AN EXPLORATION OF THE RELATIONSHIP BETWEEN MENSTRUAL PHASE AND COLLEGIATE FEMALE UPPER AND LOWER BODY ANAEROBIC CAPACITIES

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AN EXPLORATION OF THE RELATIONSHIP BETWEEN MENSTRUAL PHASE AND COLLEGIATE FEMALE UPPER AND LOWER BODY ANAEROBIC CAPACITIES (47 pp.)

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INTRODUCTION: With the rate of female collegiate and professional athletes on the rise in recent decades, fluctuations in physical performance in relation to the menstrual cycle is an important area of study. PURPOSE: The purpose of this research was to compare differences in upper and lower body maximal anaerobic capacities across a single menstrual cycle. METHODS: Participants (n=11) met a total of four times; once for familiarization and again on day 1 of menses (follicular phase), day 14 (ovulation), and day 21 (luteal phase) respectively. Upper body power was assessed using a bench press weight of ~50% of the participant’s predetermined 1-repetition maximum (1-RM) on a ballistic measurement system and variables included peak force (N), mean force (N), peak power (W), mean power (W), and peak velocity (m/s). Lower body power output was collected using a standard Wingate test. The variables of interest were anaerobic capacity (w/kg), peak power (W), mean power (W), fatigue index (W/s), and total work (J). RESULTS: Statistical significance was not observed (p>0.05) in any of the aforementioned variables after completing multiple one way of analyses of variances (ANOVAs) with repeated measures on time. CONCLUSION: Within the parameters of
this research, neither female upper nor lower body power output differed across the menstrual cycle when analyzed using 50% of a one repetition (1RM) maximal bench press and the 30 second maximal effort cycle ergometer Wingate test. Therefore, researchers should not alter their subject populations due to the incorrect assumption that power output may be influenced by the menstrual cycle.
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CHAPTER I
INTRODUCTION

Background

Collegiate female athletes have become increasingly more common in recent decades. Johnson (2014) reported that the “total number of student-athletes participating in the 23 sports that the NCAA [National Collegiate Athletic Association] sponsors also reached an all-time high at 472,625. As that number increases, the gap between male and female participation continues to narrow.” Throughout the article he discusses how every academic year since 2003-2004, nearly 53% of the teams participating in NCAA championship events were women’s teams and since 2008, the average NCAA member school sponsored an average of 17 teams- eight for men and nine for women. This evidence of an increase is female involvement calls for research to evaluate physical athletic performance specifically in women. Since women have a menstrual cycle, opposed to their male counterparts, it is prudent to explore any hormonally driven influences that the menstrual cycle may have on physical performance.

Similarly, in recent decades, the appearance of female collegiate and professional power athletes has become increasingly more frequent. This surge in female competitive athletes may be best exemplified by their participation in the highest level of athletic competition, the modern Olympic Games. Female competitors accounted for 45% of the nearly 10,000 international athletes who competed in Rio de Janeiro in 2016, a rate which
nearly doubled since the 1984 Los Angeles Olympic Games and more than tripled since the 1964 Tokyo Games (International Olympic Committee, 2016). This significant shift in the collective composition of competitive athletes continues to warrant the need for female athletic testing since most of the available research on athletes’ physical performance is conducted on male participants. This is may be due, in large part, to the presumption that the results of such studies may be influenced and impacted by the hormonal fluctuations of the female menstrual cycle.

**Purpose of Study**

The purpose of this research was to compare differences in lower body maximal anaerobic power, anaerobic capacity, and fatigue index by means of the Wingate as well as identify variations of maximal power output utilizing the bench press on Days 1 (follicular phase), Day 14 (ovulation), and Day 21 (luteal phase) of a single menstrual cycle.

**Hypothesis**

The hypothesis was that females would produce the highest overall upper and lower body power output on the days in which estrogen concentrations were highest on days 14, 21, and 1 respectively.
CHAPTER II
REVIEW OF THE LITURITURE

The Menstrual Cycle

The menstrual cycle is a term that encompasses both the uterine and ovarian cycles. The uterine cycle is divided into three phases (menstruation, proliferative, and secretory) and references the changes in thickness of the uterine wall that are driven by alterations in serum estrogen and progesterone. The ovarian cycle begins on the first day in which the female observes blood (menses) and initiates the follicular phase (days 1-13), ovulation (day 14), and the luteal phase (days 15-28), and it is regulated by fluctuating levels of the pituitary gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These phases are divided as such depending on the maturation of the female egg and whether or not it has been fertilized within a typical cycle of 28 days (Hall, 2011).

The ovarian cycle begins on the first day that a woman observes blood (menses). Menses signals the onset of the follicular phase. This phase is comprised of two sub-phases, the early-follicular phase (days 2-9) and late-follicular phase (days 9-13). It is important to note that the follicular phase lasts longer than menses, which consists of typically 5-7 days. A female with a typical 28-day ovarian cycle will eject an immature egg called an oocyte from the ovary on day 14. This is called ovulation and indicates the calendar mark of peak estrogen concentration. Finally, the luteal phase lasts post-
ovulation until the onset of another menses and may also be subdivided into mid-luteal (days 18-24) and late-luteal (days 25-28+) stages (Redman, 2003).

Previous research has examined the relationship of the potential effects of the ovarian and uterine cycles with physical performance. However, the results vary as much as the methods used within these experiments (Vaiksaar, 2011; Giacomoni, 1999; Smekal, 2007). For example, some researchers tend to use the terms menstrual cycle, uterine cycle, and ovarian cycle interchangeably when indeed they are not synonymous. However, without a consistent narrative of terminology among researchers, methodology and subsequent results have proven ambiguous. This has caused a lack of a definitive causal relationship between the cyclical feminine hormonal influences and physical performance.

