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The Effects of Menstrual Cycle Phase on Performance in Endurance Runners

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ABSTRACT

Introduction: The menstrual cycle is a widely overlooked and understudied component of female athlete training. The cycle is divided into the follicular and mid-luteal phases and is guided by the rise and fall of estrogen and progesterone. Menstrual cycle phase may have a performance effect on eumenorrheic female endurance runners, though conflicting evidence exists. The purpose of this study was to examine the effect of menstrual cycle phase on performance and associated physiological parameters in endurance runners. **Methods:** In this randomized cross-sectional study, five well-trained eumenorrheic female endurance runners completed eight weeks of data collection. Testing occurred during the early follicular (EF) and mid-luteal (ML) phases. Each subject completed two VO_2max tests, two 5 km time trials (TT) with blood lactate tests pre- and post-, and three intravenous blood collections analyzing estrogen, progesterone, and cortisol. Urine progesterone and ovulation tests were used to confirm phase. ML estrogen and progesterone, VO_2max, heart rate (HR), respiratory exchange ratio (RER), and minute ventilation (VE) during each VO_2max stage, ventilatory threshold 1 (VT1) and ventilatory threshold 2 (VT2), 5 km TT, blood lactate pre- and post-5 km TT, and plasma cortisol concentrations were analyzed between the EF and ML phases. Correlations were run between estrogen, progesterone, blood lactate, and cortisol with associated variables. **Results:** No significant differences were found in VO_2max, HR, RER, or VE during VO_2max stages, VT1 and VT2, 5 km TT, or blood lactate pre- and post-5 km TT between EF and ML phases. There was a significant difference in EF and ML plasma cortisol concentrations (EF: 11.1 ± 1.3 μg/dL, ML: 8.8 ± 2.4 μg/dL; $p = 0.04$), as well as a significant correlation between ML plasma cortisol and progesterone concentrations ($p = 0.005$). No significant correlations were found between any other measures. **Conclusions:** The findings of this study did not support the hypothesis. No significant differences were found between exercise performance or associated parameters between EF and ML phases, though a significant difference was found between EF and ML plasma cortisol concentrations, with EF cortisol measuring significantly higher. The findings of the current study raise questions as to how menstrual cycle-based cortisol fluctuations may affect performance in endurance athletes and should provoke future investigation.

KEYWORDS: Estrogen, Progesterone, Cortisol, Blood Lactate, Training.

Introduction

In 2002, elite endurance runner Paula Radcliffe ran 2:17:18 in the Chicago marathon to break the women's world record – all while bleeding and suffering from menstrual cramps the last third of the race. The menstrual cycle is a well overlooked and widely misunderstood component of training and performing as a female athlete. A healthy menstrual cycle occurs over an average of 23-38 days and is broken up into two phases: The follicular phase and the luteal phase[1]. The cycle is governed by the rise and fall of female sex hormones estrogen, progesterone, follicle stimulating hormone, and luteinizing hormone[2], though function is primarily driven by estrogen and progesterone. Menstruation is the shedding of the uterine lining and occurs during days 1-7 of the follicular phase when estrogen and progesterone levels are low. Ovulation occurs immediately after luteinizing hormone peaks, around day 14 during the transition to the luteal phase. During the luteal phase, estrogen and progesterone levels both rise before dropping to stimulate menstruation and the start of a new cycle[2].

A regularly occurring menstrual cycle is classified as eumenorrhea. The hormonal fluctuations experienced throughout the menstrual cycle have many physiological effects other than exclusively menstrual function such as cardiovascular, metabolic, and respiratory functions, which may impact exercise performance. Previous studies have shown increases in heart rate, ventilation rate, fat metabolism, glycogen sparing[1, 3, 4] and delayed onset of lactate accumulation[1, 4, 5] in response to exercise in the luteal phase when compared to the follicular phase. However, studies have provided conflicting findings on the effect of the menstrual cycle on exercise performance. This study aims to provide more data for future clarity on the subject.

The follicular and luteal phases of the menstrual cycle can be further classified into early, mid-, and late stages. Much of the previous research has varied vastly between studying each of these three stages within each cycle phase, with little consistency as to which time points are being compared. The variances of hormonal concentrations throughout each cycle phase is crucial to note when deciding on specific time periods to test. As the present study aims to observe the potential physiological effects of both estrogen and progesterone, it is crucial to observe these hormones at the time periods they are most different. The early follicular (EF) phase and the mid-luteal (ML) phase were chosen to provide the most accurate representation of the effects of the rise and fall of estrogen and progesterone on the female body.

There is a lack of broad research on the menstrual cycle in female athletes. There are, however, important health and performance implications surrounding menstrual cycle function. As a prevalent occurrence in the endurance community, more attention must be given to this area of

research in women's health. The purpose of this study is to observe the effects of menstrual cycle phase on performance in endurance runners, as well as investigate associated physiological parameters related to menstrual health. It is hypothesized that eumenorrheic female athletes will experience enhanced performance during their early follicular (EF) phase compared to their mid-luteal (ML) phase.

Methods

Subjects

Five well trained female endurance runners volunteered to participate in this study (Table 1). One subject was dropped due to injury complications, resulting in four subjects completing the study. Subjects were recruited via email, social media, and flyers posted throughout the Gunnison Valley. Inclusion criteria were that all subjects must classify as low risk for heart disease when screened by the ACSM criteria, must be between the ages of 18 and 30, must have at least a recreational fitness level, and must be trained for endurance running (run at least 30 miles per week). Exclusion criteria were that subjects must not have missed a menstrual period in the past 12 months, must not have any reported thyroid dysfunction, must not have any injuries or illness preventing them from participating in moderate to strenuous physical activity, must not actively be taking any form of contraception and must not have been on any form of contraception for the past 6 months, and must not be currently or trying to become pregnant during the course of the study. Subjects met all inclusion criteria, and anyone who met any exclusion criteria was eliminated from recruitment. All testing occurred at either Western Colorado University or at Gunnison Valley Health, in Gunnison, CO (2348m). All subjects were provided written and verbal informed consent, were given ample time to review the details of the study and ask questions, and then signed the informed consent prior to the start of data collection. This study was approved by the Institutional Review Board (IRB) prior to subject recruitment [HRC-2021-01-02-R18].

Experimental Design

In this cross-sectional study, subjects were chosen based on eumenorrheic menstrual status. Eumenorrheic subjects visited the High-Altitude Exercise Physiology (HAP) Lab a total of 5 times over the course of two months. The first visit consisted of completing surveys and questionnaires, as well as baseline anthropomorphic measures. Timing of all visits for each subject thereafter were dependent on individual menstrual cycle schedule, as testing was to occur only during the early follicular and mid-luteal phases. Any testing occurring during the predicted luteal phase was preceded by taking both an ovulation test and a urine progesterone test, and each exercise testing bout was accompanied by a blood draw at Gunnison Valley Hospital. The second and third visits occurred during the early follicular and mid-luteal phases of the first month and consisted of either a VO_2max test or a 5 km time trial (TT) with blood lactate

testing. Subjects were randomly assigned into two groups by an online research randomizer (randomizer.org): One beginning with the VO_2max and one beginning with the 5 km TT. This randomization occurred in order to limit any potential researcher bias and to limit any possible training effect that could skew data and create false trends. The fourth and fifth visits occurred during the early follicular and mid-luteal phases of the second month and consisted of either VO_2max tests or 5 km TT with blood lactate testing, depending on which were completed previously. Each visit took approximately one hour. See Figure 1.

