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EFFECTS OF MENSTRUAL CYCLE PHASE ON AEROBIC PARAMETERS DURING A GRADED-EXERCISE TEST

A THESIS APPROVED FOR THE
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

BY THE COMMITTEE CONSISTING OF

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ABSTRACT

Female participants are underrepresented in exercise research, and this exclusion can largely be attributed to a lack of knowledge regarding impacts of menstrual cycle on exercise. Results from previous exercise studies that have attempted to test different phases of the menstrual cycle often exhibit contrasting methodologies, making them difficult to compare. For maximal aerobic testing, many studies have displayed conflicting evidence about whether or not menstrual cycle phase impacts variables of performance, like VO_{2max}, blood lactate concentration, respiratory exchange ratio (RER), maximal heart rate (HR), and rating of perceived exertion (RPE). **Purpose:** The purpose of this study was to examine how different phases of the menstrual cycle – menses (bleeding), the ovulatory phase, and the mid-luteal phase – may affect objective and subjective parameters during a graded exercise test (GXT) in a healthy, college-age population. **Methods:** 21 participants (12 females, 9 males), age 18-24 (mean age = 21.38 ± 1.32 years) were recruited to participate in this study. All participants completed three maximal GXTs using a relative ramp protocol and a 20-minute verification protocol on a cycle ergometer corresponding to specific menstrual cycle phases. Females used the basal body temperature method to track their menstrual cycle and came in for visits during menses (M), ovulation (O), and the mid-luteal phase (L). Visit order was dependent on their current phase at the time of enrollment. Males visit order was randomized and matched for time between visits according to an average 28-day cycle. **Results:** Though males exhibited significantly higher values for height, maximal load, and absolute and relative VO_{2max} compared to females ($p < 0.05$), no significant differences were present across the different phases for the objective parameters (height, weight, absolute and relative VO_{2max}, max HR, RER, lactate, time to failure, and maximal load). Males also did not exhibit significant differences for the subjective parameters across visits – overall RPE, localized RPE, and recovery rating. Females, however, displayed significant differences for each subjective variable. Significantly higher values for overall RPE were found during visit M compared to visits O and L ($p < 0.05$), significantly higher values for localized RPE were found for visit M compared to visit O ($p < 0.05$), but not visit L, and significantly lower values for recovery rating were found for visit M compared to visit O and visit L ($p < 0.001$). No significant differences were found between visit O and visit L in the female participants. **Conclusion:** Females felt like they were exerting more during the GXT and felt less recovered the day following visit M, or menses, compared to the other visits (O and L), while males did not exhibit any significant changes across visits for any of the parameters. More research regarding the cause of this finding is warranted and highly recommended but may be attributed to the drop in sex hormone concentration at this point in the menstrual cycle. Moving forward, exercise researchers should use extreme caution while testing during menses until more knowledge is gained in this area.

CHAPTER I: INTRODUCTION

Sex is a biological variable that researchers recommend controlling for in exercise studies; yet, the female population has been largely underrepresented in exercise physiology research (Oostheyse & Bosch, 2010). Costello et al. reports that the average percentage of female participants from a sample of 1,382 exercise science articles ranged from 35% to 37% (Costello et al., 2014). Furthermore, some exercise studies have even excluded female participants wholly due to their menstrual cycle which may alter physiological responses to exercise (Costello et al., 2014; Oostheyse & Bosch, 2010). Over the past several decades, the prevalence of females participating in athletic activities and recreational exercise has increased, which has motivated researchers to advance the current understanding on how the menstrual cycle impacts performance and subjective perceptions to exercise (Constantini et al., 2005; Hackney et al., 1991; Hall-Jurkowski et al., 1981; Cook et al., 1998). Although most researchers agree that the scheduling of testing visits should be controlled to mitigate any potential influences that hormonal fluctuations could possibly have on any exercise testing outcomes, the current evidence regarding the impact of the menstrual cycle on testing outcomes has included inconsistent results. To date however, there has been a lack of verified and reliable recommendations for testing of this sort (Oostheyse & Bosch, 2010; Dibrezzo et al., 1988; Birch, 2000; Hackney et al., 1991; Iacovides et al., 2015; Riley III et al., 1999). In fact, a study by Murphy et al. (2017) suggested that more studies investigating differences in aerobic exercise performance across the menstrual cycle are necessary to provide more specific and reliable recommendations for healthy, college-age females (Murphy et al., 2017).

The testing of aerobic exercise parameters across the menstrual cycle could reveal positive or negative implications for performance. This could, in turn, provide researchers,

recreationally active females, and possibly even athletic participants, coaches, and trainers evidence for optimizing aerobic performance programming according to menstrual cycle phase of the participants. For instance, if oxygen utilization or perceived effort are compromised during a specific phase, it may be beneficial to adjust aerobic exercise events during that time period to negate this undesirable impact. The main aim of this study was to provide more information regarding the possible cardiorespiratory, metabolic, and perceptual changes during aerobic maximal exercise testing across specific menstrual cycle phases in order to make recommendations for further researchers and others interested in female performance during aerobic exercise.

The female gonadal sex hormones – estradiol and progesterone – may be the basis for influencing physiological and perceptual variables seen in response to exercise in females compared to males (Hackney et al., 1991; Iacovides, 2015; Riley III et al., 1999; Veldhuijzen et al., 2013; Ring et al., 2009; Teepker et al., 2010; Kowalczyk et al., 2006; Kowalczyk et al., 2010; Cook et al., 1998). These hormonal fluctuations across the menstrual cycle have elicited research from many scientific fields, such as brain imaging across different points of the cycle (Veldhuijzen et al., 2013; Choi et al., 2006). Many variables that may impact aerobic exercise according to the Fick Equation, have been found to be influenced by menstrual cycle (Laszlo, 2004). The Fick Equation can be manipulated to say:

$$VO_2 = Q \cdot (A - V)O_2$$

These variables involved include fluid shifts, heart rate, and thermoregulation. Studies by Stachenfeld (2008) and Wenner & Stachenfeld (2012) found that the sex hormones estradiol and progesterone slightly shift capillary fluid dynamics and sodium retention in

young healthy women (Stachenfeld, 2008; Wenner & Stachenfeld, 2012). Tanaka et al. (2003) observed significant correlations between estradiol concentrations and baroreflex sensitivities, indicating that estradiol may impact the control of heart rate (Tanaka et al., 2003). Additionally, a study by Godbole et al. (2016) determined that resting heart rate was significantly higher and that oxygen utilization was significantly lower prior to menses when estradiol levels are at their lowest concentration; moreover, these authors suggest that cardiorespiratory responses to aerobic exercise are reduced prior to menses (Godbole et al., 2016). Pivarnik et al. (1992) found that resting and maximal exercise rectal temperature and heart rate were significantly higher during the luteal phase compared to the follicular phase, and these authors determined that thermoregulation and cardiovascular strain were influenced during the luteal phase, when progesterone concentration is high (Pivarnik et al., 1992). Each of these studies indicate that these variables fluctuate across menstrual cycle and may impact aerobic performance according to the Fick equation, generating a need for more research to better identify any possible impacts on exercise performance (Laszlo, 2004).

Thus far, literature reviews have reported conflicting evidence regarding specific hormonal concentrations, their individual actions, interactions, and their influence on aerobic exercise parameters (Dibrezzo et al., 1988; Smekal, et al., 2007; Hackney et al, 1991; Riley III et al., 1999; Iacovides et al., 2015; Stachenfeld, 2008). Some researchers have attempted to identify how performance outcomes and physiology interact across the menstrual cycle, but differing procedures complicate identifying associated results among these studies (Riley III et al., 1999; Teepker et al., 2010; Iacovides et al., 2015). Some researchers have elected to measure submaximal performance, like a study by Hackney et al. (1991) which reported that respiratory exchange ratio (RER), which indicates what fuel source is being metabolized by

the participant, was significantly lower in the ovulatory phase compared to the follicular phase, while Smekal et al. (2007) found no difference in RER during a maximal exercise protocol between the follicular and luteal phases (Hackney et al., 1991; Smekal et al., 2007). Also, some researchers like Bemben et al. (1995) and Caldwell Hooper et al. (2011) have elected to use a treadmill to assess maximal aerobic performance, as opposed to a cycle ergometer which was utilized by Gordon et al. (2018), while Godbole et al. (2016) elected to use a step test and a prediction equation to assess VO_{2max} (Bemben et al., 1995; Caldwell Hooper et al., 2011; Gordon et al., 2018; Godbole et al., 2016). These conflicting methodologies of assessing aerobic performance, either maximally or submaximally, make results extremely difficult to compare and draw definitive conclusions between.

Another hindrance of comparing previous menstrual cycle research is the inconsistent results, reliability, or relative lack of reporting perceptive variables between studies. A review by Iacovides et al. (2015) reported that measures of pain sensitivity, using electrical, thermal, pressure stimulation and several other modalities, across the menstrual cycle have been widely conflicting – with some authors finding these values to be lowest during menstruation (bleeding), some during ovulation, and some during the "pre-menstrual" phase, while a large number of studies have reported no variability in pain sensitivity across the menstrual cycle (Choi et al., 2006; Teepker et al., 2010; Iacovides et al., 2015). Bailey et al. (2000) found no significant changes in rating of perceived exertion (RPE) between the follicular and luteal phases during a prolonged exercise protocol on an ergometer at 70% of the participants' VO_{2max} (Bailey et al., 2000). Similarly, Lara et al. (2019) found that RPE was unchanged in their placebo group across the early follicular, preovulatory, and mid-luteal phases during a staged maximal GXT on a cycle ergometer (Lara et al., 2019). Goldsmith and Glaister (2020)

also found that RPE was unchanged between early follicular, late follicular, and mid-luteal during maximal and submaximal treadmill tests (Goldsmith & Glaister, 2020). Alternatively, Caldwell Hooper et al. (2011) concluded that RPE was higher in the follicular phase than the luteal phase in moderate intensity exercise, and Pivarnik et al. (1992) reported that RPE during a maximal cycle GXT was increased during the luteal phase (Caldwell Hooper et al., 2011; Pivarnik et al., 1992). However, some studies have failed to measure or report perceptual variables like RPE altogether (Bemben et al., 1995; Gordon et al., 2018). The current study attempted to provide more information regarding changes in RPE across the menstrual cycle, amidst these variations in the current literature.

In regards to timing of testing, most studies that aim to test variability in exercise responses usually test within the middle of the follicular and luteal phases and compare only these two time points; likewise, most results have shown no difference in several different measured parameters, including isometric strength, submaximal aerobic performance, and oxygen utilization (Hackney et al., 1991; Hall-Jurkowski et al., 1981; Smekal, et al., 2007; Lebrun et al., 1995; Ring et al., 2009; Choi et al., 2006; Teepker et al., 2009). These timepoints were likely chosen out of convenience to the researchers and/or participants, as these points tend to be the easiest to identify and are the longest lasting phases. Unfortunately, this study design leaves several portions of the menstrual cycle untested, and these parts happen to be when cycling female sex hormones estradiol and progesterone fluctuate most significantly, like during menses, when the concentrations of estradiol and progesterone are at their lowest, and during ovulation, when estradiol and luteinizing hormone (LH) are at their highest (Barbieri, 2014).

Another difference found in previous literature includes the methodology for attempting to identify the menstrual cycle phases. A common method to accomplish this without access to hormonal assays is with basal body temperature (BBT) tracking. Tracking BBT in females that exhibit eumenorrhea (normal menstrual cycle including all three phases) is relatively simple and makes the point of ovulation easy to identify by observing a slight increase in BBT, attributed to be about 0.3°C on average. (McClure Browne, 1973). Ovulation, or release of the oocyte, corresponds to this slight increase in temperature, which contrasts to the relative consistency of BBT across the remainder of the menstrual cycle (Janse de Jonge, 2003; Buxton & Atkinson, 1948; Royston & Abrams, 1980). Although this method has the advantage of being simple and inexpensive to perform, it may not be the most reliable method to confirm ovulation. Guermandi et al. (2001) reported that more advanced methods of menstrual cycle tracking, such as blood or urinary hormone analyses, were preferable when compared to the BBT method, though this method accurately predicted ovulation in approximately 75% of cases (Guermandi et al., 2001).

Many studies have also attempted to gain information about menstrual cycle changes using participants with different physical activity levels. One example of this issue is apparent when comparing a study from Caldwell Hooper et al. (2011) which measured RPE among other variables in sedentary women (Caldwell Hooper et al., 2011). These authors reported that RPE and pain are higher during the follicular phase when compared to the luteal phase in this sample of sedentary females, while another study by Stephenson et al. (1981) who reported that RPE was unchanged across several testing days in healthy females, but did not attempt to control for activity level (Caldwell Hooper et al., 2011; Stephenson et al., 1981). The possible variety and failure to identify fitness level of the participants makes it so that these results

cannot directly be compared to one another since fitness level could impact findings. Another example of this issue occurs in a study which measured VO_2 in "elite" female runners aged 18-41 years with a VO_{2max} > 50 ml/kg/min (Lebrun et al., 1994; Kaminsky et al., 2015). A standardized average value for females age 20-29 years is 37.6 ± 10.2 ml/kg/min and even lower for females age 30-39 years (Kaminsky et al., 2015). The study by Lebrun et al. (1994) using elite athletes makes these results difficult to generalize to the general population and runs the risk of possible amenorrheic effects, or absence of a normal menstrual pattern, which is common among heavily endurance trained females. Characteristics of participants, like fitness level, methodology used to identify menstrual cycle phase, and exercise modality used within studies of this kind have differed largely in the past, which makes it hard to pinpoint the impact menstrual cycle phase has on certain exercise parameters.

In summary, a large gap in the literature exists surrounding the particular cardiovascular and metabolic responses to aerobic exercise during progressive phases of the menstrual cycle – specifically, menses, the early follicular phase where bleeding occurs, and the quick, challenging-to-identify ovulatory phase where BBT increases due to changing levels of the sex hormones. Additionally, eumenorrheic females may also individually experience highly variable negative perceptions during menses and/or ovulation, such as fatigue and physical discomfort (cramping, bloating, etc.), which promotes further testing of perceptive variables during and following exercise according to these phases (Constantini et al., 2005; Gordon et al., 2018; Giacomoni et al., 2000; Hackney et al., 1991; Choi et al., 2006; Caldwell Hooper et al., 2011). The inconsistencies within previous studies including menstrual phase identification, phase(s) tested, exercise mode and measurement protocols used, and sample

selection and control warrant more detailed research which led to the conception of this particular study.

Purpose

The purpose of this study was to examine how different phases of the menstrual cycle – specifically during menses (day 0-3), the ovulatory phase (according to BBT spike), and the mid-luteal phase (day 20-24) – may affect metabolic, cardiovascular, and subjective parameters during a maximal graded exercise test (GXT) in a healthy, college-age population.

Significance

Since there is a scarce amount of literature surrounding how menses and ovulation can impact aerobic exercise performance, the results of this study could elucidate the magnitude of these changes. Understanding these differences may help future researchers to more effectively standardize the design of their studies when utilizing female participants (Herzberg et al., 2017). More specifically, it may reveal if menses and ovulation impact either objective or subjective performance measures associated with aerobic exercise (Constantini et al., 2005). This study aimed to include several subjective measures, such as rating of perceived exertion (RPE) and rating of recovery, which are less commonly evaluated in exercise research, to provide more information on how the participant feels during and after maximal exercise (Cook et al., 1998; Caldwell Hooper et al., 2011). It will be of interest to see how the results from this study are comparable to the current literature selection. Most importantly, these findings could be used to optimize aerobic training programs or periodization cycles for enabling further female participation in exercise studies, and also possibly for female aerobic athletes by accounting for physiological differences over the course of the menstrual cycle.

Research Questions

1. Will there be significant differences in the following objective parameters measured during a graded-exercise test between menses, the ovulatory phase, and the mid-luteal phase?

    a. Maximal rate of oxygen utilization (VO_{2max})
    b. Respiratory exchange ratio (RER)
    c. Blood lactate concentration change (max – rest)
    d. Time to failure
    e. Maximal workload
    f. Peak heart rate (HR)

2. Will there be significant differences in the subjective parameters measured during a graded-exercise test between menses, the ovulatory phase, and the mid-luteal phase?

    a. Overall rating of perceived exertion
    b. Localized rating of perceived exertion
    c. Recovery rating post-testing

Hypotheses

3. **H_0:** There will be no significant difference in the objective parameters measured between menses, the ovulatory phase, and the mid-luteal phase.

    a. Maximal rate of oxygen utilization (VO_{2max})
    b. Respiratory exchange ratio (RER)
    c. Blood lactate concentration change (max – rest)
    d. Time to failure
    e. Maximal workload
    f. Peak heart rate (HR)

4. **H_1:** There will be significant differences in the objective parameters measured between menses, the ovulatory phase, and the mid-luteal phase. Values for the objective parameters will be higher during the ovulatory phase due to the spike in basal body temperature and increased sex hormone concentration.

a. Maximal rate of oxygen utilization (VO_{2max})
b. Respiratory exchange ratio (RER)
c. Blood lactate concentration change (max – rest)
d. Time to failure
e. Maximal workload
f. Peak heart rate (HR)

5. **H_0:** There will be no significant difference in the subjective parameters measured between menses, ovulatory phase, and mid-luteal phase.

    a. Overall rating of perceived exertion
    b. Localized rating of perceived exertion
    c. Recovery rating post-testing

6. **H_1:** There will be significant differences in the subjective parameters measured between menses, ovulatory phase, and mid-luteal phase. Values for the subjective parameters will be lower for perceived exertion and higher for recovery rating during the ovulatory phase due to the spike in basal body temperature and increased sex hormone concentration.

    a. Overall rating of perceived exertion
    b. Localized rating of perceived exertion
    c. Recovery rating post-testing

Delimitations

This study includes the following delimitations:

1. Healthy males and females between 18-24 years of age.
2. Healthy females exhibiting a normal menstrual cycle (eumenorrhea).
3. Healthy females without hormonal oral contraceptive, intrauterine device, implant, injection or other hormonal birth control method.
4. Participants without external hormonal influences (antidepressants or other medications)
5. Participants without metabolic, respiratory, neurological, or cardiovascular diseases.

