Exercise Performance Is Impaired during the Midluteal Phase of the Menstrual Cycle

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[1]H.H. Morris Human Performance Laboratories, Department of Kinesiology, School of Public Health, Indiana University, Bloomington, IN; [2]School of Public Health, Sackler Faculty of Medicine and Sylvan Adams Sports Institute, Tel Aviv University, Tel Aviv, ISRAEL; and [3]Shaare Zedek Medical Center, affiliated to the Hebrew University, Jerusalem, ISRAEL

ABSTRACT

FREEMAS, J. A., M. N. BARANAUSKAS, K. CONSTANTINI, N. CONSTANTINI, J. T. GREENSHIELDS, T. D. MICKLEBOROUGH, J. S. RAGLIN, and Z. J. SCHLADER. Exercise Performance Is Impaired during the Midluteal Phase of the Menstrual Cycle. *Med. Sci. Sports Exerc.*, Vol. 53, No. 2, pp. 442–452, 2021. **Purpose:** This study aimed to test the hypothesis that aerobic exercise performance is impaired in the midluteal (ML) compared with the midfollicular (MF) phase of the menstrual cycle. **Methods:** Twelve recreationally active eumenorrheic women (25 ± 6 yr) completed exercise sessions during the MF and the ML phases. Each session consisted of an 8-km cycling time trial that was preceded by 10 min of cycling performed at a constant power below and above gas exchange threshold. Heart rate, ventilation, and oxygen uptake were continuously measured. RPE and ratings of fatigue were assessed during the time trial using visual analog scales. Total mood disturbance was calculated from the POMS questionnaire administered before and 20 min postexercise. **Results:** Salivary progesterone concentration was 578 ± 515 pg·mL^{-1} higher in ML compared with MF phase ($P < 0.01$), whereas estradiol concentration did not differ between phases (167 ± 55 vs 206 ± 120 pg·mL^{-1}, $P = 0.31$). Total mood disturbance before exercise was greater during the ML phase compared with the MF phase ($P < 0.01$), but this difference was abolished postexercise ($P = 0.14$). Mean power output was lower during the ML phase (115 ± 29 vs 125 ± 28 W, $P < 0.01$), which led to a slower time trial in the ML phase (18.3 ± 2.0 min) compared with the MF phase (17.8 ± 1.7 min, $P = 0.03$). Ratings of fatigue were greater during the ML phase from 2 to 8 km ($P \leq 0.01$), whereas no differences in RPE were observed. Heart rate ($P = 0.85$), minute ventilation ($P = 0.53$), and oxygen uptake ($P = 0.32$) did not differ between phases during the time trial. **Conclusion:** Aerobic exercise performance is worse in the ML phase compared with the MF phase in recreationally active women, which was accompanied by a more negative mood state preexercise and increased ratings of fatigue. **Key Words:** MENSTRUAL CYCLE, EXERCISE PERFORMANCE, MOOD STATE, PERCEPTION OF FATIGUE

Over 40% of women indicate that their menstrual cycle has a negative effect on their exercise training and performance (1). The menstrual cycle is defined by fluctuations in sex hormone levels across the average menstrual cycle, which lasts ~28 d, in eumenorrheic women. For example, progesterone levels remain low during the midfollicular (MF) phase (6–9 d postmenses) and increase 12- to 20-fold postovulation during the midluteal (ML) phase (4–9 d postovulation), whereas estradiol remains low during the early follicular phase and begins to increase during the MF phase until it peaks in the late follicular and rises a second time during the ML phase (2). These hormonal oscillations affect various physiological and psychological parameters, which may affect exercise performance and/or adaptation to exercise training (3). For instance, higher levels of progesterone in normally menstruating women are associated with increased heart rate, minute ventilation, and core body temperature at rest (4,5) and during exercise (5,6). Furthermore, women have been shown to have lower running economy during high-intensity running during the ML phase, which is a factor commonly associated with reductions in exercise performance (5).

Evidence also indicates that during the ML phase, psychological changes such as irritability, anger, mild depression, and anxiety are heightened compared with the MF phase (7,8). In fact, over 80% of women have reported a more negative mood state during the luteal phase compared with the follicular phase of their menstrual cycle (7). Negative mood states may interfere with the motivation to engage in exercise (9), which may accelerate the onset of exercise fatigue (10). Notably, physiological and/or psychological alterations could impair the ability to maintain a given exercise intensity during certain phases of the menstrual cycle (3). Thus, it is often posited that exercise performance is reduced during the ML phase (2).

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Although exercise performance in women between the follicular and the luteal phases of the menstrual cycle has been examined, the literature remains inconclusive because of a lack of consistency in methods used to track the menstrual cycle. Of 18 studies published in the last 10 yr investigating the effects of the menstrual cycle on exercise performance, only 7 objectively confirmed menstrual cycle phase via the measurement of hormone concentrations and 3 found significant differences in exercise performance between phases (11). Notably, the majority of studies have examined the question of whether menstrual cycle phase alters exercise performance using time to exhaustion tests (6,12,13). However, time to exhaustion tests may not provide a reliable measure of performance because they lack fixed end points (14). This contrasts with self-paced time trial scenarios, in which individuals attempt to complete a fixed task (e.g., distance, work, etc.) as quickly as possible. Importantly, self-paced exercise time trials exhibit greater within-subject reliability than time to exhaustion tests (14,15) and allow participants to freely vary their pacing strategy, which may more closely resemble a performance scenario.

To our knowledge, only three prior studies have examined self-paced time trial performance across the menstrual cycle (16–18). Oosthuyse et al. (16) demonstrated no difference in cycling time trial efforts lasting ~30–60 min between the ML phase and the early follicular or late follicular phase in untrained and trained women. However, the effect of menstrual cycle phase on shorter time trial efforts lasting <30 min is unclear. Although Campbell et al. (17) reported a 13% improvement in 4 $kJ \cdot kg^{-1}$ time trial performance lasting ~20–30 min during the follicular compared with the luteal phase in endurance-trained women, McLay et al. (18) found no difference in the 16-km time trial performance (26.2 ± 1.4 min) between the MF and the ML phases in moderately trained females. Both time trials in Campbell et al. (17) and McLay et al. (18) were preceded by 2 h and 75 min of submaximal cycling, respectively. Given these methodological considerations, it remains unclear if exercise performance lasting <30 min is influenced by hormonal fluctuations that occur throughout the menstrual cycle. Therefore, our primary purpose was to clarify the effect of menstrual cycle phase (MF vs ML) on shorter time trial cycling performance lasting ~15–20 min in young, recreationally active, eumenorrheic women. We hypothesized that an 8-km cycling time trial performance would be slower in the ML phase compared with the MF phase and that this would coincide with a more negative mood state during the ML compared with the MF phase.