Table 1. Descriptive characteristics of subjects. Values are reported as mean ± SD.

Characteristic	N=4
Age (yr)	23.8 ± 6.6
Height (cm)	168.6 ± 3.0
Weight (kg)	54.2 ± 6.2
Body Fat %	13.2 ± 5.1
RHR (bpm)	82 ± 11
SpO2 (%)	90.8 ± 7.8
SBP (mmHg)	106 ± 10
DBP (mmHg)	72 ± 9
Stress Fracture (% n)	
Y	25
N	75
Mileage (mi/wk)	40 ± 13.5
Cross Training (hr/wk)	3.8 ± 4.4
Strength Training (hr/wk)	1.3 ± 0.5

Note: Resting heartrate. SpO2 = Oxygen saturation. SBP = Systolic blood pressure. DBP = Diastolic blood pressure.

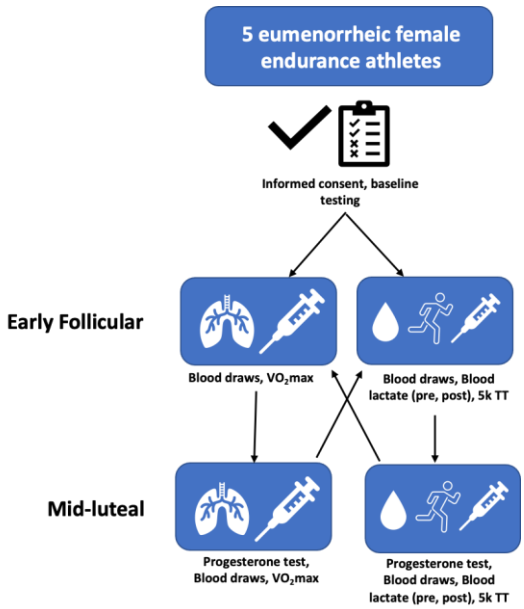

Figure 1. Experimental flowchart depicting order of procedures of all testing for each subject.

Procedures

Surveys/Questionnaires

Prior to their first visit at the HAP Lab, each subject was emailed a health and menstrual history questionnaire to be completed and returned to the researcher. Questions included information about regularity of menstrual cycle, history of birth control, pregnancy status, usage of hormonal supplements or medications, history of thyroid dysfunction, current injury status, and history of stress fractures. Subjects were also asked to report their running mileage per week, hours spent aerobic cross training per week, and hours spent doing strength and resistance training. Information from this questionnaire was used to determine if the subjects met inclusion and exclusion criteria for the study. Questions regarding stress fractures and training were to be used to compare between eumenorrheic and amenorrheic groups, but no amenorrheic subjects were able to be recruited for this study. Upon arrival at the lab, each subject completed the Physical Activity Readiness Questionnaire (PAR-Q+) to confirm that each were classified as low risk before exercise testing.

Anthropomorphic Measures

Height and Weight

Each subject was measured for height (HT) and weight (WT) via the HAP Lab scale (WB3007301, Tanita Corporation, Arlington Heights, IL). Subjects removed their shoes prior to measurements and were wearing light workout clothes. Anthropomorphic measures were each taken once.

Body Fat Percentage

Body fat percentage (BF%) was measured via OMRON fat loss monitor (HBF-306c, OMRON, Bannockburn, IL). Subjects stood still while holding the handheld transmitters until the measurement was recorded.

Resting Heart Rate and Resting Oxygen Saturation

Resting heart rate (RHR) and resting oxygen saturation (SpO_2) were taken via HAP Lab Polar heart rate straps (Polar, Lake Success, NY) and pulse oximeter (American Diagnostic Corporation, Hauppauge, NY). Subjects sat quietly in a chair for a few minutes before taking these measurements. Heart rate strap was fitted snugly around the torso with the transmitter located just below the sternum.

Resting Blood Pressure

Resting blood pressure was taken manually using a stethoscope and sphygmomanometer after the subject sat quietly for a few minutes. Blood pressure cuff was secured around the upper arm, over the brachial artery.

Blood Draws

Each subject reported to the Gunnison Valley Hospital lab for blood draws four times throughout the study: Once during their early follicular phase and once during their mid-luteal phase, for two months. Each blood draw was scheduled to coincide with scheduled exercise testing and was performed at least 2 hours after testing commenced. Eight ml of blood was drawn

each visit by a trained phlebotomist and was analyzed for estrogen, progesterone, and cortisol during the mid-luteal phase, and only cortisol during the early follicular phase using chemiluminescent microparticle immunoassays. Over the two-month period, each subject had a total of 32 ml of blood drawn.

Menstrual Tracking

Each eumenorrheic subject was asked to download the app FitrWoman (Orreco Ltd, Los Angelos, CA) to immediately begin tracking their menstrual cycle and report this information to the researcher throughout the study. Two days before predicted ovulation, each subject took a Clearblue ovulation test (Swiss Precision Diagnostics GmbH, Bedford, United Kingdom) to detect luteinizing hormone surge occurring just prior to ovulation. During their predicted mid-luteal phase, each eumenorrheic subject took a Proov Pdg test in the morning prior to exercise testing. Subjects were informed to take the test strip with their first urination of the day. Menstrual cycle phase was confirmed as mid-luteal via blood draws showing progesterone concentration greater than 7 ng/mL[6].

VO_2max

VO_2max tests were performed using the Parvo Medics metabolic cart (TrueOne 2400, Parvo Medics, Sandy, UT) and treadmill (Trackmaster, Newton, KS). Prior to arrival, subjects were asked to prepare for the test appropriately, including restricting caffeine consumption, no alcoholic drinks the night before, no food 3 hours in advance, avoiding exercise beforehand, no hard exercise the day before, and obtaining adequate sleep. Upon arrival to the HAP lab during the projected mid-luteal phase, subjects used a Proov Pdg test (MFB Fertility Inc., Boulder, CO) to determine correct menstrual phase. If during the early follicular phase, or if the subject was amenorrheic, the Pdg test was not performed. Prior to the VO_2max test, subjects were informed about what a VO_2max test is, what would be required of them, completed a pre-exercise testing questionnaire, and were fitted with a Polar heart rate strap. Subjects then performed an exercise warm-up period of five minutes at a self-selected running pace. Following their warm-up, subjects were fitted with a mask/mouthpiece with a breathing valve to collect expired gases. Subjects were instructed to use hand signals to notify researchers about the need to stop the test. The test began two minutes following the conclusion of the warm-up.

The test was performed as a graded exercise testing protocol (GXT) in two-minute increments. Stage one began at a self-selected running pace at 0% grade. Stage two increased by 0.5 mph and 1% grade. Stage three increased by 0.5 mph, at 2% grade. Each subsequent stage then continued to increase speed by 0.5 mph, while maintaining a 2% grade throughout. The Borg Scale (1-10) for rating of perceived exertion (RPE) was communicated via hand signal and recorded every two minutes along with HR.

The test was terminated if the subject signaled to stop, stopped on their own, reached a HR greater than their age predicted maximal HR, or if their respiratory exchange ratio (RER) exceeded 1.15. Immediately upon termination, the treadmill was returned to a 0% grade and the speed was brought down. The mask was then removed, and the subject was allowed to begin a slow cool-down at a self-selected pace. Data obtained from the VO_2max tests were smoothed with 15 second averaging and used for analyses.