6. Participants free from any recent or debilitating musculoskeletal injury.
7. Participants will maintain current activity level for the duration of the study.

Limitations

This study includes the following limitations:

1. Results of this study will not apply to the entire population.
2. Results of this study will not be generalizable to females with abnormal menstrual cycles, hormonal disorders, or reproductive anatomical malformations.
3. Results of this study will not apply to heavily endurance trained individuals.
4. Results of this study will not apply to birth control users.
5. Results of this study regarding menstrual cycle phase will not apply to males.
6. Participants will be recruited from the Norman and Oklahoma City metropolitan area.

Assumptions

This study includes the following assumptions:

1. All participants will present accurate and honest medical history, physical activity, and background information.
2. All female participants will present accurate and honest menstrual cycle history.
3. All participants will meet inclusion criteria.
4. All participants will be honest and consistent in reporting BBT and recovery rating.
5. All participants will perform graded exercise tests to their maximal effort.
6. All participants will answer questionnaires truthfully.

7. Female participants will be tested during their true menses (day 0-3 corresponding to 0 = onset of bleeding), ovulatory phase (within 24 hours of BBT spike), and mid-luteal phase (day 20-24).
8. All participants will complete the required visits of the study.

Operational Definitions

1. **Eumenorrheic:** normal menstrual cycle; presence of menses, ovulation, and all sex hormones and appropriate function (Birch, 2000)
2. **Menses:** sub-phase of follicular phase where bleeding occurs, averages 3-7 days in most eumenorrheic females, data will be collected within days 0-3 (Barbieri, 2014)
3. **Follicular phase:** phase defining follicle development in the ovary, from onset of menses where sex hormone concentration decreases to ovulation, variable in length but averages 14 days (Barbieri, 2014)
4. **Ovulatory phase:** point where the membrane of the developed follicle deteriorates and releases oocyte due to high levels of estradiol, usually occurs mid-way through cycle, data will be collected within 24 hours of BBT spike (Barbieri, 2014)
5. **Luteal phase:** progesterone increases rapidly and uterine lining experiences changes, always lasts 14 days until progesterone is signaled to decrease and follicle development begins, data will be collected in days 20-24 (Thiyagarajan & Jeanmonod, 2018)
6. **Basal body temperature (BBT):** waking internal body temperature, can be used to identify ovulation (Buxton & Atkinson, 1948; Royston & Abrams, 1980)
7. **Respiratory exchange ratio (RER):** VCO_2/VO_2 indicates non-protein fuel source being metabolized (Hackney et al., 1991)

8. **Rating of perceived exertion (RPE):** a subjective measure of how the participant feels; typically, a Borg scale (6-20) is used, but this study will used a modified Borg scale (0-10) (Stephenson et al., 1981)

CHAPTER II: LITERATURE REVIEW

Introduction

In order to get a full background on relevant literature with purposes related to menstrual cycle phase and exercise performance, search engines such as CINAHL Complete, Google Scholar, PubMed, and SPORTDiscus with full text were utilized using specific search terms to access textbooks and peer reviewed journal articles. The following literature review is organized to briefly introduce the endocrinology that regulates the menstrual cycle, define the phases of the menstrual cycle and recognize what makes each phase distinguishable, summarize the methods that previous researchers have used to identify these different phases within the menstrual cycle, and report objective and subjective findings from studies that have controlled for menstrual cycle phase previously. It will encompass literature from gynecology, exercise physiology, molecular biology, neuroscience, and statistics. The purpose of this chapter is to relay the current gaps and variations in the literature regarding the extent to which metabolic, cardiovascular, and subjective parameters are influenced by menstrual cycle phases.

Menstrual Cycle Background

In eumenorrheic females, gonadotropin releasing hormone (GnRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, oestrogen, and progesterone are the hormones that interact and create a rhythm that manages the menstrual cycle and its associated physiological changes (Birch, 2000). A normal cycle lasts between 21-35 days, and averages 28 days for most eumenorrheic females (Thiyagarajan & Jeanmonod, 2018). The cycle operates as both a negative and positive feedback system and occurs in three distinct

phases: follicular, ovulatory, and luteal. An overview of the menstrual cycle can be seen visually in Figure I (Reed & Carr, 2000).

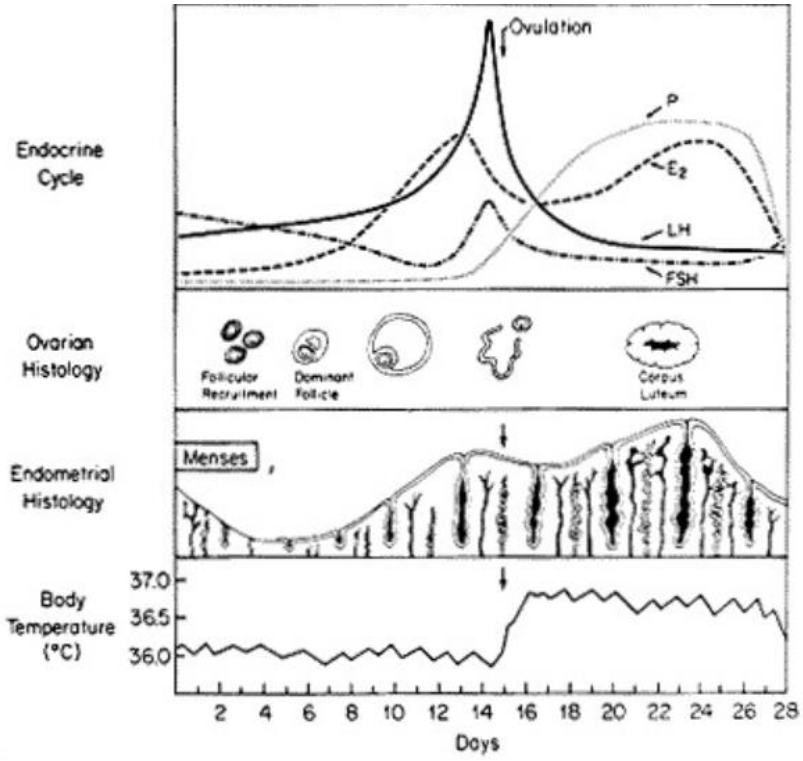


Figure I: Overview of the menstrual cycle (Reed & Carr, 2000)

It is important to note that the menstrual cycle requires complicated interactions between roles of hormones, physiology, and anatomy to function in the proper way. If each of these components do not function and interact appropriately, there are many possibilities for improper functioning of the menstrual cycle. A few of these possibilities include impaired hormone release or function, oocyte release, or anatomical malformations each of which could impact the menstrual cycle in different ways, such as irregular and unpredictable cycles, anovulation (absence of ovulation), or amenorrhea (absence of menses). Figure I and the

remainder of this section attempt to illustrate properties of eumenorrhea, or a normally functioning menstrual cycle.

Follicular Phase

The first phase in the menstrual cycle is the follicular phase. In the average 28-day cycle, this phase occurs from day 0 to 14, but has no definitive length, which contributes to menstrual cycle variability seen between females. The technical start of the menstrual cycle occurs at the onset of menses or bleeding, which is actually a sub-phase of the follicular phase. In the average cycle, it is generally accepted that the occurrence of menstruation is from days 0-5, yet it can have a fairly high variability between females, with the average lasting around 3-7 days in duration (Barbieri, 2014). Menses is said to occur when the levels of progesterone and estradiol decrease significantly, stimulating the endometrial or inner layer of the uterus to be sloughed off and exit through the cervix. Simultaneously, precursor follicles begin forming in the ovaries. Menses is usually considered part of this follicular phase and not its own phase due to the growth of these pre-follicles (Birch, 2000). As menses is ending, the hypothalamus secretes GnRH, which travels to the anterior pituitary gland. It attaches to its own 7-transmembrane G-protein receptor which triggers the anterior pituitary gland to release FSH and LH at different rhythms throughout the cycle. (Thiyagarajan & Jeanmonod, 2018; Hawkins & Matzuk, 2008). These hormones are then transported to the ovarian follicle where they interact with specific cells located there as the follicle begins to mature. Theca cells are the first to mature as they are sensitive to the presence of LH. If LH is detected, the theca cells will activate cholesterol desmolase, an enzyme, and secrete androstenedione and progesterone, seen below in Figure II (Barbieri, 2014; Reed & Carr, 2000). Then, androstenedione diffuses into granulosa cells near the theca cell it was produced by. This second specific type of cell,

the granulosa cell, is sensitive to FSH. If FSH is detected, the cell is stimulated and converts androstenedione into testosterone and then directly into a type of estrogen, 17-β-estradiol or estradiol, via the aromatase enzyme. This rise in estradiol is a characteristic of the follicular phase. The concentration of estradiol then reaches a threshold value in the bloodstream (around 200 pg/mL of plasma) (Thiyagarajan & Jeanmonod, 2018). After, it activates the kisspeptin (KISS1) system by binding to KISS1 receptors in the hypothalamus to release more GnRH and thus, initiates a critical 48-hour long LH surge (Barbieri, 2014). This critical surge of LH commences ovulation, the next phase of the menstrual cycle.

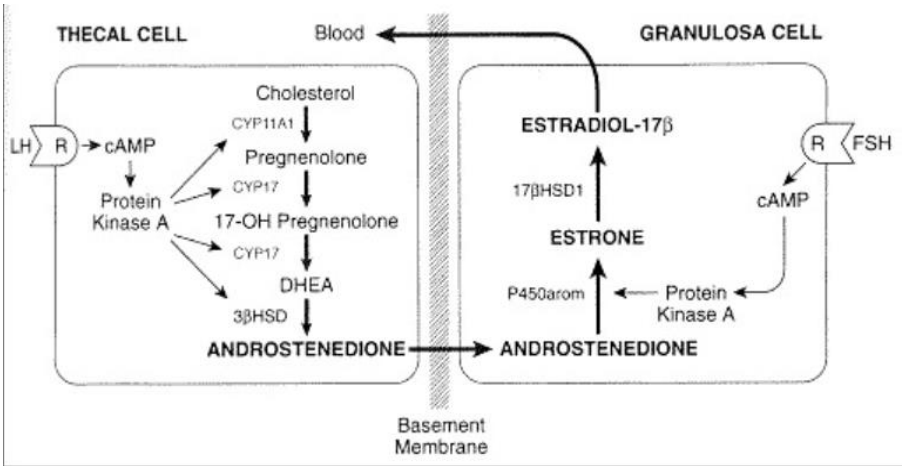


Figure II: Example of LH activation and estradiol release within the female ovary (Reed & Carr, 2000)

Ovulatory Phase

The ovulatory phase concludes the follicular phase and almost always occurs 14-days prior to the onset of menses of the next cycle; hence, in a eumenorrheic average 28-day cycle, it will occur 14-days into the cycle. The LH surge causes: 1) an increase in concentration of enzymes that deteriorate the membrane of the follicle to promote release of the oocyte, 2)

luteinizes the theca and granulosa cells which increases the concentration of progesterone in the blood, 3) increases blood flow to the follicle, 4) increases basal body temperature, and 5) stimulates epidermal growth factor (EGF)-like hormones to encourage the oocyte, a different type of specialized cell within the ovary, to begin cell division and growth. The oocyte, also known as the reproductive egg cell, is released shortly after from the mature follicle within the ovary (Barbieri, 2014). This release usually commences around 12-48 hours after the considerable increase in body temperature and LH concentration.

Luteal Phase

Immediately following ovulation, the luteal phase begins and lasts for around 14-days, unless some of the system is malfunctioning, but in eumenorrheic females, this phase makes up the remainder of the average 28-day cycle. The leftover follicle is converted into the corpus luteum, which secretes progesterone throughout the luteal phase. Most significantly, this increased concentration of progesterone increases activity of the hypothalamus, which maintains an increase in body temperature and a loss of body water (Birch, 2000; Royston & Abrams, 1980; Forman et al., 1987; Buxton & Atkinson, 1948; Hawkins & Matzuk, 2008; Murphy et al., 2017). The presence of a corpus luteum will do several other things – including causing mucous along the cervix to thicken and the endometrial lining of the uterus to prepare for detachment by transforming into a secretory tissue before beginning menses again. If the oocyte is fertilized by a sperm and implants along the uterine wall during this phase, the progesterone from the corpus luteum will help the now fertilized oocyte, or ovum, remain attached to the uterus and hold hormone levels stable to encourage completion of the early days of pregnancy before the placenta is fully formed and connects the ovum and endometrium (Thiyagarajan & Jeanmonod, 2018). If the oocyte is not fertilized and does not attach to the

endometrium, the corpus luteum will continue to secrete progesterone and will eventually shrink due to the effects of a negative feedback system. High progesterone levels decrease release of FSH and LH from the anterior pituitary gland, which will eventually discourage estradiol and progesterone secretion from the theca and granulosa cells and will decrease the concentrations of these hormones drastically. This drop in gonadal sex hormones returns the cycle to menses/bleeding and thus, the follicular phase begins again (Thiyagarajan & Jeanmonod, 2018).

Methods of Estimating Menstrual Cycle Phase

A few studies have attempted to identify and measure the physiological parameters that are affected by the menstrual cycle and the corresponding hormones involved. Previous research has encountered difficulties in attempting to correctly identify which menstrual phase is happening at a given point in time. A study by Janse de Jonge (2003) suggests several methods to verify the different menstrual phases such as retrospective counting, basal body temperature (BBT) tracking, urinary LH concentration, and blood serum or salivary assays to physically measure hormone levels, and lists the problems that arise with each method (Janse de Jonge, 2003). The most difficult and least reliable way to track a menstrual phase is by having the participant or research team count backward to the onset of the participant's last menses, also known as retrospective counting. Several problems of this method include inaccuracy of recollection, not having knowledge of anovulation or another sort of hormonal condition, and variability of the follicular phase impeding accuracy of each cycle (Janse de Jonge, 2003).

Though not the most highly accurate, the most popular, convenient, and affordable way of tracking menstrual cycle phase among the physical performance field is using BBT charts. This method only provides the participant a minor inconvenience of tracking body temperature every morning immediately upon waking and does not involve any expensive technology. Typically, a pattern can be noticed from plotting these BBT values along with corresponding days of the menstrual cycle, beginning with onset of bleeding. The average pattern of a BBT chart is biphasic (Buxton & Atkinson, 1948) and shows temperature as stable but relatively low during the follicular phase with a visible spike following the LH surge preceding ovulation by 36-44 hours (Barbieri, 2014). This temperature spike (around 0.3°C (Janse de Jonge, 2003) or $\frac{3}{5} - 1^\circ$ F (Buxton & Atkinson, 1948)) is commonly used to identify ovulation (McClure Browne, 1973). As stated previously and represented above in Figure I, ovulation is also connected to an increase in progesterone, which stays elevated throughout the luteal phase and is said to have thermic effects on the body; therefore, in the BBT chart, temperature remains relatively high throughout the luteal phase compared to the follicular phase, but then falls prior to menses, the start of the follicular phase of the next cycle (Royston & Abrams, 1980; McClure Browne, 1973). A study by Buxton and Atkinson aimed to identify the cause behind the thermic increase when using a BBT chart. This study provided 6 amenorrhoeic female participants with various exogenous ovarian hormones to see which produced any sort of thermic effect. They gave participants estrogen for 2 weeks to reach a baseline temperature and then administered 10-25 mg of progesterone over 7-14 days. They also injected a bilaterally ovariectomized female, missing the critical ovarian cells to ovulate, with exogenous gonadotropin to see if this hormone would cause an increase in BBT, since another study reported that this hormone did create an increase in BBT. Their results showed that the

amenorrhoeic females given progesterone had an average increase of > 0.5°F and the ovariectomized female showed no increase in BBT, which led to the conclusion that progesterone is the main ovarian hormone that produces a thermic increase, though they did not report any statistical results (Buxton & Atkinson, 1948). A few studies recognize the problems with this method including relative variability between and within each female, simply from one cycle to another (Buxton & Atkinson, 1948; Royston & Abrams, 1980; Janse de Jonge, 2003), as well as inaccuracy of monitoring progesterone levels (Janse de Jonge, 2003; Guermandi et al., 2001). A study by Forman et al. suggests that it may not be progesterone alone that causes an increase in BBT, but rather the increase is due to an oestrogen-progesterone synergism or the action of another substance, like norepinephrine, which might enhance the natural thermogenic effect of progesterone (Forman et al., 1987). This study also recognizes that an upward shift in BBT only requires 4ng/ml of progesterone, but discounts that progesterone alone could be responsible for the increase in BBT since it does not increase linearly in proportion to progesterone levels and plateaus 48 hours post-spike in BBT (Forman et al., 1987). Although the concerns with using the BBT are established, this method remains commonly used for performing analyses across the follicular and luteal phases in eumenorrheic women.

