THE EFFECTS OF ESTROGEN PEAKS ON THE STRETCH REFLEX RESPONSE DURING THE MENSTRUAL CYCLE

KAYLA RUFFNER

Bachelors Exercise Science

University of Mount Union

May 2017

Submitted in partial fulfillment of requirements for the degree

MASTER OF EDUCATION IN EXERCISE SCIENCE

at the

CLEVELAND STATE UNIVERSITY

December 2019

We hereby approve this thesis for

KAYLA LYNN RUFFNER

Candidate for the Master of Education in Exercise Science degree

For the Department of Health and Human Performance

and

CLEVELAND STATE UNIVERSITY'S

College of Graduate Studies by

_____

Thesis Chairperson, Dr. Douglas Wajda

_____

Department & Date

_____

Thesis Committee Member, Dr. Kenneth Sparks

_____

Department & Date

_____

Thesis Committee Member, Dr. Emily Kullman

_____

Department & Date

_____

Thesis Committee Member, Dr. Kathleen Little

_____

Department & Date

_____

Associate Dean of Academic Affairs, Dr. Brian Yusko

_____

Department & Date

Date of Defense: December 4, 2019

ACKNOWLEDGEMENT

I would like to thank my professors for providing me with the knowledge and experience who encouraged and helped get me to where I am today. Dr. Sparks, thank you for your wisdom and patience you provided me during this entire process. Dr. Kullman, thank you for your insight and knowledge during the entirety of my thesis. Dr. Little, thank you for helping me become the writer I am today and your knowledge of how to conduct research properly. Dr. Wajda, thank you for our constant support and encouragement throughout the entire thesis process, I couldn't have gotten this far without you.

I would also like to thank my subjects who participated in my study for their time and hard work they put in. I would like to thank my mentors/ past professors from my undergrad program at the University of Mount Union; Dr. Lowery, Dr. Scanlon, Dr. Mendel and Dr. Clark. You are all the reason why I am where I am today with constant knowledge and academic support you offered during my undergraduate career as well as after graduation. Dr. Lowery you shined my research light and taught me so much and I cannot thank you enough for what you have taught me. Dr. Scanlon, you showed me how hard work pays off and how researching women is not easy but should be done in today's world. Dr. Mendel, you guided me all four years of my undergraduate program and taught me how to run and love statistics and to do it correctly. Dr. Clark, you taught me the importance of human movement and how to conduct testing sessions in an efficient matter and showed me my loved for special population clients being the ones I want to work with.

Finally, I would like to thank my mother, Jennifer, my aunt, Kimberly, my uncle, John, my stepdad, Mark, my brother, Christian and the rest of my family who taught that working hard is the only way to succeed. Thank you for your constant support and words of encouragement for me to keep going. If it wasn't for you all I probably would not have had the motivation to get this far. Your never-ending love and constant motivation and life sessions are the core of my hard work.

THE EFFECTS OF ESTROGEN PEAKS ON THE STRETCH REFLEX RESPONSE DURING THE MENSTRUAL CYCLE

KAYLA RUFFNER

ABSTRACT

Purpose. The purpose of this research was to conduct a multifactorial investigation to examine potential ACL injury mechanisms and physiological changes across the phases of the menstrual cycle.

Methods. Eighteen menstruating, non-pregnant females, ages 18-35 years old participated in this study. Nine females were on an oral contraceptive were on the same dosage for the last six months and the other nine females were not on any form of birth control at all. All subjects came in for three testing sessions during their follicular, ovulation and menses phases of the menstrual cycle. Each testing session included motor control testing using Neurocom Balance Master, EMG stretch reflex testing of the knee, isometric strength, T-Test drill for agility and blood draw for estrogen and relaxin Analysis. Non- birth control subjects were given ovulation sticks to measure when they were in ovulation phase.

Results. Independent samples T-Test showed no overall difference in age (t=-.932, p=.37) or BMI (t=-1.24, p=.24) between birth control and non-birth control groups. A significant main effect for time within the cycle was observed ($F = 5.2$, $p = .031$) for strength which was highest during menses. No main or interaction effects were observed for agility (Cycle Phase: $F = 0.87$, $p = 0.43$, Group: $F = 0.91$, $p = 0.36$, Time*Group: $F = 1.9$, $p = 0.17$), backward motor control translations (Cycle Phase: $F = 0.56$, $p = 0.58$, Group: $F = 0.83$, $p = 0.38$, Time*Group: $F = 0.63$, $p = 0.54$), forward motor control

translations (Cycle Phase: $F = 1.8$, $p = 0.19$, Group: $F = 0.96$, $p = 0.34$, Time*Group: $F = 2.2$, $p = 0.16$), response latencies (Cycle Phase: $F = 0.28$, $p = 0.76$, Group: $F = 0.99$, $p = 0.35$, Time*Group: $F = 0.42$, $p = 0.66$).

Conclusion. In conclusion, the current study did not provide any concrete findings on the relationship of menstrual cycle with the stretch reflex and potential ACL or other soft tissue injuries in the active female population. Strength was greatest during menses, which is not in line with the literature showing strength is greatest during ovulation.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Overview

Researchers have observed the effects of the menstrual cycle on aerobic and anaerobic exercise, musculoskeletal injuries and strength. Predominantly, the research into potential injury differences during the menstrual cycle has focused on multiple musculoskeletal injuries, especially those of the anterior cruciate ligament (ACL). Through this work, the most striking discovery is that ACL injuries occur 2.8 and 3.6 times more often in female soccer and basketball players, respectively, compared to males (Agel, Arendt, Bershadsky 2005). Based on these findings, an abundance of studies was performed to try and determine the specific mechanisms for the observed increase in ACL injury rate for females.

An extensive number of factors including hormone levels, muscle strength and the menstrual cycle have been studied as potential moderators of injury susceptibility in females. Behan, Maden-Wilkinson, Pain & Folland, (2018) observed that there are sex differences in knee flexor and extensor muscle size ratio and proportion to the constituent muscles between males and females.

In general, many previous studies have aimed to investigate the impact of changing hormone levels during the menstrual cycle on the physiological processes related to performance and injury. To date, these studies have predominantly focused on one parameter each, disregarding a more thorough multifactorial approach. Observing the effect of the menstrual cycle on fitness performance from a neuromuscular, musculoskeletal and hormonal level may allow researchers to provide crucial information to both females, athletes and females who actively exercise.

Purpose. The purpose of this research was to conduct a multifactorial investigation to examine potential injury mechanisms and physiological changes across the phases of the menstrual cycle.

Hypothesis. The muscle stretch reflex, muscle strength and neuromuscular control will vary throughout the menstrual cycle due to hormonal changes.

CHAPTER II

REVIEW OF LITERATURE

Menstrual Cycle

The menstrual cycle consists of three phases known as the follicular phase, ovulatory phase and the luteal phase. The follicular phase is the onset of menses and can last up to nine days. During this phase, estrogen levels slowly increase inducing the secretion of luteinizing hormone (LH). Then as estrogen levels increase further within the phase surge, LH is secreted introducing the ovulatory phase. Ovulation lasts about five days and is the phase where the endometrial lining increases in preparation to receive the ovum (Luteinizing Hormone, n.d).

A typical menstrual cycle consists of 28 days with the follicular phase on days 1-9, ovulatory phase on days 10-14, and menses around days 15-28 (Figure 1). Once the follicle has released the ovum, it is transformed into the progesterone-secreting corpus luteum, initiating the luteal phase, which lasts about 14 days. During this phase, progesterone supports the endometrium until the embryo can implant into the placenta. At the end of the luteal phase, progesterone secretion from the corpus luteum ceases, the endometrium is no longer supported, and menstrual bleeding occurs. The menstrual cycle

is mainly controlled by the pituitary hypothalamus-ovarian axis involving estrogen, progesterone, relaxin and testosterone (Zazulak, Paterno, Myer, Romani, Hewett, 2006).

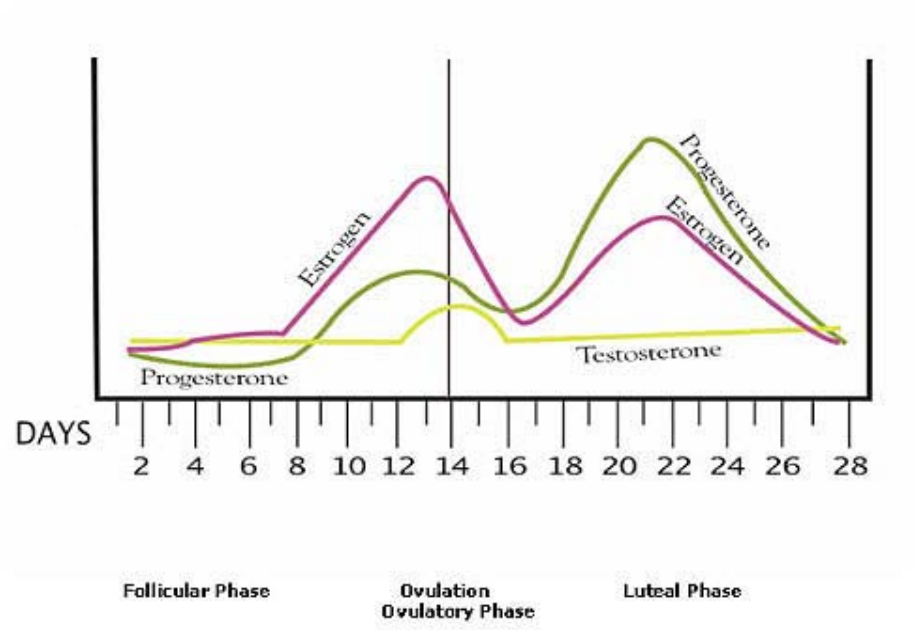

Figure 1. Women in Balance Institute. (2019). Hormone imbalance, menstrual cycles & hormone testing. [online] Available at: https://womeninbalance.org/about-hormone-imbalance/ [Accessed 27 Nov. 2019].

Oral Contraceptive

Oral contraceptives consist of exogenous steroid hormones which inhibit ovulation and result in low endogenous sex hormone concentrations (Carcia, Shultz, Granata, Gansneder, & Perrin, 2004; Chandrashekar, Slauterbeck, & Hashemi, 2005). Different types of birth control include the vaginal ring, intrauterine device (IUD) and patches, but most commonly used is the oral contraceptive. It is important to understand that the type and concentration of estrogen and progestin varies between preparations of oral contraceptives that may influence physiological responses (Elliott-Sale et al., 2013; Godsland et al., 1990).