Ventilatory Thresholds

Ventilatory threshold 1 (VT1) and ventilatory threshold 2 (VT2) were calculated manually after completion of the VO_2max test. VT1 was determined via visual interpretation by plotting ventilation (VE) against oxygen consumption (VO_2) and finding the point at which the first steep change in slope occurred, indicating the time point at which the rate of respiration must increase disproportionately to compensate for the rapid increase in oxygen demand. VT2 was determined via visual interpretation by plotting VE against carbon dioxide output (VCO_2) and finding the point at which the first steep change in slope occurred, indicating the time point at which hyperventilation can no longer compensate for the increasing levels of CO_2 with intense exercise. The change in slope was determined by fitting two linear regression lines to the data points shown in the graph and locating the coordinates where the intersection occurred.

5 km Time Trial

The 5 km TT was performed at the Western Colorado University Mountaineer Field House in Gunnison, CO (2348m). Subjects were asked to prepare for the TT appropriately, including restraining from new or strenuous exercise the day prior to the test, following a normal week of training as if they were leading up to a race, eating a normal diet the day before and the day of the TT, staying well hydrated the week leading up to the TT as well as the day of, eating no less than 3 hours prior, and avoiding alcohol the night before the TT as well as avoiding caffeine the day of. Subjects were instructed to arrive 30-45 minutes prior to the start of their TT to complete a pre-exercise testing questionnaire and perform their usual warm-up and pre-race routine. Subjects then changed into their desired racing shoes as the TT was explained to them. Prior to the start, pre-testing blood lactate was measured via disposable lancet (ReliOn, Novo Nordisk Pharmaceutical, Johnston County, NC), blood lactate strips (Lactate Plus, Nova Biomedical, Waltham, MA), and blood lactate monitor (Lactate Plus, Nova Biomedical, Waltham, MA) and recorded. The subject then commenced their 5 km TT at a race effort, with each lap split recorded by the researcher. Immediately upon completion, the total time and post-testing blood lactate were recorded. The subject then completed a self-dictated cool-down around the track.

Blood Lactate

Blood lactate was recorded prior to and

immediately following the 5 km TT. After calibration, a clean lactate test strip was inserted into the monitor. The subject's finger was first cleaned with an alcohol pad, then was pricked with a disposable lancet at the tip of the finger. The forearm was massaged to instigate blood flow into the finger, if needed. The first droplet of blood was wiped away with gauze, and the proceeding blood was collected by placing the test strip into the sample, perpendicular to the skin. The measurement reported on the monitor was then recorded, and all contaminated items were disposed of accordingly.

Statistical Analyses

Data were analyzed via SPSS version 27 (IBM-SPSS, Boston, MA). Data were tested for normality and homogeneity of variance, and statistical significance was set at $p < 0.05$. A paired samples T-test was used to compare VO_2max, 5 km TT, blood lactate pre- and post 5 km TT, plasma cortisol concentration, HR at VT1 and VT2, and VO2 at VT1 and VT2 between EF and ML phases. A repeated measures ANOVA was run between HR, RER, and VE at each incremental VO_2max stage during EF and ML phases and statistical significance was determined using the Greenhouse-Geisser correction for the violation of sphericity for each data set. Pearson's bivariate correlations were run between 5 km TT and blood lactate concentration post-5 km TT, cortisol and blood lactate pre- and post-5 km TT, cortisol and 5 km TT, cortisol and VO_2max at each EF and ML phases, estrogen and ML VO_2max, ML 5 km TT, blood lactate pre- and post-ML 5 km TT, and ML cortisol, and progesterone and ML VO_2max, ML 5 km TT, blood lactate pre- and post-ML 5 km TT, and ML cortisol. Data are reported as means ± standard deviation.

Results

Subjects

Subjects were considered well-trained endurance athletes based off reported weekly running mileage. Subjects were considered to be eumenorrheic based off self-reported regular menstrual cycles averaging between 28-30 days for the past six months. No subject was on any form of birth control for at least six months prior to the start of this research. Five subjects were recruited at the start of this study. One subject was dropped due to injury complications and an inability to complete exercise testing at the time required, resulting in four subjects completing the entire study (n=4).

Estrogen and Progesterone

Plasma estrogen and progesterone concentrations during the ML phase are shown in Table 2. All subjects were within normal range for estrogen concentration during this phase of the menstrual cycle (21-312 pg/mL). All but one subject was within normal range for progesterone concentration during this phase (1.2-15.9 pg/mL). One subject reported elevated progesterone levels during her second blood collection date. Normal ranges were defined by Gunnison Valley Hospital.

VO_2max

Figure 2 reports VO_2max values obtained during the EF and ML phases. Three of four subjects completed six full stages while one subject completed four stages during her ML phase and five stages during her EF phase. No significant differences were found between VO_2max during EF and ML phases ($p = 0.727$). Mean difference between EF and ML VO_2max scores was 0.8 ml/kg/min. No significant correlations were found between ML VO_2max and estrogen or progesterone ($p = 0.058, 0.666$). See Table 3.

HR, RER, and VE

Figures 3, 4, and 5 report HR, RER, and VE obtained at each incremental stage of the VO_2max test. Three subjects completed six full stages during each cycle phase while one subject completed five stages during her EF phase and four stages during her ML phase. Group means were used to fill missing data points. Six stages were included in this graph as no subject was able to complete a full seventh stage. No significant differences were found between EF and ML HR at each incremental VO_2max stage ($p = 0.060$). HR increased as expected by 38.3 % from 133 bpm to 184 bpm in the EF phase and by 26.3% from 153 bpm to 188 bpm in the ML phase. See Figure 3. Predicted max HR in this subject group ranged from 187-201 bpm. No significant differences were found between EF and ML RER at each incremental VO_2max stage ($p = 0.067$). RER increased as expected by 33.8% from 0.80 to 1.07 in the EF phase and by 30.9% from 0.81 to 1.06 in the ML phase. See Figure 4. No significant differences were found between EF and ML VE at each incremental VO_2max stage ($p = 0.200$). Ventilation increased as expected by 137.2% from 44.3 L/min to 105.1 L/min in the EF phase and by 164.8% from 44.3 L/min to 117.3 L/min in the ML phase. See Figure 5.

VT1 and VT2

Figures 6 and 7 report HR and VO_2 obtained at VT1 and VT2. No significant differences were found between EF and ML HR or VO_2 at VT1 ($p = 0.267, 0.325$) and VT2 ($p = 0.166, 0.080$). There was a 2.9% difference in HR at VT1 and a 2.1% difference in HR at VT2 between EF and ML phases. See Figure 6. There was a 5.3% difference in VO_2 at VT1 and a 2.4% difference in VO_2 at VT2 between EF and ML phases. See Figure 7.

5 km TT

Figure 8 reports 5 km TT at EF and ML phases. No significant differences were found between EF and ML 5 km TT performance ($p = 0.141$). Mean difference in 5 km times was 0.22 minutes. Times were approximately one to two minutes slower than on a typical race day. No significant correlation was found between ML 5 km performance and estrogen or progesterone ($p = 0.124, 0.570$). See Table 3.