The third method, urinary LH measurement, has been shown to have strong accuracy in predicting when ovulation will occur, mean time of 20 ± 3 hours and a 95% confidence interval of 14-26 hours (Miller & Soules, 1996). A study by Guermandi et al. (2001) suggested that urinary analysis predicted ovulation in 97% of 101 cases, compared to BBT tracking which predicted 74% (Guermandi et al., 2001). This method does require purchase of a urinary

LH kit, such as Ovuquick, and the process of a using 24-hour sample is a bit impractical and may cause error, as reported by Janse de Jonge (Janse de Jonge, 2003).

The last known method of attempting to classify menstrual phase is through saliva or blood serum samples and measuring hormone concentration, though the most popular of these in previous literature is blood serum measurements. This method may be the most accurate at reporting concentrations of progesterone and oestrogen, but currently, it is the most invasive and there is no standardized value of progesterone that correlates to a confirmation of ovulation (Janse de Jonge, 2003). Birch suggests that there is a thermoregulatory set point, but it has yet to be identified across our field (Birch, 2000). None of the methods listed above have been recognized and accepted as a gold standard for identifying menstrual cycle phase in obstetrics or exercise physiology, though some methods are inherently more reliable than others due to accuracy of measurement and human error.

Physical Performance and Menstrual Cycle

The above introduction to the menstrual cycle, the ways to distinguish between each phase, and methods used to examine body temperature changes suggest that hormonal fluctuations might cause changes to physiological function, especially during exercise. When considering maximal anaerobic performance, the results of most studies agree that menstrual cycle phase has no effect on isometric strength or anaerobic threshold parameters, though most of these tests are only measured during the mid-follicular and mid-luteal phases (Dibrezzo et al., 1988; Giacomoni et al., 2000; Petrofsky et al., 2007). The remainder of this section will attempt to summarize current literature regarding aerobic performance, both submaximally and maximally.

In an attempt to map how submaximal exercise variables change across all days of the menstrual cycle, a pair of studies by Stephenson et al. first examined VO_{2max} in a small sample of active female participants (n = 6, age 19-47 years) on a bicycle ergometer and calculated four levels of intensity for them to complete every 6 days at different points in their menstrual cycle (day 2, 8, 14, 20, and 26, with day 0 corroborating to the onset of menses) (Stephenson et al., 1982). The researchers measured several metabolic, cardiorespiratory, and temperature variables at each submaximal exercise level and at rest. These variables included VO_2, VCO_2, total expiratory volume (V_E), mean tidal volume (V_T), respiratory rate (F_B), respiratory exchange ratio (RER), mean skin temperature (T_s), rectal temperature (T_{re}), and total body temperature (T_b) , and RPE with a modified 9-point Borg scale and anaerobic threshold in the other study. The first study reported that peak O_2 uptake, VCO_2, V_E, V_T, F_B, and RER were unchanged throughout the menstrual cycle, but thermoregulatory data did report significant differences. It was found that resting T_{re} on days 14 and 20 was significantly higher than days 2 and 8, and that T_s and T_b were significantly lower on days 2 and 26 when compared to day 20 (Stephenson et al., 1982). They also reported that T_{re} on days 2 and 8 averaged was significantly lower than on days 14 and 20 averaged during each exercise phase and at rest ($p < 0.05$). They attributed this 0.01°C per 100 ml· min^{-1} increase in VO_2 rise in core body temperature to increased progesterone levels (Stephenson et al., 1982). The second study measured RPE and anaerobic threshold in the same group of participants. They performed the same exercise protocol, with four submaximal exercise intensities, on specific days that were thought to correspond with different cycle phases and reported that RPE was not significantly different between days, though there was an upward trend around day 20 (Stephenson et al., 1981). The researchers attributed that RPE naturally increases with increases in heart rate (HR)

and does not depend on menstrual cycle phase to offer changes. The problems of this study include a small sample size with an incredibly large age range and failure to confirm ovulation in the participants. Though this study simply observed a significant rise in core body temperature at what they believed to be the ovulatory and luteal phase, there was no confirmation of menstrual cycle phase or normal ovulatory function in the participants, which impeded their internal validity.

Many more studies have attempted to measure how different factors involved in endurance performance, such as heart rate, lactate concentration, time to failure, etc., may vary across menstrual cycle, yet the data are quite inconsistent. A study by Gordon et al. placed 16 active females into two groups, an oral contraceptive group who had been consuming mono-phasic contraceptives for at least 3 months prior to the study (n = 6, age = 21.7 ± 2.16 years) and a non-oral contraceptive group (n = 10, age = 20.6 ± 1.6 years) (Gordon et al., 2018). They assumed menstrual cycle phase by having participants diary menstrual cycle patterns and performed testing for estradoil and progesterone through salivary analysis at each visit. Testing involved four graded exercise tests on a cycle ergometer to measure VO_{2max} and cardiac output, among other cardiorespiratory variables, during four phases of the menstrual cycle: menstruation (days 1-3), mid-follicular (days 9-11), mid-luteal (days 19-20), and premenstrual (days 27-28). The results showed non-significant differences of VO_{2max}, cardiac output, HR, and stroke volume between groups and between phases. This study also reports that a change in VO_{2max} of 1.6 ± 0.85 ml/kg/min between phases is the largest observed change and that approximately 0.75 ml/kg/min is the minimal practically significant change, though these values are only reported between the luteal and follicular phases (Gordon et al., 2018).

Another study by Hall-Jurkowski et al. (1981) aimed to test blood lactate, O_2 delivery and exercise performance across different menstrual cycle phases (Hall-Jurkowski et al., 1981). Ovulation was confirmed with a 0.3°C increase in BBT and an increase of plasma progesterone in 9 females (age 20-24, mean VO_{2max} = 41.8 ml/kg/min) (Hall-Jurkowski et al., 1981). They tested these participants in the follicular and luteal phase, but not ovulatory. When comparing the results from the two phases, there were no differences in O_2 delivery, HR, VCO_2, or cardiac output. On the other hand, results showed significantly longer time to failure, higher ventilation, and significantly lower lactate levels throughout exercise and at rest during the luteal phase ($p < 0.05$) (Hall-Jurkowski et al., 1981). Though these researchers found some differences in cardiorespiratory parameters between the luteal and follicular phase, the most significant finding was that "differences in aerobic performance were not apparent between the two phases" (Hall-Jurkowski et al., 1981, p. 1496). This finding has been widely cited, like in a study done by Hackney et al. (1991), which began by attempting to confirm ovulation in aerobically fit eumenorrheic females (n = 6, age = 26 ± 6 years, VO_{2max} = 44 ± 4 ml/kg/min) by using the BBT charting method 2-3 months prior to testing and periodically collecting urine and blood samples to test for an increase in LH, which elicits ovulation (Hackney et al., 1991). These participants were measured at three different phases – mid-follicuar (days 7-8), ovulatory (days 14-16), and mid-luteal (days 22-23) – which were confirmed with resting blood draws and hormonal analysis. They found that RER was significantly lower during the ovulatory phase ($p < 0.03$) when compared to the mid-follicular phase, and the mid-luteal RER approached significance when compared to mid-follicular values ($p = 0.07$). They also found that the rating of percieved exertion in the legs during exercise, or L-RPE, was significantly greater during the ovulatory phase ($p < 0.05$) and that the total-RPE was slightly higher during

the ovulatory phase, though not statistically significant (Hackney et al., 1991). This finding suggests that exercise felt more difficult to the participants during the ovulatory phase. A clinical assumption to name what is responsible for this phenomenon has yet to be determined; therefore, this elicits the need for more studies during the ovulatory phase and comparing them to other phases that are more commonly tested, like the mid-follicular or mid-luteal phases.

A study by Smekal et al. (2007) recognized the difficulty of confirming ovulation and completing testing within this short phase prior to the luteal phase (Smekal et al., 2007). The majority of their results were similar to earlier studies and show no difference in power output, VO_2, HR, lactate concentration, and RER at any exercise intensity between the follicular and luteal phases (Smekal et al., 2007). Despite finding only one significant difference, a higher ventilatory drive in the luteal phase during submaximal cycling ($p < 0.01$), this study makes recommendations for future studies to test eumenorrheic females during the ovulatory phase (Smekal et al., 2007). A more recent study by Godbole et al. (2016) measured weight, resting heart rate, and respiratory rate, and utilized the Queen's college step test to estimate VO_{2max} with a prediction equation during the premenstrual (day 20-25) and postmenstrual (day 5-10) time period across three consecutive menstrual cycles (Godbole et al., 2016). Results of this study showed statistically significantly higher weight, resting pulse rate, and respiratory rate during the premenstrual period compared to the postmenstrual. Their results also showed significantly lower VO_{2max} prior to menses, as opposed to after (Godbole et al., 2016).

Summary

A variety of studies have attempted to analyze how the different phases in the menstrual cycle and the hormones involved may impact physical performance. There are

disagreements and inconsistencies in measurement and identification of menstrual cycle phases, and an unclear knowledge of how hormonal actions involved in the menstrual cycle may impact different parameters of performance. There are also several dissimilarities of results within studies that have similar methods and purposes to the current study. The majority of previous studies show non-significant changes in oxygen utilization in varying exercise intensities, usually in the luteal and follicular phase and in a semi-active to active population. A prominent problem with a number of these previous studies is a small sample size, which not only can be problematic statistically, but can be difficult to generalize to the greater population (Stephenson et al., 1981; Stephenson et al., 1982; Hackney et al., 1991; Hall-Jurkowski et al., 1981). Some researchers using an aerobic exercise protocol have only tested aerobically fit females (Hackney et al., 1991; Lebrun et al., 1994), whereas alternavtive researchers have used too large of an age range in which pre-menopausal symptoms could interfere with significant results (Stephenson et al., 1981; Stephenson et al., 1981). Another problem is that few researchers have attempted to perform these tests during ovulation or menses. Since the LH surge and small increase in BBT happen shortly prior to ovulation, it can be difficult for the participant and the researcher to pinpoint; however, further research of how metabolic and cardiovascular factors are affected during this shift in hormonal concentration and the increase in BBT is quite necessary in order to make conclusions for studies with female participants in the future. These quandaries within previous studies and reviews incited motivation which led to the research questions that the current study attempted to answer.

CHAPTER III: METHODOLOGY

Introduction

The purpose of this study was to determine how objective and subjective outcome measures during aerobic exercise may differ across the menses, ovulatory, and mid-luteal phases of the menstrual cycle. This chapter will first aim to outline the sample of participants, research design for each visit, data collection procedures and instrumentation. It will then establish the specific objective and subjective variables that were recorded, explain data collection, data management, and data analyses techniques necessary for interpretation and further application of results.

Sample

This study includes results from 21 participants, 9 males and 12 females. The participants in this study were recruited with convenient recruiting materials including flyers, electronic mail, and word of mouth. All were students or alumni of the University of Oklahoma and residents of the surrounding area. Upon performing a sample size calculation in G*Power for a 2 ml/kg/min ΔVO_{2max} , a 2 (group: males vs females) x 3 repeated measures (menstrual cycle phases) between/within ANOVA and partial $\eta^2 = 0.30$ (effect size), and an α-level = 0.05, showed that a sample size of n = 6 per group reflected statistical power of 0.80 (Lebrun et al., 1995; Gordon et al., 2018). Therefore, we sought to recruit at least 9 participants for both groups to account for participant attrition and compliance. Following the inclusion criteria listed below and sample size calculation, this study includes data from all 21 participants.

The following indicate the inclusion criteria for male participants (n = 9):

1. Between 18-24 years of age.
2. Not using external hormonal influences (antidepressants or other medications)
3. Free from metabolic, respiratory, neurological, or cardiovascular diseases.
4. Free from any recent or debilitating musculoskeletal injury (within 1 year).
5. Will maintain current activity level for the duration of the study.

The following indicate the inclusion criteria for female participants (n = 12):

1. Between 18-24 years of age.
2. Exhibiting a normal menstrual cycle (eumenorrhea).
3. Without hormonal oral contraceptive, intrauterine device, implant, injection or other hormonal birth control method.
4. Not using external hormonal influences (antidepressants or other medications)
5. Free from metabolic, respiratory, neurological, or cardiovascular diseases.
6. Free from any recent or debilitating musculoskeletal injury.
7. Will maintain current activity level for the duration of the study.

Research Design

This study utilized a repeated measures design, across three specific time points – menses (M), ovulation (O), and the mid-luteal phase (L). Males were treated as the control group in this experiment, due to the absence of fluctuations in progesterone and estradiol, which are responsible for phase changes throughout the menstrual cycle (Barbieri, 2014).

Using males as a control group was intended to increase the internal validity of this study and eliminate any problems that would arise from the absence of a control group.

The female group was monitored to determine menstrual cycle phases and data collection sessions corresponded to menses, the ovulatory phase, and the mid-luteal phase. All participants received and completed an informed consent, physical activity readiness questionnaire (PAR-Q), medical history questionnaire, International Physical Activity Questionnaire (IPAQ), Profile of Mood States (POMS), and a Health Insurance Portability and Accountability Act (HIPPA) form. Females also received a menstrual cycle history questionnaire. Additionally, the remainder of the first visit included a familiarization with the exercise protocol and equipment. Upon completion of this familiarization visit, all participants were provided a thermometer for BBT tracking and sent text messages directly to the researcher reporting BBT for each morning throughout their enrollment in the study. Further visits for data collection were scheduled depending on whichever phase occurred following the familiarization visit, according to the menstrual cycle questionnaire and BBT tracking method. Participants were informed that their participation in the study is entirely voluntary and can be self-terminated at any point, even prior to finishing all visits. This study received funding from the Graduate Student Senate and the Robberson Research Grant, both from the University of Oklahoma, to cover the cost of necessary equipment.

Measurement Procedures

This study consisted of four visits which included the informed consent and familiarization during the first visit, followed by three additional data collection visits corresponding to menses, the ovulatory phase, and the mid-luteal phase. The length between

visits was variable due to the range of phases when female participants could come in for familiarization and variability of cycle timing from female to female (Barbieri, 2014). Visits for males were randomly generated to assign a phase their first exercise visit and the following visits paralleled the length of time between visits for the average female 28-day cycle, according to estimated menstrual cycle phase changes. For example, if a male was assigned visit M first, his visit O occurred about 14 days after visit L was about 6-10 days later.

In order to randomize testing across the menstrual cycle, the time at which a female participant enrolled in the study set the track for her following visits. For example, a female participant that enrolled prior to menses (bleeding day 0-3) had visit M as her first exercise visit. Conversely, if a female participant enrolled during the early luteal phase (day 16-20), the first exercise session would serve as her visit L. Therefore, the visit number corresponding to menses, ovulation, and mid-luteal phase was relatively randomized across female participants, which served to eliminate any chances of accumulating a learning effect. Data measured from male and female participants over the course of these three visits also aimed to reflect the reliability of the protocol and measurement of VO_{2max} stability over a wide time frame.

Since the participants engaged in a graded exercise test (GXT) until volitional fatigue, they were asked to refrain from vigorous exercise for 48 hours and not have eaten 3 hours prior to data collection (Macfarlane, 2001). Participants were also encouraged not to consume any caffeine on the day of testing or within 6 hours prior to testing to ensure proper control of ergogenic aids throughout testing. Additionally, prior to any data collection, the TrueOne® 2400 Metabolic Measurement System (ParvoMedics Ind., Sandy, UT, USA) was properly calibrated following the operator's manual instructions. The Lactate Plus Meter (Nova Biomedical Corporation, Waltham, MA, USA) was also calibrated before data collection per

instructions of the operator's manual. Following the familiarization trial, all testing visits were scheduled to control for time of day (within a 2-hour start range).

Visit 1

All participants were thoroughly informed of the study protocol and possible risks prior to any measurement. If the participant wished to continue with participation, he/she voluntarily signed an informed consent form, which was approved by the local Institutional Review Board at the University of Oklahoma Norman campus (IRB # 11034). Following confirmation that the participant met all inclusion criteria and was approved for further participation, demographic information of each participant, including age, sex, mood, and physical activity level, were collected from these questionnaires and recorded. Participants were assigned a personal participant identification (ID) number, which they were referred to as for the remainder of the study to maintain confidentiality of each participant. Following the assignment of a participant ID, the participant began familiarization. Baseline measurements for the following characteristics were then taken – height, weight, current menstrual cycle phase (females only), and temperature at the time of visit, heart rate (HR), blood pressure (BP), and resting blood lactate concentration. Height was measured to the nearest 0.5 centimeter with a Stadiometer (Seca Model 242, Chino, CA, USA), weight was measured to the nearest 0.1 kilogram on an electronic scale (Tanita Model BWB-800, Tokyo, Japan), and blood pressure was measured using an automatic occlusion cuff (BP742 HEM-7200-Z, OMRON Healthcare Inc., Lake Forest, IL, USA). Basal body temperature each morning and temperature at time of testing were measured to the nearest 0.1°C with a digital thermometer (iProvèn Model BBT-113Ai, Beaverton, OR, USA) and blood lactate concentration was measured to the nearest 0.1 mmol/L using the Lactate Plus Analyzer and test strips (Hart et al.,

2013). Participants were fitted with a Polar Heart Rate Sensor and elastic band (Polar Inc. Model H1, Bethpage, NY, USA) that measures heart rate via Bluetooth sensor on the bike. The participants were also fitted to the Excalibur Sport Lode Cycle Ergometer (Lode BV, Groningen, NED) and seat and handlebar height and location were recorded on his/her data sheet to insure comfortable consistency between visits. Next, participants were shown a laminated copy of a modified Borg scale ranging from 0 indicating "no exertion at all" to 10 indicating "maximal exertion" (Haddad et al., 2017). They were instructed to point to numbers corresponding to their overall RPE rating then instructed to "zoom in and really focus on how their lower limbs are feeling" to report localized RPE rating of the lower limbs. Dunbar et al. (1992) found this method of collecting RPE to be more reliable in maximal GXTs using a cycle ergometer compared to a treadmill and stated that this method can provide a valid method of regulating exercise (Dunbar et al., 1992).