METHODS

Participants

Fifteen eumenorrheic women between the ages of 18 and 35 yr were recruited to participate in the present study. Participants qualified if they self-reported having normal menstrual cycles (between 25 and 36 d in length) (16), had abstained from hormonal contraceptive use for at least 6 months before entering the study and throughout the study duration, and met minimum physical activity requirements of participating in at least 150 $min \cdot wk^{-1}$ of aerobic exercise (19). We refrained from recruiting endurance-trained cyclists because the physiological response to fluctuations in hormones throughout the menstrual cycle may be blunted compared with the recreationally active/untrained woman. Participants who qualified were then tracked for at least three consecutive menstrual cycles. Participants were excluded if 1) they were currently taking hormonal contraceptives, 2) ovulation was not present (see details below), and/or 3) their body mass index was <18 $kg \cdot m^{-2}$ or they had experienced a weight change of ≥2 kg during the 3 months before and/or throughout study enrollment. Before participation, participants completed the Physical Activity Readiness Questionnaire and provided written informed consent to participate in study procedures approved by the Indiana University Institutional Review Board.

Participants were instructed to refrain from consumption of caffeine in any form (e.g., coffee, energy drink) for at least 12 h and to avoid high-intensity exercise for at least 24 h before exercise testing. We chose to standardize the use between visits by having each subject refrain from any caffeine before each visit to the laboratory. Although this may have resulted in caffeine withdrawal, it is expected that the extent of this withdrawal would have been the same in each visit. Participants were also encouraged to maintain their normal exercise routines and diet throughout the duration of the experimental period. Percent body fat and body mass were monitored throughout the study duration. All participants were nonsmokers, with no history of cardiovascular or pulmonary disease, and had normal lung function as assessed by pulmonary function testing in accordance with the American Thoracic Society or European Respiratory Society guidelines (20).

Measurements

Anthropometric. Height was measured at the beginning of each visit using a wall-mounted stadiometer and recorded to the nearest centimeters, and body mass was measured to the nearest grams using a digital scale. Skinfold thickness measurements were taken by the same investigator with Lange Skinfold calipers (Beta Technology, Santa Cruz, CA) at three sites (triceps, suprailiac, and abdomen) (21). Duplicate measurements were taken at each site, with a third obtained if the first two were not within 5 mm of each other (22). Skinfold measurements were calculated as the sum of all sites, and percent body fat was calculated from body density (23) using the Siri formula (24). Body mass index was calculated as weight (kg)/height (m^2).

Respiratory. All resting pulmonary function tests were performed using a metabolic cart (Vmax-Encore System; CareFusion, Yorba Linda, CA) after the participants had been seated and rested for at least 5 min (20). Measurements were obtained before the start of exercise in both the MF and the ML phases of the third month. The test included a forced vital capacity (FVC) maneuver, from which the fraction of expired volume in 1 s (FEV_1), peak expiratory flow rate (PEF), and

fraction of expiratory flow between 25% and 75% expiration ($FEF_{25–75}$) were determined. Spirometry was performed in triplicate according to the American Thoracic Society recommendations, and the largest value of FVC, FEV_1, PEF, and $FEF_{25–75}$ are reported (20).

Mood state. POMS (25) was used to assess the psychological responses to exercise and was administered before exercise and 20 min postexercise. POMS is a 65-item questionnaire that assesses six specific mood state subscales (tension–anxiety, depression, anger–hostility, vigor–activity, fatigue, and confusion–bewilderment) and provides a general measure of total mood disturbance (TMD). POMS has been extensively used in exercise and sport research (26). Participants filled out the questionnaire using the Qualtrics survey software (Qualtrics, Provo, UT), and the measures assessed by POMS have each been validated (25). In the present study, the standard instructional set of POMS (i.e., "last week including today") was modified to assess the immediate psychological state of the participants by asking them to complete the questionnaire on the basis of how they felt "right now." A TMD score was calculated by adding the scores of the five subscales and then subtracting "vigor" score and adding a constant of 100 to prevent the occurrence of negative values [(confusion + tension + depression + fatigue + anger – vigor) + 100]. Because the range of scores possible is not equivalent across the six subscales, the values have been graphed as *T*-scores. The *T*-score distribution for the TMD is based on normative values for each subscales, and raw scores were converted into *T*-scores with a mean equal to 50 and an SD equal to 10 (25), with the *T*-score TMD calculated as noted above. A lower total score indicated less mood disturbance (better mood) and vice versa.

Perceptual. A written script was used in the laboratory while verbally assessing analog measures of RPE (6–20 Borg scale [27]) rating of perceived breathlessness (1–10, Modified Borg scale [27]), and rating of fatigue (scale 0–10 [28]) during exercise. The written script was used to administer each measure of perception in each individual testing session and to differentiate between each scale so the subject could make the distinction. RPE, rating of perceived breathlessness, and rating of fatigue were assessed in the last 10 s of the last minute during each constant load exercise bout. Every 2 km during the 8-km time trial, participants were asked to rate their rating of fatigue on a scale of 0–10 (28), where 0 implies "no fatigue" and 10 indicates "maximum fatigue." In addition, every 2-km test, participants were asked to rate their RPE where 6 in implies "no effort" and 20 indicates "maximal effort" (27), which they were also familiarized with along with the rating of perceived breathlessness scale. Participants were familiarized with this scale before each test and were told that 0 implies no "noticeable breathing effort above what occurs at rest" and 10 indicates "maximal ventilatory effort."

