finger stick drawing of ~1.0 µL of blood was used to perform the lactate analysis. A portable lactate analyzer (Lactate Plus, Nova Biomedical, Waltham, MA, USA) was used to analyze the samples. Blood lactate was collected before, at the end of each stage, and immediately after the LT protocol.

The LT visual (LT_{vis}) method consisted of plotting blood lactate concentrations against power output and was defined as the highest exercise intensity before the occurrence of a sharp increase in blood lactate concentration. In addition, there are two key points of interest as lactate levels increase: lactate threshold one (LT1), which occurs when lactate accumulation exceeds 2 mmol/L, and the onset of blood lactate accumulation (LT2 or OBLA), which happens when blood lactate concentrations surpass 4 mmol/L. These thresholds are commonly used to estimate and prescribe endurance exercise intensity across various exercise modalities to improve training and enhance performance.[6] The power corresponding to blood lactate concentrations of 2.0 mmol/L ($LT_{2.0mmol}$) and 4.0 mmol ($LT_{4.0mmol}$) were calculated by plotting power against blood lactate concentrations.[6,29]

A modified Borg scale ranging from 0 (no exertion at all) to 10 (maximal exertion) was used to indicate the rates of perceived exertion (RPE) during the test.[30] RPE was collected before, at the end of each stage, and immediately following the LT protocol.

Perceived recovery was assessed using the Perceived Recovery Scale (PRS),[31] which ranges from 0 (very poorly recovered and extremely tired) to 10 (very well recovered and highly energetic). The researcher collected the PRS scores of each participant via email 24 hours after each test.

Before engaging in exercise, the participants completed the Profile of Mood States (POMS) and a Visual Analog Scale (VAS) for perceived pain. For the study, the short form of the POMS was used.[32] The questionnaire consisted of 30 words with ratings from 0 (not at all) to 4 (extremely) describing how the participants were feeling at the time of the visit. Feelings of fatigue and vigor were the categories of interest for this study. The questionnaire data was utilized to analyze changes in self-reported energy levels throughout the menstrual cycle for female participants and over time for male participants.

The VAS was used to gather information regarding the participant's rating of perceived pain before the test.[33] A 100 mm line with the words "no pain at all" and "worst imaginable pain" were anchored at opposite ends of the line. The participants were instructed to draw a line perpendicular to the line representing their perceived pain while seated. The VAS was used as a method to examine self-reported perceived pain throughout the menstrual cycle for female participants and over time for male participants.

Statistical Analysis

SPSS version 29 (IBM Corp., Armonk, NY, USA) was used to perform statistical analysis. A 2 group (females/males) by 3 time point (EF/O/ML) repeated measures ANOVA was performed for all variables. Bonferroni corrections were applied as further pairwise comparisons. Effect sizes were calculated using partial eta squared (ηp^2), where effect sizes of 0.01-0.05 were considered small, 0.06-0.13 were considered moderate, and greater than 0.14 were considered large.[34]

Results

There were no observed main effects of time ($F_{2,28}=1.413$, $p=0.260$, $\eta p^2=0.092$) or time by sex interactions ($F_{2,38}=0.727$, $p=0.492$, $\eta p^2=0.042$) for body weight (Table 1). However, there was a main effect of sex with a large effect size ($F_{1,14}=10.862$, $p=0.005$, $\eta p^2=0.437$) on body weight, with the male participants weighing more than the female participants (Mean Difference: 16.50 kg) (Table 1). For pre-exercise feelings of fatigue, there were no observed main effects of time ($F_{2,28}=0.334$, $p=0.719$, $\eta p^2=0.023$) or time by sex interactions ($F_{2,38}=0.281$, $p=0.757$, $\eta p^2=0.020$) (Table 1). However, there was an observed effect of sex on fatigue levels pre-exercise ($F_{1,14}=6.275$, $p=0.025$, $\eta p^2=0.310$), with the male participants reporting lower levels of fatigue than the female participants (Mean Difference: 2.46) (Table 1). There were no observed main effects of time ($F_{2,28}=0.187$, $p=0.831$, $\eta p^2=0.013$), sex ($F_{1,14}=0.136$, $p=0.718$, $\eta p^2=0.010$), or time by sex interactions ($F_{2,28}=2.667$, $p=0.087$, $\eta p^2=0.160$) for vigor (Table 1). For pre-exercise perceived pain, there was a sex-by-time interaction, with a large effect size, for pre-exercise perceived pain ($F_{2,28}=3.896$, $p=0.032$, $\eta p^2=0.218$) (Table 1). The female participants had a higher perceived pain before exercise than the male participants during the EF time point (Mean Difference: 14.86 mm, $p=0.031$, $\eta p^2=0.291$) (Table 1). While not statistically significant, there was a large effect size for perceived pain between the female and male participants during both the O (Mean Difference: 1.33 mm, $p=0.116$, $\eta p^2=0.167$) and ML (Mean Difference: 2.53 mm, $p=0.138$, $\eta p^2=0.150$) visits (Table 1). For the female participants, pre-exercise perceived pain was higher