Participants were then fitted with a mouthpiece, two-way non-rebreathing valves (Hans Rudolph Inc. Series 2700, Shawnee, KS, USA), noseplugs, and appropriately sized headgear. Participants were then informed of the cycle protocol used in this study – a ramp protocol that originated at 0.5 watt per kilogram of the participant's body weight and increased every 60 seconds by 0.5W/kg body weight (Larson et al., 2015). Participants were instructed to maintain a comfortable cadence around 60 rpm, but no lower than 40 rpm. This protocol is normally used to establish VO_{2max}, but the participants completed only a portion of the protocol at this familiarization visit and stopped once his/her overall RPE using the modified Borg scale (0-10) score was 5-6. This gave them the ability during this first visit to adequately grasp the physical demand of the future visits. HR, VO_2, RER, overall RPE, and localized RPE were collected at the end of each phase, and participants were told that lactate concentration

will be measured via a finger prick at the end of the cycle protocol on regular testing days. This second measure of lactate concentration was omitted during the familiarization visits since the value would not be representative of a true maximal effort and to minimize any unnecessary discomfort from another finger stick.

Post-Visit 1

Both male and female participants were instructed to take their BBT measurements using the provided thermometer each morning immediately after waking and prior to performing any other actions in order to ensure the most accurate reading. BBT was tracked between 1-4 weeks before data collection begun, depending upon the menstrual cycle phase that the participant was currently experiencing at the time of enrollment in the study. This process established a baseline of BBT values for each individual and aided in the confirmation of ovulation in the eumenorrheic female participants (McClure Browne, 1973).

Visit 2, 3, and 4

The next three visits were dependent on the BBT reading and determination of menstrual cycle phase per each individual from the menstrual cycle questionnaire. Each visit was identical in protocol but corresponded to each identified menstrual cycle phase of interest. The female participants were asked to come in during menses (day 0-3), the day of or within 24 hours of the spike in BBT $\geq 0.3°C$ indicating ovulation, and around 7 days following ovulation to indicate the middle of the luteal phase, entitled in this study mid-luteal (approximately day 20-24). Male participants were randomly matched to come in on analogous days according to a predicted 28-day cycle.

Upon arrival, participants completed the IPAQ and POMS-B questionnaires. Then, the participant's height, weight, resting HR, temperature, BP, and resting blood lactate concentration were measured. The female participants' BBT morning measure and menstrual cycle phase was recorded. Next, the mouthpiece, headgear, and cycle ergometer seat and handlebars were fitted to the participant. Participant ID and characteristics were input into the metabolic cart software. The cycle protocol consisted of the same ramp protocol that was demonstrated at Visit 1 which begins with 0.5 watt per kilogram of the participant's body weight for a 2-minute warm-up and increases every 60 seconds by 0.5W/kg body weight (Larson et al., 2015). Overall RPE, localized RPE, VO_2, RER, and HR were measured and recorded at the conclusion of each 60-second exercise stage. Participants were instructed to cycle against the increasing resistance until he/she reached volitional fatigue, or the cadence fell below 40 rpm. Motivational music and encouragement from the researcher were given to each participant evenly throughout each test. Maximal lactate concentration was measured and recorded as soon as the participant reached volitional fatigue. It was assumed that this graded-exercise test protocol will reflect a true VO_{2max} according to the American College of Sports Medicine. The standardized criteria for confirming a true VO_{2max} include most importantly, 1) a plateau in VO_2 with an increase in work load, 2) an RPE > 17 on the original Borg scale (~ 8 on the modified scale), 3) HR reaching 90% of age-predicted max using the calculation 220 – age, 4) RER > 1.15, and/or 5) blood lactate levels > 8 mmol/L (ACSM, 2010). Following volitional fatigue from the participant, measurements for maximal lactate concentration, overall RPE, localized RPE, VO_2, HR, and time to failure were collected and recorded before the participant entered a cool-down period at 25 watts. The participant cycled comfortably (cadence around 30-50 rpm) until his/her HR was ≤ 130 bpm. Then the researcher removed the

headgear and mouthpiece at this time for comfort. To ensure participants did achieve a maximal test the researcher then started a 20-minute period for the participant to rest before administering a verification test on the cycle ergometer. The load was set at the wattage corresponding to the final stage the participant had just completed when their maximal GXT concluded. The verification test began with a 10-second warm-up at 0.5 watts per kilogram of the participant's body weight and increased in every 5 seconds to ensure that the participant has reached the maximal set load (W) within 30 seconds of cycling. If the participant maintained a cadence of at least 40 rpm for 5 minutes or the majority of 5 minutes, or if the participants VO_2 value visibly exceeded the previous measure, then the participant's previous maximal graded exercise test was deemed invalid and the visit was rescheduled. If these criteria were not met and the participant stopped cycling or cycled at a cadence < 40 rpm within 5 minutes, then the original maximal test was considered valid and all measured parameters during both the maximal and verification test were recorded. The participant entered a similar cool down protocol at 25 watts and was instructed to dismount the bike when his/her HR measured ≤ 130 bpm and he/she felt comfortable to walk and exit the lab safely. Following this verification test, the researcher removed the mouthpiece, headgear, and heart rate monitor to begin cleaning and sterilizing the equipment according to manufacturer recommendations.

Post-Visits 2, 3, and 4

Twenty-four hours after each visit, the participant was asked to complete a Perceived Recovery Status (PRS) scale. This involved the participant reporting a single value back to the researcher to attempt to assess how recovered he/she felt 24 hours following the visit. Optional answers for this scale range from 0 indicating "very poorly recovered and extremely tired" to

10 indicating “very well recovered and highly energetic” (Laurent et al., 2011). The researcher recorded this value and instructed the participant to continue to collect and report accurate BBT in the mornings as well as to complete the PRS questionnaire the day following every data collection visit. Following the final visit (Visit 4 – M, O, or L), the participant was no longer required to monitor BBT and returned the thermometer to the laboratory, but the recovery questionnaire was still completed the next day and reported to the researcher.

Data Management

Once participant information and questionnaires were completed during the familiarization visit (Visit 1), the participant ID was assigned, and this ID was the only way to identify the participant for the remainder of his/her involvement. Completed questionnaires and forms were labeled with participant ID and kept in a locked file cabinet within the Body Composition and Human Performance Laboratory. Following the measurement of all variables after each visit, the data was input into a password-protected Microsoft Excel file on a password-protected desktop computer. Only the primary researcher and secondary data collectors acknowledged on the IRB form had key access to the locked file cabinet, and only the researcher and primary investigator had knowledge of the passwords to the desktop and the separately locked file. This password protected the confidentiality of each participant and followed HIPPA, CITI, and IRB guidelines.

Data Analyses

Statistical analysis for this study was performed using IBM SPSS Statistics (Version 26). The alpha level of significance will be set at $\alpha = 0.05$ for all tests. First, descriptive statistics were run to calculate demographic characteristics of the sample, and evaluate

objective and subjective parameters, which will further be expressed as mean ± standard deviation (SD). Then, an analysis of variance (ANOVA) with repeated measures for time between the sexes [2 x 3] was utilized to analyze the differences in height, weight, maximal HR, the difference between resting and maximal blood lactate concentration, absolute and relative values for VO_{2max}, difference in VO_{2max} between the maximal test and the verification test for both relative and absolute values, RER, time to failure, maximal load, maximal overall RPE, maximal localized RPE, and recovery rating between visits M, O, and L. Results from the "vigor" and "fatigue" categories from the POMS mood questionnaire were analyzed to screen for changes in mood prior to exercise using the same 2 x 3 ANOVA. Results from the ANOVA included main effects of phase and sex and a phase*sex interaction. Then, the file was split between the sexes and a repeated-measures ANOVA for the three phases was run to further identify differences. If the p-value from the ANOVAs were significant, pairwise comparisons using a Bonferroni post-hoc test compared each phase time point to pinpoint where significant differences were shown – both in the whole sample file and the split file version of the data. Effect size for each variable were calculated using partial eta squared (η^2) in order to quantify the statistical results. A small effect size was considered 0.01-0.05, medium effect size is 0.06-0.13, and a large effect size is $\geq$ 0.14 (Lakens 2013).

CHAPTER IV: RESULTS

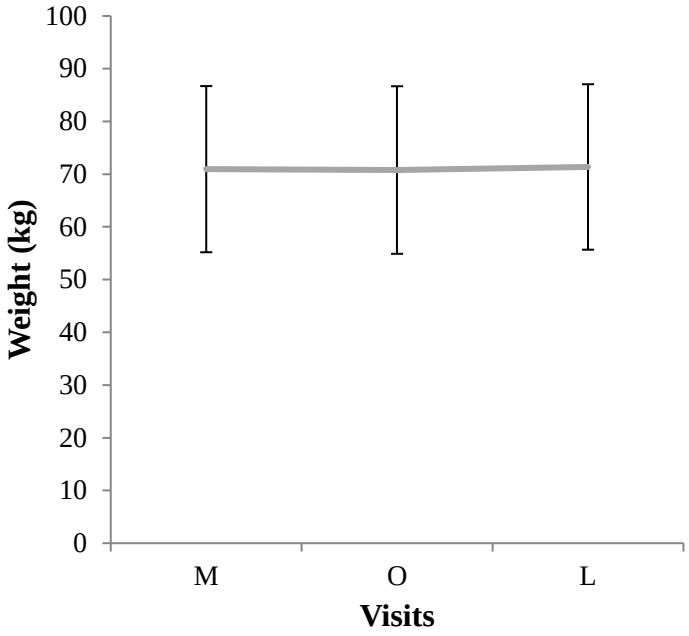
Participant Characteristics

Descriptive data for the entire sample – including age, height, and weight – as well as length of participation are reported below in Table 1 as mean ± SD. Results of the 2 x 3 repeated measures ANOVA for sex and three phases showed that there were no statistically significant main effects from phase or sex, but that there was a significant phase*sex interaction for weight ($p = 0.026$). There was also a significant difference in height between the sexes ($p = 0.009$) indicating that males were taller, but no significant differences for phase or a phase*sex interaction were found. Results of the 2 x 3 ANOVA showed no significant differences for the "fatigue" or "vigor" categories when scoring the POMS mood status questionnaire, which signifies that there were no alterations in mood prior to exercise between cycle phases. For visit order, three males and three females completed the visits starting with Visit M, then O, and Visit L last. Four males and two females completed the order Visit O, L, and M. Two males and six females completed the order starting with Visit L, M, then O. One female participant completed the visits out of order, beginning with Visit M, then L, then O due to out-of-state travel during her spike in BBT. Female participants were tested between day 0-3 of menses (mean = 2.08 ± 1.16 days) and had an average gap between of 14.67 ± 2.57 days between Visit M and Visit O. Females had an average BBT of 36.21 ± 0.25°C on the morning of Visit M and 36.27 ± 0.38°C the morning of Visit L. On the morning of Visit O, females had an average BBT 36.62 ± 0.32°C with an average spike in BBT of 0.41 ± 0.07°C from the previous day's morning temperature recording, which exceeds the standard of 0.3°C from prior literature. Male BBT recordings on the mornings of Visit M, O, and L were 36.18 ± 0.21°C, 35.99 ± 0.34°C, and 35.92 ± 0.29°C, respectively.

Table 1: Participant Characteristics

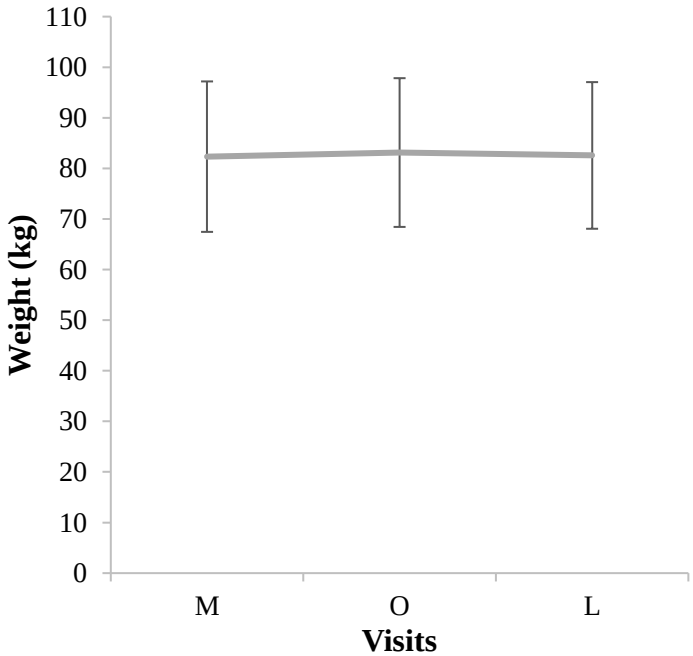
Variable	Whole Sample (n = 21)	Females (n = 12)	Males (n = 9)
Age (years)	21.38 ± 1.32	21.25 ± 1.06	21.56 ± 1.67
Height (cm)	172.05 ± 8.50	167.88 ± 5.99*	177.61 ± 8.38*
Weight † (kg)	76.05 ± 16.06	70.93 ± 15.80	82.89 ± 14.47
Length of Participation (days)	42.71 ± 9.95	45.92 ± 11.33	38.44 ± 5.94

Values are displayed as mean ± SD.
(*) indicates a significant sex difference ($p < 0.05$).
(†) indicates a significant phase*sex interaction ($p < 0.05$).



No significant phase difference ($p > 0.05$).

Figure III: Weight (kg) ± SD across visits for females (n = 12)



No significant phase difference ($p > 0.05$).

Figure IV: Weight (kg) ± SD across visits for males (n = 9)

Objective Parameters

Results for absolute VO_{2max} (L/min), absolute VO_2 difference (value from maximal test – verification test) (L/min), relative VO_{2max} (ml/kg/min), relative VO_2 difference (maximal test – the verification test) (ml/kg/min), HR max (bpm), RER, lactate increase from resting to maximal (mmol/L), time to failure (seconds), and maximal load (watts) are separated by sex and are displayed below – females in Table 2-A, and males in Table 2-B. No significant differences were found for any of these variables as phase differences within-subjects. In regard to sex differences, males showed statistically significantly higher values than females for absolute VO_{2max} ($p < 0.001$), relative VO_{2max} ($p = 0.026$), and maximal load in watts ($p = 0.001$). Since there were no phase differences for relative VO_{2max} (ml/kg/min), stability between the maximal GXT and the verification test are visually represented below for females (Figure V-A) and males (Figure V-B) between Visit M, Visit O, and Visit L.

Effect sizes using partial eta squared (η^2) are reported for the sex differences below in Table 3. Effect sizes for these sex differences were considered large for absolute VO_{2max}, relative VO_{2max}, maximal workload ($\eta^2 = 0.49$, 0.24, and 0.47 respectively). Large effect sizes for sex differences were also found for weight, absolute VO_2 difference, and time to failure ($\eta^2 = 0.14$, 0.16 and 0.19). Relative VO_2 difference, maximal HR, and lactate difference exhibited a medium effect size for sex differences ($\eta^2 = 0.13$, 0.13, and 0.11). A large effect size was shown for the phase*sex interactions of weight ($\eta^2 = 0.17$). Moderate effect sizes were seen in relative VO_{2max}, lactate difference, and time to failure ($\eta^2 = 0.06$, 0.08, and 0.10).

Once the file was split between the sexes, there were still no apparent statistically significant differences in these variables between the three phase time points. Reported in

Table 2-A, large effect sizes for phase differences were seen in females for maximal HR and RER (η^2 = 0.18 and 0.16). Moderate effect sizes between phase differences were found in females for the following variables: absolute VO_{2max} (η^2 = 0.13), absolute VO_2 difference (η^2 = 0.11), relative VO_{2max} (η^2 = 0.10), relative VO_2 difference (η^2 = 0.10), and lactate difference (η^2 = 0.12), though there were no statistically significant phase differences of these objective parameters. Similarly, in Table 2-B, males had large effect sizes for phase differences in maximal HR and time to failure (η^2 = 0.14 and 0.18), while medium effect sizes were shown in relative VO_{2max}, relative VO_2 difference, and lactate difference (η^2 = 0.08, 0.10, and 0.08), though none exhibited statistical significance.