Hormonal. Upon arrival to the laboratory, saliva was immediately collected from participants, which was collected by instructing participants to direct their accumulated saliva through a miniature straw that led into a salivary collection tube. Saliva was collected while in an upright seated position before any exercise. Participants were instructed to arrive to the laboratory having abstained from food for at least 3 h prior and consumed only water before salivary collection. Evidence suggests that progesterone peaks during the ML phase compared with the MF phase, wherein it remains at a relatively low concentration (4,16). Progesterone and estradiol were retroactively analyzed through Saliva ELISA Assay Kits (Steroid ELISA; Eagle Biosciences, Amherst, NH) with a measurement sensitivity of 20 $pg \cdot mL^{-1}$ for progesterone and a sensitivity of 10 $pg \cdot mL^{-1}$ for estradiol. The intra-assay variation for progesterone was less than 13.3% and 10.3% for estradiol. The interassay variation for progesterone was less than 12.7% and 13.6% for estradiol. Salivary collection of progesterone and estradiol has been shown to be a reliable and highly accurate method to determine menstrual cycle phase (29). The molar ratio of estradiol to progesterone (E_2/Pg ratio) was calculated as the quotient of estradiol ($pmol \cdot L^{-1}$) to progesterone ($nmol \cdot L^{-1}$).

Experimental Procedures

Maximal aerobic capacity test. Participants performed an incremental exercise test to volitional exhaustion on a Velotron cycle ergometer (RacerMate Inc., Seattle, WA) to determine peak oxygen uptake ($\dot{V}O_{2peak}$) during the MF phase of the cycle. The seat and handlebar settings were adjusted during the initial visit and remained consistent throughout all test sessions for each subject. The highest 30-s average data of the $\dot{V}O_{2peak}$ test were used to determine power at the gas exchange threshold for each subject using the V-slope method (30). The graded exercise protocol began at 25 W and increased by 20 W each minute until volitional exhaustion. Participants could self-select pedaling cadence but were asked to maintain constant cadence (60–80 rpm) throughout the test. $\dot{V}O_{2peak}$ was calculated as the highest 30 s average oxygen uptake ($\dot{V}O_2$) attained. Ventilatory and metabolic measurements were collected using breath-by-breath analysis (Vmax-Encore System, CareFusion) while participants breathed through an oronasal facemask (7450 Series; Hans Rudolph, Kansas, MO) attached to a mass flow sensor that measured inspired and expired flow rates. The O_2 and the CO_2 analyzers were calibrated before each test with room air and calibration gases within the physiological range, and the mass flow sensor was calibrated at varying flow rates (30–360 $L \cdot min^{-1}$) using a 3.0-L syringe. During all trials, the mass flow sensor was attached to a two-way, non-rebreathing valve (2700 Series, Hans Rudolph). In addition, heart rate was continuously monitored using a heart rate monitor (Model FT1; Polar, Stamford, CT).

Constant load cycle tests. Physiological variables are subject to change based on power output; thus, we used a preloaded time trial with fixed workloads to accurately assess gas exchange and ventilatory parameters. Participants were asked to perform two 5-min constant load cycling bouts at a power output equivalent to 10% below (i.e., moderate intensity domain) and 10% above (i.e., heavy intensity domain) their gas exchange threshold, respectively, to be able to compare these physiological parameters at two distinct relative

exercise intensity domains. Before the first cycle bout, 2 min of seated rest on the bike were administered to collect baseline minute ventilation and heart rate. Five minutes of passive rest were given between the two constant load bouts. RPE, ratings of fatigue, and ratings of perceived breathlessness were recorded within the last 10 s of completing each constant load bout. Ventilatory and metabolic data were continuously collected, and heart rate, metabolic, and ventilatory data are reported as averages of the last minute of each constant load trial.

8-km time trial. After 10 min of passive rest after the constant load trials, participants were asked to complete an 8-km time trial. An 8-km time trial was chosen as the optimal distance for our untrained subject population given the expected duration (i.e., 15–22 min). For instance, a longer duration could have increased the variation in performance responses because these participants are not trained cyclists.

Before the start of each time trial, participants were reminded that the objective of the test was to complete the distance as quickly as possible. Participants were able to self-select cadence and resistance using an electronic gearing system connected to the Velotron cycle ergometer. During the 8-km time trial, participants could view their completed distance only and were blinded to additional variables such as power output, exercise time, and cadence. Scripted, verbal encouragement was provided to the participants every 2 km. Ventilatory parameters, gas exchange variables, and heart rate were continuously monitored and recorded using methods previously mentioned and were subsequently averaged and analyzed for every 2 km. In addition, RPE, rating of fatigue, and rating of perceived breathlessness were assessed at each 2-km marker. Heart rate, metabolic, and ventilatory data were analyzed every 2 km. An index of pacing strategy was calculated by normalizing power output at each 2 km to the average power output during the 8-km time trial during each experimental trial.

Study Design

Menstrual cycle phase was assessed through collection of basal body temperature, ovulation kits and salivary progesterone concentrations. All participants recorded their basal body temperature using an oral digital thermometer (Medical Thermometer; B. Weiss Personal Care, Shenzhen, China) upon waking each morning throughout the duration of enrollment. Participants were instructed to test for ovulation at home on days 13–15 of their cycle using an over-the-counter ovulation prediction kit (Easy@ Home Ovulation Test; Easy Healthcare Corporation, Burr Ridge, IL). These kits were used to detect the surge in urinary luteinizing hormone that precedes ovulation and is indicative of a normal, monthly hormonal profile. In addition, during visits 2–5, participants provided a salivary sample for a retrospective analysis of progesterone concentration.

Each subject participated in three familiarization and two experimental testing sessions over the course of three complete menstrual cycles (4). Only data collected during the third month were analyzed. Testing was carried out during the MF phase (days 6–9 of a normal menstrual cycle) and during the ML phase (4–9 d postovulation) (2). These phases were initially determined by a sustained rise in oral temperature of 0.2°C–0.3°C measured daily by the participants and ovulation tests each month, which were retrospectively confirmed through analysis of salivary samples of progesterone.

All data were collected in the morning and the time of day was standardized within each subject (±1 h) to minimize any possible confounding effects from diurnal variations in hormone concentrations (15). Visit 1 was completed during the MF phase for every subject and included body composition measures, a maximal aerobic capacity ($\dot{V}O_{2peak}$) exercise test, and a familiarization of the 8-km time trial. The phase during which the subsequent visit was performed was randomized for each subject (see Fig. 1 for a schematic of the study design randomization). Visits 2–5 each included body composition measures, saliva collection, two constant load cycle bouts, an 8-km time trial, and POMS questionnaires administered before exercise and 20 min postexercise. This ensured that participants were well familiarized with all experimental procedures. In addition, baseline pulmonary function tests were performed during the last two visits, i.e., only in the third month of testing for each subject (the two experimental test sessions).