However, most researches agree that the menstrual phases can be identified in three ways: the first, by counting the days since the first day of the previous menstrual period. This calendar-based method may increase the likelihood of improper phase categorization because females tend to have average cycles ranging 28±2 days. If the participant fluctuates phases within that “normal” 48 hour window, the research findings may be faulty. The second way to categorize phases of the menstrual cycle is by using the basal body temperature method. This measurement is often collected by the participant herself by taking her temperature with an oral or temporal thermometer immediately upon waking before getting out of bed in the morning. The final way to categorize menstrual phase timing is through the use of hormonal confirmation through
chemical analysis. Though the latter is suggested to be the most accurate method, it is also the most costly and requires advanced laboratory skills and lengthy analysis.

There is also conclusive evidence reporting that menstrual irregularities among female athletes are far greater than the general population (Dušek, 2001; Timmerman, 1996; Şahin, 2011). Some of the most frequently cited theories behind this phenomenon include low to very low percentages of body fat, chronic levels of hypocaloric intake, excessive energy expenditure, psychological stress, and hormonal changes resulting from intense exercise (Şahin, 2013). Additionally, in a 12-week resistance training study consisting of collegiate physically active females, Şahin, F. N., & Korkusuz (2013) suggested that athletic amenorrhea may also be associated with increased serum prolactin hormone concentrations following chronic resistance training protocols.

**Estrogens**

The endocrine system aids in the maintenance of homeostasis through a variety of mechanisms. It regulates the chemical concentrations within the blood, participates in the metabolism of macronutrients, and contributes in chemical reactions that allow the body to cope with stress. Additionally, it regulates growth and development that encourage sexual maturation and reproduction.

In non-pregnant females, estrogens are secreted in large quantities primarily by the thecal cells within the ovaries. However trace amounts are also produced within the cortices of the adrenal glands and adipose tissue. Androgens, such as dehydroepiandrosterone (DHEA) are anabolic steroids that are produced within the
adrenal cortex. Derived from cholesterol, these androgens serve as precursors to estrogens, an important female sex hormone group. In pregnant females, estrogens are also produced in large quantities within the placenta to aid in the proliferation and development of fetal cells. The human body secretes three types of estrogens: estrone, estriol, and estradiol (E2) which is also commonly referred to as 17β-estradiol. Estradiol is the most influential estrogen due to its potency accounting for 12 times that of estrone and 80 times that of estriol in females of childbearing age (Hall & Guyton, 2011). Estriol is the primary estrogen during pregnancy and estrone is the only estrogen secreted after menopause.

Estrogens function at the level of multiple tissues, including aiding in cell proliferation to develop female sexual characteristics such as breasts, endometrium thickness, and regulation of the menstrual cycle. In non-pregnant, premenopausal women, estrogen levels change throughout the month. They are highest in the middle of the menstrual cycle during ovulation (day 14 of 28) and lowest during menses. If serum estrogen concentrations are limited or elevated at inappropriate times of the menstrual cycle, females may experience a loss of sexual desire, extreme fatigue, and menstrual problems such as abnormally light or heavy bleeding, amenorrhea, non-cancerous tumors within the uterus or breasts and/or symptoms of depression (Home Healthy Network, 2016).
Estrogen and Skeletal Muscle

Estrogens’ influence on skeletal muscle is still somewhat misunderstood, thus further, well-controlled research is needed. However, research based on gender comparisons suggests that while males have a greater capacity to produce maximum force, females have a greater ability to resist muscular fatigue and recover faster than their male counterparts (Glenmark, 2004, Häkkinen, 1993). Though the mechanisms behind these gender-related differences have not been explicitly defined, Glenmark (2004) and colleagues suggest that it may be due to differences in hormonal statuses.

In males and females alike, the effects of estrogen are mediated by ligand-activated transcription factor estrogen receptors: ERα, ERβ and GPER. In humans, these receptors have been identified within skeletal muscle at the cellular level of mRNA, but only ERβ has been identified at the protein level (Wiik, 2003). Estradiol’s affinity to these alpha and beta receptors is quite similar according to Kuiper et al. (1998), but there are differences in the affinity of other compounds such as genistein, a weak phytogestrogens commonly found in soybeans that prefers to bind to ERβ rather than ERα. Other than skeletal muscle, tissues with high concentrations of ERα include the uterus, mammary glands, placenta, central nervous system, cardiovascular system and bone with lower concentrations found within non-classical target tissues such as the prostate, testis, ovaries, adrenals, pancreas, skin, and urinary tract (Wiik, 2003, Ciocca & Vargas, 1995). Additionally, ERβ may have antiproliferative effects and oppose the actions of ERα in reproductive tissue (Weihua, 2000).
The presence of estrogen and progesterone receptors in bone, skeletal muscle, ligaments, and the nervous system suggest that hormonal fluctuations may affect the structure and function of these tissues (Ciocca & Vargas, 1995). Casey and colleagues (2014) examined the muscle stretch reflex within the rectus femoris and vastus medialis across the menstrual cycle in hopes of identifying hormonally influenced monthly motor control differences. The study concluded that the rectus femoris muscle stretch reflex response was 2.4 times lower during the peri-ovulatory phase (days 12-14) when compared with the luteal phase post ovulation. The same trend was seen in the analysis of the vastus medialis, but statistical significance was not observed. This significant data (p=0.007) indicates that the rectus femoris muscle produced the lowest stretch reflex response when estrogen concentrations were at their highest which contrary to previous data may suggest a decrease in maximal anaerobic power output.

**Estrogen and Lactate**

Conflicting results are present when analyzing the influence of female hormones relating to anaerobic activities such as sprinting via cycle ergometer or running on foot (Dombovy, 1987; McCracken, 1994; Bonen, 1983). This may be partially due to the indistinct relationship of the menstrual cycle’s influence on blood lactate accumulations. For example, two studies reported a higher blood lactate concentration in response to exercise during the mid-follicular phase opposed to other times of testing during the month (Sutton, 1981; Forsyth, 2005). However, McCracken et al. (1994) speculated that estrogens were responsible for spared glycogen use and enhanced lipid oxidation thus
lowering the lactate response during the mid-luteal phase. This is supported by the evidence that estrogens influence the release nitric oxide, a potent vasodilator which could increase the amount of blood available for circulation and therefore improve overall cardiovascular health (Chambliss, 2002). The decrease in lactate response to exercise during the mid-luteal phase was suggested to have been due to differences in subjects’ nutritional status, such as time and substance of feeding prior to testing and the positive observed effects on consuming carbohydrates prior to anaerobic exercise.