Table 2-A: Female VO_2 data (n = 12)

Variable	Visit M (Menses)	Visit O (Ovulation)	Visit L (Luteal)	Phase Effect Size (η^2)
Absolute VO_{2max} (L/min)	2.05 ± 0.39*	2.09 ± 0.43*	2.12 ± 0.45*	0.13 φ
Absolute VO_2 Difference (L/min)	0.02 ± 0.11	0.11 ± 0.24	0.12 ± 0.15	0.11 φ
Relative VO_{2max} (ml/kg/min)	29.59 ± 6.26*	30.23 ± 6.80*	30.42 ± 6.95*	0.10 φ
Relative VO_2 Difference (ml/kg/min)	0.20 ± 1.52	1.49 ± 3.62	1.53 ± 2.09	0.10 φ
HR max (bpm)	175.96 ± 9.04	179.63 ± 7.18	179.42 ± 7.63	0.18 ¥
RER	1.23 ± 0.08	1.26 ± 0.07	1.22 ± 0.09	0.16 ¥
Lactate Difference (mmol/L)	8.34 ± 1.59	8.83 ± 1.54	9.08 ± 2.08	0.12 φ
Time to Failure (seconds)	521.50 ± 68.75	526.08 ± 69.15	521.67 ± 75.02	0.03
Maximal Load (watts)	202.42 ± 40.68*	206.67 ± 38.27*	201.25 ± 41.41*	0.05

Values are displayed as mean ± SD. "Lactate Difference" indicates value from maximal lactate value – resting value. "Absolute VO_2 Difference" and "Relative VO_2 Difference" indicate values from maximal GXT – verification test. Positive values indicate that the maximal GXT was higher than the verification test.
(*) indicates a significant sex difference ($p < 0.05$).
(φ) indicates medium effect size (0.06 – 0.13).
(¥) indicates large effect size (≥ 0.14).

Table 2-B: Male VO_2 data (n = 9)

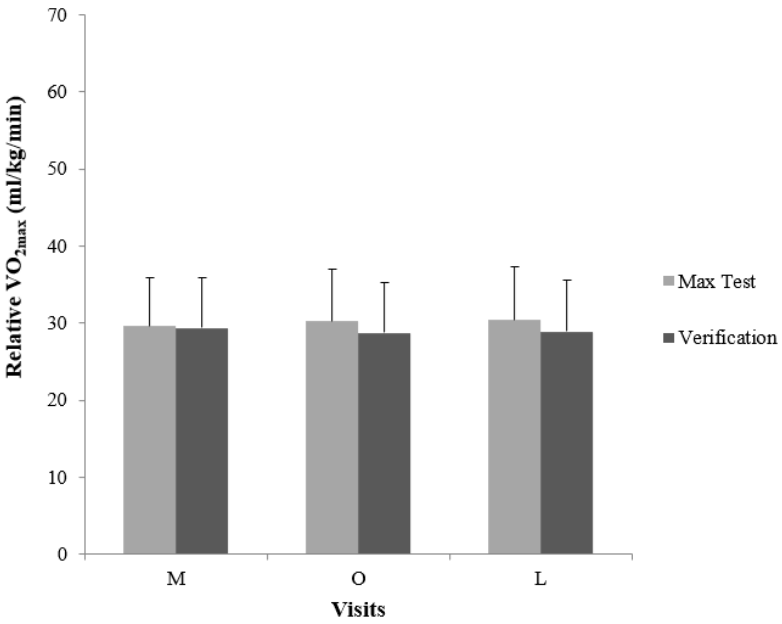
Variable	Visit M (Menses)	Visit O (Ovulation)	Visit L (Luteal)	Phase Effect Size (η^2)
Absolute VO_{2max} (L/min)	3.21 ± 0.82*	3.17 ± 0.73*	3.20 ± 0.71*	0.03
Absolute VO_2 Difference (L/min)	0.26 ± 0.22	0.25 ± 0.44	0.21 ± 0.21	0.02
Relative VO_{2max} (ml/kg/min)	39.77 ± 11.64*	38.99 ± 10.57*	39.79 ± 11.68*	0.08 φ
Relative VO_2 Difference (ml/kg/min)	3.01 ± 2.50	2.84 ± 5.15	2.64 ± 2.65	0.10 φ
HR max (bpm)	175.56 ± 12.88	172.89 ± 8.29	170.06 ± 6.34	0.14 ¥
RER	1.23 ± 0.08	1.23 ± 0.05	1.23 ± 0.05	0.00
Lactate Difference (mmol/L)	10.00 ± 1.76	9.43 ± 1.42	9.68 ± 1.34	0.08 φ
Time to Failure (seconds)	607.33 ± 113.80	592.00 ± 92.90	607.44 ± 109.22	0.18 ¥
Maximal Load (watts)	288.78 ± 65.90*	290.00 ± 54.77*	292.44 ± 56.82*	0.02

Values are displayed as mean ± SD. "Lactate Difference" indicates value from maximal lactate value – resting value. "Absolute VO_2 Difference" and "Relative VO_2 Difference" indicate values from maximal GXT – verification test. Positive values indicate that the maximal GXT was higher than the verification test.
(*) indicates a significant sex difference ($p < 0.05$).
(φ) indicates medium effect size (0.06 – 0.13).
(¥) indicates large effect size (≥ 0.14).

Table 3: Objective variables effect size for sex differences and phase*sex interactions for whole sample (n = 21)

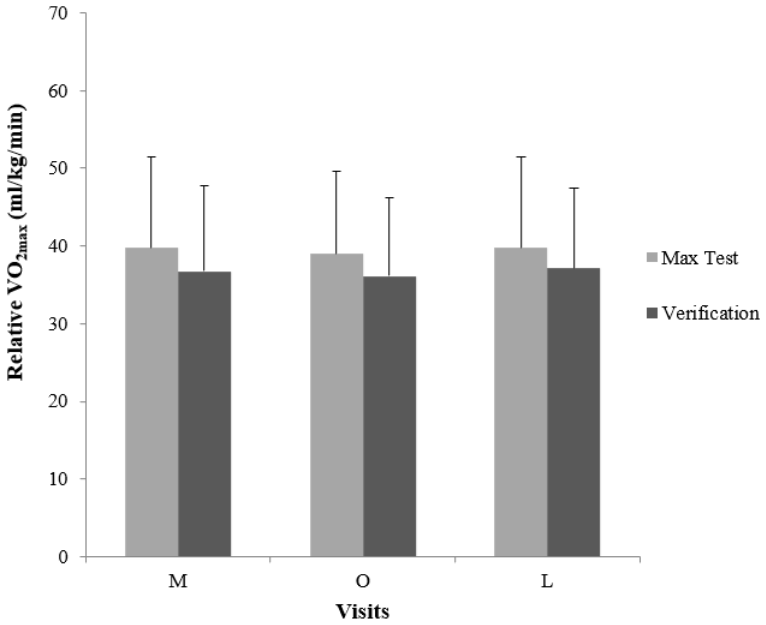
Variable	Sex Effect Size (η^2)
Weight (kg)	0.14 ¥
Absolute VO_{2max} (L/min)	0.49 ¥
Absolute VO_2 Difference (max – ver)	0.16 ¥
Relative VO_{2max} (ml/kg/min)	0.24 ¥
Relative VO_2 Difference (max – ver)	0.13 φ
HR max (bpm)	0.13 φ
RER	0.00
Lactate Difference (mmol/L)	0.11 φ
Time to Failure (seconds)	0.19 ¥
Maximal Load (watts)	0.47 ¥

"Lactate Difference" indicates value from maximal lactate value – resting value. "Absolute VO_2 Difference" and "Relative VO_2 Difference" indicate values from maximal GXT – verification test.
(φ) indicates medium effect size (0.06 – 0.13).
(¥) indicates large effect size (≥ 0.14).



No significant phase difference ($p > 0.05$).

Figure V-A: Stability of relative VO_{2max} (ml/kg/min) for females (n = 12) at each visit (mean ± SD)



No significant phase difference ($p > 0.05$).

Figure V-B: Stability of relative VO2max (ml/kg/min) for males (n = 9) at each visit (mean ± SD)

Subjective Parameters

The subjective parameters measured during this study, which were overall rating of perceived exertion (RPE), localized RPE of the lower limbs, and rating of recovery the following day according to the perceived recovery scale (PRS) (Laurent et al., 2011). These values are displayed below for females (Table 4-A) and males (Table 4-B) as mean ± SD, along with effect size for phase differences of these subjective variables. Results of the 2 x 3 ANOVA for overall RPE in the whole sample (n = 21) showed that there was a statistically significant phase difference ($p = 0.039$) and phase*sex interaction ($p = 0.039$), but there was not a significant sex difference, seen in Figure VI. Overall RPE showed a small effect size for sex differences ($\eta^2 = 0.02$). Pairwise comparisons using a Bonferroni correction showed that when comparing Visit M to O, M to L, and O to L no significant differences were present between the phases for overall RPE even though there were significant phase differences and a phase*sex interaction. Localized RPE (Figure VII) showed no significant differences between the sexes or the phases. Recovery rating, however, showed that males had statistically higher ratings than females for recovery 24 hours post-test at $p = 0.031$ with a large effect size ($\eta^2 = 0.22$), and phase differences and a phase*sex interaction both at $p < 0.001$. Pairwise comparisons for recovery rating in the whole sample showed that Visit M was significantly lower than both Visit O and Visit L ($p < 0.001$), but visit O was not different from visit L, seen below in Figure VIII.

Once the data file was split for sex, pairwise comparisons showed that the female sample had phase differences for several subjective parameters (Table 4-A). Results from overall RPE exhibited that Visit M was significantly higher than Visit O ($p = 0.005$) and Visit L ($p = 0.007$) and Visit O and L were not different from one another. These phase differences

for overall RPE exhibited a rather large effect size at $\eta^2 = 0.46$. Localized RPE of the lower limbs was significantly higher on Visit M compared to Visit O ($p = 0.038$) but not Visit M to Visit L or Visit O to Visit L. These differences also exhibited a large effect size at $\eta^2 = 0.31$. Recovery rating was also significantly lower during Visit M compared to Visit O and Visit L ($p < 0.001$) and not between Visit O and Visit L, with a rather large effect size of $\eta^2 = 0.79$ (Table 4-A). The male sample had no significant phase differences and small effect sizes for each of these subjective parameters (Table 4-B).

Table 4-A: Female subjective parameters (n = 12)

Variable	Visit M (Menses)	Visit O (Ovulation)	Visit L (Luteal)	Phase Effect Size (η^2)
Overall RPE€ †	8.92 ± 0.79	7.67 ± 1.23	7.75 ± 1.06	0.46 ¥
Localized RPE€	9.25 ± 0.75	8.58 ± 0.99	8.83 ± 1.03	0.31 ¥
PRS Rating€ †	6.83 ± 0.94*	8.83 ± 1.12*	8.67 ± 0.65*	0.79 ¥

Values are displayed as mean ± SD.
(*) indicates a significant sex difference ($p < 0.05$).
(€) indicates a significant phase difference ($p < 0.05$).
(†) indicates a significant phase*sex interaction ($p < 0.05$).
(¥) indicates large effect size (≥ 0.14).

Table 4-B: Male subjective parameters (n = 9)

Variable	Visit M (Menses)	Visit O (Ovulation)	Visit L (Luteal)	Phase Effect Size (η^2)
Overall RPE †	8.33 ± 1.23	8.33 ± 1.00	8.33 ± 1.00	0.00
Localized RPE	9.11 ± 1.36	9.33 ± 0.50	9.22 ± 0.67	0.02
PRS Rating †	9.00 ± 1.00*	8.89 ± 0.78*	9.00 ± 1.12*	0.02

Values are displayed as mean ± SD.
(*) indicates a significant sex difference ($p < 0.05$).
(†) indicates a significant phase*sex interaction ($p < 0.05$).

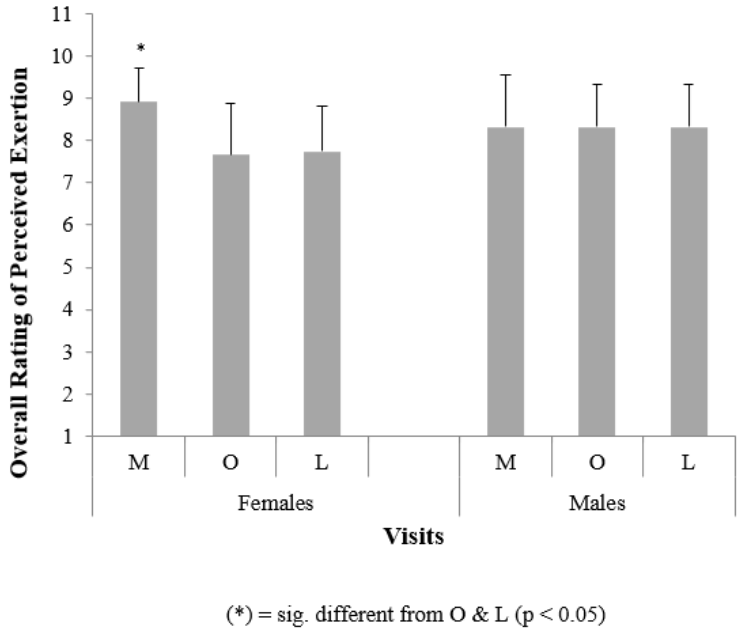


Figure VI: Comparison between females (n = 12) and males (n = 9) for overall rating of perceived exertion (RPE) on a 10-point scale (mean ± SD)

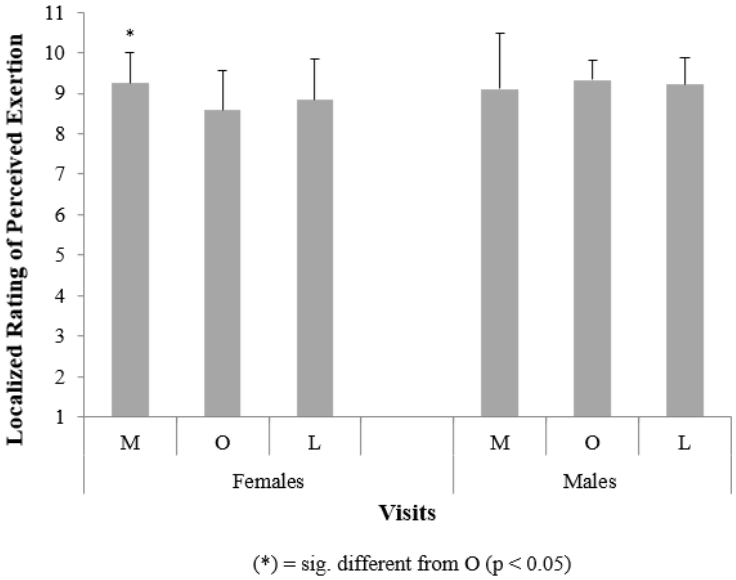


Figure VII: Comparison between females (n = 12) and males (n = 9) for localized rating of perceived exertion (RPE) on a 10-point scale (mean ± SD)

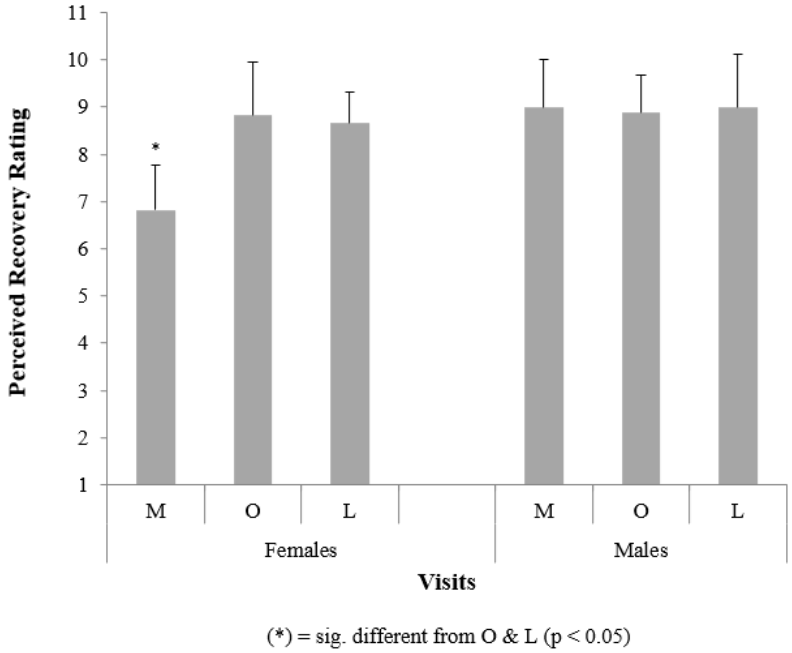


Figure VIII: Comparison between females (n = 12) and males (n = 9) for perceived recovery rating (PRS) on a 10-point scale (mean ± SD)

CHAPTER V: DISCUSSION

The purpose of this study was to use a maximal graded-exercise test to determine if there were differences in objective and subjective aerobic performance parameters across three time points in the menstrual cycle – menses, ovulation, and the mid-luteal phase. The objective variables tested in this study include height, weight, absolute and relative VO_{2max}, difference from resting to maximal blood lactate concentration, maximal HR, RER, time to failure, and maximal load. The subjective variables measured during and after exercise testing included RPE of the body as a whole, localized RPE of the lower limbs, and recovery rating twenty-four hours post-test. It was hypothesized that menstrual cycle would impact the measured variables and that there would be differences in all the measured objective and subjective parameters between the three exercise visits. Specifically, it was predicted that the ovulatory phase may positively impact performance variables by increasing VO_{2max}, decreasing ratings of perceived exertion, and increasing recovery rating due to an increase in hypothalamic activity and basal body temperature during this phase.