Data Analysis

Data analysis was performed using PRISM version 8.3.0 (GraphPad Software, La Jolla, CA). Paired sample *t*-tests were used to assess differences between the ML and the MF phases for variables measured at only one time point in each trial (e.g., 8-km time trial finishing time). Two-way repeated-measures ANOVA (phase × distance covered or phase × pre/postexercise) were used to determine differences in variables measured

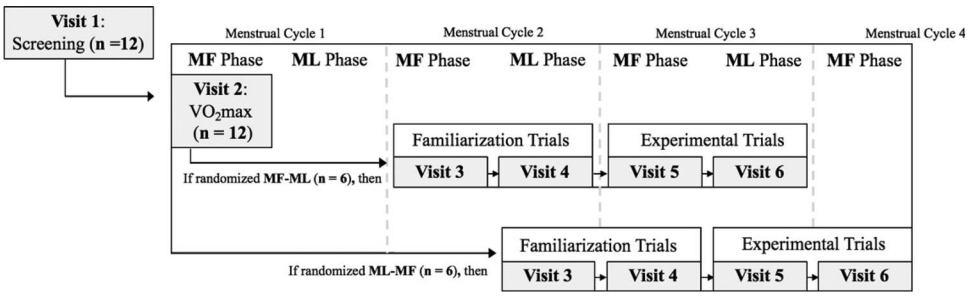

FIGURE 1—Schematic of study design randomization.

at each 2-km segment across the 8-km time trial (e.g., heart rate, $\dot{V}O_2$) and to analyze TMD and POMS subscales pre- and postexercise (e.g., vigor, confusion). All *post hoc* tests were conducted using the Sidak correction method for multiple comparisons. Two-tailed significance was set at $\alpha \leq 0.05$. Data are reported as mean ± SD and mean with 95% confidence interval (CI) when examining differences between trials. Where statistical significance was observed in the paired *t*-tests, effect sizes are included using Cohen's d_z (31).

A power analysis was carried out with G-Power version 3.1.9.4 (32) using previous literature evaluating time trial performance (17). It was estimated that seven participants were needed to detect differences in time trial performance using standard parameters of $1 - \beta = 0.80$ and $\alpha = 0.05$.

RESULTS

Participants. Of the 15 participants initially enrolled, 12 completed all study procedures. Three participants did not complete the study due to either 1) irregular menstrual cycle ($n = 1$) or 2) dropout ($n = 2$). Participant characteristics obtained during visit 1 data are displayed in Table 1.

Menstrual cycle characteristics. Oral temperature on the day of testing was 0.7°C ± 0.6°C higher in the ML phase compared with the MF phase ($P = 0.002$, 95% CI = 0.3°C–1.1°C, Cohen's $d_z = 0.91$). Progesterone concentration was 578 ± 515 pg·mL^{-1} higher in the ML phase compared with the MF phase ($P = 0.003$, 95% CI = 251–905 pg·mL^{-1}, Cohen's $d_z = 1.12$), whereas estradiol concentration did not differ between phases (167 ± 55 vs 206 ± 120 pg·mL^{-1}, $P = 0.312$). In addition, the molar ratio of estradiol to progesterone did not differ between phases ($P = 0.772$; Table 2). The average length of the menstrual cycle and the day of LH surge are also shown in Table 2 along with measures of body mass, body fat (%), baseline heart rate, and baseline ventilation differentiated by ML and MF phase.

Gas exchange variables during constant load cycling. During the constant load performed at an intensity of 10% below gas exchange threshold, minute ventilation ($P = 0.029$, 95% CI = 0.3–5.3 L·min^{-1}, Cohen's $d_z = 0.72$) and absolute $\dot{V}O_2$ ($P = 0.031$, 95% CI = 0.01–0.17 L·min^{-1}, Cohen's $d_z = 0.71$) were higher during the ML phase. There were no differences in heart rate ($P = 0.677$) or $\dot{V}CO_2$ ($P = 0.085$) between phases.

TABLE 1. Participant characteristics ($n = 12$).

Age (yr)	24.8 ± 5.6
Height (cm)	165 ± 5
Body mass (kg)	61.1 ± 6.3
BMI (kg·m^2)	22.4 ± 2.2
$\dot{V}O_{2peak}$ (L·min^{-1})	2.3 ± 0.5
$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	40.1 ± 11.0
Peak HR (bpm)	179 ± 11
Peak work rate (W)	189 ± 37
Power at 10% below GET (W)	88 ± 19
Power at 10% above GET (W)	108 ± 19

Values are presented as mean ± SD. Peak heart rate and work rate were obtained during maximal aerobic capacity test.
BMI, body mass index; $\dot{V}O_{2peak}$, maximal O_2 uptake; GET, gas exchange threshold.

TABLE 2. Menstrual cycle characteristics ($n = 12$).

	MF	ML
Oral temperature	36.1 ± 0.4	36.5 ± 0.7*
Body mass (kg)	61.0 ± 6.3	61.2 ± 6.0
Body fat (%)	25.6 ± 5.6	26.5 ± 5.2
Progesterone (pg·mL^{-1})	380 ± 138	958 ± 506*
Estradiol (pg·mL^{-1})	167 ± 55	206 ± 120
E_2/Pg ratio	589 ± 328	528 ± 662
Baseline HR (bpm)	71 ± 9	77 ± 11*
Baseline $\dot{V}_E$ (L·min^{-1})	10.3 ± 1.1	10.6 ± 1.1
Cycle length (d)	29 ± 3	
Day of LH surge	14 ± 0	

Values are presented as mean ± SD.
*$P < 0.05$, compared with MF.
HR, heart rate; $\dot{V}_E$, minute ventilation; LH, luteinizing hormone. Pg/E_2 ratio, progesterone to estradiol concentration ratio.

During the constant load performed at an intensity of 10% above gas exchange threshold, there were no differences in heart rate ($P = 0.502$), absolute $\dot{V}O_2$ ($P = 0.238$), $\dot{V}CO_2$ ($P = 0.153$), or minute ventilation ($P = 0.110$) between phases. Table 3 displays physiological and perceptual parameters during the constant load cycle bouts.