Female sex hormones. There has been an abundance of research analyzing how female sex hormones affect exercise and nervous system function. There is also evidence that sex hormones also modulate non-reproductive tissues within the body (Turgeon, Carr, Maki, Mendelsohn, Wise, 2006). Progesterone and estrogen receptors can be found in skeletal muscle, bone, ligaments and the nervous system, which suggests that hormonal fluctuations can influence the function and structure of these tissues (Ciocca & Vargas, 1995). It is less clear what the role of female sex hormones is on neuromuscular function.

Estrogen

In its various forms, estrogen is responsible for the development of female secondary sexual characteristics and typical female pattern of fat deposition (Constantini, Dubnov & Lebrun, 2005). It helps control the menstrual cycle, and also positively affects cholesterol and bones in females. The ovaries are the main source of estrogen which is transported by blood throughout entire body. The three types of estrogen are estradiol (E2), the main hormone on premenopausal women, estriol (E3), the main hormone during pregnancy and estrone (E1), the only estrogen the body secretes after menopause ()

Levels of estrogen change during the menstrual cycle, with Reed, B. G., & Carr, B. R. (2018) found that the highest level of estrogen is during the middle of the menstrual cycle, and the lowest during menstruation (Figure 1). At menopause estrogen level remain very low.

Relaxin

The peptide hormone relaxin belongs to insulin family of hormones (Sherwood, 2004; Van der Westhuizen et al., 2008). Research has shown that relaxin has three forms

in humans (Hayes, 2004) with a broad range of physiologic effects throughout the body. Relaxin is transcriptionally regulated by progesterone and glucocorticoid hormones (Garibay-Tupas, Okazaki, Tashima, Yamamoto & Bryant-Greenwood, 2004). Relaxin-2 has been studied in pregnant women but is also found in the serum of nonpregnant women (Glock, Nakajima, Stewart, Badger & Brumsted, 1995; Weiss et al., 1978) and effects on nonreproductive tissues merit further investigation.

It has been shown that relaxin is associated with increased knee joint laxity in pregnant women (Blecher & Richmond, 1998), along with decreased ACL strength in animal models (Dragoo, Padrez, Workman & Lindsey, 2009). Presence of relaxin receptors in the female ACL (Dragoo, Lee, Benhaim, Finerman & Hame, 2003; Glary, Konieczko, Arnold & Cooney, 2003) showed that relaxin- mediated remodeling of female ligaments is an important mechanism. There have been no studies to date that have described the serum relaxin concentrations (SRC) in young, healthy female elite athletes. It is important to understand SRC within this population because relaxin has been shown to increase ligament laxity in women (Belcher & Richmond, 1998; Damen, Burruk, Guler-Uysal, Lotgering, Snijders & Stam, 2001; Dumas & Reid, 1997). Relaxin is secreted during both the follicular and luteal phases but reaches peak levels in the luteal phase (Wreje, Kristiansson, Aberg, Bystrom & VonSchoultz1995).

ACL injury. ACL injuries are more prominent in females compared to males, with a 4-6-fold higher incidence of knee injury than males in the same sports, particularly sports requiring a high degree of jumping and cutting (Chandy & Grana, 1985; Ferretti, Papandrea & Conteduca & Mariani, 1992; Gray et al., 1985). One study reported that serious knee injury was about 6-fold higher in female indoor soccer players compared to

male players (Lindenfeld et al., 1994). Numerous studies have corroborated the higher incidence of serious knee injury in females involved in sports with jumping and cutting movements in comparison to males involved in the same sports (Huston & Wojtys, 1995; Whiteside, 1980; Malone, Hardaker & Garrett et al., 1993).

The three general theories for differences between male and female knee injury rates are anatomical, neuromuscular and hormonal. The anatomical theory consists of two different parts. The first part of the anatomical theory is in relation to the quadriceps angle (Q-angle). There have been arguments on differences in pelvic structure and lower extremity alignment, which possibly account for differences in knee injury rates in males and females (Zelisko, Noble & Porter, 1982; Haycock & Gillette, 1976). Gray et al. (1985), who reported that injury rate differences could not be due to anatomical differences. The second part of the anatomical theory for ACL injury in females, is attributed to females having a smaller femoral notch width compared to males (Emerson, 1993; Hutchinson & Ireland, 1995).

In regard to the Neuromuscular theory, Haycock and Gillette (1976), observed and reported similar injury rates for collegiate male and female athletes although it was indicated that females had higher rates of injury including the knee cap. Along with the theory on neuromuscular involvement, it is important to note that neuromuscular training helps to decrease the differences between athletic males and females (Hewett, Stroupe & Nance et al., 1996) which can help to lead a significant decrease serious knee injury rates in female athletes (Hewett, Lindenfeld, Riccobene, Noyes, 1999). Haycock and Gillette (1976), speculated that gender differences were due to differing levels of training and coaching, and not to anatomical or physiological differences. The neuromuscular theory

is based on neuromuscular alterations which physiologically alter muscular movement and can happen in both knee and ankle joints (Hart, Ko, Konold & Pietrosimione, 2010).

The female hormones estrogen, progesterone and relaxin are part of the third hormone theory influencing the neuromuscular and musculoskeletal systems. There have been reports that show injury rate differences due to estrogen-related differences such as increased joint laxity in women (Huston et al., 1996; Zelisko, Noble & Porter 1982). However, there have been studies that contradict increased laxity as a possible result from fluctuation of estrogen and relaxin on ligament collagen. Estrogen has been known to have a direct and indirect effect on the female neuromuscular system, (Sarwar et al.,1996) reported that quadriceps strength increases and there is a significant slowing of relaxation of the muscle during the ovulatory phase of the menstrual cycle.

Knee Laxity

Research has shown that increased joint laxity is one of the risk factors for ACL injury in women compared to men, it is believed that increased laxity causes changes in biomechanical factors which increase the pressure on knee ligaments, which in return increases the rate of ACL rupture (Park et al., 2009; Zazulak et al. 2006). It is also believed that the hormones estrogen and progesterone which control the menstrual cycle affect the overall integrity of the ACL by altering its structure. Generally, the hormones estrogen decreases tensile properties of the ACL by binding to specific receptors (Warren, Liu, Hatch, Panossian & Finerman, 1999). When estrogen is bound to receptors on the ACL, it's been shown to decrease fibroblast proliferation, which then decreases collagen production. This theory is supported by a case-control study where female recreational skiers who had sustained an ACL non-contact injury demonstrated a two-

fold increase in injury rates during their pre-ovulatory menstrual phase compared to uninjured controls (Ruedl et al., 2009).

Sensorimotor control. The myotatic reflex, the formal term for the muscle stretch reflex, is the contraction of a muscle in response to passive stretching (Bhattacharyya, 2017). When a muscle is stretched, the stretch reflex regulates the length of the muscle by increasing contractility. Muscle lengthening results in stretching of the spindle, which results in an increased rate of muscle spindle afferents fired to the given muscle being lengthened (Bhattacharyya, 2017). This then relays to the alpha motor neurons, which cause muscle fibers to contract to resist the stretch (Eccles & Lundberg, 1959). Gamma motor neurons regulate sensitivity of the stretch by contracting or relaxing the spindle fiber. Eccles and colleagues (1959), were some of the first researchers to use the stretch reflex as a model to observe the synaptic transmission within the peripheral nervous system using the quadriceps muscle.

For as long as the stretch reflex has been identified, it has been thought to be a spinal reflex of short latency (Liddell & Sherrington, 1924). Liddell & Sherrington (1924), also demonstrated the tonic response to stretch in cats, which elicited evidence to suggest that the stretch reflex is a spinal process, following similar experiments in dogs. Extensive credit for the spinal cord and mediation of reflex, as well as the reflex activity of the spinal cord, was produced by Creed, Denny-Brown, Eccles, Liddell & Sherrington (1938).

It is also believed that the stretch reflex is evoked by passive stretch of a muscle, while the tendon reflex is elicited by tapping the muscle tendon, with both being spinal in character. However, disparities in the status of the two reflexes in certain clinical

conditions led Marsden, Merton & Morton (1973) who were a neurologist, physiologist, and engineer, to carry out a set of experiments during the 1960s and 1970s to help establish that both reflexes had different anatomical pathways.

Along with the stretch reflex cycle, proprioception is involved as well. It is crucial to understand sensorimotor control of posture and balance to understand mechanical events in the body (Chiel, Ting, Ekeberg & Hartman, 2009; Ting, Chvatal, Safavynia & Mckay, 2012). The muscle spindle proprioceptive receptors play the main role in signaling the body, firing action potentials indicating changes in muscle contraction or muscle length and shortening velocity (Matthews, 1963; Matthews & Stein, 1969). Researchers have proposed that transient, history-dependent properties of muscle spindle firing have been due to history-dependent muscle forces arising from non-steady-state cross bridge formation (Proske & Stuart, 1985; Proske, Tsay & Allen, 2014).

ACL and Myotatic Reflex. Research has been performed on female sex hormones to examine a possible interaction with musculoskeletal disorders such as ACL injuries (Agel et al., 2005). Liu et al (1996) were the first to recognize the presence of estrogen receptors in the human ACL. Estrogen can modulate the structural and mechanical properties of this ligament, which can affect the myotatic reflex. Liu et al. (1996) also discovered that estrogen and progesterone modulate protein synthesis in ligaments. Hansen et al. (2011) showed a reduction in skeletal muscle protein synthesis in women taking oral contraceptives. These hormones might have nonsteroidal effects which can have the potential to alter neural transmission through the peripheral nervous system, spinal cord, and brain (Schmitt & Kaufman, 2005). There have been studies which showed no changes on motor control throughout the menstrual cycle (Friden,

Saartok, Backstrom, Leanderson & Renstrom, 2003; Hertel, Williams, Olmstead-Kramer, Leidy & Putukian, 2006), while others have shown fluctuations in muscle activation patterns and knee loads in certain sport activities (Dedrick et al, 2008; Park et al., 2009). Females have been reported to experience changes in knee laxity throughout the menstrual cycle, due to cyclic hormonal concentrations during the cycle (Schmitz & Shultz, 2013).