at EF compared to both O (Mean Difference: 14.20 mm, p=0.021) and ML (Mean Difference: 14.99 mm, p=0.041) (Table 1).

Table 1. Descriptive statistics.

		EF	O	ML
Weight (kg)				
	Males	77.46 ± 11.33†	78.50 ± 11.09†	77.56 ± 11.15†
	Females	61.41 ± 8.39	61.48 ± 9.08	61.15 ± 8.83
POMS-B Fatigue				
	Males	1.25 ± 1.04†	1.00 ± 1.07†	1.13 ± 0.64†
	Females	3.38 ± 4.10	3.13 ± 2.42	4.25 ± 4.10
POMS-B Vigor				
	Males	4.88 ± 2.80	4.63 ± 2.83	3.25 ± 2.12
	Females	3.63 ± 2.72	3.38 ± 3.25	4.50 ± 2.20
VAS-Pain (mm)				
	Males	0.92 ± 2.21†	0.26 ± 0.74	0.28 ± 0.51
	Females	15.79 ± 17.40	1.59 ± 2.11*	2.80 ± 4.52*

EF: Early Follicular, O: Ovulatory, ML: Mid-Luteal, POMS: Profile of Mood States. * - Different from EF, † - Different from Females. $p<0.05$. All values are Mean ± SD.

For VO2max, there was no statistically significant main effect of time ($F_{2,38}=3.365$, $p=0.050$, $\eta p^2=0.194$), sex ($F_{1,14}=2.821$, $p=0.115$, $\eta p^2=0.168$), or time by sex interactions ($F_{2,38}=1.518$, $p=0.237$, $\eta p^2=0.098$) (Table 2). However, the male participants tended to have a higher VO_{2max} than the female participants (Mean Difference: 9.88 ml/kg/min) (Table 2). There were no observed significant main effects of time ($F_{2,38}=0.713$, $p=0.499$, $\eta p^2=0.048$), sex ($F_{1,14}=0.241$, $p=0.631$, $\eta p^2=0.017$), or time by sex interactions ($F_{2,38}=3.283$, $p=0.052$, $\eta p^2=0.199$) for RER (Table 2). For HRmax, there were no statistically significant main effects of time ($F_{2,28}=0.306$, $p=0.739$, $\eta p^2=0.021$) or sex ($F_{1,14}=0.800$, $p=0.386$, $\eta p^2=0.054$) (Table 2). However, there was an observed time-by-sex interaction ($F_{2,38}=5.017$, $p=0.014$, $\eta p^2=0.264$), though when examining the pairwise comparisons from the Bonferroni correction, no within-sex or between-sex differences could be found at any time points (Table 2). Nevertheless, the female participants tended to have a higher HRmax during the O visit compared to the males (Mean Difference: 8.63 bpm, $p=0.133$, $\eta p^2=0.169$) (Table 2). In addition, the female participants tended to have a lower HRmax during the EF visit compared to the O (Mean Difference: 6.50 bpm, $p=0.089$) and ML (Mean Difference: 6.13 bpm, $p=0.107$) visits (Table 2). There were no observed main effects of time ($F_{2,28}=1.550$, $p=0.230$, $\eta p^2=0.100$) or time-by-sex interactions ($F_{2,28}=0.888$, $p=0.423$, $\eta p^2=0.060$) for end-test power (Table 2). There was a main effect of sex on end-test power ($F_{1,14}=6.417$, $p=0.024$, $\eta p^2=0.314$), where the male participants were observed to have a higher end-test power than the female participants (Mean Difference: 78.771 W) (Table 2). There were no observed significant main effects of time ($F_{2,28}=0.615$, $p=0.548$, $\eta p^2=0.042$), sex ($F_{1,14}=1.822$, $p=0.198$, $\eta p^2=0.115$), or time by sex interactions ($F_{2,28}=1.110$, $p=0.344$, $\eta p^2=0.073$) for peak blood lactate (Table 2). There were no observed significant main effects of time ($F_{2,28}=3.178$, $p=0.055$, $\eta p^2=0.166$), sex ($F_{1,14}=0.454$, $p=0.512$, $\eta p^2=0.031$), or sex by time interactions ($F_{2,28}=0.117$, $p=0.890$, $\eta p^2=0.008$) for end-test perceived exertion (Table 2). However, both the male and female participants tended to rate exercise as more difficult during the EF visit compared to O and ML (Table 2). In addition, there were no observed main effects of time ($F_{2,28}=1.023$, $p=0.373$, $\eta p^2=0.068$), sex