Differences in the 8-km time trial performance times between MF and ML. On average, the women completed the 8-km time trial in 18.3 ± 2.0 min during the ML phase and in 17.8 ± 1.7 min during the MF phase. There was, on average, a difference of 26 ± 36 s (2.6%) between phases ($P = 0.030$, 95% CI = 3–49 s, Cohen's $d_z = 0.72$). Figure 2A displays mean power output throughout the time trial.

Mean power output was significantly lower during the ML phase (115 ± 29 vs 125 ± 28 W, $P = 0.007$, 95% CI = −6.2 to −3.2 W, Cohen's $d_z = 1.00$). Figure 2B shows the mean power output per 2 km between the ML and the MF phases. At the 2-km splits, differences in power between phases were observed at 2 km ($P = 0.016$), 4 km ($P = 0.016$), 6 km ($P = 0.028$), but not 8 km ($P = 0.252$). Pacing strategy represented as percentage of average pace is displayed in Figure 2C, no differences between phases were observed ($P = 0.218$).

There were no differences between phases in absolute $\dot{V}O_2$ ($P = 0.317$), $\dot{V}CO_2$ ($P = 0.232$), minute ventilation ($P = 0.534$), or heart rate ($P = 0.849$) across any 2-km segment during the 8-km time trial.

TABLE 3. Constant load exercise variables ($n = 12$).

	MF	ML
10% below GET		
HR (bpm)	126 ± 13	127 ± 15
$\dot{V}_E$ (L·min^{-1})	35.4 ± 4.7	38.2 ± 5.0*
$\dot{V}O_2$ (L·min^{-1})	1.3 ± 0.2	1.4 ± 0.2*
RPE (6–20)	11.0 ± 2.0	11.0 ± 2.3
Rating of fatigue (0–10)	2.0 ± 1.4	3.0 ± 1.6*
Rating of perceived breathlessness (0–10)	2.0 ± 1.2	2.0 ± 1.6
10% above GET		
HR (bpm)	143 ± 13	144 ± 13
$\dot{V}_E$ (L·min^{-1})	44.5 ± 7.6	47.8 ± 7.5
$\dot{V}O_2$ (L·min^{-1})	1.6 ± 0.3	1.6 ± 0.2
RPE (6–20)	12.2 ± 1.9	13.0 ± 1.7
Rating of fatigue (0–10)	3.3 ± 1.6	4.5 ± 1.8
Rating of perceived breathlessness (0–10)	2.5 ± 2.0	2.8 ± 2.0

Values are presented as mean ± SD.
*$P < 0.05$, compared with MF.
GET, gas exchange threshold; HR, heart rate; $\dot{V}_E$, minute ventilation; $\dot{V}O_2$, O_2 uptake.

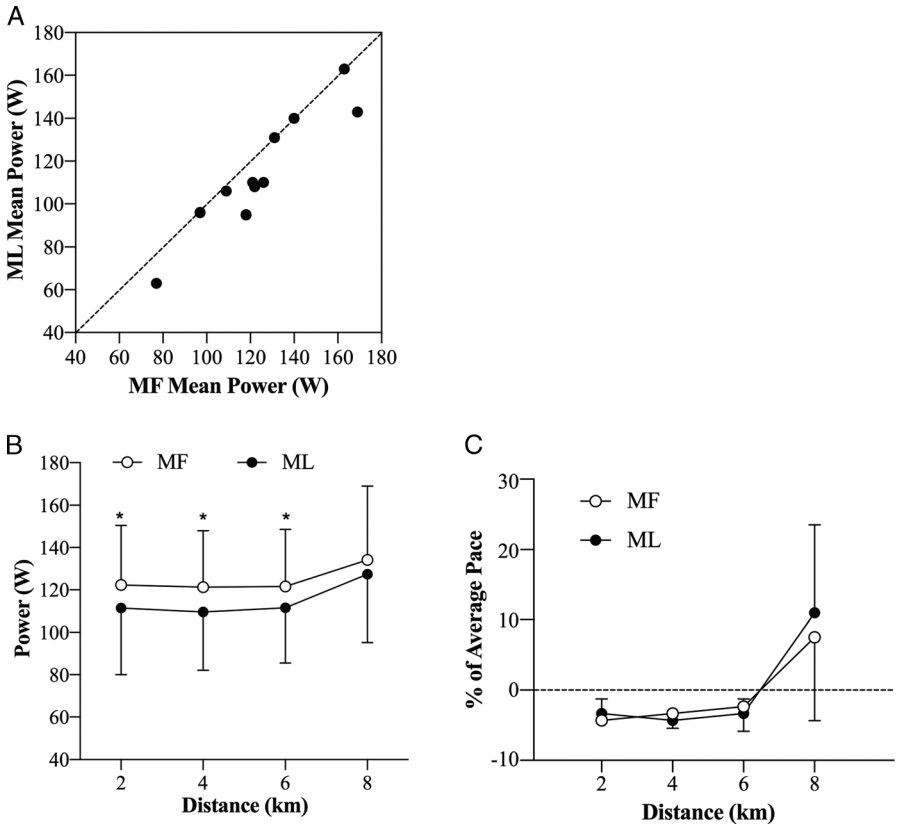

FIGURE 2—A, Individual time trial power outputs (W) between MF and ML phases. *Dotted line* represents line of identity (equal performances). B, Mean power output at each 2 km between the MF and the ML phases. C, Normalized pacing per 2 km between MF and ML phases. Positive values represent a faster-than-average pace; negative values a slower-than-average pace. *Dotted line* at zero indicates the average power output across the time trial. *Significantly different between phases ($P < 0.05$).

Perceptual measures. There was a difference between phases in rating of fatigue during constant load cycling at the intensity below gas exchange threshold ($P = 0.039$, 95% CI = 0.1–1.9, Cohen's $d_z = 0.68$), but not above gas exchange threshold. Ratings of fatigue was greater during the ML phase at 2 km ($P = 0.003$), 4 km ($P < 0.001$), 6 km ($P < 0.001$), and 8 km ($P < 0.001$) of the 8-km time trial.