Blood Lactate

Figure 9 reports EF and ML blood lactate pre- and post-5 km TT. No significant differences were found between EF and ML blood lactate pre- or post-5 km TT ($p = 0.967, 0.481$). Blood lactate increased as expected from 1.7 mmol/L to 8.6 mmol/L during the

EF phase and from 1.7mmol/L to 7.9 mmol/L during the ML phase. No significant correlation was found between EF or ML blood lactate post-5 km TT and 5 km TT performance (p = 0.437, 0.360). See Table 3.

Cortisol

Figure 10 depicts cortisol concentration between EF and ML phases. A significant difference was found between EF and ML cortisol concentrations (p = 0.04). Mean EF cortisol measured 11.1 mg/dL while mean ML cortisol measured 8.8 mg/dL, with a difference of 2.3 mg/dL. See Figure 10. No significant correlations were found between EF and ML cortisol and 5 km TT performance (p = 0.445, 0.481) or EF and ML cortisol and VO_2max (p = 0.929, 0.569). See Table 3. No significant correlation was found between ML plasma cortisol and estrogen concentrations (p = 0.890), but a significant correlation was found between ML plasma cortisol and progesterone concentrations (p = 0.005). See Table 3.

Table 2. Plasma estrogen and progesterone concentrations during ML phase. Values are reported as mean ± SD.

Hormone	Concentration (pg/mL)	Normal Range (pg/mL)
Estrogen 1	153.3 ± 42.5	21-312
Estrogen 2	109.0 ± 47.5	21-312
Progesterone 1	11.97 ± 3.1	1.2-15.9
Progesterone 2	10.9 ± 8.0	1.2-15.9

Note: "1" indicates first ML phase blood test. "2" indicates second ML phase blood test. Normal range as defined by Gunnison Valley Hospital.

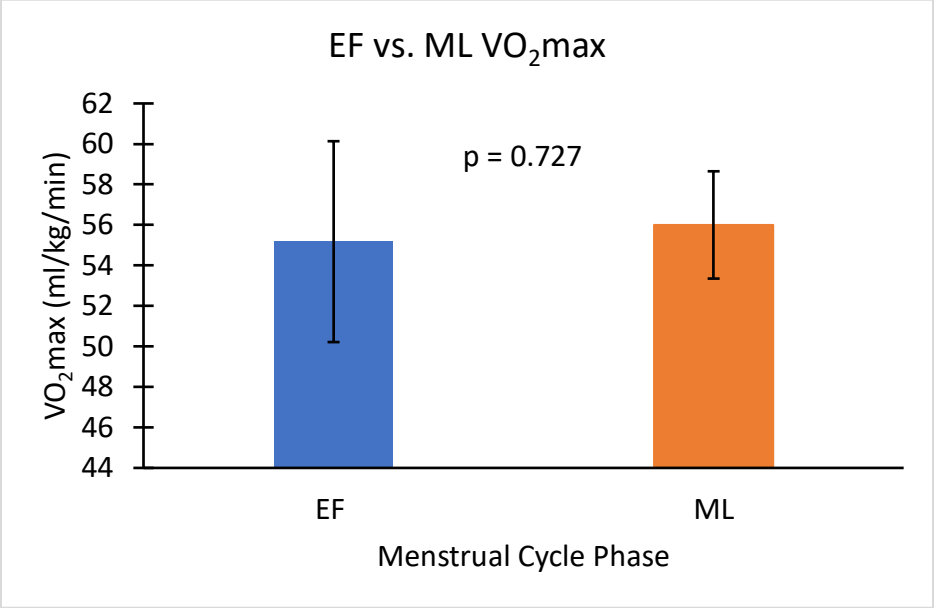

Figure 2. Bar graph depicting VO_2max during EF and ML phases. Data are presented as group mean and standard deviation.

Table 3: Correlations between hormones and exercise testing metrics.

Estrogen	**Regression**	**R^2**	**P value**
ML VO_2max	y = 44.177x - 389.82	0.8869	0.058
ML 5 km TT	y = -16.168x + 185.51	0.9027	0.124
ML Cortisol	y = 10.497x - 97.168	0.4986	0.890
Progesterone			
ML VO_2max	y = 3.3763x + 18.692	0.3304	0.666
ML 5 km TT	y = -1.24x + 36.048	0.3387	0.570
ML Cortisol	y = 1.6979x - 9.9946	0.8319	0.005*
EF Lactate post-5 km TT			
EF 5 km TT	y = 1.3218x + 10.828	0.3164	0.437
ML Lactate post-5 km TT			
ML 5 km TT	y = 1.0892x + 13.74	0.4096	0.360
EF Cortisol			
EF 5 km TT	y = -1.1603x + 34.951	0.3078	0.445
EF VO_2max	y = 0.3796x + 52.085	0.0051	0.929
ML Cortisol			
ML 5 km TT	y = -0.5945x + 27.557	0.2697	0.481
ML VO_2max	y = 1.36x + 44.077	0.1857	0.569

Note: * Indicates significance at $p < 0.05$.

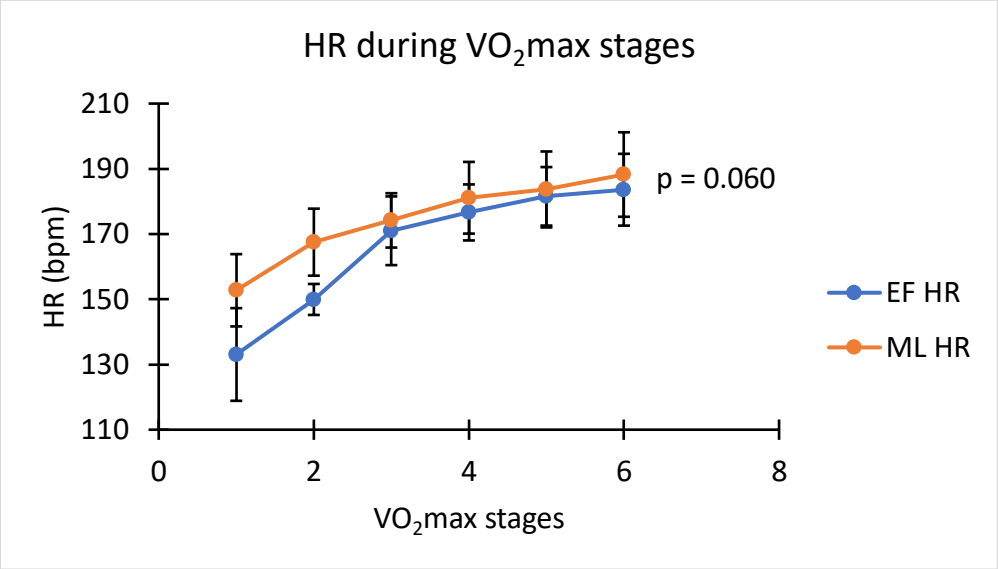

Figure 3. Line graph depicting HR during each incremental stage of VO_2max test during EF and ML phases. Data are presented as group mean and standard deviation.