Objective Parameters

Despite testing across three specific time points within the menstrual cycle including ovulation and menses, our results were in agreement with several previous studies for the objective parameters measured (Gordon et al., 2017; Bemben et al., 1995; Smekal et al., 2007). This study found that each of the aerobic performance and cardiovascular factors in healthy, college-aged eumenorrheic females were unchanged across the three menstrual cycle phases that were tested. These results are conflicting with the finding from Lebrun et al. (1994), whom reported absolute VO_{2max} was lower in the luteal phase than in the follicular phase and

Godbole et al. (2016) whom reported that VO_{2max} was significantly decreased during the premenstrual phase compared to the postmenstrual phase due to a decreased efficiency of the cardiorespiratory system during this time (Lebrun et al., 1994; Godbole et al., 2016). These findings were, however, consistent with previous findings from Gordon et al. (2017), Stephenson et al. (1982), Bemben et al. (1995), and Smekal et al. (2007), even though each of these studies examined different menstrual cycle phases and used different maximal aerobic exercise testing protocols opposed to the ones used in this study (Gordon et al., 2017; Stephenson et al., 1982; Bemben et al., 1995; Smekal et al., 2007). Hall-Jurkowski et al., (1981) did not examine VO_{2max}, but did find that other objective variables, such as blood lactate, maximal HR, and RER were unchanged during maximal graded-exercise tests across the menstrual cycle, as did Lebrun et al. (1994) (Hall-Jurkowski et al., 1981; Lebrun et al., 1994).

Results showed that there were significant sex differences with large effect sizes for absolute VO_{2max}, relative VO_{2max}, and maximal load, each of which males recorded higher values. This finding is not unexpected according to similar findings from Kaminsky et al. (2015), who established average values for relative VO_{2max} (ml/kg/min) in males and females across six age groups, separated by decade (Kaminsky et al., 2015). Results of our study showed that male and female participants ages 19-24 years obtained lower relative VO_{2max} values compared to Kaminsky et al.'s recommended findings of average 20-29-year old's, though the average for both sexes was within the average range reported. This study showed that males achieved a relative VO_{2max} of 39.5 ± 10.9 ml/kg/min, compared to 47.6 ± 11.3 mLO_2/kg/min, and that females achieved a relative VO_{2max} of 30.1 ± 6.5 ml/kg/min, compared to 37.6 ± 10.2 mLO_2/kg/min (Kaminsky et al., 2015). This lower finding is likely due to the

fact that we performed graded-exercise tests on a cycle ergometer, whereas Kaminsky et al. used a different protocol on a treadmill, which usually elicits a higher VO_{2max}.

Results for maximal HR in this study for males and females were found to be reliably and noticeably low throughout the exercises test compared to the age-predicted maximal value. This is a reasonably common finding in maximal GXTs that utilize a cycle ergometer compared to a treadmill and is consistent with findings from Tanaka et al. (1991) and Larson et al. (2015), who established the protocol used in this study (Tanaka et al., 1991; Larson et al., 2015).

Subjective Parameters

The important findings from this study include how overall RPE, localized RPE, and recovery in the female participants significantly varied across the menstrual cycle, each with rather large effect sizes. Results showed that females presented significantly lower scores across all visits for recovery rating compared to males, though this finding may not be relevant according to Figure VIII. It was also interesting to see that females reported significantly higher maximal overall RPE scores during the menstrual visit, when compared to the ovulatory and mid-luteal visits. Females also exhibited a significantly higher localized RPE score of the lower limbs during the menstrual visit compared to the ovulatory but not the luteal visit, and the ovulatory and luteal visits were not significantly different. Lastly, females reported significantly lower PRS (recovery) values during the menstrual visit compared to both the ovulatory and luteal visits. These results imply that during menses, females felt as if they were exerting more during the maximal graded-exercise test and felt less recovered twenty-four hours following their maximal aerobic exercise bout. This finding contrasts the

findings of Stephenson et al., (1981), Lara et al. (2019), and Bailey et al. (2000) who each claimed there were no changes in RPE related to different testing days during the menstrual cycle, though they each measured different exercise intensities and menstrual cycle phases (Stephenson et al., 1981; Lara et al., 2019; Bailey et al., 2000). These results also contradict the findings of Hackney et al. (1991) which determined that RPE of the lower limbs, or L-RPE, was significantly greater during the ovulatory phase compared to the follicular and luteal phases (Hackney et al., 1991). On the other hand, these findings are analogous to results found by Caldwell Hooper et al. (2011), who measured RPE and perceived pain during a treadmill exercise protocol at 65% of measured VO_{2max} in regularly menstruating females (Caldwell Hooper et al., 2011). These authors concluded that RPE and perceived pain ratings were higher in females during the early follicular phase when compared to the late follicular and luteal phases. In an attempt to rationalize findings from the current study, a deeper look into physiology of the sex hormones is required.

A study by Tousignant-Laflamme and Marchand (2009) claims that only inhibitory mechanisms of pain fluctuate throughout the menstrual cycle and operate more effectively around the time of ovulation (Tousignant-Laflamme & Marchand, 2009). Similarly, Veldhuijzen et al. (2013) found that lower pain thresholds were found in the mid-follicular phase compared to the ovulatory and mid-luteal phases (Veldhuijzen et al., 2013). They also measured activation in different areas of the brain associated with pain using functional brain imaging and believe that fluctuations in pain sensitivity across the menstrual cycle, specifically the follicular phase, are due to cognitive pain and higher bodily awareness (Veldhuijzen et al., 2013). Another study by Teepker et al. (2010) tested pain using several different stimuli – cold, pressure, and electrical – and found that each had higher threshold levels outside of

menses, specifically on days 14 or 22 of the menstrual cycle (Teepker et al., 2010). However, results from a study by Choi et al. (2006) conflict with each of these findings, claiming that pain and unpleasantness ratings were significiantly higher during the luteal phase, not the follicular phase, while Ring et al. (2009) reported that pain ratings were unchanged between the luteal and follicular phases (Choi et al., 2006; Ring et al., 2009). The findings from Tousignant-Laflamme and Marchand (2009), Veldhuijzen et al. (2013), and Teepker et al. (2010) are minimally akin to results from this study, due to the obscurity of comparing different physiological expressions of discomfort, like comparing "pain" to "exertion". These results may not be fully comparable to the results of this study, but they may provide some possible explanations of the current results. These authors suggest that the justification for higher discomfort ratings, may be due to increased sensitivity, decreased effectiveness of the inhibitory pathways, or a higher awareness to any kinesthetic discomforts during menses, or the early follicular phase (Tousignant-Laflamme & Marchand 2009; Veldhuijzen et al., 2013; Teepker et al., 2010). The previously mentioned study by Caldwell Hooper et al. (2011) which measured RPE and perceived pain during treadmill exercise concluded that RPE and perceived pain ratings were higher in females during the early follicular phase, and attributed this change to a drop in concentrations of both the sex hormones, estradiol and progesterone, during this phase (Caldwell Hooper et al., 2011). This suggestion provides a reasonable explanation in congruence with the finding of Teepker et al. (2010) and may support claims of varying menstrual cycle physiology impacting rating of perceived exertion and recovery rating in this study and previous other studies; however, the cause of this finding is still widely undetermined and elicits a need for more comprehensive research. In actuality, there may be a more complicated interaction of physiological elements and hormonal function that may be

responsible for the influence behind this finding (Caldwell Hooper et al., 2011; Teepker et al., 2010).

Summary of Key Findings

The aim of this study was to observe if there are differences in objective and subjective parameters of a maximal graded-exercise test between phases of the menstrual cycle. The results of this study demonstrated no significant differences for the objective variables collected from the maximal exercise test between the phases; therefore, the first null hypothesis failed to be rejected. An interesting finding from this study, which strays from the original purpose, but is still worth noting, is the stability of VO_{2max} values for both males and females across 3-6 weeks of exercise testing. Though there were no significant changes across the testing days for males or females, it is important to note that the average relative VO_{2max} values for males and females only fluctuated about 1.0 ml/kg/min. This is a smaller value than a study by Katch et al. (1982), which claimed that total within-subject variation was around 3.2 ml/kg/min (Katch et al., 1982). When comparing the difference of VO_2 values from the maximal GXT and the verification test (max – ver), the average change was 0.15 ± 0.25 L/min (min = -0.16 L/min) for absolute VO_2 and 1.83 ± 3.11 ml/kg/min (min = -2.15 ml/kg/min) for relative VO_2.

Female participants exhibited significant differences between the phases for the subjective parameters during and following the maximal exercise tests. Results showed that females had statistically higher ratings of perceived exertion during the menstrual phase, both overall and for the lower limbs, and a statistically lower recovery rating corresponding to the menstrual phase as well. Explanations for this finding are still unknown but could be attributed

to variation of perceived physical symptoms related to premenstrual syndrome, higher kinesthetic awareness in the brain, decreases of inhibitory pathway action, drop in sex hormone concentration and basal body temperature, or other physical phenomena that may happen during menstruation that is not known to the scientific community yet. The null hypothesis is rejected in this case, but the research hypothesis was partially correct. This hypothesis stated that Visit O would be higher in each variable due to the subsequent thermogenic activity of the hormones involved during this phase. Results of this study found that Visit M was significantly different from Visit O for all three subjective parameters measured; however, Visit O was not significantly different from Visit L for any of the subjective parameters.

Limitations

This study included several limitations before being able to generalize the results to other healthy, college-age eumenorrheic females. The most pressing limitation may be the method of tracking basal body temperature (BBT) to distinguish ovulation in the female participants. This method does not directly measure hormone concentration and therefore, cannot technically confirm the presence of ovulation in this group of participants, like assays of blood and urine collection would be able to do. This methodology raised the assumption that ovulation was accurately being tested following the spike in BBT. As mentioned previously in Chapter I, there are many things that could malfunction in regard to the hormonal and physiological actions of the menstrual cycle that may not be disclosed by using this BBT method; therefore, some of the participants in the current study may suffer from irregular menstrual function and timing or hormonal interaction, which may have impacted the current findings. It has been articulated that BBT tracking may not be the most reliable method

of tracking menstrual cycle and estimating ovulation, according to Guermandi et al. (2001); however, the cost and practicality of this method reinforced its selection of use to schedule the ovulatory visit in this study (Guermandi et al., 2001).

Another limitation is the methodology used in this study. The ramp cycle protocol used may not be a popularly used ramp protocol, but it provided work rates relative to body weight of each individual participant instead of absolute work rates, and Larson et al. (2015) previously studied and established these guidelines for recreating the protocol (Larson et al., 2015). It was recommended that this study utilize the 20-minute verification protocol to distinguish VO_{2max} from VO_{2peak} during each GXT. This may not be the most popular methodology to do so, but in congruence, VO_{2max} was also confirmed using ACSM's recommendations to establish a plateau of VO_2 values within 2 ml/kg/min on average within the last 30 seconds of testing.

The measurement of RPE is another controversial matter. Haddad et al. (2017) reported that measures of RPE may be influenced by gender, age, fitness level, and familiarity with the testing equipment and protocol (Haddad et al., 2017). Psychological factors possibly including leadership, psychological resistance, and endurance level were previously found to have influences on RPE rating (Coquart et al., 2012). This study did attempt to control for psychological impacts on the measured parameters, including perceived exertion rating by using the POMS-B mood questionnaire as a screening tool. Another small limitation may include that one female participant did not complete all visits in the correct order based off of her enrollement in the study due to out-of-state travel. Though she completed all three visits and her results were not significantly different, this stray from the correct scheduling is important to mention.

The results of this study can only be generalized to a small population of healthy college-aged, eumenorrheic females and healthy, college-aged males, due to the strict inclusion criteria. This criteria omitted participants who used external hormonal influences, participants who were highly endurance trained to eliminate a training effect, participants older than 24 years of age, and females that may be using hormonal contraceptives or have irregular menstrual cycles. Each of these conditions may further influence maximal exercise performance outside of menstrual cycle effects and should be considered when referencing results from this study.

CHAPTER IV: CONCLUSION

The results of this study concluded that measures of maximal oxygen utilization (VO_{2max}), maximal heart rate (HR), respiratory exchange ratio (RER), lactate difference (maximal – resting), time to failure, and maximal work load were not significantly influenced by menstrual cycle phase – specifically menses, ovulation, and the mid-luteal phase – in normally menstruating, college-aged female participants. This could be attributed to possible stability of stroke volume and peak heart rate during vigorous aerobic exercise overcoming hormonal influences of menstrual cycle to keep performance stable. The parameters measured in this study also did not significantly vary across the three time points for college-aged males, indicating that maximal aerobic performance is relatively stable across 3-6 weeks in participants with this specific inclusion criteria.

The results also illustrate that during maximal aerobic exercise, female participants currently experiencing menses (the earliest portion of the follicular phase of the menstrual cycle where female sex hormone concentrations have declined and bleeding occurs) percept that exercise feels more rigorous and that recovery following testing takes longer compared to aerobic exercise testing directly following ovulation and during the luteal phase. This could be due to an increase in kinesthetic awareness during the follicular phase, as suggested by Veldhuijzen et al. (2013), a decrease in inhibitory activity during menses, as suggested by Tousignant-Laflamme and Marchand (2009), a dramatic drop in estradiol and progesterone as suggested by Caldwell Hooper et al. (2011), an interaction of each of these influences, or something unknown (Veldhuijzen et al., 2013; Tousignant-Laflamme & Marchand, 2009; Caldwell Hooper et al., 2011). These findings warrant more in-depth study of neural and hormonal physiology of the menstrual cycle, especially during menses.

This study recommends controlling for menstrual cycle phase in exercise research and periodization of exercise practice and training. Testing methods that could initiate physical discomfort for the participant may be elucidated during menses for eumenorrheic female participants; therefore, it is recommended that further studies should track, account for, and schedule visits based on menstrual cycle phase and particularly use caution or avoid testing during menses since the participant may experience a higher level of discomfort and need a longer amount of time to recover during this time period. It is also recommended that a time trial, or endurance test, with a similar protocol is used in the future to see if changes between the phases become apparent across the phases of interest in this study.

REFERENCES

American College of Sports Medicine. (2010). *ACSM's Guidelines for Exercise Testing and Prescription.* Philadelphia: Lippincott Williams & Wilkins.

Bailey, S. P., Zacher, C. M., & Mittleman, K. D. (2000). Effect of menstrual cycle phase on carbohydrate supplementation during prolonged exercise to fatigue. *Journal of Applied Physiology 88*, 690-697.

Barbieri, R. L. (2014). The endocrinology of the menstrual cycle. *Methods of Molecular Biology 1154*, 145-169.

Bemben, D. A. (1995). Ventilatory blood lactate responses to maximal treadmill exercise during the menstrual cycle. *Journal of Sports Medicine and Physical Fitness 35(4)*, 257-262.

Birch, K. (2000). Circamensal Rhythms in Physical Performance. *Biological Rhythm Research 31*, 1-14.

Buxton, C. L., & Atkinson, W. B. (1948). Hormonal factors involved in the regulation of basal body temperature during the menstrual cycle and pregnancy. *Journal of Clinical Endocrinology 8(7)*, 544-549.

Caldwell Hooper, A. E. (2011). Menstrual Cycle Effects on Perceived Exertion and Pain During Exercise Among Sedentary Women. *Journal of Women's Health 20(3)*, 439-446.

Choi, J. C. (2006). Different Brain Activation Patterns to Pain and Pain-related Unpleasantness during the Menstrual Cycle. *Anesthesiology 105*, 120-127.

Constantini, N. W., Dubnov, G., & Lebrun, C. M. (2005). The Menstrual Cycle and Sport Performance. *Clinics in Sports Medicine 24(2),* 51-82.

Cook, D. B. (1998). Sex Differences in Naturally Occurring Leg Muscle Pain and Exertion During Maximal Ergometry. *International Journal of Neuroscience 95(3)*, 183-202.

Coquart, J. B. J., Dufour, Y., Groslambert, A., Matran, R., & Garcin, M. (2012) Relationships Between Psychological Factors, RPE and Time Limit Estimated by Teleoanticipation. *The Sport Psychologist 26,* 359-374.

Costello, J. T., Bieuzen, F., & Bleakley, C. M. (2014). Where are all the female participants in Sports and Exercise Medicine research? *European Journal of Sport Science 14(8),* 847-851.

Dibrezzo, R., Fort, I. L., & Brown, B. (1988). Dynamic strength and work variations during the three stages of the menstrual cycle. *Journal of Orthopaedic and Sports Physical Therapy 10(4),* 113-116.

Dunbar, C. C., Robertson, R. J., Baun, R., Blandin, M. F., Metz, K., Burdett, R., & Goss, F. L. (1992) The validity of regulating exercise intensity by ratings of perceived exertion. *Med Sci SPorts Exercise 24(1),* 94-99.

Forman, R. G., Chapman, M. C., & Steptoe, P. C. (1987). The effect of endogenous progesterone on basal body temperature in stimulated ovarian cycles. *Human Reproduction 2(8),* 631-634.

Giacomoni, M., Bernard, T., Gavarry, O., Altare, S., & Falgairette, G. (2000). Influence of the menstrual cycle phase and menstrual symptoms on maximal anaerobic performance. *Medicine and Science in Sports and Exercise 32(2),* 486-492.

Godbole, G., Joshi, A. R., & Vaidya, S. M. (2016). Effect of female sex hormones on cardiorespiratory parameters. *Journal of family medicine and primary care*, *5*(4), 822–824.

Goldsmith, E. & Glaister, M. (2020). The effect of the menstrual cycle on running economy. *The Journal of Sports Medicine and Physical Fitness.* DOI: 10.23736/S0022-4707.20.10229-9

Gordon, D., Scruton, A., Barnes, R., Baker, J., Prado, L., & Merzbach, V. (2018). The effects of menstrual cycle phase on the incidence of plateua at VO2 max and associated cardiorespiratory dynamics. *Clinical Physiology and Functional Imaging 38(4),* 689-698.

Guermandi, E. V. (2001). Reliability of ovulation test in infertile women. *Obstetrics & Gynecology 97(1),* 92-96.