RPE and rating of perceived breathlessness did not differ between phases after constant load cycling at intensities below ($P = 0.999$; $P = 0.276$) or above gas exchange threshold ($P = 0.069$; $P = 0.600$). In addition, there were no differences in RPE ($P = 0.094$) or rating of perceived breathlessness ($P = 0.678$) across the 8-km time trial. However, within each phase, RPE was greater at 4 km ($P = 0.001$), 6 km ($P < 0.001$), and 8 km ($P < 0.001$) compared with 2 km. Similarly, within each phase, rating of perceived breathlessness was greater at 6 km ($P < 0.001$) and 8 km ($P < 0.001$) compared with 2 km. The development of RPE, rating of perceived breathlessness, and rating of fatigue across the 8-km time trial is displayed in Figure 3A, B, C with differences between phases indicated.

Profile of mood state. Subscale scores for each of the six POMS components taken during each phase before exercise are shown in Figure 4. Raw scores of TMD were greater preexercise during the ML compared with the MF phase (123 ± 19 vs 114 ± 22 a.u., $P = 0.002$) but improved postexercise in the ML phase with no differences between phases (100 ± 12 vs 96 ± 11 a.u., $P = 0.138$) (T-scores displayed in Fig. 4C). Preexercise differences between phases were also observed in the subscales of vigor ($P = 0.016$), tension ($P = 0.001$), and anger ($P = 0.048$). However, after exercise, no differences between phases were observed (vigor, $P = 0.095$; tension, $P = 0.752$; anger, $P = 0.697$). Subscales of confusion ($P = 0.512$), depression ($P = 0.733$), and fatigue ($P = 0.659$) showed no differences at the pre– or post–time points between phases. Confusion (ML and MF, $P < 0.001$), tension (ML and MF, $P < 0.001$), depression (ML and MF, $P < 0.001$), fatigue (ML, $P = 0.003$; MF, $P = 0.012$), vigor (ML, $P < 0.001$; MF, $P = 0.035$), and TMD (ML and MF, $P < 0.001$) decreased from pre- to postexercise in both the ML and the MF phases. Changes pre- to postexercise in anger were observed in the ML phase ($P = 0.005$) but not the MF ($P = 0.118$) phase. In addition, results showed a greater decrease in tension from pre- to postexercise in the ML phase compared with the MF phase ($P = 0.017$).

DISCUSSION

In the present study, an 8-km cycling time trial was used to examine whether aerobic exercise performance differed between

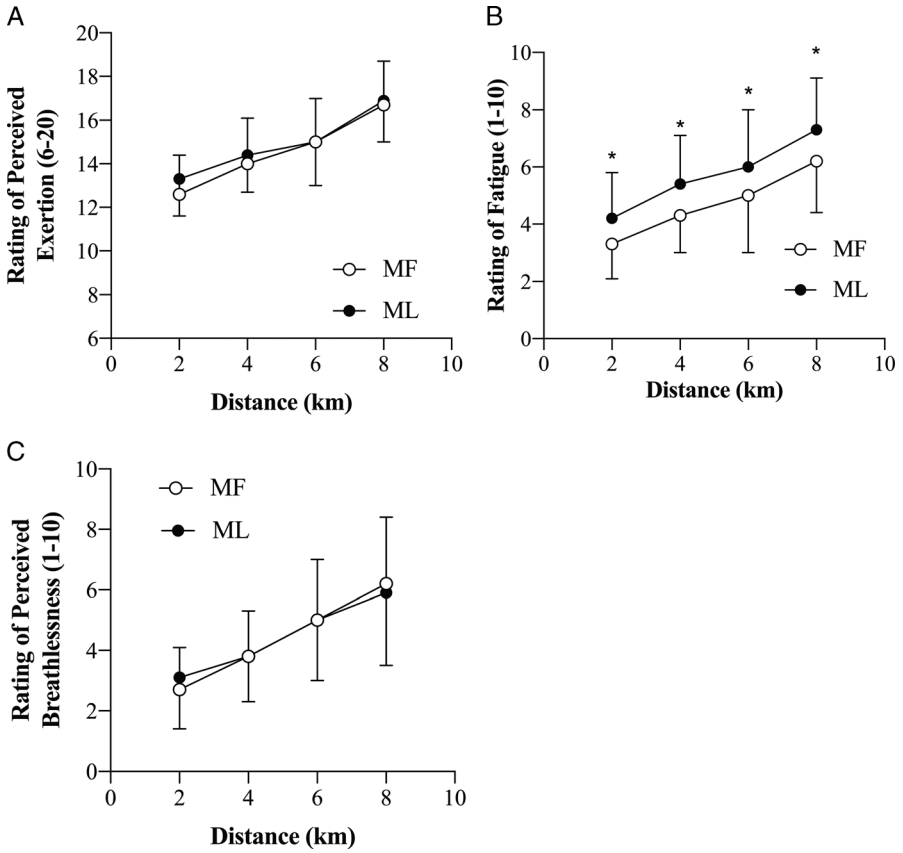

FIGURE 3—A, RPE every 2 km between MF and ML phases. B, Rating of fatigue every 2 km between MF and ML phases. C, Rating of perceived breathlessness every 2 km between MF and ML phases. *Significantly different between phases ($P < 0.05$).

the MF and the ML phases of the menstrual cycle in recreationally active eumenorrheic women. We assessed selected physiological and psychological variables that have previously demonstrated variation across the menstrual cycle. Our primary finding was that the 8-km time trial cycling performance was slower in the ML phase compared with the MF phase. The slower performance times in the ML phase occurred alongside a more negative mood state preexercise and heightened perceptions of fatigue during the 8-km time trial in the ML phase compared with the MF phase. A secondary finding was that exercise improved postexercise mood, such that the negative mood state observed preexercise in the ML phase was abolished after exercise. Thus, our data support the possibility that a more negative mood state and/or increases in perceived fatigue may play a role in determining the lower initial exercise workload in the ML phase during self-paced exercise, and that exercise improves mood state irrespective of the relative performance achieved by the subject.

Pacing strategy between the MF and the ML phases. We observed a lower self-selected power output in the ML compared with the MF phase that persisted through 6 km of the 8-km time trial (Fig. 2B). This difference in exercise workload throughout most of the time trial is unlikely attributed to changes in standard physiological variables (e.g., heart rate, oxygen uptake, etc.) across the menstrual cycle because the average selected power outputs attained during the time trial were above the power at gas exchange threshold, an exercise workload of which we did not identify any differences in physiological responses (Table 3). Thus, it is plausible that the lower power outputs throughout most of the time trial were likely due to the more negative mood state preexercise (Fig. 4A) and/or increased ratings of fatigue during exercise (Fig. 3B). The precise physiological mechanisms mediating these psychological constructs remain unclear from the data collected in the current study.