Biomechanics/anthropometrics. The quadriceps femoris angle (Q Angle) is defined as the angle between a line drawn to the center of the patella from the anterior superior iliac spine, and another drawn from the center of the patella to the center of the tibia. This angle is a skeleton based measurement and is in the detection of joint function and lower extremity alignment (Holmes et al., 1998; Schulthies, 1995). There is no definite diagnosis for normal values of the Q angle, but 15 degrees and greater for men and 20 degrees greater for women has been reported (Horton & Hall, 1989). Women have a greater Q angle (Krivickas, 1997) due to a wider pelvis which happens during puberty with due to the hormonal influence.

Oral contraceptive and ACL injury and laxity. Estradiol and progesterone are at their lowest during the follicular phase of the menstrual cycle (Adachi, Nawata, Maeta & Kurozawa, 2008; Agel, J., Bershadsky, B., & Arendt, E. A., 2006; Belanger et al., 2004). Estradiol is at peak concentration around the time of ovulation (days 10-14) (Cheung, Boguszewski, Joshi, Wang & McAllister, 2015; Deie, Sakamaki, Sumen, Urabe & Ikuta, 2002) with a second slight rise during the luteal phase (Gray, Gugala, & Baillargeon, 2016; Hashemi, Chandrashekar, Gill, Beynnon, Slauterbeck, Schutt Jr & Dabezies, 2008; Hashemi, Mansouri, Chandrashekar, Slauterbeck, Hardy, & Beynnon,

2011). Progesterone has a gradual rise during the late follicular phase just before ovulation, with highest levels reached at the mid-luteal phase (Ford, Myers, Hewett, 2003; Hertel, Williams, Olmsted-Kramer, Leidy, & Putukian, 2006). These periodic hormonal fluctuations during the menstrual cycle have been postulated to cause joint laxity, increasing the risk for ACL injury (Warren et al., 1999; Warren, Panossian, Hatch, Liu, & Finerman, 2001). Four studies were conducted in the US, Australia, France and Denmark on the effect of oral contraceptives on ACL injury. In a total of 65-51,348 subjects, (Gray et al., 2016; Rahr-Wagner, Thillemann, T. Mehnert, Pedersen, & Lind, 2014). nearly a 20% reduced risk for ACL injury was found in females taking oral contraceptives.

CHAPTER III

METHODS

Research Design

The research design for this study was experimental, using a repeated measures design to investigate physiological changes across the menstrual cycle including hormones, sensorimotor control and physical performance including agility and strength. The independent variables were menstrual cycle phase (follicular, ovulation, menses) and birth control status (non-birth control vs. oral contraceptive users) and the dependent variables included knee extension strength, patellar stretch reflex, agility, postural reaction time, and hormone levels.

All testing was conducted in the Human Performance Laboratory of Cleveland State University. Eligibility criteria were non-pregnant in the past year, not lactating, non-smokers, no diagnosed eating disorder, no menstrual dysfunction or ovarian disease, and no current musculoskeletal injury within the past year. Participants must have had a regular menstrual cycle which aligned with the 28- day average cycle for those off birth control, and females on birth control must be taking their pill each day. Subjects had to have a physical activity level that was above sedentary. Physical activity level was based on how they scored on their Godin Leisure-Time Exercise questionnaire (see appendix).

Based on this questionnaire score less than 14 units is insufficiently active/sedentary, 14-23 units is moderately active, and 24 or more units is active. Participants were selected as long as they had an activity level of 15 or more.

Participants

The sample was composed of 18 menstruating, non-pregnant females, ages 18-35 years. Recruitment was completed via flyers (see appendix E) verbal announcements at meetings and classes for females on campus, and flyers distributed across the Cleveland area. This study was approved by the Institutional Review Board at Cleveland State University and all participants signed an Informed Consent prior to testing (see appendix A).

Nine females were recruited for the study were not on any form of contraceptive. The other nine females had been taking an oral contraceptive that was combination pill (estrogen and progesterone) for at least six months, and continued to be on it during the study, and were considered the control group.

Procedures

The participants met a total of three times for the study in the Human Performance Laboratory. The first session consisted of participants becoming familiarized with the testing protocol, filling out the Informed Consent and determining where each participant was within their menstrual cycle and approximately when their next one would occur. Testing sessions were completed with session one being the follicular phase, session two the ovulation phase, and session three was menses for non-birth control subjects. Birth control participants completed session one follicular, session two follicular, and session three menses in a randomized order. Each subject completed

motor control testing using the Neurocom Balance Master, Electromyography testing/stretch reflex testing of the knee, isometric strength testing, T-Test agility drill and blood draws for future analysis of the hormone's estrogen and relaxin. Subjects off birth control were provided ovulation sticks to determine ovulation, so LH could be measured. To minimize the effect of fluctuations of hormone levels, testing was conducted at a consistent time of day throughout the cycle.

There was a detailed pre-screening process for participants to be eligible to participate in the study. Participants were provided a demographic survey, inclusion/exclusion criteria, and a physical activity questionnaire (Godin Leisure-Time Exercise Questionnaire). Importance for selection was age, birth control status, smoking status, musculoskeletal injury, diagnosed eating disorder, ovarian disease history, and pregnancy in the past six months. Activity level was assessed with a self-report questionnaire. Questionnaires for prescreening can be found in the appendices (see appendices B,C,D).

To determine ovulation for non- birth control subjects, each subject was provided luteinizing hormone Ovulation strips to self-test at home to determine when they were in the ovulation phase. The subjects were informed to urinate into a cup and then stick an ovulation testing strip into the urine for the amount of time given in the instructions until a positive test, being both the control and test line becoming pink.

Day one of testing consisted of familiarization with the study and how testing sessions will be conducted. Subjects had a blood draw, followed by isometric strength testing, EMG/stretch reflex testing, Neurocom motor control testing and lastly, the T-Test Drill for agility. Blood was centrifuged and serum was drawn off prior to being stored in

a -80-degree Celsius freezer for further analysis using commercially available ELISA kits. Testing sessions day two and three were the same protocol, during different menstrual cycle phase.

Hormone Levels

During each testing session, subjects had their blood drawn to test estrogen and relaxin levels during each cycle phase. Blood draws were collected from the antecubital vein with the use of a 25-gauge needle and a 3.5 mL serum tube (BD, Franklin Lakes, New Jersey). Blood samples were mixed well and allowed to clot for 30 minutes. Samples were then centrifuged in a Heraeus Multifuge 3S Plus (Thermo Fisher Scientific) and serum was stored in a -80C freezer. Future analysis of hormone levels will be accomplished using Enzyme-linked immunosorbent assay (ELISA, Thermo Fisher Scientific).

Knee Extension Strength Testing

Maximal isometric contractions of the quadriceps were measured using a strength cable tensiometer (Figure 2). Testing was done each session on the same knee (dominant side) each time for three trials. Instructions for this test were that the subject was asked which her dominant leg was with the question "If you were to kick a soccer ball which foot would you use?" Once the dominant leg was established, the cable tensiometer was attached to the ankle. Subjects were instructed to cross their arms across their chest to make sure they did not use their hands to assist. Subjects were instructed to push against

the resistance and try to reach the highest score they could for three consecutive trials. The maximal value was calculated for the trials during each testing sessions.

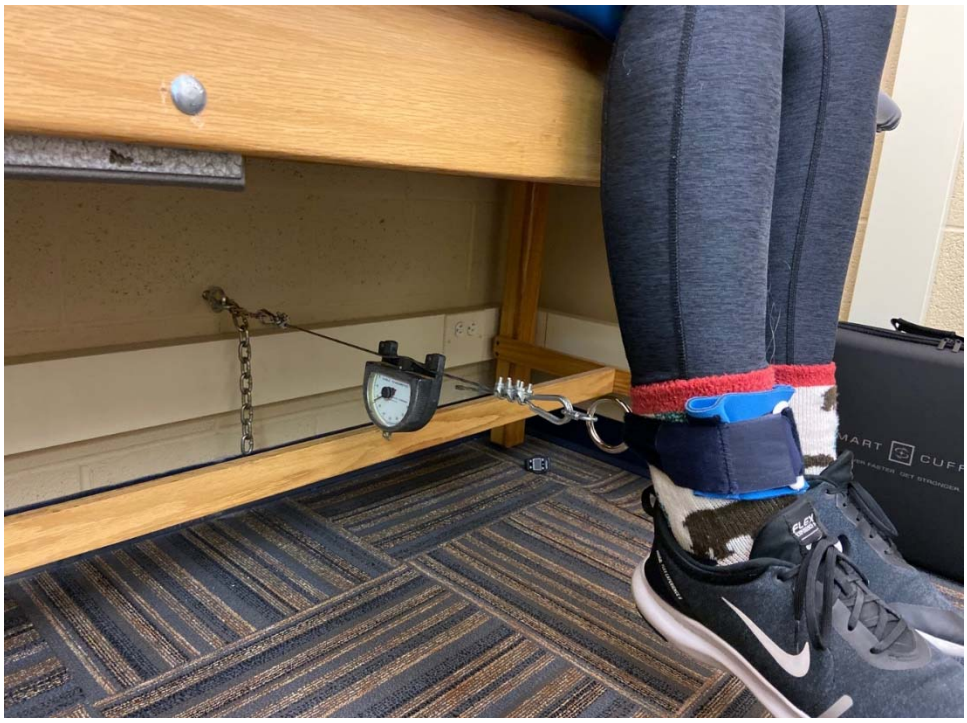

Figure 2: Isometric Knee Extension Strength Configuration

Myotatic Reflex Testing

Testing was conducted using the same leg used for isometric strength testing. Three surface EMG sensors were placed on the rectus femoris, vastus medialis, and vastus lateralis. A reflex hammer was attached to torsional spring device that was incorporated into a standard mat table (Figure 2). The device was constructed by study personnel, which allowed the hammer to hit the same spot on the patella tendon each time with consistent force. The test was conducted 10 times to allow for multiple recordings of latency. Subjects were instructed to look up at the ceiling during the entirety of testing to keep from intentionally inhibiting the reflex prior to hammer contact.