Hackney, A. C., Curley, C. S., & Nicklas, B. J. (1991). Physiological responses to submaximal exercise at the mid-follicular, ovulatory, and mid-luteal phases of the menstrual cycle. *Scandanavian Journal of Medicine in Science and Sports*, 94-98.

Haddad, M., Stylianides, G., Djaui, L., Dellal, A., & Chamari, K. (2017). Session-RPE Method for Training Load Monitoring: Validity, Ecological Usefulness, and Influencing Factors. Neuroscience 11, doi: 10.3389/fnins.2017.00612

Hall-Jurkowski, J. E., Jones, N. L., Toews, C. J., & Sutton, J. R. (1981). Effects of menstrual cycle on blood lactate, O2 delivery, and performance during exercise. *Journal of Applied Physiology: Respiratory, Environmental, and Exercise Physiology 51(6)*, 1493-1499.

Hart, S., Drevets, K., Alford, M., Salacinski, A., & Hunt, B. E. (2013). A method-comparison regarding the validity and reliability of the Lactate Plus analyzer. *BMJ Open (3)*.

Hawkins, S., & Matzuk, M. (2008). The menstrual cycle: basic biology. *Annals of the New York Academy of Sciences 1135*, 10-18.

Herzberg, S. D., Motu'apuaka, M. L., Fu, R., Brady, J., & Guise, J. M. (2017). The effect of menstrual cycle and contraceptives on ACL injuries and laxity: A systematic review and meta-analysis. *Orthopaedic Journal of Sports Medicine 5*, 1-10.

Iacovides, S. A. (2015). Does pain vary across the menstrual cycle? A review. *European Journal of Pain 19*, 1389-1405.

Janse de Jonge, X. A. (2003). Effects of the menstrual cycle on exercise performance. *Sports Medicine 33(11)*, 833-851.

Kaminsky, L. A. (2015). Reference Standards for Cardiorespiratory Fitness Measured With Cardiopulmonary Exercise Testing: Data From the Fitness Registry and the Importance of Exercise National Database. *Mayo Clinic Proceedings 90(11)*, 1515-1523.

Katch, V. L. (1982). Biological variability in maximum aerobic power. *Medicine and Science in Sports and Exercise 14(1)*, 21-25.

Kowalczyk, W. J. (2006). Sex Differences and Hormonal Influences on Response to Cold Pressor Pain in Humans. *The Journal of Pain 7(3),* 151-160.

Kowalczyk, W. J. (2010). Sex Differences and Hormonal Influences on Response to Mechanical Pressure Pain in Humans. *The Journal of Pain 11(4),* 330-342.

Lakens D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Frontiers in psychology, 4,* 863. https://doi.org/10.3389/fpsyg.2013.00863

Lara, B., Gutierrez-Hellin, J., Garcia-Bataller, A., Rodriguez-Fernandez, P., Romero-Moraleda, B., Del Coso, J. (2019). Ergogenic effects of caffein on peak aerobic cycling power during the menstrual cycle. *European Journal of Nutrition.* https://doi.org/10.1007/s00394-019-02100-7

Larson, R. D., Cantrell, G. S., Ade, C. J., Ferrell III, J. W., Lantis, D. J., Barton, M. A., Larson, D. J., et al. (2015). Physiological Responses to Two Distinct Maximal Cardiorespiratory Exercise Protocols. *International Journal of Sports and Exercise Medicine 1(13).*

Laszlo, G. (2004). Respiratory measurements of cardiac output: from elegant idea to useful test. *Journal of Applied Physiology (96).* 428-437.

Laurent, C. M., Green, J. M., Bishop, P. A., Sjokvist, J., Schumacker, R. E., Richardson, M. T., et al. (2011). A practical approach to monitoring recovery: development of a perceived recovery status scale. *Journal of Strength and Conditioning Research 25(3),* 620-628.

Lebrun, C. M., McKenzie, D. C., Prior, J. C., & Taunton, J. E. (1995). Effects of menstrual cycle phase on athletic performance. *Medicine and Science in Sports and Exercise 27(3),* 437-444.

Macfarlane, D. J. (2001). Automated Metabolic Gas Analysis Systems: A Review. *Sports Med 31(12),* 841-861.

McClure Browne, J. C. (1973). *Postgraduate Obstetrics and Gynaecology (4th ed.).* London, UK: Butterworth.

Miller, P. B., & Soules, M. R. (1996). The usefulness of a urinary LH kit for ovulation prediction during menstrual cycles of normal women. *Obstetrics and Gynaecology 87(1),* 13-17.

Murphy, P. G. (2017). Performance Related Hemodynamic Responses to an Aerobic Exercise Across Different Stages of the Menstrual Cycle. *International Journal of Exercise Science: Conference Proceedings 2(9).*

Oostheyse, T., & Bosch, A. (2010). The effect of the menstrual cycle on exercise metabolism: Implications for exercise performance in eumenorrheic women. *Sports Medicine 40(3),* 207-227.

Petrofsky, J., Al Malty, A., & Suh, H. (2007). Isometric endurance, body and skin temperature and limb and skin blood flow during the menstrual cycle. *Medical Science Monitor 13(3),* 111-117.

Pivarnik, J. M., Marichal, C. J., Spillman, T., & Morrow, J. R. (1992) Menstrual cycle phase affects temperature regulation during endurance exercise. *Journal of Applied Physiology 72(2),* 543-548.

Reed, B. G., & Carr, B. R. (2000-updated 2018). The Normal Menstrual Cycle and the Control of Ovulation. *Endotext [Internet], MDText.com.* Available from: https://www.ncbi.nlm.nih.gov/books/NBK279054

Riley III, J. L. (1999). A meta-analytic review of pain perception across the menstrual cycle. *Pain 81*, 225-235.

Ring, C. V. (2009). Effects of sex, phase of the menstrual cycle and gonadal hormones on pain in healthy humans. *Biological Psychology 81,* 189-191.

Royston, J. P., & Abrams, R. M. (1980). An objective method for detecting the shift in basal body temperature in women. *Biometrics 36,* 217-224.

Smekal, G., Von Duvillard, S. P., Frigo, P., Tegelhofer, T., Pokan, R., & Hofmann, P. (2007). Menstrual Cycle: No Effect on Exercise Cardiorespiratory Variables or Blood Lactate Concentration. *Medicine in Science and Sports and Exercise 39(7),* 1098-1106.

Stachenfeld N. S. (2008). Sex hormone effects on body fluid regulation. *Exercise and sport sciences reviews*, *36*(3), 152–159. https://doi.org/10.1097/JES.0b013e31817be928

Stephenson, L. A., Kolka, M. A., & Wilkerson, J. E. (1981). Perceived exertion and anaerobic threshold during the menstrual cycle. *Medicine and Science in Sports and Exercise 14(3),* 218-222.

Stephenson, L. A., Kolka, M. A., & Wilkerson, J. E. (1982). Metabolic and thermoregulatory responses to exercise during the human menstrual cycle. *Medicine and Science in Sports and Exercise 14(4)*, 270-275.

Tanaka, H., Fukumoto, S., Osaka, Y., Ogawa, S., Yamaguchi, H., & Miyamoto, H. (1991). Distinctive Effects of Three Different Modes of Exercise on Oxygen Uptake, Heart Rate and Blood Lactate and Pyruvate. *International Journal of Sports Medicine 12(5).* 433-438.

Tanaka, M., Sato, M., Umehara, S., & Nishikawa, T. (2003). Influence of menstrual cycle on baroreflex control of heart rate: Comparison with male volunteers. *American Journal of Physiology*. Regulatory, integrative and comparative physiology. 285. R1091-7. 10.1152/ajpregu.00162.2003.

Teepker, M. P. (2010). Menstrual Variation in Experimental Pain: Correlation with Gonadal Hormones. *Neuropsychobiology 61*, 131-140.

Thiyagarajan, D. K., & Jeanmonod, R. (2018). *Physiology: Menstrual Cycle.* Treasure Island, FL: StatPearls Publishing.

Tousignant-Laflamme, Y. M. (2009). Excitatory and inhibitory pain mechanisms during the menstrual cycle in healthy women. *Pain 146*, 47-55.

Veldhuijzen, D. S. (2013). The role of circulating sex hormones in menstrual cycle dependent modulation of pain-related brain activation. *Pain 154(4)* , 548-559.

Wenner, M. M., & Stachenfeld, N. S. (2012) Blood pressure and water regulation: understanding hormone effects within and between men and women. *Journal of Physiology 590(23)*, 5949-5961.

APPENDIX

1. IRB Outcome Letter

Institutional Review Board for the Protection of Human Subjects

Approval of Initial Submission – Expedited Review – AP01

Date: August 19, 2019 **IRB#:** 11034

Principal Investigator: Rebecca Larson, PhD **Approval Date:** 08/18/2019

Status Report Due: 07/31/2020

Study Title: Effects of Menstrual Cycle Phase on Aerobic Parameters during a Graded-Exercise Test in Eumenorrheic Females

Expedited Category: 4

Collection/Use of PHI: Yes

On behalf of the Institutional Review Board (IRB), I have reviewed and granted expedited approval of the above-referenced research study. To view the documents approved for this submission, open this study from the *My Studies* option, go to *Submission History*, go to *Completed Submissions* tab and then click the *Details* icon.

Requirements under the Common Rule have changed. The above-referenced research meets one or more of the circumstances for which continuing review is not required. However, as Principal Investigator of this research, you will be required to submit an annual status report to the IRB.

As principal investigator of this research study, you are responsible to:

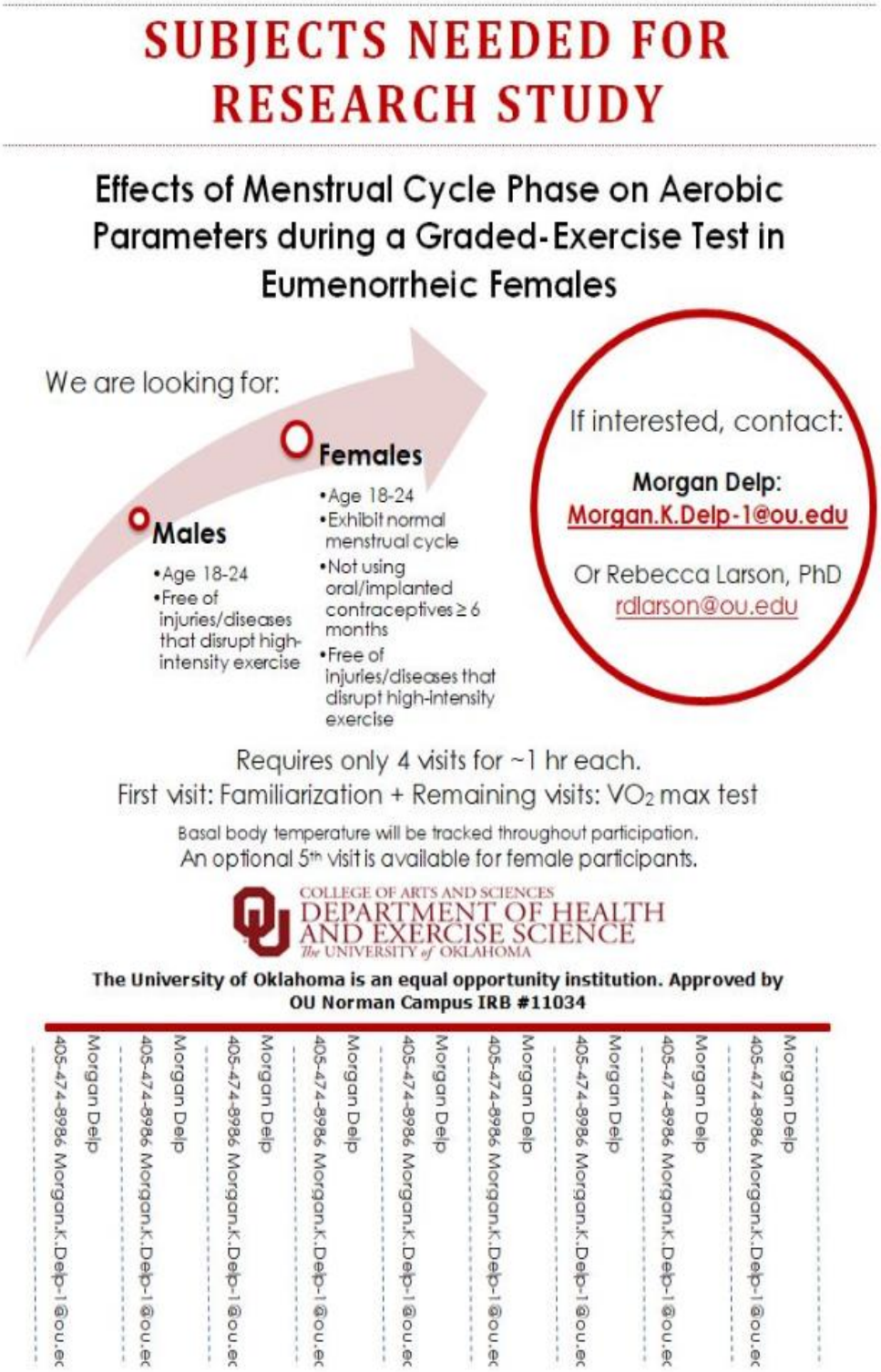
- Conduct the research study in a manner consistent with the requirements of the IRB and federal regulations 45 CFR 46.
- Obtain informed consent and research privacy authorization using the currently approved, stamped forms and retain all original, signed forms, if applicable.
- Request approval from the IRB prior to implementing any/all modifications.
- Promptly report to the IRB any harm experienced by a participant that is both unanticipated and related per IRB policy.
- Maintain accurate and complete study records for evaluation by the HRPP Quality Improvement Program and, if applicable, inspection by regulatory agencies and/or the study sponsor.
- **Submit an annual status report to the IRB to provide the study/recruitment status and report all harms and deviations that may have occurred.**
- **Submit a final closure report at the completion of the project.**

If you have questions about this notification or using iRIS, contact the IRB @ 405-325-8110 or irb@ou.edu.

Cordially,

Sara Mayeux

2. Recruitment Flyer



3. Consent Form

701-A-1

Signed Consent to Participate in Research

Would you like to be involved in research at the University of Oklahoma?

I am Morgan Delp, an Exercise Physiology Master's student from the Health and Exercise Science Department and I invite you to participate in my research project entitled Effects of Menstrual Cycle Phase on Aerobic Parameters during a Graded-Exercise Test in Eumenorrheic Females. This research is being conducted at the University of Oklahoma in the Body Composition and Human Performance Laboratory. You were selected as a possible participant because you are a female that exhibits a normal menstrual cycle and does not use any oral/implanted contraceptives and meets the inclusion criteria or you are a male that meets the inclusion criteria – meaning you are also free from any lower-limb or neuromuscular abnormalities and any cardiovascular diseases. You must be at least 18 years of age to participate in this study.

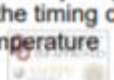
Please read this document and contact me to ask any questions that you may have BEFORE agreeing to take part in my research.

What is the purpose of this research? The purpose of this research is to examine how different phases of the menstrual cycle, specifically during menses, the ovulatory phase, and the mid-luteal phase, may affect metabolic, cardiovascular, and subjective parameters during a graded exercise test in a eumenorrheic, college-age population.

How many participants will be in this research? About 50 people, 25 males and 25 females, aged 18-24, from a variety of physical activity levels will take part in this research.

What will I be asked to do? If you agree to be in this research, you will be asked to attend four visits. Females will also have an optional fifth visit. The first visit consists of completing paperwork including health, physical activity, and menstrual cycle background questionnaires and familiarization with the equipment, procedures, and measurements used in this study. You will be provided with a thermometer following this visit and will be asked to track and report via text message your waking body temperature immediately upon waking up for 2-4 weeks prior to your first exercise visit. The following three to four visits will correspond to specific menstrual cycle phases based on your waking body temperature values, and you will complete a mood questionnaire and an exercise test on a bicycle. During this exercise test, you will be asked to ride against increasing resistance until you cannot meet the desired intensity. In addition, during the test, we will be collecting data involving the heart and breathing with specific equipment. Other measures will be assessed at the beginning of each visit and throughout the test, including: height, weight, temperature, heart rate, blood pressure, and blood lactate concentration through a finger prick. Objective parameters revealing oxygen utilization and metabolic rate will be measured through software during the test. Subjective measures to reveal your comfort/effort level will also be assessed throughout the test. The day after each visit, fatigue will be tracked with a questionnaire. After completion of the third exercise visit, female participants will be given the option to continue to track waking body temperature and complete a fourth exercise test according to a specific menstrual cycle phase that was previously tested. Following the last visit, all participants will no longer need to track their waking body temperature.

How long will this take? Your participation will take about 1 hour per visit for 4-5 visits – equating to 4-5 hours. Temperature tracking will cover at least 1 menstrual cycle (about 28 days) and could last a maximum of 3 cycles (~84 days) depending on the timing of participation and presence of ovulation during the menstrual cycle. Temperature



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measurements will take about 2 minutes every day. The fatigue questionnaire following each visit will take about 5 minutes.