(F1,14=0.887, p=0.365, ηp^2=0.059), or sex by time interaction (F2,28=0.772, p=0.472, ηp^2=0.052) for perceived recovery 24 hours following exercise (Table 2).

Table 2. Aerobic performance variables.

		EF	O	ML
VO_{2max} (ml/kg/min)				
	Males	42.05 ± 15.38	39.24 ± 17.00	41.06 ± 15.64
	Females	31.53 ± 5.77	30.75 ± 4.92	30.43 ± 4.38
RER				
	Males	1.14 ± 0.05	1.11 ± 0.04	1.14 ± 0.07
	Females	1.09 ± 0.06	1.15 ± 0.07	1.13 ± 0.05
Heart Rate Max (bpm)				
	Males	182.00 ± 9.45	177.87 ± 11.00	178.50 ± 12.50
	Females	180.00 ± 12.55	186.50 ± 9.37	186.13 ± 12.90
End-Test Power (W)				
	Males	232.13 ± 82.97†	227.63 ± 89.19†	233.25 ± 84.44†
	Females	158.50 ± 31.22	149.13 ± 12.93	149.06 ± 24.51
Peak Lactate (mmol/L)				
	Males	9.56 ± 2.50	8.94 ± 2.66	9.55 ± 2.58
	Females	7.73 ± 1.91	8.14 ± 0.80	8.28 ± 1.44
RPE				
	Males	8.50 ± 1.69	7.50 ± 1.69	7.38 ± 2.62
	Females	9.00 ± 1.20	7.88 ± 1.89	8.12 ± 1.73
PRS				
	Males	8.75 ± 1.49	9.25 ± 0.89	8.88 ± 0.99
	Females	8.00 ± 2.00	8.38 ± 1.19	8.88 ± 1.81

RER: Respiratory Exchange Ratio, LT: Lactate Threshold, RPE: Rating of Perceived Exertion, PRS: Perceived Recovery Scale. † - Different from Females. p<0.05. All values are Mean ± SD.

Table 3. Lactate threshold.

		EF	O	ML
LT_{vis} (W)				
	Males	111.36 ± 64.06	107.38 ± 67.99	119.17 ± 71.63
	Females	73.75 ± 18.72	70.41 ± 16.66	68.03 ± 17.58
$LT_{2.0mmol}$ (W)				
	Males	113.41 ± 66.23	114.38 ± 59.65	121.58 ± 69.05
	Females	76.51 ± 18.63	68.43 ± 18.36	70.40 ± 12.45
$LT_{4.0mmol}$ (W)				
	Males	165.69 ± 70.28	160.95 ± 70.63	162.26 ± 78.77
	Females	112.33 ± 18.80	104.57 ± 15.09	104.18 ± 15.74

All values are Mean ± SD.

There were no observed main effects of time (F2,28=0.784, p=0.466, ηp^2=0.053), sex (F1,14=2.948, p=0.108, ηp^2=0.174), or any sex by time interactions (F2,28=2.056, p=0.147, ηp^2=0.128) on power output at LT_{vis} (Table 3). While not statistically significant, the male participants tended to have a higher power output at LT_{vis} than the female participants (Mean Difference: 41.91 W) (Table 3). For $LT_{2.0mmol}$, there were no observed main effects of time (F2,28=0.509, p=0.607, ηp^2=0.035),

sex (F1,14=3.735, p=0.074, ηp^2=0.211), or any sex-by-time interactions (F2,28=1.148, p=0.332, ηp^2=0.076) (Table 3). Similar to LT_{vis}, the male participants tended to have a higher power output at $LT_{2.0mmol}$ compared to the female participants (Mean Difference: 44.68 W) (Table 3). Finally, there were no statistically significant main effects of time (F2,28=2.414, p=0.108, ηp^2=0.147), sex (F1,14=4.514, p=0.052, ηp^2=0.244), or any sex-by-time interactions (F2,28=0.284, p=0.755, ηp^2=0.020) for $LT_{4.0mmol}$ (Table 3). Again, the male participants tended to have a higher power output at $LT_{4.0mmol}$ compared to the female participants (Mean Difference: 55.94 W) (Table 3). Individual data for power output at LT can be seen in Figure 1.