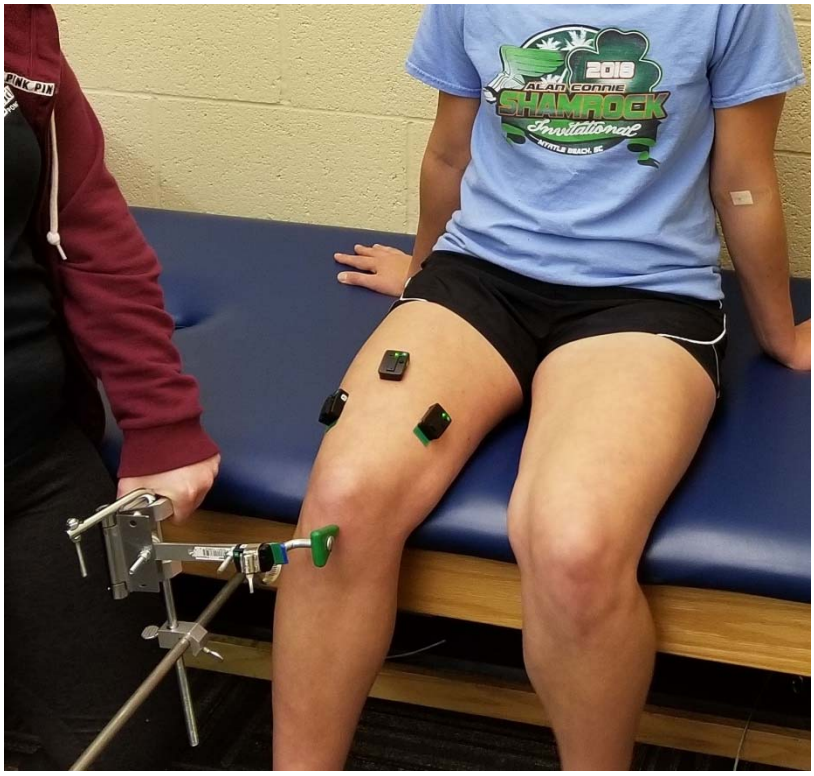

Figure 3: Patellar stretch reflex testing configuration

T-Test Agility Drill

The T-Test (Figure 4) was used to measure agility. Cones were set up in a T formation with the bottom of the T to the middle of the cross of the T being 10 yards apart; five yards from the middle were the ends of the T. Subjects began at point A at the bottom of the T, then sprinted to point B (middle of the top of the T) touching the cone, shuffled to the right and touched the cone at point D, shuffled all the way to the left to point C touching the base of the cone, then shuffled right to touch point B and then ran backwards past point A to complete the test. Participants completed the test three times at each testing session and the fastest time was used.

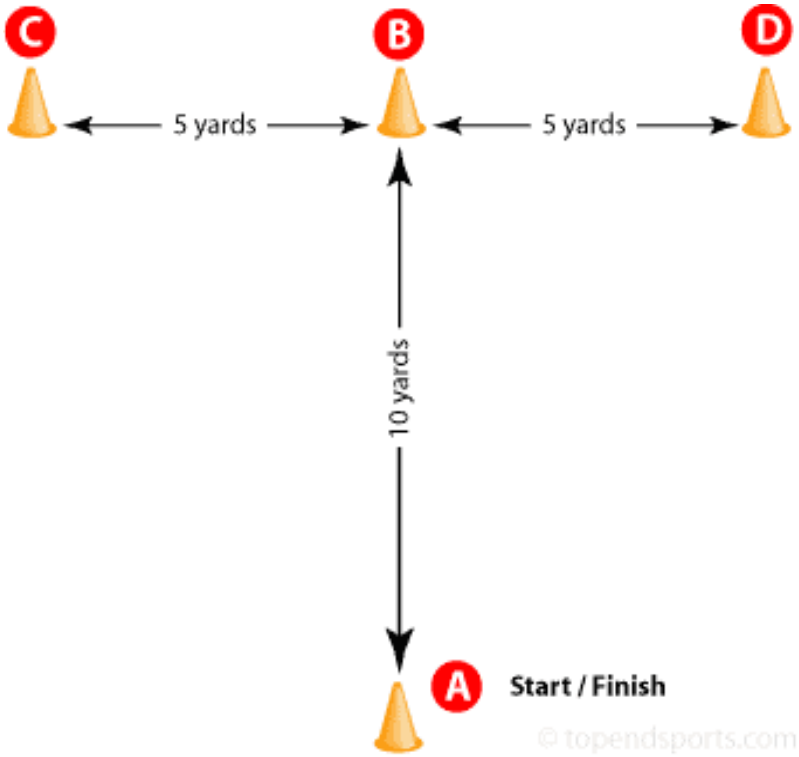

Figure 4: T-test agility test course

Neurocom Testing

The Neurocom Balance Master (Figure 5) was used to test postural response to perturbation by quantifying the subject's ability to quickly recover following an unexpected external disturbance. Small, medium and large platform translations, which are scaled to each subject's height, moved in forward and backward directions. The balance system measures latency between theses translations and the participant's postural responses to them. This test was completed during each session to observe the difference of response latency throughout the menstrual cycle.

Figure 5. Neurocom Balance Master

Data Analysis

Descriptive statistics were obtained. Inferential statistics (repeated measures ANOVA) were used to assess differences due to the independent variables (menstrual cycle phase and birth control status) on the dependent variables (strength, reflex latency, agility and balance response). SPSS (Version 26) was used for all analyses with an alpha of .05 used as the level of significance. In the event of a significant main or interaction effect, post hoc testing was performed. P values for multiple comparisons were corrected using the Bonferroni method.

CHAPTER IV

RESULTS

This research study consisted of 18 females ages 18-35 years, with nine females on oral contraceptives and nine not on any form of birth control. Based on study factors including attrition due to voluntary withdrawal from participation or inability to complete all testing procedures, the non-birth control group consisted of seven individuals with complete data for analysis. Descriptive statistics for age and BMI between the two groups are presented in Table 1.

Table 1. Age and BMI

Group	Age (Mean ± SD)	BMI (Mean ± SD)
Non-Birth Control	24.1 ± 5.4	23.5 ± 1.6
Birth Control	26.2 ± 3.5	25.0 ± 3.0

An independent samples t-test was performed to investigate potential differences in the demographic variables between the groups. Overall, there were no differences in age ($t = -0.932$, $p = .37$) or BMI ($t = -1.24$, $p = .24$) between the birth control and non-birth control group.

Strength

A 3x2 repeated measures ANOVA was completed for maximum strength values with cycle phase as the within subject factor and group as the between subject factor. Descriptive statistics of strength values for each group at the three time points are provided in Table 2.

Table 2. Group Strength Measurements by Cycle Phase ($\underline{x}$+ SD)

Group	Phase 1 Follicular	Phase 2 Ovulation	Phase 3 Menses
Non-Birth Control	38.1 ± 6.3	42.0 ± 10.0	42.6 ± 10.4
Birth Control	35.4 ± 10.3	37.4 ± 11.2	38.2 ± 11.8

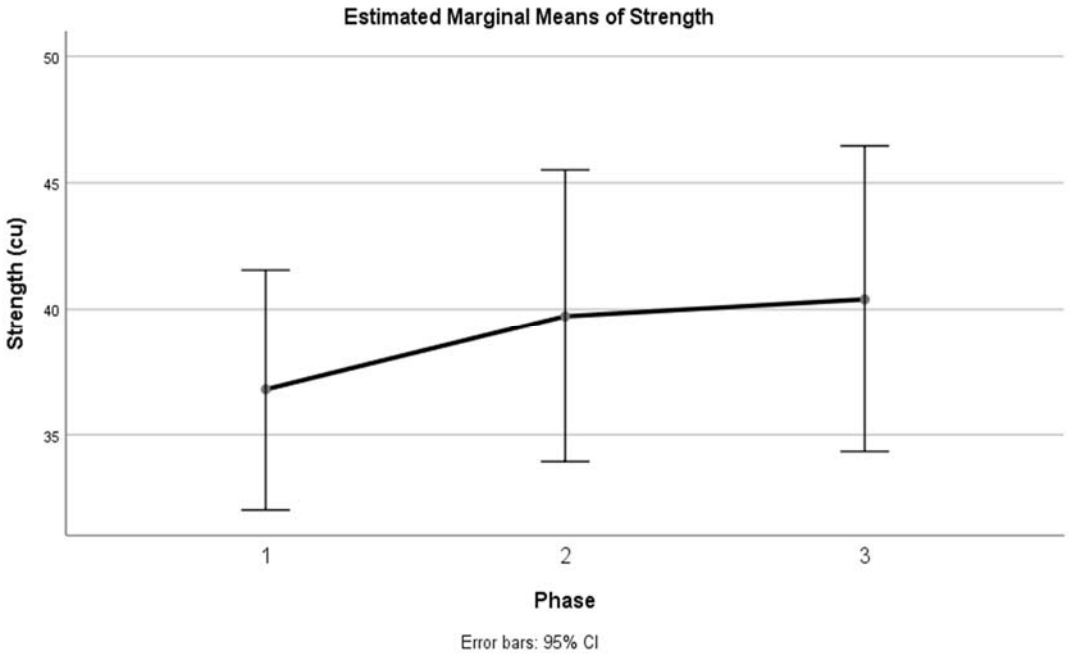

Figure 6. Main effect of cycle phase for knee extension strength

Overall, a significant main effect for cycle phase was observed (F = 3.3, p = .05). This suggests that regardless of birth control group, strength significantly differed between the three phases of the cycle.