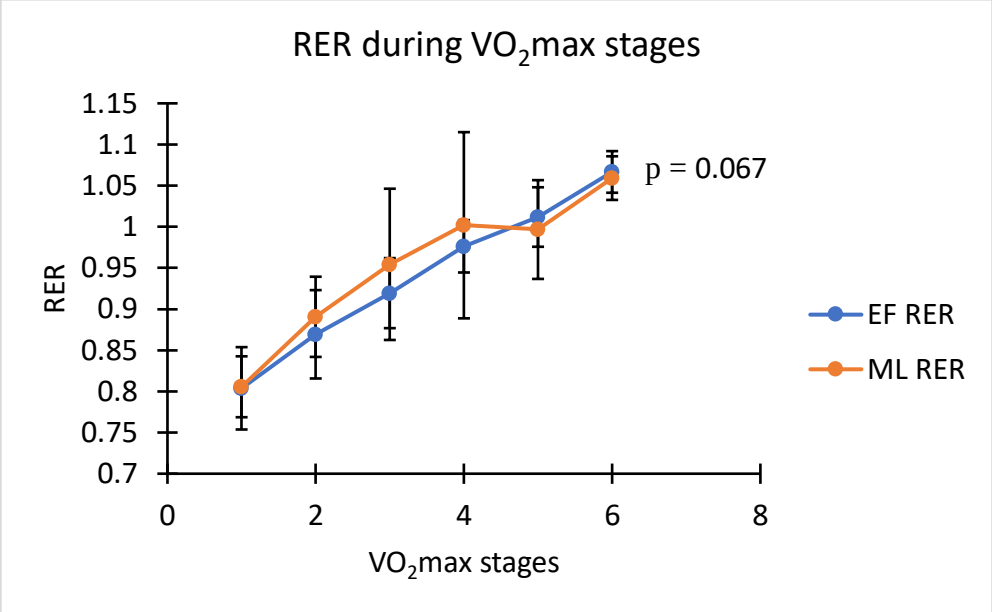

Figure 4. Line graph depicting RER at each incremental stage of VO_2max test during EF and ML phases. Data are presented as group mean and standard deviation.

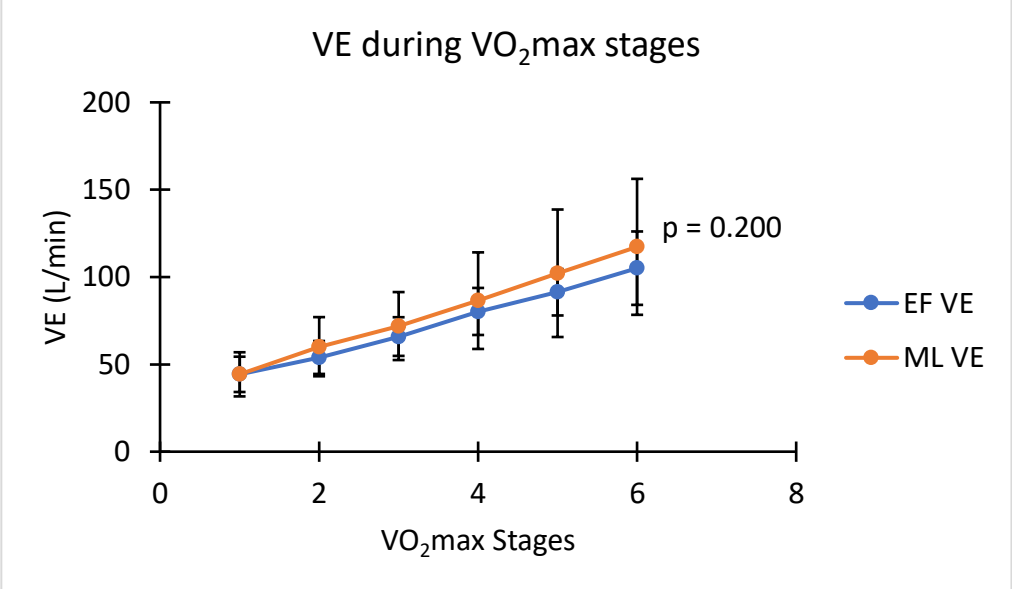

Figure 5. Line graph depicting VE at each incremental stage of VO_2max test during EF and ML phases. Data are presented as group mean and standard deviation.

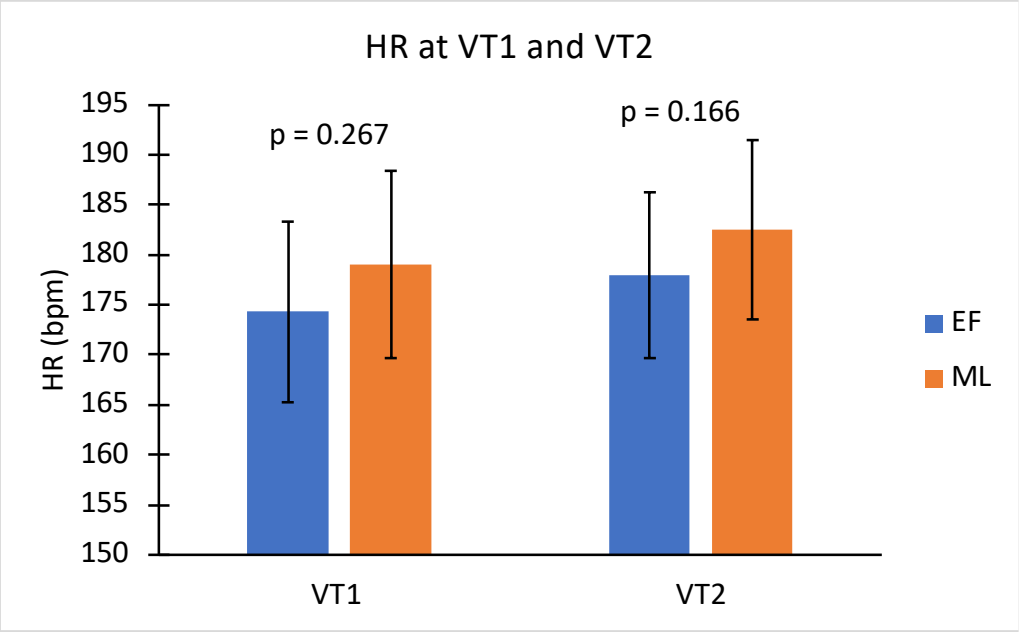

Figure 6. Bar graph depicting HR at VT1 and VT2 during both EF and ML phases. Data are presented as group mean and standard deviation.

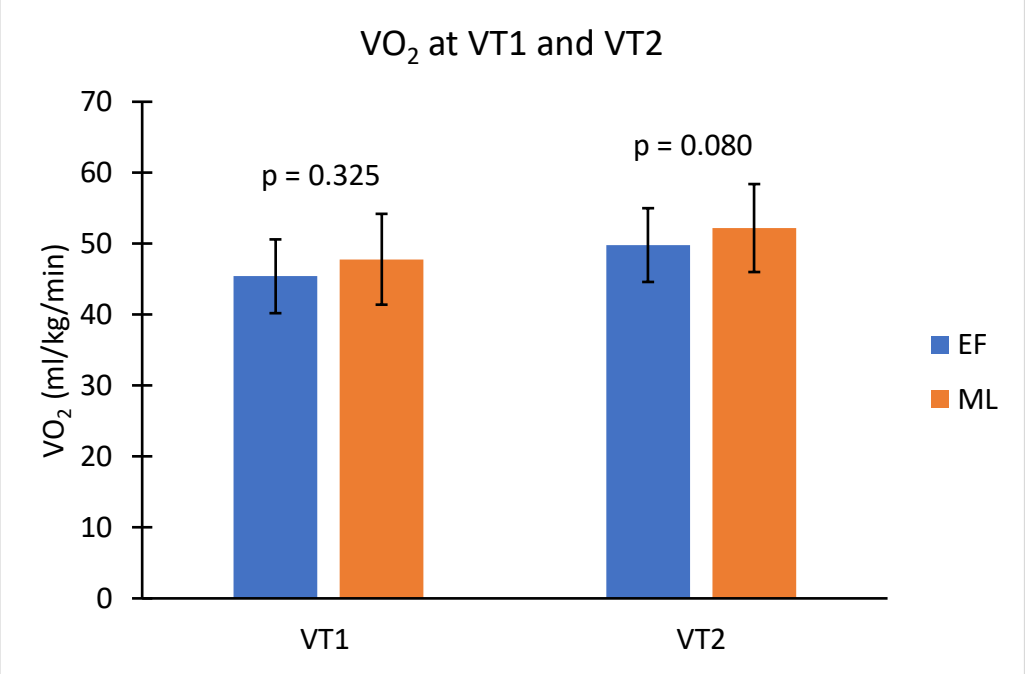

Figure 7. Bar graph depicting VO_2 at VT1 and VT2 during both EF and ML phases. Data are presented as group mean and standard deviation.