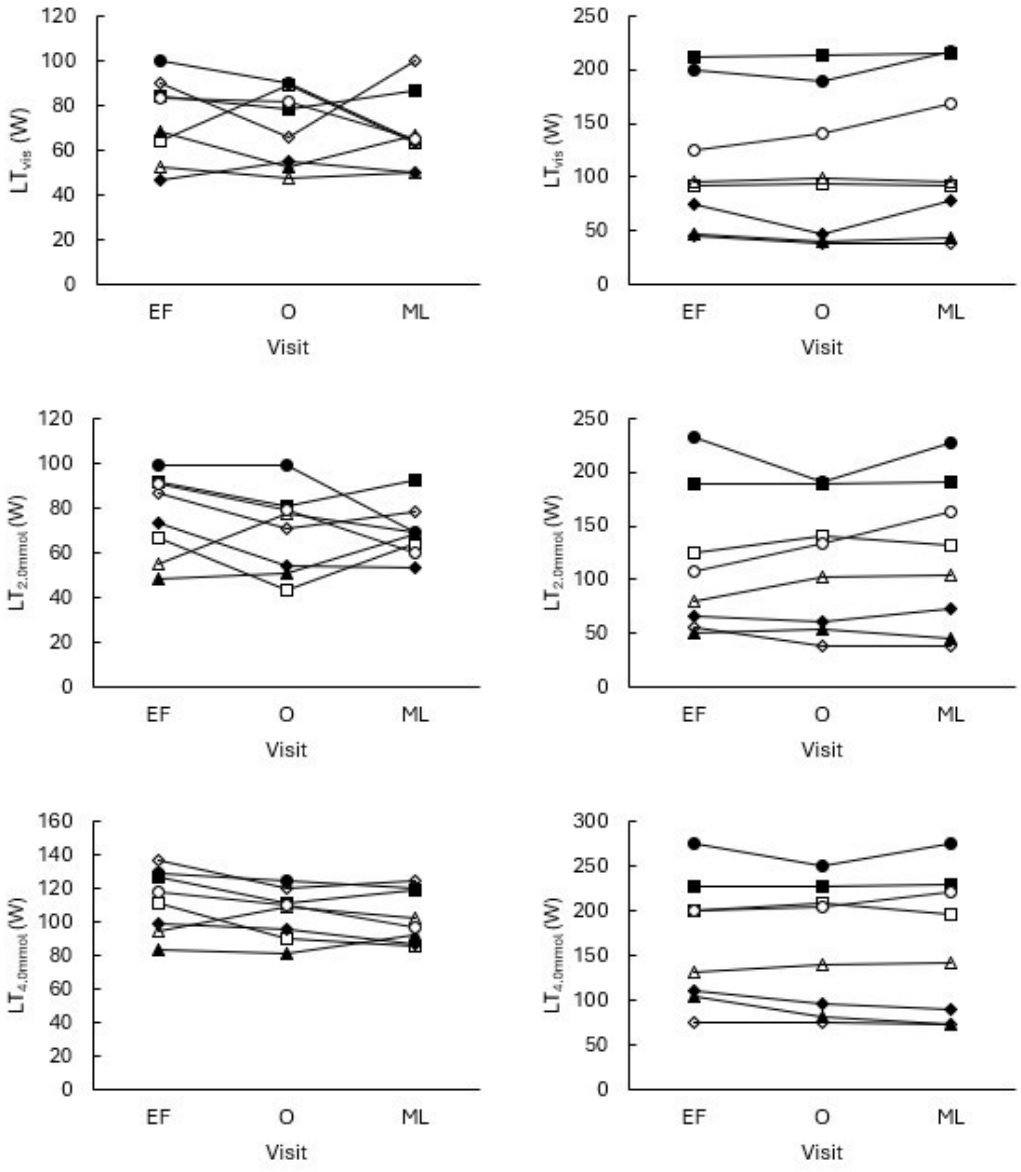

Figure 1. Power at lactate threshold individual data. Left column: females, right column: males.

Discussion

The primary objective of this study was to examine how lactate threshold varies across three distinct phases of the menstrual cycle (EF, O, and ML) in the female participants and the "phase" matched male participants. The major finding of the study was that there were no statistically significant differences observed in any of the LT measurements between sexes and/or across phases.