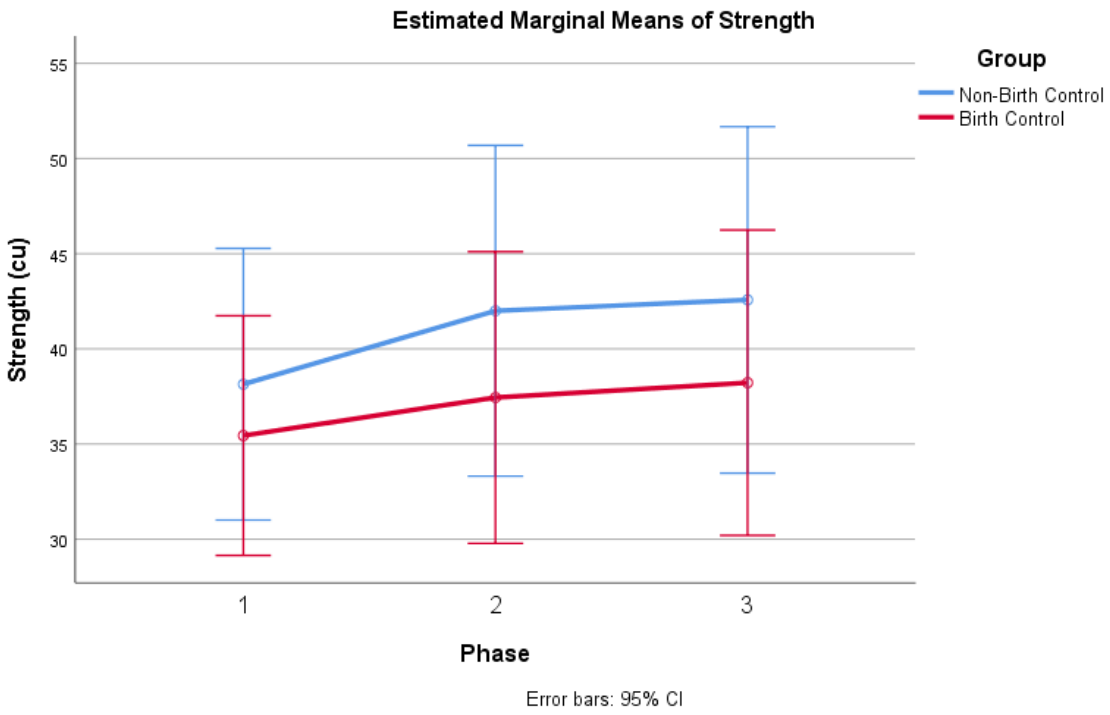

Figure 7. Strength Performance by Group and Cycle Phase

A post hoc analysis was performed, however there was no statistical differences to show where the differences occurred between the phases. Examining the averaged values in Figure 6, shows that strength was greatest during ovulation and menses compared to the follicular phase. Group breakdowns of strength are shown in Figure 7. Overall, no group or interaction effects were observed for strength (Group: $F = 0.62$, $p = 0.44$, Phase*Group: $F = .23$, $p = 0.79$).

EMG

A 3x2 repeated measures ANOVA was completed for the stretch reflex latencies during patellar tendon reflex testing with cycle phase as the within subject factor and group as the between subject factor. Descriptive statistics of response latency values for each group at the cycle phases are provided in Table 6.

Table 3. Patellar Stretch Reflex Latencies (ms) by Cycle Phase ($\underline{x}$+SD)

Group	Time 1 Follicular	Time 2 Ovulation	Time 3 Menses
Non-Birth Control	22.6 ± 5.4	21.3 ± 6.3	21.4 ± 4.0
Birth Control	23.6 ± 5.5	25.6 ± 4.3	22.8 ± 4.4

Figure 8 shows the group line plots for the stretch reflex response latencies. Overall, no main or interaction effects were observed for stretch reflex (Cycle Phase: F = 0.28, p = 0.76, Group: F = 0.99, p = 0.35, Phase*Group: F = 0.42, p = 0.66).

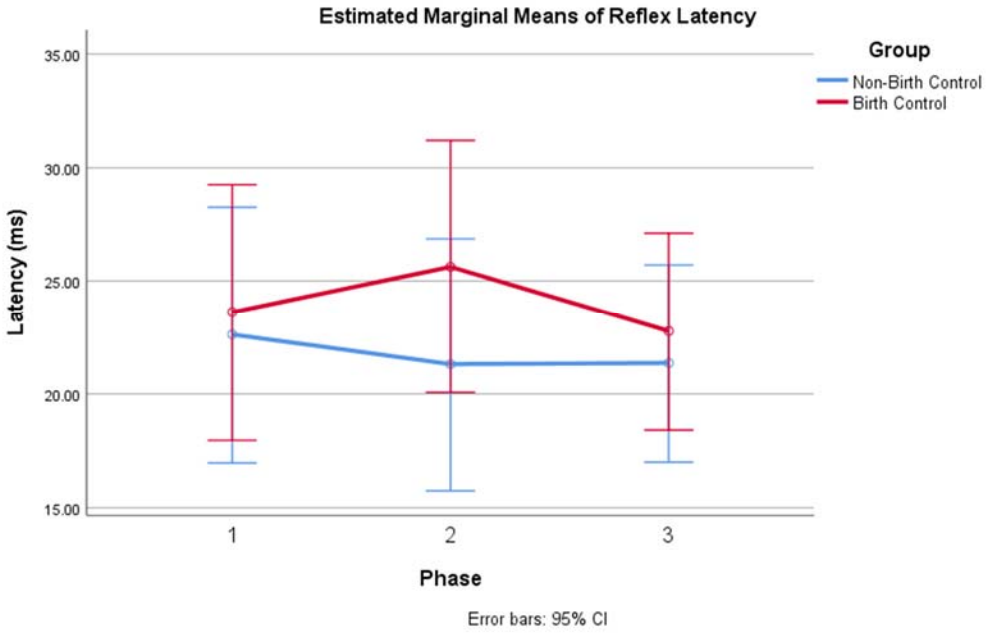

Figure 8. Stretch Reflex Latency by Group and Cycle Phase

Agility

A 3x2 repeated measures ANOVA was completed for the agility test times with cycle phase as the within subject factor and group as the between subjects' factor. Descriptive statistics of agility values for each group at the three cycle phases are provided in Table 4.

Table 4. Agility Test Times (s) by Cycle Phase ($\underline{x}$+SD)

Group	Time 1 Follicular	Time 2 Ovulation	Time 3 Menses
Non-Birth Control	13.1 ± 1.5	13.3 ± 1.6	13.4 ± 1.5
Birth Control	14.1 ± 2.1	14.5 ± 2.0	13.5 ± 1.3

Figure 9 shows the group line plots for the agility test. Overall, no main or interaction effects were observed for agility (Cycle Phase: F = 1.05, p = 0.36, Group: F = 0.82, p = 0.38, Phase*Group: F = 1.9, p = 0.17).

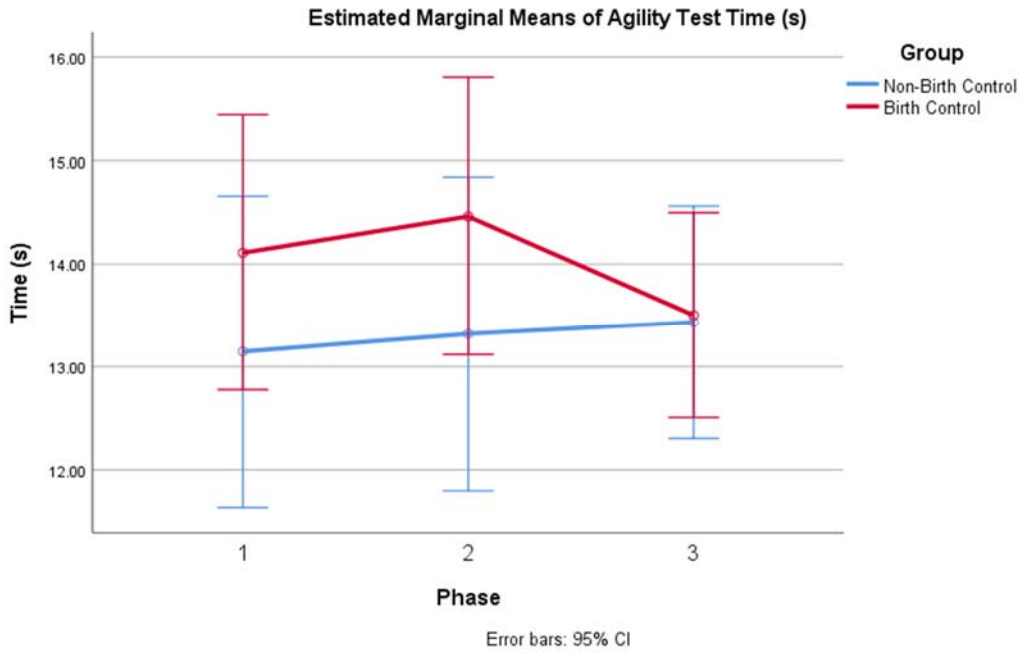

Figure 9. Agility Performance by Group and Cycle Phase

Motor Control Test, Backward Translations

A 3x2 repeated measures ANOVA was completed for the motor control test latencies during backwards translations with cycle phase as the within subject factor and group as the between subject factor. For brevity, descriptive statistics of response latency values for each group at the three menstrual phases are provided only for the large translation condition in Table 5.

Table 5. Group Response Latencies (ms) During Backward Translations by Cycle Phase ($\underline{x}$+SD)

Group	Time 1 Follicular	Time 2 Ovulation	Time 3 Menses
Non-Birth Control	104.3 ± 46.6	111.4 ± 25.3	89.3 ± 53.0
Birth Control	111.7 ± 24.4	112.2 ± 19.2	112.8 ± 23.7

Figure 10 shows the group line plots for the backward translation response latencies. Overall, no main or interaction effects were observed for backward motor translations (Cycle Phase: F = 0.56, p = 0.58, Group: F = 0.83, p = 0.38, Time*Group: F = 0.63, p = 0.54).

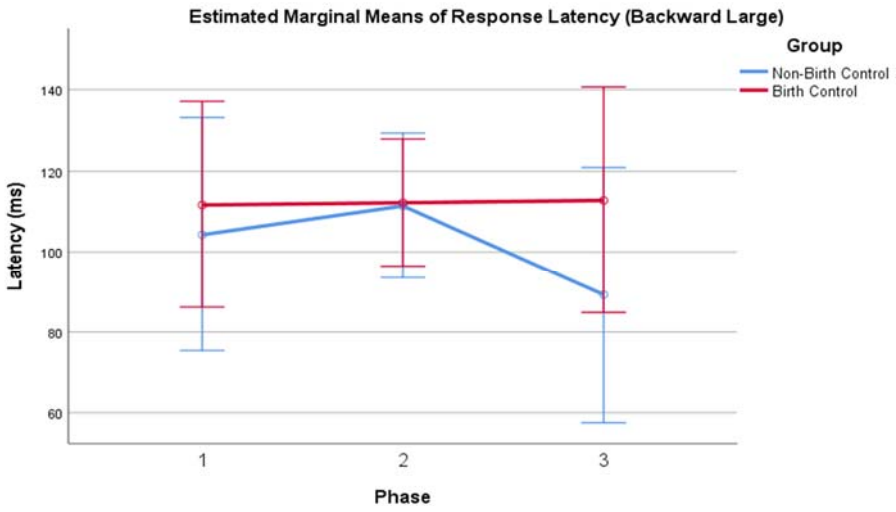

Figure 10. Response Latency to Backward Translations by Group

Motor Control Test, Forward Translations

A 3x2 repeated measures ANOVA was completed for the motor control test latencies during forward translations with cycle phase as the within subject factor and group as the between subjects' factor. For brevity, descriptive statistics of response latency values for each group at the cycle phases are provided only for the large translation condition in Table 6.