Bonen et al. (1983) compared substrate and hormonal responses of females to a session of 30 minutes at 40% of predetermined VO$_{2\text{max}}$ followed by an additional 30 minutes at 80% during to follicular and luteal phases of the menstrual cycle. Though estradiol was not measured within this study, the researchers found no significant changes in lactate response between the exercise completed within the two phases. Similar research has also concluded similar findings. (Dombovy; 1987 and Nicklas; 1989). Upon discussion, the noted decrease in lactate response to exercise during the mid-luteal phase was suggested to have been due to differences in subjects’ nutritional status, such as time and substance of feeding prior to testing and the positive observed effects on consuming carbohydrates prior to anaerobic exercise. In contrast to the aforementioned studies, many have used oral contraceptives as a hormonal control rather than relying on the menstrual/ovarian cycles as the sole independent variable(s) when studying anaerobic effects and have yielded either lactate threshold or blood lactate concentrations vary dependent upon menstrual phase of testing as well (Forsyth, 2005;
Sunderland, 2011). Therefore, according to this controlled research, it seems most likely that the menstrual cycle does not affect blood lactate concentration in response to anaerobic exercise.

**Luteinizing Hormone**

Luteinizing hormone (LH) performs a key function in a healthy reproductive system in both males and females. It is a gonadotropic hormone that is produced and released in the anterior pituitary gland within the brain. In females, it is responsible for the stimulation of the ovaries to produce estrogens and measured at its greatest concentrations 24-48 hours prior to ovulation. This surge is responsible for allowing the ovaries to release an egg during ovulation. If fertilization occurs, luteinizing hormone will stimulate the corpus luteum, producing progesterone to prepare the endometrium for embedding of the fertilized ovum (Hall & Guyton, 2011).

If females of childbearing age have abnormally high serum concentration of luteinizing hormone, infertility may occur. Additionally, elevated concentrations are connected with the diagnosis of polycystic ovary syndrome, which create an environment where testosterone, the primary male gonadotropic hormone, secretions are increased. Furthermore, genetic conditions that affect the X chromosome such as Turner syndrome effecting only females and Klinefelter’s syndrome, effecting only males, are linked with elevated levels of LH. In Turner syndrome specifically, the elevated LH levels may be linked to a lack of negative feedback from the ovarian hormones. Furthermore,
individuals diagnosed with these syndromes are naturally unable to reproduce (National Library of Medicine, Turner Syndrome, and Home Health Network- LH).

**Luteinizing Hormone, Estrogen, and Athletics**

Within athletics, competitive females that frequently engage in high intensity training sessions and chronically limit caloric intake with the intention to increase leanness, have been shown to suppress their gonadotropin-releasing hormone (GnRH) pulsatility. Secondly, they exhibit hyperandrogen and hypoestrogen hormonal profiles (Warren, 2001). Limited GnRH will subsequently limit the secretion of luteinizing hormone and result in ovarian stimulation to produce estradiol. Reduced levels of luteinizing hormone have been shown to result in delayed menarche or amenorrhea (Warren, 1980; Baker, 1981; Loucks, 1989). Chronic reduced estrogen may inhibit a young female athlete from reaching the normal levels of peak bone mass, which in turn predisposes her to osteoporosis, osteopenia, and other forms of suboptimal bone mineral density as well as the possibility of infertility (Warren, 2001; Cumming 2001).

In a 12-week resistance training program, Şahin and Korkusuz (2013) measured hormone levels in female participants against controls. They concluded that the exercising group increased their prolactin levels (p=0.02) from baseline to the conclusion of the study, but estrogen, follicle stimulating hormone, luteinizing hormone, and progesterone remained unchanged. Additionally, Nakamura et al. (2011) investigated the effects of acute resistance training on the ovarian and anabolic hormones of young women during the different phases of their menstrual cycles and claimed that serum
estradiol and progesterone levels increased after acute resistance exercise in the mid-luteal phase of the cycle, but not within the early follicular phase. Copeland (2002) compared the acute effects (over 7 days) of upper and lower body resistance exercise as well as aerobic exercise on hormones in 6 healthy recreationally active subjects, ages 19-31 years old, with regular menstrual cycles for a minimum of 1 year. Exercises were performed in the mornings of the luteal phase of their menstrual cycles and findings indicated a significant increase in estradiol, growth hormone, testosterone, and cortisol at the conclusion of the study in comparison to baseline measurements. Though the aforementioned studies had varying conclusions regarding overall serum fluctuations in sexual hormones following acute resistance training, the aim of this research is to explore differences in anaerobic power output that are associated with known variances in estradiol during the early follicular, ovulation, and mid-luteal phases of the menstrual cycle respectively.
CHAPTER III

METHODOLOGY

Participants

The sample was composed of 11 non-pregnant collegiate females age 20.27 ± 1.27 years who were either on the same prescription of birth control for at least six months or not on a prescription at all for the past six months. They had an average height of 162.44 ± 6.08 cm, weight of 62.07 ± 9.95 kg, and body mass index of 23.45 ± 3.13 kg/m². Each of the 11 participants self-identified as being free from metabolic and menstrual-related diseases or disorders and having had menstrual cycles consisting of 28 ±2 days for the past three months. All participants reported that they were free of any physical limitations that would impact the ability to perform a maximally loaded bench press as well as cycling against 7.5% of their body weight for 30 seconds. Participants were recruited via word of mouth and oral advertising at sorority meetings, during lecture class announcements, and at the university exercise science club meetings. This study was approved through the Institutional Review Boards of both Kent State University and University of Mount Union, and all participants signed informed consent documents prior to any experimental testing.