Participants arrived feeling less vigorous during the ML phase compared with the MF phase, which corresponded with a slower, more conservative selection of power output as early as the first 2 km of the time trial. By contrast, RPE and perceived breathlessness did not differ between phases (Fig. 3A, C). This leads us to believe that participants self-selected a lower power output in the ML exercise trial so that their perceived exertion responses would be similar to the MF phase trial, presumably because a lower power felt equivalent to the effort of a higher power attained during the MF phase, when scores of vigor were significantly greater. This is supported by our findings that the pacing strategy (i.e., the distribution of the rate of mechanical work over the entire duration of the time trial) used did not differ between the ML and the MF phases (Fig. 3C). In additional support, we also found that ratings of perceived fatigue were higher during the entire 8-km time trial performed during the ML phase compared with MF, despite the fact that participants were cycling at a lower power

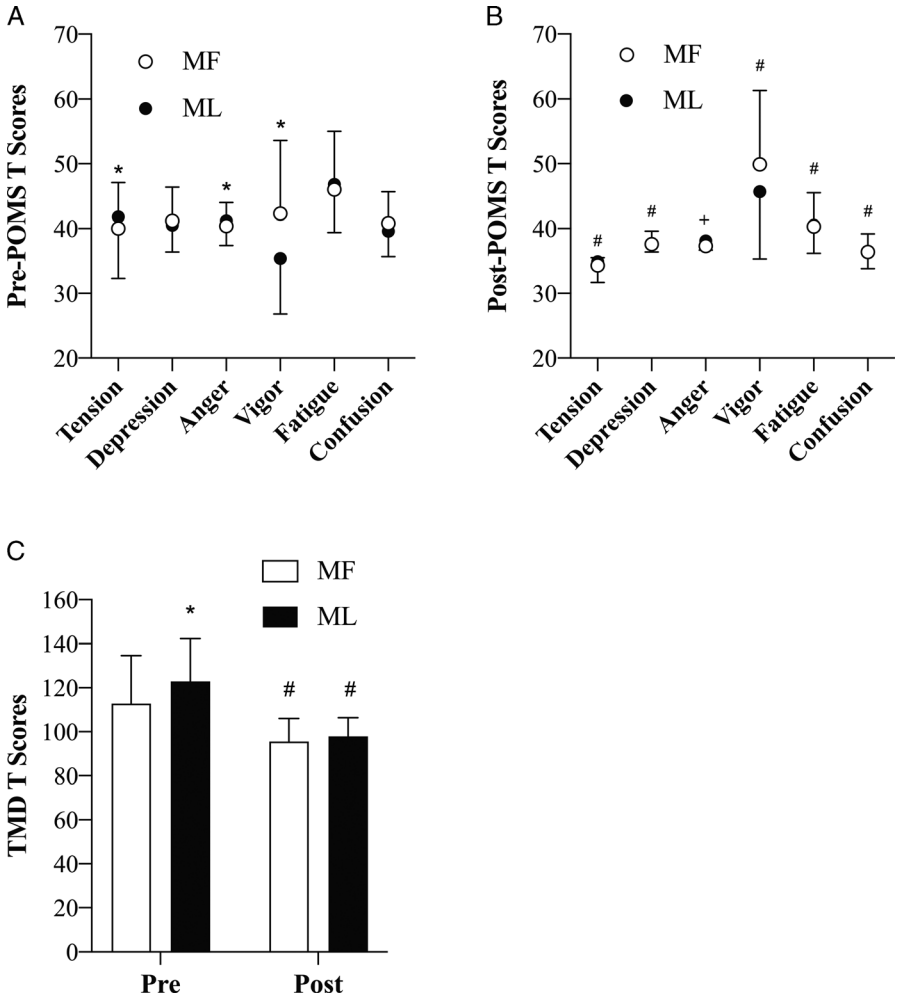

FIGURE 4—A, *T*-scores of POMS subscales preexercise between the MF and the ML phases. B, *T*-scores of POMS subscales postexercise between the MF and the ML phases. C, TMD from pre- to postexercise between the MF and the ML phases. *Significantly different between phases. #Significantly different pre- to postexercise in both the MF and ML phases. +Significantly different pre- to postexercise in the ML phase. Data are presented as *T*-scores.

output during these time points and overall performed worse during the ML phase (Fig. 2B). This finding is consistent with the mental health model of sport performance, which posits that desirable psychological factors including low mood disturbance and high vigor are associated with better athletic performance, which is what we saw during the MF phase trial (33).

The fact that we found slower time trial performance during the ML compared with the MF phase (17) whereas other researchers have not (16,18) is likely a consequence of differences in experimental design and the exercise test administered. Other researchers assessed exercise performance using time to exhaustion exercise tests (6,12,13) that typically exhibit poor within-subject test–retest reliability of over 26% (14), whereas the within-subject reliability of time trial performance tests typically ranges from 1% to 3% (14,15). Furthermore, time to exhaustion tests performed at submaximal intensity (14,34) provide a measure of endurance capacity, which favors the metabolic profile of the ML phase (16), rather than assess exercise performance, likely reducing the reliability of that protocol across the menstrual cycle. For these reasons, and because the aim of this study was to identify mechanisms that could explain difference in a true performance measure between phases, we chose to use a time trial performance test.

Although a few other studies have used a time trial to examine exercise performance across the menstrual cycle (16–18), results differ due to discrepancies in the distance (or duration) of the self-paced exercise, the training status of participants, and the specific timing of menstrual cycle phase when the test was performed (4). For example, Campbell et al. (17) administered a fixed work time trial (~20–30 min in duration) in endurance-trained women using a protocol specific to body mass between the follicular phase and the luteal phase without differentiating early/middle/late, whereas Oosthusye et al. (16) compared trained and untrained women in a 15-km time trial and 30-km time trial during the early follicular, late follicular, and ML phases of the cycle. Our study involved young, recreationally active women spanning a wide range of aerobic fitness levels (25–57 $mL \cdot kg^{-1} \cdot min^{-1}$). Because this sample is more representative of the general population range (19) compared with a trained athlete population, we propose that the 2.6% difference in time trial performance observed between phases may also be experienced monthly, by the "average/

every day" woman performing exercise throughout the menstrual cycle. However, formal evidence is required before potentially translating our findings to the general population.