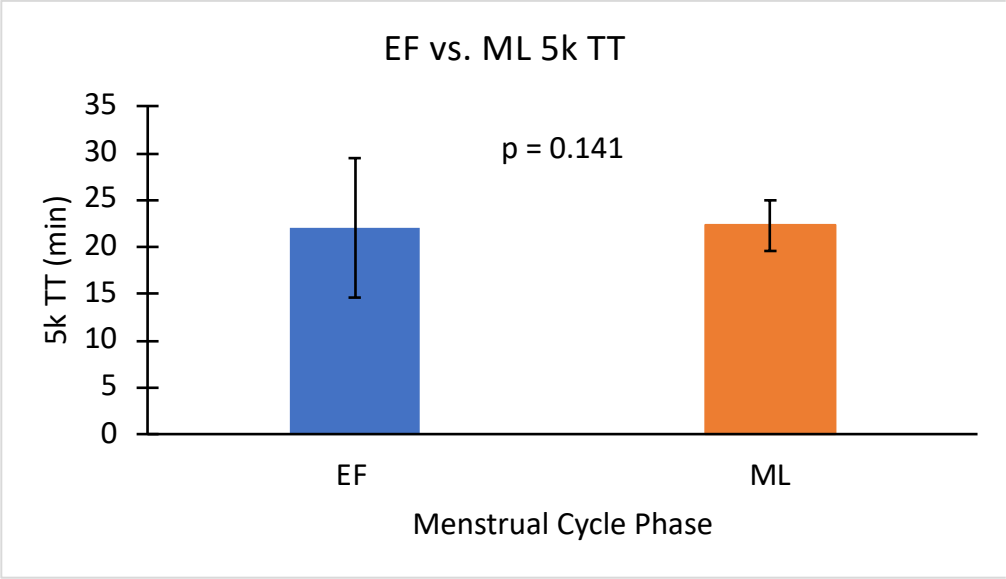

Figure 8. Bar graph depicting 5 km TT performance during both EF and ML phases. Data are presented as group mean and standard deviation.

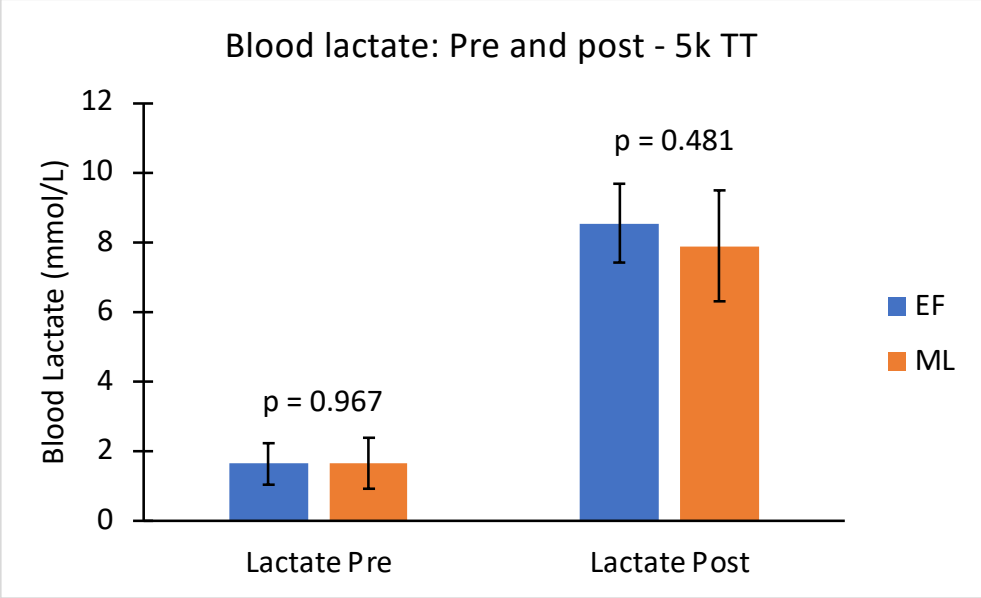

Figure 9. Bar graph depicting blood lactate pre- and post-5 km TT during both EF and ML phases. Data are presented as group mean and standard deviation.

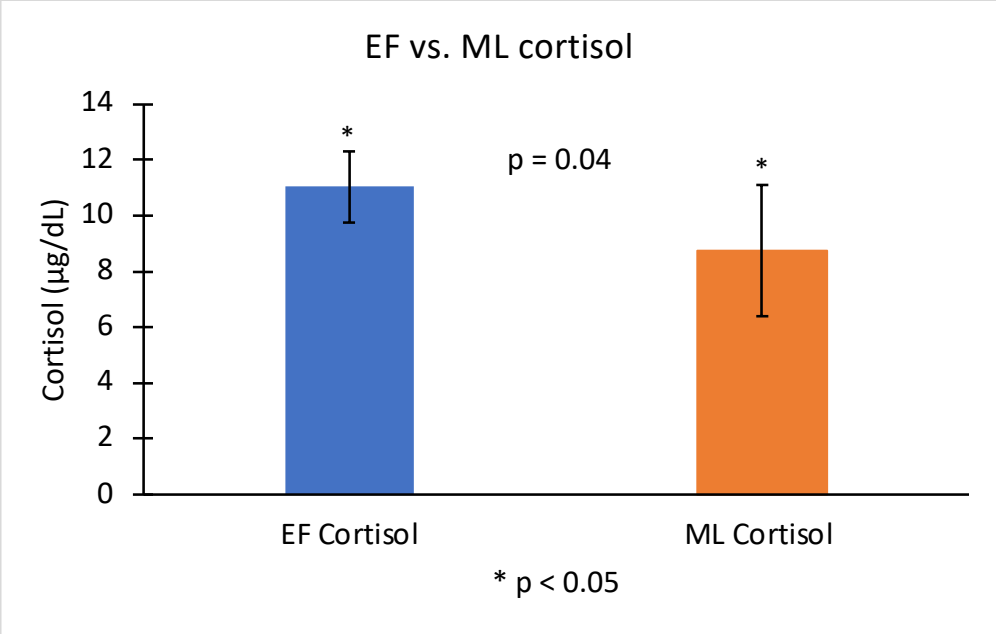

Figure 10. Bar graph depicting plasma cortisol concentration during both EF and ML phases. Data are shown as group mean and standard deviation. * Indicates significance at $p < 0.05$.