Experimental Design

The participants met a total of four times throughout this study in the musculoskeletal exercise science lab of the university. The first meeting included a familiarization session of exercises to reduce a learning effect on the days of data
collection. Subsequent testing was completed on days 1, 14, and 21 of each participant’s individual menstrual cycle and upper and lower body anaerobic capacities were compared.

**Familiarization**

The first meeting with all of the participants was conducted at any time period prior to the onset of menses. It included a familiarization session of the standardized warmup that was to be completed at the beginning of each testing session. Anthropometric data was collected to be used for the descriptive statistics including height in centimeters via a stadiometer (North Bend, WA) and weight in kilograms via a calibrated digital scale (COSMED, Chicago, IL). The participants then completed a 1 repetition maximum (1RM) bench press to assess upper body strength on a counterbalanced Smith Machine (Columbus, OH).

**Standardized warmup.** The standardized warmup included peddling on a stationary cycle ergometer against zero resistance for 5 minutes, completing 10 body weight jump squats and 10 modified (knees as the fulcrum) push-ups at their own pace. After the warmup, they were asked to subjectively rate their motivation using a scale from 1-7. A rating of 1 indicated they were not at all motivated; 2, poorly motivated; 3, fairly motivated; 4, moderately motivated; 5 somewhat strongly motivated; 6, strongly motivated; and 7, extremely motivated.

**One repetition maximum protocol.** The 1RM protocol was to first instruct the proper technique to the participant including adjusting for a comfortable hand position
shoulder width apart. The bar was initially loaded to 45lbs no matter the resistance training status of the participant. She was instructed press the bar upwards up to three times and then rest. Depending on her comfort level, weight was increased 2.5, 5, 10, or 15 lbs at a time. She was instructed to press the weight no more than twice if possible the second time and was given a full 5 minute break afterwards. This cycle continued until the participant could only press the weight to full extension one time. The weight to be used as the resistance on the subsequent testing days was calculated to be as close to 50% as possible to the nearest 2.5lb of the 1RM for even weight distribution on the barbell. If her weight had to be slightly altered for even bar distribution, she pressed less than 50%, not more.

**Follicular Phase Testing: Day 1 of Menstrual Cycle**

Each participant contacted the principal investigator via text message at the onset of her menses. Testing was then scheduled to occur as soon as possible within 12 hours of the onset. The participants were instructed to keep a food diary of every item consumed prior to testing that day as well as the time of each consumption. Text messages were sent to the participants 24 hours prior to scheduled testing days with a reminder of the pre-testing requirements. Food was not restricted, but the females had to consume everything they had eaten prior to Day 1 testing on the subsequent days of testing for consistency purposes. However, participants were required to refrain from food, drink, chewing gum, and brushing their teeth a minimum of 1 hour prior to testing as those factors may have influenced the estradiol saliva-based analysis. Upon arrival to
the laboratory, the participants spit into a 1.5mL plastic microcentrifuge test tube until it was full to be used for estradiol analysis. They then completed the standardized warmup and rated their motivation to exercise on a scale from 1-7. A score of 1 indicated they were not at all motivated; 2, poorly motivated; 3, fairly motivated; 4, moderately motivated; 5 somewhat strongly motivated; 6, strongly motivated; and 7, extremely motivated.

The equipment used to analyze upper body power was the Ballistic Measurement System (Innervations, Toledo, OH). The Ballistic Measurement System (BMS) consists of a linear position transducer PT5A which contains a thin bungee cord out of the top of the box that ends with a Velcro strap. The linear position transducer PT5A was directly aligned using an electronic level underneath one side of the barbell and the Velcro strap was then wrapped around the barbell so that when the barbell traveled in a linear plane, the results would yield values without the need of mathematical correction. A phone cord then attached the rest of the BMS equipment from the transducer into a laptop computer with the system software (Innervations Version: 2016.3.0.) to record the force and velocity of the moving bar. To complete the upper body power test, participants first laid supine on a flat weight bench and adjusted their hand position to where it was comfortable shoulder width apart. They were instructed to lower the bar all the way to their chest and hold it there. As this point the BMS was calibrated in the bottom position by clicking a calibration button on the laptop computer. They were then instructed to lift the weight to their highest extension and the barbell was calibrated in the top position.
The weight was then racked and the researcher explained the protocol to the participant again.

When the participant was ready, the researcher clicked a data collection button on the laptop computer and the participant moved the bar to her chest. From there, she lifted the weight for three strict presses slowly raising the bar to a four second verbal count from the researcher to reach full extension and lowered at her leisure against a resistance of their calculated ~50% of the predetermined 1RM. After the third strict slow press, the participant was instructed to pause with the weight in the bottom (chest) position and then explosively press maximally and rack the bar. The specific variables of interest recorded were peak force (N), mean force (N), peak power (W), mean power (W), and peak velocity (m/s). Participants completed three sets of the exercise protocol with three minutes of rest in between each set and the set that produced the greatest power output was used for statistical analysis.

Participants were then escorted to the Velotron cycle ergometer (Seattle, WA) for the Wingate test. The seat was adjusted for their comfort and they were reminded of the testing procedures. The Wingate procedure includes the subject peddling against zero resistance for two minutes on a designated mechanical stationary bike. After this warm up, they were given a verbal 20 second countdown followed by “3-2-1-GO!” The participant then peddled as quickly as possible for 30 seconds against a resistance of 7.5% of their recorded body weight recorded immediately prior to the test. Verbal positive encouragement was given throughout the test. Data was used to assess maximal
anaerobic power, peak watts, mean watts, fatigue index, and total work from the 30 seconds of exercise. Upon immediate conclusion of the test, the participant was asked to rate her perceived exertion on a standard Borg RPE scale. The Borg RPE scale ranges from values of 6-20 and linearly increases with intensity. A rating of 6 indicates “no exertion at all” and a rating of 20 indicates “maximal exertion” (McArdle, 2010).