Psychological factors. The mechanisms underlying reduced exercise performance that may be modulated by negative mood state during the ML phase remain largely unexplored. We speculate, however, that the observed psychological effects on exercise performance may be related to changes in dopaminergic signaling. For instance, during the ML phase when progesterone is elevated, the dopaminergic system is depressed (35). Thus, it is possible our participants experienced a similar phenomenon where slower performance times during the ML phase could be caused by the metabolic changes of dopamine in the brain (10). This supports the hypothesis that suppressed dopamine signaling reduces motivation and motor coordination, which could lead to lethargy and fatigue (10) and, in our case, increased perception of fatigue and reduced exercise performance during the ML phase.

The physiological mechanisms underlying reduced vigor preexercise and/or increased perceptions of fatigue during exercise during exercise in the ML phase remain largely unclear. However, we believe the underlying cause of these changes may be related to the differences in progesterone between phases. Recent work has revealed that the concentration of estradiol relative to the concentration of progesterone (E_2/Pg ratio) may have a greater influence on physiological and psychological deviations than the fluctuations in a single hormone (36), but our findings at the group level do not support this conjecture given that only progesterone differed between the ML and the MF phases, whereas the estradiol and the E_2/Pg ratio remained unchanged between these two time points (Table 2). However, of important note, the E_2/P_g ratio was higher in 8 of the 12 women tested.

It is well established that mood state preexercise alters pacing (37,38). However, to our knowledge, we are the first study to examine pacing profiles across the menstrual cycle. Prior fatigue induced by a 6-d intensive training period resulted in a slower starting pace during the initial 4 km of a 40-km time trial, which led to slower overall performance times in male cyclists (38). In agreement with our findings, no difference was observed in RPE, or in pacing strategy during the later portion of the time trial (38). In addition, others have found greater TMD, fatigue, and reduced vigor as assessed by POMS accompanying impaired time trial performance (39).

As far as we know, we are the first study to examine mood state after exercise across the menstrual cycle. We found that TMD was greater before exercise during the ML phase but that regardless of phase, significant improvements in mood state were found postexercise (Fig. 4A, B), consistent with a large body of research. Importantly, mood state in the ML phase essentially became normalized to the MF phase 20 min after exercise because the differences seen before exercise in TMD were abolished between phases postexercise. Thus, exercise may be a therapeutic modality to improve mood across the menstrual cycle.

Practical implications. Our findings may have important implications in determining readiness to engage in routine exercise across the menstrual cycle as a consequence of its influence on mood state, particularly during the ML phase. The lack of vigor felt by our participants in the ML phase was associated with a lower power output and slower performance than observed during the MF phase. Thus, it may be that women may face more challenges to adhere to their exercise regimen during the ML phase of their menstrual cycle. Therefore, these findings can also be applied in designing strategies to enhance recreationally active women's engagement in exercise during certain times in which their mood state may be negative (e.g., ML phase). Moreover, instead of "believing" the menstrual cycle to be a barrier to following a consistent exercise routine, women can start adapting their training regimen based on the cycle changes (i.e., peak training could occur during the MF phase, taper days during the ML phase). Furthermore, because we saw improvements in mood postexercise, perhaps exercise (i.e., moderate- to high-intensity aerobic activity) (10) should be used specifically as a therapeutic technique to increase feelings of vigor and reduce anger, tension, fatigue, and depression during the ML phase.

Methodological considerations. It is substantially more difficult to carry out research studies on women compared with studies that exclusively recruit men. They take considerably longer time to complete as each subject must be tracked for at least two full menstrual cycles before any experimental testing and analysis can take place. Throughout these 3 months of cycles, body (oral) temperature, hormones, ovulation status, body composition, and dietary and training logs should be considered. Our study has succeeded in following this protocol with the exception that we did not receive daily training and dietary logs from our cohort of participants. However, RER was not different during out constant load cycle bouts (data not shown), and previous research indicates that a different metabolic response/use of fuel substrates between the phases will most likely occur only if the E_2/Pg ratio is significantly elevated, which we found to be the same between phases in our participants (40), and we did measure percent body fat and ensured the women in our study were body weight stable, suggesting training status and dietary intake did not vary much while participants were enrolled in the study. However, we acknowledge that diet could be subject to change based on menstrual cycle phase, and we were not able to control for the influence of diet and other factors (e.g., travel, stress, etc.) on exercise performance throughout a cycle. Considering our subject cohort was recreationally active women and not trained cyclists, we chose to familiarize each subject with the 8-km time trial three times, which we believe is one of the strengths of this study. Notably, in our study, the coefficient of variation for time trial performance within a given subject and phase of the menstrual cycle was 2%, which was smaller than the magnitude of the observed difference in exercise performance between the MF and the ML phases of the menstrual cycle (~3%). We chose to analyze saliva opposed to blood because of the noninvasive nature of this measurement and because hormone concentrations measured through saliva more accurately represent the biologically available fraction of

progesterone (i.e., in its unbound form) (29). However, we acknowledge that salivary collection may be less sensitive compared with serum measurements. Although there is a high amount of variability in progesterone and estrogen between women when measuring serum or salivary hormone levels (29), our participants' absolute values for progesterone and estradiol were within the accepted ranges (29). Lastly, we tested for ovulation between days 13 and 15 for each subject, which also coincided with a sustained rise in oral temperature. That said, there is a small chance we missed the ovulation window in the subject we had to exclude. Thus, we recommend that future studies aim to test for ovulation starting earlier and lasting longer in a woman's cycle (e.g., days 10–20).

CONCLUSION

Aerobic exercise performance was discovered to be worse in the ML phase, when progesterone levels are elevated, compared with the MF phase of the menstrual cycle. This reduction in exercise performance is likely driven by increased perceptions of fatigue and an overall negative mood state, which was largely caused by reductions in perceptions of vigor. Moreover, aerobic exercise acutely eliminated menstrual cycle–dependent differences in vigor, anger, and tension, fatigue, and depression, suggesting that aerobic exercise may be a therapeutic modality to improve mood across the menstrual cycle. Future studies should consider examining women who take contraceptives, particularly those involving exogenous progesterone, such as the intrauterine device, as the effects of steady progesterone levels on mood state and exercise performance are unknown.

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No conflicts of interest, financial or otherwise, are declared by the authors.

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