What are the risks and/or benefits if I participate? Risks involved in this study include moderate soreness. You will be asked to cycle on a stationary bike against increasing resistance until you cannot anymore, which may result in fatigue and soreness the following days if you are unfamiliar with this exercise protocol. You may find the seat of the bike or the headgear/mouthpiece necessary for assessing oxygen utilization uncomfortable. This protocol involves increased breathing rate and heart rate, which may result in dizziness or faintness. Also, there may be some discomfort associated with the finger prick protocol. Your safety is up the upmost importance, so you will be closely monitored during testing and thoroughly screened beforehand to assure that no complications, injuries, or unnecessary discomfort occurs during your participation. There is no medical benefit for participating in this research study.

What do I do if I am injured? If you are injured during your participation, report this to a researcher or the principal investigator, Rebecca Larson, immediately. Dr. Larson can be reached at 352-359-8432 (cell) or 405-325-6325 (work). Emergency medical treatment is available. However, you or your insurance company will be expected to pay the usual charge from this treatment. The University of Oklahoma Norman Campus has set aside no funds to compensate you in the event of injury.

Will I be compensated for participating? You will not be reimbursed for your time and participation in this research.

Who will see my information? In research reports, there will be no information that will make it possible to identify you. Research records with identifiable information will be stored securely in locked file cabinets and research computers, and only approved researchers and the OU Institutional Review Board will have access to the records. You will be assigned a subject identification number, so your identifiable information will be kept confidential.

You have the right to access the research data that has been collected about you as a part of this research. However, you may not have access to this information until the entire research has completely finished and you consent to this temporary restriction.

Do I have to participate? No. If you do not participate, you will not be penalized or lose benefits or services unrelated to the research. If you decide to participate, you don't have to answer any questions and can stop participating at any time.

What will happen to my data in the future? After removing all identifiers, we might share your data with other researchers or use it in future research without obtaining additional consent from you.

Will I be contacted again? The researcher might like to contact you to gather additional data or recruit you into new research.

I give my permission for the researcher to contact me in the future. ___Yes ___ No

Who do I contact with questions, concerns or complaints? If you have questions, concerns or complaints about the research or have experienced a research-related injury, contact me at Morgan.K.Delp-1@ou.edu or on my cell at 405-474-8986, or you may contact the principal investigator Rebecca Larson at rdlarson@ou.edu , on her cell at 352-359-8432 or in her office at 405-325-6325.

You can also contact the University of Oklahoma – Norman Campus Institutional Review

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Board (OU-NC IRB) at 405-325-8110 or irb@ou.edu if you have questions about your rights as a research participant, concerns, or complaints about the research and wish to talk to someone other than the researcher(s) or if you cannot reach the researcher(s).

You will be given a copy of this document for your records. By providing information to the researcher(s), I am agreeing to participate in this research.

Participant Signature	Print Name	Date
Signature of Researcher Obtaining Consent	Print Name	Date

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4. HIPAA Form

AUTHORIZATION TO USE or SHARE
HEALTH INFORMATION[1] THAT IDENTIFIES YOU FOR RESEARCH
An Informed Consent Document for Research Participation may also be required.

Title of Research Project: **Effects of Menstrual Cycle Phase on Aerobic Parameters during a Graded-Exercise Test in Eumenorrheic Females**

IRB Number:

Leader of Research Team: **Rebecca Larson**

Address: **Department of Health and Exercise Science, 1401 Asp Avenue SJSC 117, Norman, OK 73019**

Phone Number: **405-325-6325**

If you decide to sign this document, University of Oklahoma (OU) researchers may use or share information that identifies you (protected health information) for their research. Protected health information will be called PHI in this document.

PHI To Be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your PHI. If you give permission, the researchers may use or share with the people identified in this Authorization any PHI related to this research from your medical records and from any test results. Information used or shared may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form; medical records and charts; name, address, telephone number, date of birth, race, government-issued identification numbers, and can include physical findings from questionnaires, basal body temperature readings, anthropometric measures, and graded-exercise tests.

Purposes for Using or Sharing PHI. If you give permission, the researchers may use your PHI to examine how different phases of the menstrual cycle impact cardiovascular, metabolic, and subjective parameters during a graded-exercise test in eumenorrheic females. Males are included for comparison.

Other Use and Sharing of PHI. If you give permission, the researchers may also use your PHI to develop new procedures or commercial products. They may share your PHI with other researchers, the research sponsor and its agents, the OU Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Department of Health and Human Services

[1] **Protected Health Information includes all identifiable information relating to any aspect of an individual's health whether past, present or future, created or maintained by a Covered Entity.**

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(HHS), and when required by law. The researchers may also share your PHI with no one outside of the main research team.

Confidentiality. Although the researchers may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. The law does not require everyone receiving the information covered by this document to keep it confidential, so they could release it to others, and federal law may no longer protect it.

YOU UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING A COMMUNICABLE OR NONCOMMUNICABLE DISEASE.

Voluntary Choice. The choice to give OU researchers permission to use or share your PHI for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OU researchers to use or share your PHI if you want to participate in the research and, if you cancel your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care unrelated to this study from OU.

Canceling Permission. If you give the OU researchers permission to use or share your PHI, you have a right to cancel your permission whenever you want. However, canceling your permission will not apply to information that the researchers have already used, relied on, or shared or to information necessary to maintain the reliability or integrity of this research.

End of Permission. Unless you cancel it, permission for OU researchers to use or share your PHI for their research will never end.

Contacting OU: You may find out if your PHI has been shared, get a copy of your PHI, or cancel your permission at any time by writing to:

Privacy Official
University of Oklahoma
PO Box 26901
Oklahoma City, OK 73190

or Privacy Board
University of Oklahoma
201 Stephenson Pkwy, Suite 4300A
Norman, OK 73019

If you have questions, call: (405) 271-2511 or (405) 325-8110

Access to Information. You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study is completely finished. You consent to this temporary restriction.

Giving Permission. By signing this form, you give OU and OU's researchers led by the Research Team Leader permission to share your PHI for the research project listed at the top of this form.

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Participant Name (Print): _____

_____ _____
Signature of Participant Date
or Parent if Participant is a minor

Or

_____ _____
Signature of Legal Representative** Date

**If signed by a Legal Representative of the Participant, provide a description of the relationship to the Participant and the authority to act as Legal Representative:

_____
OU may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Participant or the Legal Representative at the time this signed form is provided to the researcher or his representative.

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5. Medical History Questionnaire

Date:_____

Medical History Questionnaire

Name: _____ Date of Birth: _____
Address: _____ Phone Number: _____
_____ alt #: _____
Email: _____
Age: _____
Dominant side: Left Right (circle)
Blood Pressure: _____/_____
Height: _____ Weight: _____ Shoe Size: _____
Sex: Male Female (circle) Gender: Male Female (circle)
Ethnicity: Caucasian African American Hispanic Asian Other: _____
Emergency contact name and number: _____

Please answer the following questions:

1. Have you ever been diagnosed with diabetes?
   Y N If "yes," when where you diagnosed? _____
2. Have you ever been told by a physician that you have Osteoporosis/Osteopenia?
   Y N
3. Have you ever had a heart attack or stroke?
   Y N If "yes," what and when? _____
4. Have you ever been diagnosed with any disease affecting the brain, spine, or nerves? (ex: Multiple sclerosis, brain tumors, epilepsy, Parkinson's disease, Neuropathy, ALS, etc.)
   Y N If "yes," what and when? _____
   _____
5. Have you ever been diagnosed with arthritis?
   Y N If "yes," when?
6. Have you had any injuries of the lower limbs specifically involving bone, tendon, or ligament damage?
   Y N If "yes," what and when? _____
   _____
7. Have you had any injuries of the lower limbs specifically involving the muscles?
   Y N If "yes" what and when? _____
   _____
8. Do you experience frequent pain in your lower limbs?
   Y N If "yes," where and how often?_____

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9. Do you have a decreased range of motion or mobility in your hips, knees, or ankles?

   Y N If "yes," how much and where? _____

10. Do you use an assistive device for walking?

    Y N If "yes," what? _____

11. Do you experience any difficulties producing and maintaining rapid and repetitive movements?

    Y N If "yes," then describe. _____

12. Are you currently on any kind of medications?

    Y N If "yes," what medication, amount taken, time on medication, and reason.

    _____

    _____

13. Is there anything else you feel that the researchers should be aware of?

_____

_____

_____

I certify that these answers are accurate and complete

_____

Your Signature: Date:

Witness: _____ Date:_____

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6. Menstrual Cycle Questionnaire

Department of Health and Exercise Science
University of Oklahoma

MENSTRUAL HISTORY QUESTIONNAIRE

Participant ID:_____Date:_____

We are asking you to give us as complete a menstrual history as possible. All information is strictly confidential.

Are you pregnant (circle your response)
YES- Do not complete the rest of this form
NO- Continue to section A.

SECTION A: CURRENT MENSTRUAL STATUS

1. Approximately how many menstrual periods have you had during the past 12 months?
(please circle what months you have had a period. This means from this time last year to the present month)

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

2. What is the usual length of your menstrual cycle (first day of your period to the next onset of your period)?

_____days. Today is day _____ of your present menstrual cycle.

3. When was the date of the onset of your last period?

4. When do you expect your next period?

5. What is the average length (number of days) of your menstrual flow? _____ days

How many of these days do you consider "heavy"?_____days

6. Do you take oral contraceptives or any other medication that includes estrogen and/or progesterone?

If yes, how long have you been taking this medication?_____

What is the brand name and dosage of this medication?_____

Has this medication affected your menstrual cycle (regularity, length and amount of flow)? If yes, indicate changes.

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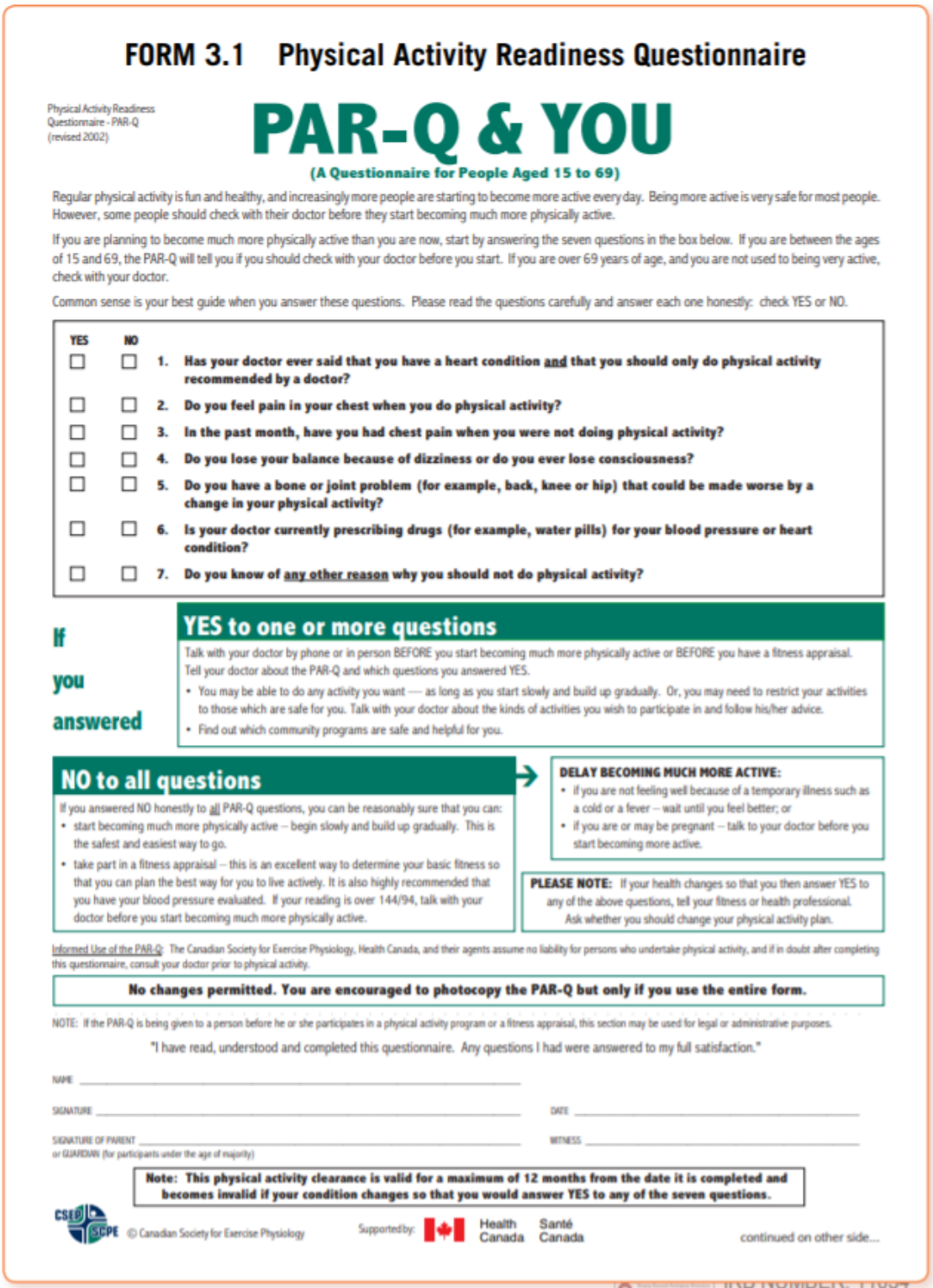
7. Physical Activity Readiness Questionnaire



FORM 3.1 Physical Activity Readiness Questionnaire

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

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IRB APPROVED

8. Physical Activity Questionnaire

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ **days per week**

   ☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

LONG LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised October 2002.

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

9. POMS-B Mood Questionnaire

I.D. Number: _____ **Session Number:** _____ **Date:** _____

POMS-B QUESTIONNAIRE

INSTRUCTIONS: Below is a list of words that describe feelings that people have. Please read each word carefully. Then circle the number that best describes:

☐ How you have been feeling during the PAST WEEK, INCLUDING TODAY.

☐ How you feel RIGHT NOW.

	Not At All	A Little	Moderately	Quite a Bit	Extremely
1. Tense	0	1	2	3	4
2. Angry	0	1	2	3	4
3. Worn out	0	1	2	3	4
4. Lively	0	1	2	3	4
5. Confused	0	1	2	3	4
6. Shaky	0	1	2	3	4
7. Sad	0	1	2	3	4
8. Active	0	1	2	3	4
9. Grouchy	0	1	2	3	4
10. Energetic	0	1	2	3	4
11. Unworthy	0	1	2	3	4
12. Uneasy	0	1	2	3	4
13. Fatigued	0	1	2	3	4
14. Annoyed	0	1	2	3	4
15. Discouraged	0	1	2	3	4

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PLEASE ANSWER QUESTIONS ON OTHER SIDE

I.D. Number: _____ **Session Number:** _____ **Date:** _____

POMS-B QUESTIONNAIRE

INSTRUCTIONS: Below is a list of words that describe feelings that people have. Please read each word carefully. Then circle the number that best describes:

☐ How you have been feeling during the PAST WEEK, INCLUDING TODAY.

☐ How you have been feeling during the PAST 24 HOURS.

	Not At All	A Little	Moderately	Quite a Bit	Extremely
16. Nervous	0	1	2	3	4
17. Lonely	0	1	2	3	4
18. Muddled	0	1	2	3	4
19. Exhausted	0	1	2	3	4
20. Anxious	0	1	2	3	4
21. Gloomy	0	1	2	3	4
22. Sluggish	0	1	2	3	4
23. Weary	0	1	2	3	4
24. Bewildered	0	1	2	3	4
25. Furious	0	1	2	3	4
26. Efficient	0	1	2	3	4
27. Full of Pep	0	1	2	3	4
28. Bad-tempered	0	1	2	3	4
29. Forgetful	0	1	2	3	4
30. Vigorous	0	1	2	3	4

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10. Robberson Research Grant Funding Outcome Letter

Robberson Research Grant Application

Congratulations! The Graduate College is pleased to award you a Robberson Research Grant in the amount of $783.48. This funding may be used for research expenses incurred through May 31, 2021, as long as you remain enrolled at OU when expenditures are incurred. Please request reimbursement by July, 31, 2021.

If human subjects will be involved in your study, approval must be obtained from the Institutional Review Board prior to the beginning of the study. Once IRB approval is received, your research funds may be transferred to your department account for your use.

Please consult with your academic unit on the proper procedures for reimbursement. When you are ready to have the funds transferred, your department should send the following to Shared Business Services (sbsc@ou.edu):

1. A departmental request for reimbursement
2. A copy of this email award letter
3. A copy of your IRB approval, when applicable

We hope that this financial support will be beneficial to your research activities. If you have any questions, feel free to contact me.

Sincerely,

Abigail Shelton, M.Ed. | Director of Operations
Graduate College | University of Oklahoma
Tel: 405.325.7715 | Fax: 405.325.5346 | aokoe@ou.edu

11. Graduate Student Senate Funding Outcome Letter

GSS Grant Notification

Dear Graduate Student:

The Graduate Student Senate (GSS) has awarded you a Research Grant for fall 2019 in the amount of $600.00. Your next steps in the process are:

1. Submit this award letter to your department immediately.
2. You will then need to work with your department to figure out the best route to make your purchases/reimbusements.

Your department will then need to:

1. Provide documentation of purchases/reimbursements, along with a copy of this award letter, to the Student Government Association Accountant, Curt Swanson, at coffeemonkey@ou.edu in order to initiate a departmental transfer from GSS to reimburse your department *up to* the amount of the award. **The deadline for this step is December 6, 2019, but should take place as soon as possible prior to that date to ensure timely processing.**
2. Contact Mr. Swanson with any concerns.

Thank you for your application and congratulations!

Ways and Means Committee
Graduate Student Senate
University of Oklahoma
gsswam@ou.edu