**Ovulation Testing: Day 14 of Menstrual Cycle**

Fourteen calendar days after the first testing session, participants returned to the lab mimicking the time of day and previous food intake, exercise, and sleep patterns prior to the first meeting. Participants were then escorted to the ladies’ restroom to urinate on a New Choice ovulation predictor one-time-use test (Salexess Enterprises, Frederik, MD) for insight as to if their body was still in the follicular phase or approaching the luteal phase of the ovarian cycle. The ovulation predictor test screened for elevated levels of luteinizing hormone (LH) and should have tested positive if the female was ovulating or within a 48-hour window of approaching ovulation. However, since the subject matter of this research was aimed to focus on the correlation of estrogen levels and power output, the female participants were permitted to perform the physical tests regardless of the ovulation predictor test results. The participants then had their saliva collected to measure estrogen at a later date, performed the same prescribed dynamic warmup and all of the exercise testing that had occurred 14 days prior.
Luteal Phase Testing: Day 21 of Menstrual Cycle

Participants returned to the lab a final time on day 21 of their menstrual cycle, 7 days following the previous data collection point. All food intake, exercise, and sleep patterns that were demonstrated on the first day of data collection were mimicked in efforts to decrease external factors that may have influenced dependent variables. All of the aforementioned protocols were repeated for a third time, therefore recording a third point of power output comparison for the upper and lower body throughout the menstrual cycle.

Estradiol Analysis

The 17-β estradiol levels were extracted by means of the saliva sample collected on the three days of data collection. The sample was put into a microcentrifuge and spun at 3000 rpm for 15 minutes. The supernatant was then pipetted off of the top and frozen at -20°C overnight. The sample was then brought to room temperature the next day over ice. When it had thawed, it was spun at 3000rpm for an additional 15 minutes in the microcentrifuge. The supernatant was removed again and dispensed into a new microcentrifuge tube. The sample was then frozen again at -20°C again per manufacturer’s (DiaMetra, Perugia Italy) instructions until all of the samples were collected and ready for the enzyme-linked immunosorbent assay (ELISA) analysis.

Analytical Plan

The data was analyzed for statistical differences utilizing multiple one way of analyses of variances (ANOVAs) with repeated measures on condition. The upper body
dependent variables evaluated were: peak force (N), mean force (N), peak power (W), mean power (W), and peak velocity (m/s) and motivation to exercises. The lower body dependent variables evaluated were anaerobic capacity (w/kg), peak power (W), mean power (W), fatigue index (W/s), and total work (J) from the Wingate test as well as the subjective rating of perceived exertion (RPE) data immediately after the test. Estradiol levels coinciding with the test day were to be set as a covariate, but this was not possible due to a limitation explained in the discussion section of this dissertation. A-priori significance was set at $\alpha \leq 0.05$ and all data was analyzed using the Statistical Package for the Social Sciences (SPSS) Version 24.
CHAPTER IV

ANALYSIS OF FINDINGS

Results

Table 1 presents descriptive statistics for the participants including their determined 1-repetition maximum on the bench press during the familiarization session prior to menstrual phase-related data collection. Additionally, it includes mean calculations and standard deviations of the weight lifted as well as the calculation percentage of the maximum allowing for even distribution on the barbell to the nearest 2.5lbs. The multiple ANOVAs revealed a trend (p=0.077) in peak power across the three time points. However, the remaining variables did not yield statistical significance. Peak force (p=0.859), mean force (p=0.384), mean power (p=0.397), peak velocity (p=0.936). Table 2 describes this data by means of averages and standard deviations.

For the lower body exercise, there were no significant effects of time from any of the ANOVAs across the menstrual cycle: anaerobic capacity (p=0.356), peak power (p=0.342), mean power (p=0.347), fatigue index (p=0.249), and total work (p=0.341). Additionally, there were no differences between participants’ motivation to exercise on each of the testing days (p=0.114) nor did the participants differ in their subjective ratings of perceived exertion after the maximal lower body power test (p=0.149). Of the 11 subjects, six tested positive on the ovulation predictor test on day 14 of their individual menstrual cycles and five tested negative. Table 3 describes the data by means of averages and standard deviations.
Table 1. *Participant Physical Characteristics*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Females (N= 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20.3 ± 1.3</td>
</tr>
<tr>
<td>Height (centimeters)</td>
<td>162.4 ± 6.1</td>
</tr>
<tr>
<td>Weight (kilograms)</td>
<td>62.1 ± 9.95</td>
</tr>
<tr>
<td>Body Mass Index (kg/m^2)</td>
<td>23.5 ± 3.1</td>
</tr>
<tr>
<td>Max Bench Press (lbs)</td>
<td>79.1 ± 16.7</td>
</tr>
<tr>
<td>Resistance (lbs)</td>
<td>37.7 ± 8.2</td>
</tr>
<tr>
<td>Percent of Max (lbs)</td>
<td>47.7 ± 1.6</td>
</tr>
</tbody>
</table>

Data are presented as the means ± SD.

Table 2. *Upper Body Dependent Variables from the Three Conditions*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Follicular Phase</th>
<th>Ovulation</th>
<th>Mid-Luteal Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1 of Menses</td>
<td>Day 14</td>
<td>Day 21</td>
</tr>
<tr>
<td>Peak Force (N)</td>
<td>215.21 ± 59.16</td>
<td>212.55 ± 59.67</td>
<td>213.31 ± 56.77</td>
</tr>
<tr>
<td>Mean Force (N)</td>
<td>170.66 ± 37.77</td>
<td>170.66 ± 38.83</td>
<td>161.32 ± 47.42</td>
</tr>
<tr>
<td>Peak Power (W)</td>
<td>149.12 ± 61.95</td>
<td>135.35 ± 51.45</td>
<td>134.84 ± 59.16</td>
</tr>
<tr>
<td>Mean Power (W)</td>
<td>90.37 ± 41.49</td>
<td>83.44 ± 36.84</td>
<td>94.25 ± 45.24</td>
</tr>
<tr>
<td>Peak Velocity (m/s)</td>
<td>0.76 ± 0.28</td>
<td>0.79 ± 0.14</td>
<td>0.78 ± 0.16</td>
</tr>
</tbody>
</table>

Data are presented as the means ±SD.