Table 6. Response Latencies (ms) During Forward Translations by Cycle Phase

Group	Time 1 Follicular	Time 2 Ovulation	Time 3 Menses
Non-Birth Control	127.1 ± 7.0	105.0 ± 47.5	125.0 ± 8.2
Birth Control	126.6 ± 9.4	126.7 ± 9.0	125.0 ± 9.0

Figure 11 shows the group line plots for the forward translation response latencies. Overall, no main or interaction effects were observed for forward motor control translations (Cycle Phase: F = 1.8, p = 0.19, Group: F = 0.96, p = 0.34, Phase*Group: F = 2.2, p = 0.16).

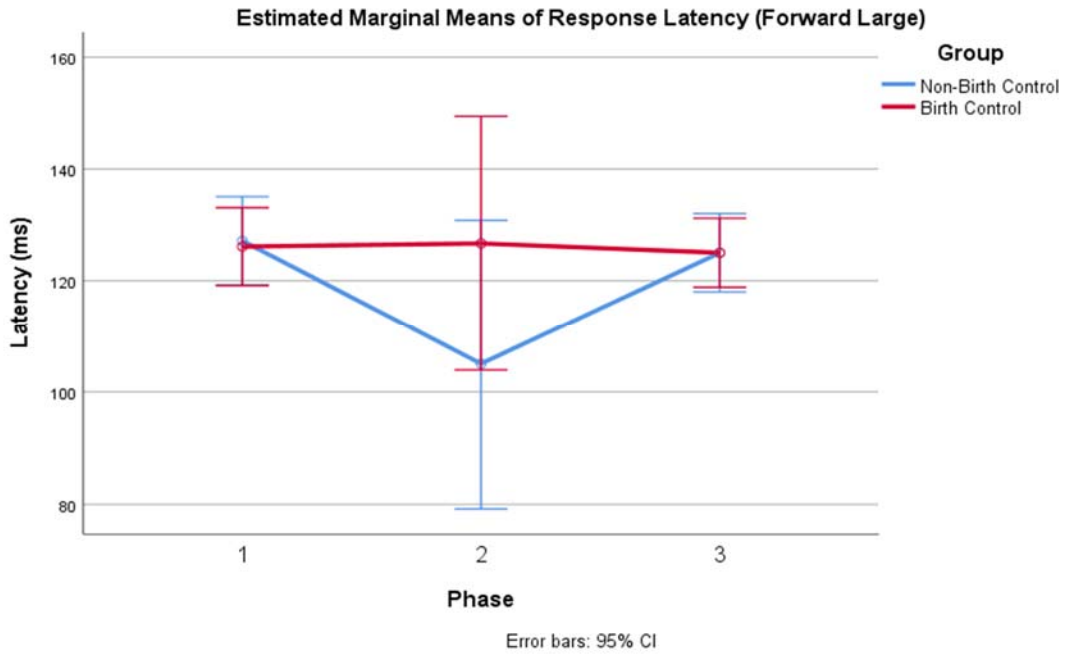

Figure 11. Response Latency to Forward Translations by Group and Cycle Phase

CHAPTER V

DISCUSSION

Discussion

The purpose of the present investigation was to conduct a multifactorial analysis of factors related to injury risk in females throughout the distinct phases of the menstrual cycle. A secondary component of the study was the inclusion of both a non-birth control group as well as a group of individuals utilizing oral contraceptives for birth control. This study found little evidence of the main effect of cycle phase (follicular phase, ovulation, and menses) or birth control status on the dependent variables of strength, stretch reflex latency, postural control, and agility. In only one case was a statistically significant main effect observed. It was shown that cycle phase had an overall effect on knee extension strength regardless of birth control status. Strength was the lowest during the follicular phase and greatest during menses. Agility, postural reaction and reflex latency displayed no significant differences based on cycle phase or birth control status.

Previous literature has shown that muscular strength does not appear to fluctuate at a significant rate during menstrual cycle (Constantini et al., 2005). Some reported an inotropic effect of estrogen on muscle (Phillips, Sanderson, Birch, Bruce, & Woledge, 1996) peaking right before ovulation with about a 10-11% magnitude (Sarwar, Niclos, &

Rutherford, 1996), with switching muscle cross-bridges from low to high force generation. One study in particular concluded that estrogen and progesterone levels during maximal voluntary muscle contractions were significantly higher during ovulation possible due to contraction properties (Iwamoto, Kubo, Ito, Takemiya, & Asami, 2002). Compared to another study (Hortobágyi, & Maffiuletti,, 2011). used electrical stimulation to show maximal neural activation and muscle contraction. This study found no significant changes in the quadriceps muscle strength, fatigue, or electrical stimulation contraction properties in women over three phases of the menstrual cycle, and no correlations of any of the strength indices with female reproductive hormone concentrations. This study showed greater strength during menses which is not in line with the literature where strength was greatest during the pre-ovulation to ovulation phases.

The findings related to agility in the present study are not in line to previous reports examining T-test performance across the menstrual cycle. Kheniser (2016) who observed that females were significantly faster on the T-test during the follicular phase compared to the ovulation phase. This study found that the females off oral contraceptives had a gradual increase in agility time each phase while the oral contraceptive group had slight increase during ovulation phase but then decreased during menses. It should be noted, however, that the mean difference in his study was ~0.26s, and thus not substantially greater than the non-significant differences observed in the present study. Potentially, the lack of a significant finding in the current study may be due to methodological and variability differences. First, the current investigation tested participants at three phases within the menstrual cycle compared to the two phases in the

previous investigation. Second, standard deviations for T-test times in the current study were greater than those observed by Kheniser (2016) indicating that the current sample had greater variability in their performances. Taken together, the magnitude of impact that the menstrual cycle has on agility cannot be fully interpreted. Further, the current results suggest that hormone differences due to use of oral contraceptives do not have a significant effect on agility performance. After analyzing the maximal values in the repeated measures test, showed that the birth control group had a major drop during the time of their placebo pill (menses).

Previous studies have examined the patellar stretch reflex throughout the menstrual cycle Liu et al. (1996) discovered that estrogen and progesterone modulate protein synthesis in ligaments. Hansen et al. (2011) were the ones who showed a reduction in skeletal muscle protein synthesis in women taking oral contraceptives. It has also previously been found that hormones might have nonsteroidal effects which have potential to alter neural transmission through the PNS, spinal cord and brain (Schmitt & Kaufman, 2005; Smith & Wooley, 2004). Within the present study, no significant effects of cycle phase or birth control usage was observed for stretch reflex latencies. Potential explanations of this finding could be the type/brand of oral contraceptive used, cycle phase not being accurate, or error in EMG software during testing. Also, it was noticed at some points with certain subjects, they barely had a reflex physically when the hammer hit the patella, but there was EMF activity on EMG.

Along the lines of stretch reflex latency, this study also examined postural response latency. Automatic postural adjustments in response to a perturbation require a combination of muscular strength, power and neuromuscular control. It was hypothesized

that differences in postural control may be due hormonal changes observed during the menstrual cycle phases. Nonetheless, no observable effects of cycle phase or birth control status were observed in the present investigation. This may be due to varied cycle phase accuracy.

One explanation for potential changes in ACL injury risk throughout the menstrual cycle are the fluctuations in hormones that occur. While the present study did not specifically analyze these hormones, samples were collected for future analysis on the potential role that relaxin plays in soft tissue laxity, and its correlation with the measures discussed above. Due to presence of relaxin receptors on the female ACL (Dragoo, Lee, Benhaim, Finerman & Hame, 2003; Glaey, Konieczko, Arnold & Cooney, 2003), suggests that the relaxin- mediated remodeling of female ligaments is an important mechanism to closely observe. There have been no studies to date that have described serum relaxin concentration (SRC) in young, healthy female elite athletes nor active females. Relaxin may be a moderator in the ACL injury due to its active role during menstruation. Research has shown that relaxin 2 plays a crucial role in altering ACL laxity and more importantly, this relaxin receptor is only found in women (Dragoo, et al., 2003; Dragoo, Castillo, Braun, Ridley, Kennedy, Golish, 2011). In non-pregnant women, relaxin 2 serum concentrations are increased during the luteal phase of the menstrual cycle (Dragoo et al., 2011) which was why no effect in laxity was observed in this study. Studies have suggested that oral contraceptives have a protectve effect against ACL injury, and relaxin 2 concentrations and significantly lower in those taking oral contraceptives compared to non-oral contraceptive users (Dragoo, Castillo, Korotkova, Kennedy, Kim, Stewart, 2011).

CHAPTER VI

CONCLUSION

Conclusion

In conclusion, the current study does not provide any concrete findings on the relationship of menstrual cycle phase with the stretch reflex and potential ACL or other soft tissue injuries in the active female population. Of all factors analyzed, it was determined that strength was greatest during menses, which is not in line with previous research. Overall, this suggests that more in depth research needs to be conducted to determine the factors most responsible for elevated ACL injury rates in females compared to males.

In line with the literature, there is a potential hormonal factor for why females are more prone to ACL injury, as well as their faster stretch reflex response. More research should be conducted on physiological/hormonal response levels to truly understand the relationship. Biopsy of soft tissues would be a more realistic way to examine this from a physiological level. While the current study only considered oral contraceptives, different types of oral contraceptives should be studied to see if varying hormonal levels have a difference on testing based on the form it is being used. Blood analysis of hormones should be completed to obtain a better understanding of hormone and

physiological effects on the outcome measures. Future studies should also focus very closely on the oral contraceptive effect on agility, since this study found a large decrease during placebo pill week (menses) of testing.

Limitations of the current study include a small sample size that potentially led to low statistical power. Additionally, subjects were enrolled based on self-report physical activity, which may have led to differences in activity levels between study participants. Future evaluations should consider screening based on a functional test such as VO2max or Wingate. Moreover, there was no exclusion criteria based on body fat percentage. This means that there was a potential lean mass difference in participants that could have had an underlying effect on the outcome measures of the current investigation. Cycle phase accuracy is another potential limitation as well as equipment accuracy.