Discussion

The purpose of this study was to observe the effects of menstrual cycle phase on performance and physiological parameters related to menstrual health in endurance runners. The findings of this study did not support this hypothesis. There were no significant differences in VO_2max, 5 km TT, or blood lactate concentration pre- and post-5 km TT. Furthermore, there were no significant correlations between blood lactate concentration post-5 km and 5 km TT performance, plasma cortisol concentration and 5 km TT, or plasma cortisol concentration and VO_2max during either EF or ML phases. There were also no significant correlations between estrogen and ML VO_2max, ML 5 km TT, or ML cortisol, or between progesterone and ML VO_2max or ML 5 km TT. Interestingly, there was a significant difference in plasma cortisol concentration between EF and ML phases, as well as a significant correlation between progesterone and ML plasma cortisol concentration.

Estrogen and Progesterone

Data falling within the normal range indicated that each subject completed exercise testing and blood collection within the target menstrual cycle phase. The subject that presented with elevated progesterone did not exhibit any other unusual data, so the reason for this and the potential performance impact is unknown. Estrogen and progesterone have been shown to have antagonistic effects on one another[1], so a further look at blood biomarkers would be interesting in future research involving subjects presenting with elevated hormone levels. Previous research has shown little to no difference in metabolic response between menstrual cycle phases when the estrogen to progesterone ratio

was low[7, 8], as was demonstrated by this subject. Though group means showed no significant differences in exercise performance, it cannot be ruled out that the elevated progesterone of this individual may have impacted her lack of significant change. The elevated progesterone experienced by this individual is likely due to individual variation in hormonal concentration, as the subject exhibited good health parameters elsewhere.

VO_2max

Three of four subjects completed six stages and reached a true VO_2max. One subject completed five stages during her EF phase and four stages during her ML phase where she did not reach a true VO_2max. VO_2max was expected to be higher during the EF phase as compared to the ML phase as a result of the hormonally influenced shift in substrate utilization. For this same reason, a negative correlation between ML VO_2max and female sex-steroid hormones was expected. Previous research has shown that increased estrogen concentration favors fat metabolism over carbohydrates[9]. Estrogen causes glycogen sparing in which glycogen is stored in the liver and muscle while fats are mobilized as the primary fuel source[9]. Conversely as estrogen levels are low during the EF phase, carbohydrates are favored as the primary fuel source during intense exercise due to the greater efficiency of glycolysis. As the short and high intensity of VO_2max tests would rely heavily on carbohydrate utilization, it was expected that subjects would perform better on their VO_2max test during their EF phase as compared to their ML phase.

HR, RER, VE

Three of the four subjects completed six two-minute stages and reached VO_2max. One subject completed five stages during her EF phase and four stages during her ML phase, so group means were used to fill missing data points. Progesterone has been shown to have stimulatory effects on the sympathetic nervous system[10]. The sympathetic nervous system primes the body for "fight or flight" by stimulating HR and respiration, so an increase in these parameters would be expected during the ML phase when progesterone levels are high. The findings of this current study, however, did not support this. The lack of significant difference in HR, RER, and VE was consistent with the findings of DeSouza et al. (1990), observing the physiological and metabolic responses to maximal and submaximal exercise in the EF and ML phase in eumenorrheic compared to amenorrheic runners[11], as well as Dokumaci and Hazir (2019), observing the effect of the menstrual cycle on running economy during the follicular and luteal phases of the menstrual cycle in eumenorrheic female athletes[12].

VT1 and VT2

Similar to previously discussed, both HR and VO_2 were expected to be higher during the ML phase at any given time point due to the stimulatory effects of progesterone on the parasympathetic nervous system[10]. Furthermore, the physiological process of fat

metabolism as favored by high estrogen concentration is a less efficient mechanism than carbohydrate utilization at high exercise intensities. This may result in greater work rates during this phase as a result of the increased oxygen demand of fat metabolism, which would lead to an increase in VO_2. The findings of this study however did not support this and were consistent with the findings of DeSouza et al (1990) and Dakumaci and Hazir (2019)[11, 12].

5 km TT

5 km TT performance was expected to be enhanced during the EF phase as opposed to the ML phase as a result of the shift in substrate utilization from favoring fats to favoring carbohydrates with decreased estrogen concentrations[9], as previously discussed. For a well-trained endurance runner, a 5 km race is a relatively short and high intensity effort so carbohydrates would be the primary fuel source to support the cellular metabolism at this level of sustained effort. As the ML phase is characterized by high levels of estrogen and progesterone, a positive correlation between ML 5 km time and hormone concentration was expected as a result of the shift towards fat metabolism, which theoretically would be detrimental to 5 km performance.

Previous research has demonstrated estrogen's stimulatory effects on peripheral vasodilation along with progesterone's stimulatory effects on peripheral vasoconstriction, which may have an impact on exercise performance[13, 14, 15]. However, no previous research was found on the combined effects of estrogen and progesterone on vascular response and exercise performance. The antagonistic effects of each hormone may work to regulate one another and mitigate any potential performance effects, which may help explain why no significant differences were found.

Blood Lactate

The lack of significant differences between blood lactate concentration pre- and post-5 km TT during each phase were not expected but were consistent with the findings of a study conducted by Forsynth and Reilly (2008) on the effect of the menstrual cycle on 2000m rowing ergometry performance[4]. Previous research has indicated a delayed onset of blood lactate accumulation during exercise in the luteal phase of the menstrual cycle as a result of overall decreased blood lactate concentrations[1, 5]. This is due to the increase in estrogen concentration causing the shift in substrate utilization from carbohydrates to fats, as lactate is a byproduct of glycolysis[9]. Though blood lactate accumulation was expected to be greater during the EF phase in the current study as a result of greater carbohydrate utilization, the lack of significant difference was consistent with the lack of significant difference in 5 km TT performance. Higher blood lactate levels were expected post-5 km TT during the EF phase, accompanied by a faster 5 km time. This was hypothesized because greater levels of blood lactate would have indicated a greater rate of

glycolysis, which would occur at the high intensity level of a 5 km race. A possible explanation for the lack of findings for this measure are that the participants were instructed to run the 5 km at a hard effort, but there were no quantitative physiological target measures. Efforts may have been inconsistent and were likely not a full race effort as each individual ran solo around the indoor track, outside of their usual race environment with competitors and spectators.

Cortisol

One subject's cortisol report was not located from the hospital upon the scheduled data pick-up day. Group means were used to fill this missing data point. The significant difference between plasma cortisol concentration in the EF and ML phases was intriguing, but was contrasted by the significant positive correlation between ML plasma cortisol and progesterone concentrations. This was further unsupported by the ack of significant correlation found between ML plasma cortisol and estrogen concentrations. It should be noted that individual stress levels across subjects may have been a confounding factor for these contrasting findings, and correlations cannot be used as evidence for an accurate cause-effect relationship between variables. Previous studies have reported contradictory results. Genazzani et al., (1975) reported that menstruating women experienced higher daily cortisol concentrations during their luteal phase as compared to their follicular phase[16], while Montero-Lopez et al. (2018) found significantly higher cortisol concentrations during the luteal phase, though only after a stress inducing task[17]. These findings indicate an interaction of sex steroid hormones with the hypothalamic-pituitary-adrenal (HPA) axis found in the brain, which regulates the stress response and consequent secretion of cortisol into the bloodstream[16]. The findings of the present study however, are consistent with a 2020 meta-analysis reporting higher circulating cortisol in the follicular as opposed to the luteal phase[18]. Hamidovic et al. (2020) describes that greater circulating cortisol concentration during the follicular phase as compared to the luteal phase may be caused by the increase in estrogen and progesterone concentration during the luteal phase[18]. The paraventricular nucleus of the hypothalamus, the portion of the brain responsible for cortisol regulation, contains estrogen receptor-□ and estrogen receptor-□, which exhibit antagonistic stimulatory and inhibitory effects on the HPA axis, while progesterone is responsible for the modulation of a specific neurotransmitter that exhibits inhibitory effects on the HPA axis[18]. The combined effects of elevated levels of estrogen and progesterone during the luteal phase would therefore be expected be inhibitory on the HPA axis, resulting in decreased circulating levels of cortisol, as was presented in the current study. Further research is warranted on the effect of the menstrual cycle on daily circulating plasma cortisol concentration in eumenorrheic female athletes.