Table 3. *Lower Body Dependent Variables from the Three Conditions*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Follicular Phase</th>
<th>Ovulation</th>
<th>Mid-Luteal Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1 of Menses</td>
<td>Day 14</td>
<td>Day 21</td>
</tr>
<tr>
<td>Anaerobic Capacity (W/kg)</td>
<td>5.42 ± 0.43</td>
<td>5.80 ± 1.25</td>
<td>5.48 ± 0.45</td>
</tr>
<tr>
<td>Peak Power (W)</td>
<td>551.73 ± 147.98</td>
<td>525.45 ± 116.48</td>
<td>533.64 ± 125.80</td>
</tr>
<tr>
<td>Mean Power (W)</td>
<td>339.09 ± 62.64</td>
<td>345.55 ± 63.48</td>
<td>346.73 ± 60.16</td>
</tr>
<tr>
<td>Fatigue Index (W/s)</td>
<td>10.60 ± 4.02</td>
<td>9.39 ± 3.15</td>
<td>9.97 ± 2.93</td>
</tr>
<tr>
<td>Total Work (J)</td>
<td>10171.55 ±</td>
<td>10373.65 ±</td>
<td>10398.86 ±</td>
</tr>
<tr>
<td></td>
<td>1878.83</td>
<td>1901.12</td>
<td>1801.86</td>
</tr>
<tr>
<td>RPE</td>
<td>17.64 ± 1.57</td>
<td>18.09 ± 1.30</td>
<td>18.45 ± 1.63</td>
</tr>
</tbody>
</table>

Data are presented as the means ±SD.
CHAPTER V
DISCUSSION, IMPLICATIONS, AND RECOMMENDATIONS

Discussion of the Findings

The participants did not statistically differ in any of the examined power output-related variables. Each testing day after the female participants completed their standard dynamic warm-up, they were asked on a scale of 1-7 how motivated they were to exercise. They never reported feeling more or less motivated as a group on any particular testing day (p=0.114), so motivation did not impact the findings of this research. Similarly, the participants gave a subjective rating of perceived exertion using the 6-20 Borg scale immediately after each lower body Wingate test. This variable did not change across the menstrual cycle (p=0.149). This indicates that the subjects did not feel as if they were working any harder after the Wingate on any particular testing day and consistently reported subjective values of 17-20 indicating maximal effort.

Pestana et al. (2016) compliments this research as they investigated the influences of the mid-follicular and late-luteal phases on the anaerobic capacity of university students. They employed the same Wingate test as this research and evaluated the variables of absolute peak power, average power, relative peak power (w/kg), and average relative power (w/kg). They did not find any statistical differences (p>0.05) among the variables between the phases. However, they did find that the participants had a higher peak heart rate (p=0.01) in the late-luteal phase (183.90 ± 12.95 bpm) in
comparison to the mid-follicular phase (180.48 ± 11.83 bpm). This increase in an average of three beats/min may be attributed to any number of factors.

In another example of similar research, Bushman et al. (2006) evaluated anaerobic power performance and the menstrual cycle on eumenorrheic and oral contraceptive users. They used both the Wingate test and the Margaria-Kalamen stair test. After familiarization, testing was completed during menses and the luteal phase as documented by a urinary luteinizing hormone test for the non-oral contraceptive users. The study concluded no significant findings (p>0.05) between the phases and determined that there were no differences in anaerobic data between the groups either.

Štefanovský et al. (2016) sought to identify differences in anaerobic power, capacity, and fatigue index in female judokas by utilizing an upper body Wingate test and a special judo fitness test which consisted of running and throws using the ippon-seoi-nage technique. Unlike this research and others that tested on specific days of the menstrual cycle, for example 1, 14, and 21, this research tested within days 6-10 (follicular phase) and within days 20-24 of the luteal phase. No significant differences were reported in power output within the upper body Wingate test between phases, but the special judo fitness test did yield a difference in the number of throws within the first 15 seconds of the test between phases (p=0.03).

In another example, Okudan and colleagues (Okudan, 2005) aimed to identify differences in peak power, mean power, and fatigue index using the lower body Wingate test to correlate to serum estradiol among other menstrual cycle driven hormones on days
1, 14, and 21 of their sedentary participant’s individual cycles. Their results yielded no significant changes in power output across the days and they concluded that anaerobic performance which requires high motivation is not affected by the menstrual cycle. Their research protocols in collecting estrogen samples and testing on days 1, 14, and 21 in a single menstrual cycle are remarkably similar to this study and these studies collectively add to the research in that menstrual phase does not impact maximal anaerobic physical performance.

This study extends the literature in this area of inquiry as it assessed upper body power out in female participants who were not all part of the same organized sports. Some of the participants were out of season track sprinters (N=2), but the rest of the participants were simply interested in partaking in the research and aerobically exercised recreationally at a moderate intensity 2-3 days a week. Additionally, it tested upper body power output during time points of a single menstrual cycle opposed to a comparison of two phases or not controlling for menstrual phase at all. One thing to note, however, for all researchers who study female populations is the limitation of truly knowing the phase prior to data collection.

**Confirmation of Menstrual Phase**

In this study, the female participants came in on days 1, 14, and 21 of their cycles. Day 1 was categorized at the onset of menstrual blood and testing was performed within 12 hours of documentation. Menstrual phase was planned to be confirmed through estrogen concentrations observed through an ELISA, but that confirmation was not
possible until the entire study was completed. The ELISA technology used in this particular research came with a 96 well plate that contained a protein to bind to the estrogen when exposed to the saliva sample. Though this method is robust in identifying estradiol concentrations, all of the samples must be run at one time since the ELISA plate cannot be reused after exposure to particular manufacture chemicals and incubation. Thus, the research must still rely on other methods of predicting menstrual phase by either utilizing the basal temperature or calendar-based protocols.