REFERENCES

Adachi, N., Nawata, K., Maeta, M., & Kurozawa, Y. (2008). Relationship of the menstrual cycle phase to anterior cruciate ligament injuries in teenaged female athletes. *Archives of orthopaedic and trauma surgery*, *128*(5), 473-478.

Agel, J., Arendt, E. A., & Bershadsky, B. (2005). Anterior cruciate ligament injury in national collegiate athletic association basketball and soccer: a 13-year review. *The American journal of Sports Medicine.* 33(4), 524-531.

Agel, J., Bershadsky, B., & Arendt, E. A. (2006). Hormonal therapy: ACL and ankle injury. *Medicine and science in sports and exercise*, *38*(1), 7-12.

Behan, F. P., Maden-Wilkinson, T. M., Pain, M. T., & Folland, J. P. (2018). Sex differences in muscle morphology of the knee flexors and knee extensors. *PloS one,* 13(1), e0190903.

Belanger, M. J., Moore, D. C., Crisco, J. J., Fadale, P. D., Hulstyn, M. J., & Ehrlich, M. G. (2004). Knee laxity does not vary with the menstrual cycle, before or after exercise. *The American journal of sports medicine*, *32*(5), 1150-1157.

Bhattacharyya, K. B. (2017). The stretch reflex and the contributions of C David Marsden. Annals of Indian Academy of Neurology, 20(1)

Blecher, AM, Richmond, JC. (1998). Transient laxity of an anterior cruciate ligament-reconstructed knee related to pregnancy. *Arthroscopy*, 14(1), 77-79.

Carcia, C. R., Shultz, S. J., Granata, K. P., Gansneder, B. M., & Perrin, D. H. (2004). Knee ligament behavior following a controlled loading protocol does not differ by menstrual cycle day. *Clinical biomechanics*, *19*(10), 1048-1054.

Chandrashekar, N., Slauterbeck, J., & Hashemi, J. (2005). Sex-based differences in the anthropometric characteristics of the anterior cruciate ligament and its relation to intercondylar notch geometry: a cadaveric study. *The American journal of sports medicine*, *33*(10), 1492-1498.

Chandy, T. A., & Grana, W. A. (1985). Secondary school athletic injury in boys and girls: a three-year comparison. *Journal of Pediatric Orthopedics*, *5*(5), 629.

Cheung, E. C., Boguszewski, D. V., Joshi, N. B., Wang, D., & McAllister, D. R. (2015). Anatomic factors that may predispose female athletes to anterior cruciate ligament injury. *Current sports medicine reports*, *14*(5), 368-372.

Chiel, H. J., Ting, L. H., Ekeberg, Ö., & Hartmann, M. J. (2009). The brain in its body: motor control and sensing in a biomechanical context. *Journal of Neuroscience*, *29*(41), 12807-12814.

Ciocca, D. R., & Vargas Roig, L. M. (1995). Estrogen receptors in human nontarget tissues: biological and clinical implications. *Endocrine Reviews*, *16*(1), 35-62.

Constantini, N. W., Dubnov, G., & Lebrun, C. M. (2005). The menstrual cycle and sport performance. *Clinics in sports medicine*, *24*(2), e51-e82.

Dedrick, G. S., Sizer, P. S., Merkle, J. N., Hounshell, T. R., Robert-McComb, J. J., Sawyer, S. F., ... & James, C. R. (2008). Effect of sex hormones on neuromuscular control patterns during landing. *Journal of Electromyography and Kinesiology*, *18*(1), 68-78.

Deie, M., Sakamaki, Y., Sumen, Y., Urabe, Y., & Ikuta, Y. (2002). Anterior knee laxity in young women varies with their menstrual cycle. *International orthopedics*, *26*(3), 154-156.

Dragoo, J. L., Castillo, T. N., Braun, H. J., Ridley, B. A., Kennedy, A. C., & Golish, S. R. (2011). Prospective correlation between serum relaxin concentration and anterior cruciate ligament tears among elite collegiate female athletes. *The American journal of sports medicine*, *39*(10), 2175-2180.

Dragoo, J. L., Lee, R. S., Benhaim, P., Finerman, G. A., & Hame, S. L. (2003). Relaxin receptors in the human female anterior cruciate ligament. *The American journal of sports medicine*, *31*(4), 577-584.

Dragoo, J. L., Padrez, K., Workman, R., & Lindsey, D. P. (2009). The effect of relaxin on the female anterior cruciate ligament: Analysis of mechanical properties in an animal model. *The Knee*, *16*(1), 69-72.

Dumas, G. A., & Reid, J. G. (1997). Laxity of knee cruciate ligaments during pregnancy. *Journal of Orthopedic & Sports Physical Therapy*, *26*(1), 2-6.

Eccles, R. M., & Lundberg, A. (1959). Supraspinal control of interneurons mediating spinal reflexes. *The Journal of physiology*, *147*(3), 565-584.

Ferretti, A., Papandrea, P., Conteduca, F., & Mariani, P. P. (1992). Knee ligament injuries in volleyball players. *The American journal of sports medicine*, *20*(2), 203-207.

Ford, K. R., Myer, G. D., & Hewett, T. E. (2003). Valgus knee motion during landing in high school female and male basketball players. *Medicine & Science in Sports & Exercise*, *35*(10), 1745-1750.

Friden, C., Saartok, T., Bäckström, C., Leanderson, J., & Renström, P. (2003). The influence of premenstrual symptoms on postural balance and kinesthesia during the menstrual cycle. *Gynecological endocrinology*, *17*(6), 433-440.

Garibay-Tupas, J. L., Okazaki, K. J., Tashima, L. S., Yamamoto, S., & Bryant-Greenwood, G. D. (2004). Regulation of the human relaxin genes H1 and H2 by steroid hormones. *Molecular and cellular endocrinology*, *219*(1-2), 115-125.

Glock, J. L., Nakajima, S. T., Stewart, D. R., Badger, G. J., & Brumsted, J. R. (1995). The relationship of corpus luteum volume to relaxin, estradiol, progesterone, 17-hydroxyprogesterone and human chorionic gonadotropin levels in early normal pregnancy. *Early pregnancy: biology and medicine: the official journal of the Society for the Investigation of Early Pregnancy*, *1*(3), 206-211.

Gray, A. M., Gugala, Z., & Baillargeon, J. G. (2016). Effects of Oral Contraceptive Use on Anterior Cruciate Ligament Injury Epidemiology. *Medicine and science in sports and exercise*, *48*(4), 648-654.

Gray, J., Taunton, J. E., McKenzie, D. C., Clement, D. B., McConkey, J. P., & Davidson, R. G. (1985). A survey of injuries to the anterior cruciate ligament of the knee in female basketball players. *International journal of sports medicine*, *6*(06), 314-316.

Hansen, M., Langberg, H., Holm, L., Miller, B. F., Petersen, S. G., Doessing, S., ... & Kjaer, M. (2011). Effect of administration of oral contraceptives on the synthesis and breakdown of myofibrillar proteins in young women. *Scandinavian journal of medicine & science in sports*, *21*(1), 62-72.

Hart, J. M., Ko, J. W. K., Konold, T., & Pietrosimione, B. (2010). Sagittal plane knee joint moments following anterior cruciate ligament injury and reconstruction: a systematic review. *Clinical biomechanics*, *25*(4), 277-283.

Hashemi, J., Chandrashekar, N., Gill, B., Beynnon, B. D., Slauterbeck, J. R., Schutt Jr, R. C., ... & Dabezies, E. (2008). The geometry of the tibial plateau and its influence on the biomechanics of the tibiofemoral joint. *The Journal of Bone and Joint Surgery. American volume.*, *90*(12), 2724.

Hashemi, J., Mansouri, H., Chandrashekar, N., Slauterbeck, J. R., Hardy, D. M., & Beynnon, B. D. (2011). Age, sex, body anthropometry, and ACL size predict the structural properties of the human anterior cruciate ligament. *Journal of orthopaedic research*, *29*(7), 993-1001.

Haycock, C. E., & Gillette, J. V. (1976). Susceptibility of women athletes to injury: myths vs reality. *Jama*, *236*(2), 163-165.

Hayes, E. S. (2004). Biology of primate relaxin: a paracrine signal in early pregnancy? *Reproductive Biology and Endocrinology*, *2*(1), 36.

Hertel, J., Williams, N. I., Olmsted-Kramer, L. C., Leidy, H. J., & Putukian, M. (2006). Neuromuscular performance and knee laxity do not change across the menstrual cycle in female athletes. *Knee Surgery, Sports Traumatology, Arthroscopy*, *14*(9), 817-822.

Hewett, T. E., Lindenfeld, T. N., Riccobene, J. V., & Noyes, F. R. (1999). The effect of neuromuscular training on the incidence of knee injury in female athletes. *The American journal of sports medicine*, *27*(6), 699-706.

Hortobágyi, T., & Maffiuletti, N. A. (2011). Neural adaptations to electrical stimulation strength training. *European journal of applied physiology*, *111*(10), 2439–2449. doi:10.1007/s00421-011-2012-2

Iwamoto, Y., Kubo, J., Ito, M., Takemiya, T., & Asami, T. (2002). Variation in maximal voluntary contraction during the menstrual cycle. *Japanese Journal of Physical Fitness and Sports Medicine*, *51*(2), 193-201.

Kheniser, K. G. (2016). *The Effects of the Menstrual Cycle on Agility* (Doctoral dissertation, Cleveland State University).

Krivickas, L. S. (1997). Anatomical factors associated with overuse sports injuries. *Sports medicine*, *24*(2), 132-146.

Liddell, E. G. T., & Sherrington, C. S. (1924). Reflexes in response to stretch (myotatic reflexes). *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, *96*(675), 212-242.

Liu, S. H., Al-Shaikh, R., Panossian, V., Yang, R. S., Nelson, S. D., Soleiman, N., ... & Lane, J. M. (1996). Primary immunolocalization of estrogen and progesterone target cells in the human anterior cruciate ligament. *Journal of orthopedic research*, *14*(4), 526-533.

Luteinizing Hormone. (n.d.). Retrieved from https://www.hormone.org/hormones-and-health/hormones/luteinizing-hormone

Marsden, C. D., Merton, P. A., & Morton, H. B. (1973). Is the human stretch reflex cortical rather than spinal? *The Lancet*, *301*(7806), 759-761.

Matthews, P. B. C., & Stein, R. B. (1969). The sensitivity of muscle spindle afferents to small sinusoidal changes of length. *The Journal of Physiology*, *200*(3), 723-743.