The results of this study agree with most that have investigated the effect of MC phase on the LT, which showed no statistically significant effects of MC phase on LT determination during cycling.[11,21,22] Two of the studies tracked the MC using blood hormone concentrations,[11,21] while the other study used basal body temperature.[22] The results of the present study are in line with studies that used both hormonal assays and non-invasive measurements. One novel finding of the present study was based on training status. The previous studies all had female participants described as trained,[11] sedentary,[21] or did not provide the training status of their participants.[22] Although the studies reported varying fitness levels among their participants, all three studies noted that their participants had a VO_{2max} in the low to mid-40s (~41-43 ml/kg/min).[11,21,22] The current study observed that the male participants had an average VO2max of approximately 40 ml/kg/min and the female participants had an average VO2max of approximately 30 ml/kg/min. This would put the two groups below the 50th percentile compared to reference values.[35]. It is useful for practitioners to know that even individuals with low aerobic fitness levels have a stable LT across the MC.

The findings of the present study conflict with those of one study that found a higher power output at LT during the ML phase.[23] The study found that power at LT4.0mmol was slightly higher (~10-15 W) at ML compared to MF. The power output at LT4.0mmol appears to be ~10-15W higher at ML compared to MF.[23] The differences in the findings could be due to three factors. First, this study used rowing while the present study used cycling.[23] Second, this study compared MF to ML, whereas the present study examined EF.[23] Finally, the previous study examined the effects of MC on the LT in trained female participants while the present study had untrained female participants.[23] Of interest, while not significant, the present study found that power at EF was slightly higher (~7W) than at O or ML, though these results were not statistically significant. However, whether or not this is enough of a difference in power output to affect endurance performance is unknown. To the best of our knowledge, only one study has demonstrated higher exercise performance during EF.[9] In both the previous study and the present study, the participants exercised at a minimum of 3h fasting state, which may have impacted the contribution of carbohydrates to total energy expenditure, increasing performance during EF.[36] The higher performance during EF may also be an artifact of the small sample size displayed in this study due to the high dropout rate in the female participants.

Although the menstrual cycle may have a minimal impact on exercise performance,[37] the psychological state can significantly affect how females feel during exercise, potentially acting as a barrier to exercise adherence.[38,39] A previous study showed a significantly higher RPE during EF (~9) compared to O (~8) and ML (~8).[5] While not statistically significant, the trends in the present study were similar for RPE and the effect size between time points was considered large (ηp^2=0.188).

Another key finding of this study was the heightened pain perception in females during EF compared to O and ML. Sex hormone receptors are distributed throughout the nervous system, potentially influencing pain modulation.[40] It has been observed that female participants had a higher pain tolerance during O, with EF and ML showing lower tolerance[41]. Estrogen has been shown to exert analgesic effects via opioid systems, and progesterone can modulate afferent sensory inputs.[40,42] These mechanisms may reduce pain sensitivity during O and ML, leaving EF as a vulnerable period when hormone levels are low. Interestingly, perceived pain was only correlated to end-task RPE during the EF phase for the female participants (r=-0.78, p=0.024). Heightened pre-exercise pain may have resulted in higher perceived effort, though interestingly this did not seem to affect maximal performance as VO_{2max} and max power output were similar across all three phases.

The small sample size in this study is a significant limitation. However, our sample is similar to most studies investigating lactate accumulation in females across the menstrual cycle where the sample sizes averaged around 10 participants per group.[22,23] Another significant limitation of this study was the lack of direct measure of blood hormone concentrations to assess the presence of adequate hormone levels during the ML phase. This could have allowed for individual disturbances in the MC, with smaller hormone fluctuations, to be included in the sample. Exercise performance and/or perception of performance could have been affected by subtle differences in the MC.[27] Finally, though the timing of eating was controlled before the LT tests, the participants did not keep a nutrition diary to ensure that their diet was consistent throughout the study.

In conclusion, natural fluctuations in sex hormones during the menstrual cycle appear to not significantly impact LT or maximal exercise performance in naturally menstruating, untrained females. Consequently, adjusting for menstrual cycle phases may not be necessary when conducting maximal exercise tests on a cycle ergometer. Our findings also suggest that, although the menstrual cycle significantly affects pain and fatigue, these states do not appear to impact maximal exercise performance. Future research should focus on evaluating psychological state changes before and after exercise to better understand how exercise influences mood profiles and perceived pain across different phases of the menstrual cycle.

Acknowledgements

The authors wish to thank the participants for their time and effort. Funding for this project was provided by the Robberson Research Grant and the Graduate Student Senate Grant from the Graduate College of the University of Oklahoma.

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