**Luteinizing Hormone**

An ovulation predictor test was given to the participants to complete on day 14 of testing to identify if there was a surge in luteinizing hormone. Elevated serum concentrations of luteinizing hormone immediately prior to ovulation are partially responsible for the surge in estrogen that occurs with ovulation. Of the 11 participants, six of the females tested positive for elevated luteinizing hormone in the urine on day 14 while five of the participants tested negative for elevated levels. Since the window for ovulation is optimal for 24 hours (Hall & Guyton, 2011), the researchers’ may have missed this narrow margin. However, based on the extended windows of the follicular phase accounting for days 1-13 and the luteal phase lasting between approximately days 15-28 of the menstrual cycle, the researchers are confident that testing occurred within those phases. Redmen (2003) made note that there is normal variability within the female menstrual cycle. This variability may be due to acute stress, heavy exercise, sickness, nutritional status or other intrinsic or extrinsic factors. Therefore, it is not
surprising that the ovulation predictor tests were not unanimous in their positive or negative results.

**Limitations**

This research examined estrogen levels in collegiate females by means of an enzyme-linked immunosorbent assay (ELISA). However, when the biochemical analysis took place after all data had been collected, it was discovered that the ELISA kit had been compromised and that the 17-β estradiol levels could not be confidently determined. Therefore, based on previous research protocols, this study can suggest the female participants were in the follicular phase on the first day of testing and the luteal phase on the third day of testing, but peak ovulation cannot be confirmed due to the lack of accurate endocrine data. Therefore, the hypothesis cannot be confirmed nor rejected due to this lack of evidence. However, this research still suggests that there is not a difference any of the upper body nor lower body dependent variables assessed across the menstrual cycle.

Secondly, within the parameters of this research, subject size may have been too small to identify significant differences across the testing days with 11 participants. However, similar research tends to focus on subject pools of 8-10 women (McCracken, 1994; O’Driscoll, 2013; Štefanovský, 2016), limited sample size is an important consideration when reviewing research of this subject matter as it appears to be common.

Thirdly, participants were asked to keep a food diary prior to any testing on day one of menses and mimic that food intake as well as timing of consumption and exercise
routines on the following testing days. Since the participants were not monitored 24
hours a day, this information was based on volunteer’s discretion and thus serves as the
third limitation for this study.
CHAPTER VI

CONCLUSION

Within the parameters of this research, neither female upper nor lower body power output differed across the menstrual cycle when analyzed using 50% of a one repetition (1RM) maximal bench press and the 30 second maximal effort cycle ergometer Wingate test. Therefore, researchers should not alter their subject populations due to the incorrect assumption that power output may be influenced by the menstrual cycle. Though similar research has produced equivocal results, other research supports these same findings. Lastly, future studies on female populations should also use a combination of the ovarian calendar method, basal temperature, and endocrine analysis to confirm for menstrual phase.
APPENDICES
APPENDIX A

LETTER OF CONSENT
Appendix A

Letter of Consent

An Exploration of the Relationship between Menstrual Phase and Collegiate Female Upper and Lower Body Anaerobic Capacities

Participant Informed Consent

Invitation to Participate
You are cordially invited to participate in this study to explore the relationship between 17-β estradiol and power output at three times during the menstrual cycle.

Purpose and Significance
Females are often excluded from exercise-based studies due to the assumption that the hormonal influences of the menstrual cycle may impact results. Nonetheless, with the increased quantity of competitive female athletes in recent decades, it is important to study populations of females who engage in chronic high intensity physical activity and the potential influences of the menstrual cycle on performance. While previous research has probed the impact of potential menstrual influences on aerobic activities, anaerobic actions and possible hormonal influences are far less discussed. The aim of this research is to better understand the correlations between anaerobic capacities and the female sex hormone, estrogen, throughout the menstrual cycle.

Athletics has seen an increase in competitive female athletes over the past decades. With this increase, it is prudent to explore times during which females may have a natural boost in anaerobic power based on their own menstrual cycle regulated hormonal concentrations. If females reportedly are able to produce more power when estrogen concentrations are at their highest, they may be able to individualize and maximize their training based on their own biochemistry. Secondly, this could give competitive anaerobic female athletes additional motivation to avoid amenorrhea in order to take advantage of this possible naturally occurring ergogenic aid. Lastly, this research could lead to the production of synthetic estrogens marketed as performance enhancers.

Basis for Subject Selection
Participants must be apparently healthy non-pregnant females between the ages of 18-30 years with a regular menstrual cycle (28±2 days for the past three months) and able to tolerate 30 seconds of maximal effort on a stationary bike and perform a maximal bench press. If they have begun a new topical, oral, or implanted form of birth control within the past 6 months, they are ineligible to participate as it may influence the hormones that determine the phases of the ovarian cycle.
Methods and Procedures.

Subjects will meet a total of four times. The first meeting will consist of familiarization of the Wingate procedures and the completion of a 1 repetition maximum (1RM) bench press. The second meeting will be scheduled within 24 hours of the onset of menses indicating the follicular phase of the ovarian cycle. Participants will have their weight and stature assessed using a standard calibrated scale and stadiometer. They will have their 17 Beta estradiol (estrogen) levels measured via a saliva sample following the ALPCO manufacturer’s directions for the enzyme-linked immunosorbent assay (ELISA). Participants will then complete a standardized dynamic warmup prior to physical activity. The warm-up will include cycling on a stationary bike against zero resistance for 5 minutes, completing 5 jump squats at their own pace, and performing 10 modified (knees on the ground) push-ups. Using a 1-7 Likert Scale, participants will rate their motivation to exercise prior to physical activity testing.