The lack of significant correlations found between plasma cortisol and 5 km TT and plasma cortisol and VO_2max during the EF and ML phases was unexpected as well. Greater cortisol concentrations may have been expected alongside greater VO_2max, lower 5 km TT time, and greater blood lactate concentration post-5 km TT during the EF phase based off of findings for each individual variable across past studies, but little research was found on a causation effect of cortisol on any of these variables. Cortisol concentration rises post-exercise as a result of the exercise-induced stress response, but the measures taken for this study were daily circulating cortisol concentrations prior to any exercise. These measures were each only taken twice per subject as well, so the values are only representative of acute concentration as opposed to chronic. A future study documenting daily cortisol concentration over a longer period of time may provide greater insight to the relationship between cortisol concentration and exercise performance.

Limitations

The main limitation of this study was a small sample size resulting in a low power for statistical analysis. A larger sample size would likely result in greater chance for significance across all variables measured. It was intended to have estrogen and progesterone concentration measured at both EF and ML phases to not only confirm cycle phase, but to allow for correlations between hormone concentration and exercise performance, plasma cortisol, and blood lactate accumulation, but this measure was limited by budget. The present study used estrogen and progesterone concentrations as a method to confirm cycle phase only.

Another limitation in this study was scheduling resulting in data collection sometimes occurring either before or after target hormone concentrations. Three of the four subjects were NCAA cross country and track athletes so testing had to be scheduled around traveling and competitions. Being competitive athletes in season, some testing occurred after strenuous training days which may have impacted results. Furthermore, one subject completed five stages during her EF phase and four stages during her ML phase while all others completed six full stages, so group means were used to fill missing data points.

Further limitations include challenges with blood collection scheduling through Gunnison Valley Hospital. Some exercise testing days were accompanied by inaccurate blood collection dates (1-2 days apart) due to limited hours at the hospital lab. Furthermore, one subject's estrogen and progesterone data as well as one cortisol measure were not found upon the coordinated data packet pick-up day. For this subject, group means were used for missing data points.

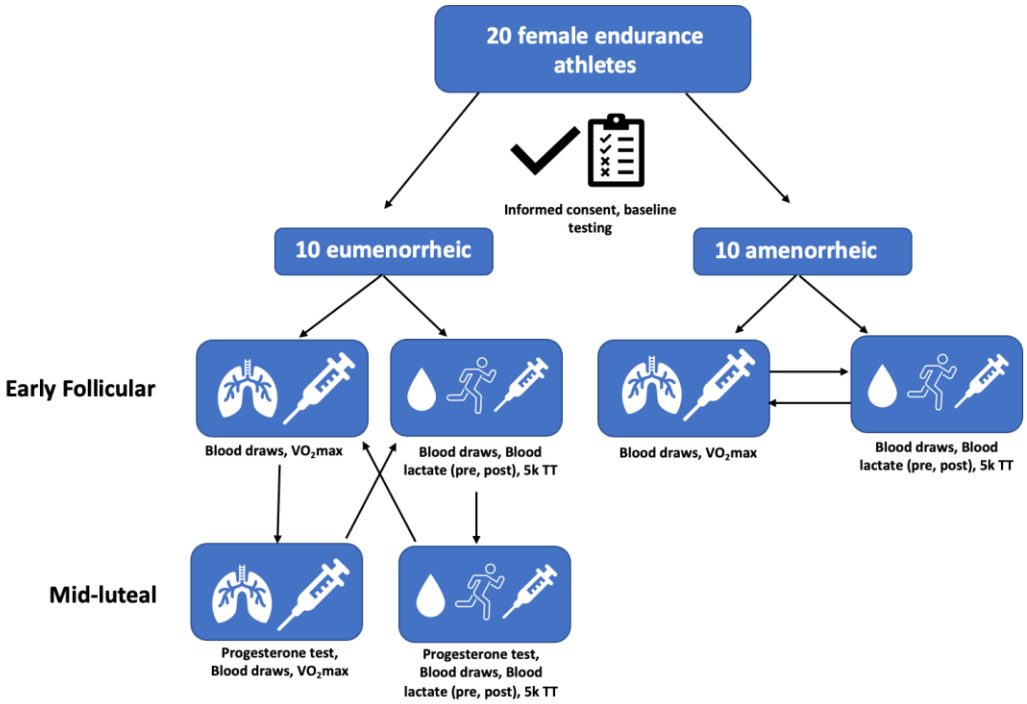

Figure 11. Theoretical experimental flowchart for future research involving both eumenorrheic and amenorrheic athletes.

Future Research

Future research on this topic should include a group of amenorrheic endurance athletes in addition to the eumenorrheic group. These athletes should all experience secondary amenorrhea as a result of training, exhibiting this by having had a regular menstrual cycle in the past and having ceased completely for at least 6 consecutive months after training. These women should also not be on any form of birth control.

The design for a potential future study should follow a similar design to the present one, though with the added group of amenorrheic athletes. See Figure 11. This group would only perform two exercise tests: One VO_2max test and one 5 km time trial with blood lactate testing, each separated by at least one week to allow for sufficient recovery. Blood draws for this group would only include one cortisol test to observe physiological stress levels. Comparative analyses would be run between the amenorrheic group and the eumenorrheic group during their early follicular phase to determine differences in VO_2max, 5 km performance, blood lactate accumulation, and cortisol concentration. The early follicular phase should be chosen to compare to the amenorrheic group because the depressed female sex steroid hormone levels during this phase will be most physiologically similar to amenorrheic athletes, who suffer from chronic depressed hormone levels.

This study would also benefit from bone mineral density analysis via DEXA scans. Budgeting did not allow for this measure in the present study, but future research should aim to include this as a key measure. Mean bone mineral density values could be compared between the eumenorrheic and amenorrheic groups as well as correlated to the reported history of stress fractures.

CONCLUSIONS

The findings of this study did not support the hypothesis that exercise performance would be enhanced during the EF phase as compared to the ML phase in eumenorrheic female endurance runners. This study did however find significantly greater cortisol concentrations during the EF as compared to the ML phase along with a contrasting significant positive correlation between ML cortisol and progesterone, raising questions as to how menstrual cycle-based cortisol concentrations may affect performance in endurance runners. Limited research exists on the effect of menstrual cycle phase on exercise performance in well-trained endurance athletes, as this area of research is a growing field. Future research on menstrual health in endurance athletes should focus on female training programs moving forward.

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