After the warm-up, participants will be escorted to a standard Smith Machine to assess upper body anaerobic capacity against a resistance of 50% of their 1RM to the nearest kilogram. They will be instructed to do 2 sets of 3 strict repetitions raising the bar for a four second count to reach full extension and lower at their leisure. The force plate transducer of the ballistic measurement system will be attached to the barbell as well as a computer to record the force and velocity of the moving bar. When the participant is ready, she will be instructed to maximally press to full extension as quickly as she can. Participants will do this a total of three times with three minutes of rest between sets. After each maximal press, she will be asked to rate her perceived exertion on a standard 6-20 Borg rating of perceived exertion (RPE) scale. The highest value of anaerobic capacity (W/kg) will be analyzed as well as peak power (watts), mean power (watts), fatigue index (watts/second), and total work (joules).

After an additional 5 minutes of rest, participants will then be seated on the Velotron cycle ergometer and instructed on the protocols for a Wingate Test. The Wingate procedure includes the subject peddling against zero resistance for two minutes on a designated stationary bike. After this warm up, they will be given a 20 second countdown followed by “3-2-1-GO!” verbal instructions. The participant will then pedal as quickly as possible for 30 seconds against a resistance of 7.5% of their recorded body weight. Verbal positive encouragement will be given throughout the test and the data will be used to assess maximal anaerobic power, peak watts, mean watts, fatigue index, and total work from the 30 seconds of exercise. Upon immediate conclusion of the test, she will be asked to rate her perceived exertion a standard 6-20 Borg RPE scale as she did after the bench-press protocol.

Fourteen calendar days after the first testing session, participants will return to the lab mimicking the time of day and previous food intake, exercise, and sleep patterns prior to the first meeting. Participants will be asked to record all of these factors so that outside influences on the results may be reduced. Participants will then be escorted to the ladies’ restroom to urinate on an ovulation predictor one-time-use test for validation that their body is in the luteal phase of the ovarian cycle. The ovulation predictor test screens for
elevated levels of luteinizing hormone (LH) and should test positive if the female is ovulating. Since the subject matter of this research is focused on the correlation of estrogen levels and power out, the female participant will be permitted to perform the physical tests regardless of the ovulation predictor test results. The participant will then have her saliva collected to measure estrogen and repeat the upper and lower body power tests in the same order as she had during menses.

Lastly, participants will return to the lab 7 days later on day 21 of their menstrual cycle. All food intake, exercise, and sleep patterns that were demonstrated on the first day of data collection should be mimicked in attempts to decrease external factors that may influence dependent variables. All of the aforementioned protocols will be repeated for a third time and thus a third point of power output comparison throughout the menstrual cycle will be recorded.

**Potential Benefits and Compensation.**

Subjects will learn their anaerobic power values and fatigue index at three points in the menstrual cycle. They will also be contributing to research that is underexplored within the field of exercise science. At this time no financial compensation will be given for participation.

**Potential Risks to the Subjects.**

Sudden maximal exercise causes an increase in respiratory rate and heart rate. Due to the immediate pulling of blood in the lower body, participants may feel light headed after the exercise concludes. They may also feel nauseous and even vomit. Muscle soreness for up to 72 hours is also a common side effect of maximal exercise.

**Elimination or Minimization of Potential Risks.**

Individuals will be given a designated area to rest and elevate their legs after the conclusion of the exercise. This modality will return blood to the brain and improve recovery. A restroom is located next to the lab and participants will be escorted if they feel the need to vomit and accompanied until they’re ready to return to the lab.

**Location of Data Collection**

Data will be collected in the Clay Exercise Science Lab at the University of Mount Union. The address is 1972 Clark Ave, Alliance, OH 44601.

**Guarantee of Confidentiality**

Participants will be guaranteed full confidentiality in accordance with HIPPA laws. The only people who will have access to the subject’s information will be the researchers. Documents will be kept in a locked filing cabinet when not in use. No names will be used throughout the study in order to ensure confidentiality standards.
If You and Questions

If you have any questions about the procedures in which you will participate, please do not hesitate to ask. If you have questions later, please feel free to contact the investigators listed below. All questions about the procedures or the study in general will be answered. However, the investigators may choose to wait to answer your questions until after you have completed the procedure, to ensure that your responses will not be affected by your knowledge of the research. If you have additional questions concerning the rights of research subjects, you may contact the University of Mount Union Human Subjects Committee at humansubjects@mountunion.edu.

You are voluntarily making a decision whether or not to participate. Your signature certifies that you have decided to participate, having read and understood the information presented. Your signature also certifies that you have had an adequate opportunity to discuss this study with the investigator and that you have had all your questions answered to your satisfaction. You will be given a copy of this consent form to keep.

_______________________________________  ____________________________
Signature of participant                   Date

_______________________________________  ____________________________
Printed name of participant                Phone Number

_______________________________________  ____________________________
Investigator Signature                     Date

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APPENDIX B

MOTIVATION TO EXERCISE LIKERT SCALE
APPENDIX B

MOTIVATION TO EXERCISE LIKERT SCALE

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
<td>Poorly Motivated</td>
</tr>
<tr>
<td>3</td>
<td>Fairly Motivated</td>
</tr>
<tr>
<td>4</td>
<td>Moderately Motivated</td>
</tr>
<tr>
<td>5</td>
<td>Somewhat Strongly Motivated</td>
</tr>
<tr>
<td>6</td>
<td>Strongly Motivated</td>
</tr>
<tr>
<td>7</td>
<td>Extremely Motivated</td>
</tr>
</tbody>
</table>
APPENDIX C

BORG RATING OF PERCEIVED EXERTION SCALE
APPENDIX C

BORG RATING OF PERCEIVED EXERTION SCALE

6……………………………………………………………………….. No exertion at all
7……………………………………………………………………….. Extremely light
8…………………………………………………………………………
9……………………………………………………………………….. Very light
10…………………………………………………………………………
11……………………………………………………………………….. Light
12…………………………………………………………………………
13……………………………………………………………………….. Somewhat hard
14…………………………………………………………………………
15……………………………………………………………………….. Hard (heavy)
16…………………………………………………………………………
17……………………………………………………………………….. Very hard
18…………………………………………………………………………
19……………………………………………………………………….. Extremely hard
20……………………………………………………………………….. Maximal exertion
REFERENCES
REFERENCES