Park, S. K., Stefanyshyn, D. J., Ramage, B., Hart, D. A., & Ronsky, J. L. (2009). Alterations in knee joint laxity during the menstrual cycle in healthy women leads to increases in joint loads during selected athletic movements. *The American journal of sports medicine*, *37*(6), 1169-1177.

Phillips, S. K., Sanderson, A. G., Birch, K., Bruce, S. A., & Woledge, R. C. (1996). Changes in maximal voluntary force of human adductor pollicis muscle during the menstrual cycle. *The Journal of physiology*, *496*(2), 551-557.

Proske, U., & Stuart, G. J. (1985). The initial burst of impulses in responses of toad muscle spindles during stretch. *The Journal of physiology*, *368*(1), 1-17.

Proske, U., Tsay, A., & Allen, T. (2014). Muscle thixotropy as a tool in the study of proprioception. *Experimental brain research*, *232*(11), 3397-3412.

Rahr-Wagner, L., Thillemann, T. M., Mehnert, F., Pedersen, A. B., & Lind, M. (2014). Is the use of oral contraceptives associated with operatively treated anterior cruciate ligament injury? A case-control study from the Danish Knee Ligament Reconstruction Registry. *The American journal of sports medicine*, *42*(12), 2897-2905.

Reed, B. G., & Carr, B. R. (2018). The normal menstrual cycle and the control of ovulation. In *Endotext [Internet]*. MDText. com, Inc.

Ruedl, G., Ploner, P., Linortner, I., Schranz, A., Fink, C., Sommersacher, R., ... & Burtscher, M. (2009). Are oral contraceptive use and menstrual cycle phase related to anterior cruciate ligament injury risk in female recreational skiers? *Knee surgery, sports traumatology, arthroscopy*, *17*(9), 1065-1069.

Sarwar, R., Niclos, B. B., & Rutherford, O. M. (1996). Changes in muscle strength, relaxation rate and fatiguability during the human menstrual cycle. *The Journal of physiology*, *493*(1), 267-272.

Schmitt, P. M., & Kaufman, M. P. (2005). Erratum: Estrogen's attenuating effect on the exercise pressor reflex is more opioid dependent in gonadally intact than ovariectomized female cats (Journal of Applied Physiology (February 2005) 98 (633-639)). *Journal of Applied Physiology*, *99*(2), 782.

Schmitz, Randy J., and Sandra J. Shultz. "Anterior knee stiffness changes in laxity “responders” versus “nonresponders” across the menstrual cycle." *Journal of athletic training* 48.1 (2013): 39-46.

Sherwood, O. D. (2004). Relaxin’s physiological roles and other diverse actions. *Endocrine reviews*, *25*(2), 205-234.

Smith, S. S., & Woolley, C. S. (2004). Cellular and molecular effects of steroid hormones on CNS excitability. *Cleveland Clinic journal of medicine*, *71*(2), S4.

Turgeon, J. L., Carr, M. C., Maki, P. M., Mendelsohn, M. E., & Wise, P. M. (2006). Complex actions of sex steroids in adipose tissue, the cardiovascular system, and brain: Insights from basic science and clinical studies. *Endocrine reviews*, *27*(6), 575-605.

Van der Westhuizen, E. T., Halls, M. L., Samuel, C. S., Bathgate, R. A., Unemori, E. N., Sutton, S. W., & Summers, R. J. (2008). Relaxin family peptide receptors–from orphans to therapeutic targets. *Drug discovery today*, *13*(15-16), 640-651.

Warren, D. Y., Liu, S. H., Hatch, J. D., Panossian, V., & Finerman, G. A. (1999). Effect of estrogen on cellular metabolism of the human anterior cruciate ligament. *Clinical Orthopedics and Related Research®*, *366*, 229-238.

Warren, D. Y., Panossian, V., Hatch, J. D., Liu, S. H., & Finerman, G. A. (2001). Combined effects of estrogen and progesterone on the anterior cruciate ligament. *Clinical Orthopaedics and Related Research®*, *383*, 268-281.

Weiss, G. E. R. S. O. N., O'Byrne, E. M., Hochman, J. O. S. E. P. H., Steinetz, B. G., Goldsmith, L. A. U. R. A., & Flitcraft, J. G. (1978). Distribution of relaxin in women during pregnancy. *Obstetrics and gynecology*, *52*(5), 569-570.

Wreje, U., Kristiansson, P., Åberg, H., Byström, B., & von Schoultz, B. (1995). Serum levels of relaxin during the menstrual cycle and oral contraceptive use. *Gynecologic and obstetric investigation*, *39*(3), 197-200.

Zazulak, B. T., Paterno, M., Myer, G. D., Romani, W. A., & Hewett, T. E. (2006). The effects of the menstrual cycle on anterior knee laxity. *Sports medicine*, *36*(10), 847-862.

Zelisko, J. A., Noble, H. B., & Porter, M. (1982). A comparison of men's and women's professional basketball injuries. *The American journal of sports medicine*, *10*(5), 297-299.

APPENDIX A

INFORMED CONSENT

Cleveland State University

College of Education and Human Services
Department of Health and Human Performance

Informed Consent

Effects of Menstrual Cycle on Injury Risk

Introduction

Thank you for considering this project. It is being conducted by College of Education researchers from the department of Health and Human Performance at Cleveland State University. The study location is the Human Performance Laboratory (HPL). The research will be led by graduate student Kayla Ruffner and Dr. Douglas Wajda.

Before you decide if you would like to participate in this study, there are a few things you need to know. Please read this document carefully. Ask any questions you may have.

The purpose of this study is to determine the effect of hormone changes during the Menstrual cycle on body movements and injury risk in females. There is evidence that changes in hormones like estrogen may impact strength and movement. This could result in worse sports performance or increased risk of injury.

By performing this study, we can attempt to develop specific training programs. These will be aimed at improving performance throughout the menstrual cycle. This could lead to better standards regarding injury prevention in female athletes.

You will be asked to complete three testing sessions as part of this study at the HPL. Each session will consist of a brief warm-up. This will be followed by tests of agility, strength, balance and muscle reflex.

Testing Procedures

You will be asked to track your menstrual cycle for one complete cycle using a questionnaire. You will also use a home ovulation test kit. It monitors levels of Luteinizing Hormone in your urine. The kits will be provided to you by the study team to complete in your own home. We will use this to decide when your test sessions will be scheduled.

For each of the testing dates you will be asked to come to the HPL. A venous blood sample will be obtained to verify hormone levels. Next, you will perform a brief warm up consisting of 5 minutes of light jogging and 5 minutes of dynamic stretching.

The first session will be the first day of your menstrual cycle. The first test is a muscle reflex test that will be completed on your patellar tendon (area by you knee). This test is like having a physician test your reflexes. The next test is a balance control test. The third test will examine the strength of your thigh muscles. The final test will test agility. The other two testing sessions will be on days 14 and 21 of your Menstrual cycle. The same tests from the first session will be performed on these days.

The knee muscle stretch reflex testing requires you to be seated while we measure your muscle response to a tap on the patellar tendon. Muscle response will be measured with a sensor placed

The purpose and risks of the study have been explained to me. If I have any questions about the procedures I can contact Dr. Wajda (216-687-4873) or Ms. Ruffner (330-272-4680). I have read the consent form, or it has been read to me, and I understand it. I acknowledge that I am at least 18 years old and agree to participate in this study. I have been given a copy of this consent form.

Participant Name (Please Print): _____

Signature: _____ Date: _____

Witness Name (Please Print): _____

Witness: _____ Date: _____

APPENDIX B

INCLUSION/EXCLUSION QUESTIONNAIRE

You have expressed interest in participating in a study conducted at Cleveland State University by Graduate Student Kayla Ruffner. In order to see if you qualify for participation in the Menstrual cycle and injury risk study, there are a few questions we would like you to answer to the best of your ability:

1. When is your birthday (mm/dd/yyyy)? _____

   **If not between the ages of 18-35 years then excluded.

2. Are you currently on any form of birth control? _____

   a. If no move on

   b. If yes: What type? _____

   c. If yes how long have you been on this dose and birth control type?

      _____

   **If on birth control other than oral contraceptives then exclude

3. Have you ever been diagnosed with an eating disorder? By whom (hospital/doctor/self-report)?

   _____

   _____

   **If diagnosed by hospital/doctor or self-reported then excluded.

4. Have you ever had a significant musculoskeletal injury (e.g. broken bone, muscle strain, torn tendon/ligament)? If yes when was the injury?

   _____

   a. Did you participate in therapy for injury? _____
   b. Was surgery needed for injury if so when was it? _____

   **If less than one year then excluded.

5. Do you partake in regular physical activity?

   _____

   **If no (i.e. sedentary) then exclude

6. Are you currently pregnant or been pregnant in the previous year? _____

   **If yes then excluded.

7. Are you currently lactating? _____

   **If yes then excluded.

8. Do you have any history of ovarian disease? _____

   **If yes then excluded.

9. Do you currently smoke? _____

   **If yes then excluded.

We appreciate your time and interest in this study.

**If individual passes the inclusion/exclusion portion of the study then they will continue onto the scheduling phase of the project

APPENDIX C

PARTICIPANT DEMOGRAPHICS SURVEY

Please complete the following information about yourself.

1. Gender (Circle one) Female Male

2. Marital Status (Circle one)

    a. Married

    b. Single

    b. Divorced/Separated

3. Date of Birth (mm/dd/yyyy) _____

4. Race (Circle one)

    a. American Indian

    b. Asian

    c. Black or African American

    d. Native Hawaiian

    e. Other Pacific Islander

    f. Caucasian

    g. Latino/a

    h. Other:_____

9. Height: _____ inches

10. Weight: _____ pounds

13. Are you currently or have you previously utilized birth control?

a. No

b. Yes, type:_____

- Time on current dosage of oral contraceptive:_____

14. Have you previously had a serious musculoskeletal injury that required medical attention?

a. No

b. Yes, Type and affected area: _____

When did the injury occur? _____

Did the injury require surgery and/or physical therapy? _____

15. Have you been pregnant or have been lactating for the year?

a. yes

b. no

16. Do you plan on becoming pregnant during this study?

a. yes

b. no

APPENDIX D

PHYSICAL ACTIVITY ASSESSMENT

Godin Leisure-Time Exercise Questionnaire

INSTRUCTIONS

In this excerpt from the Godin Leisure-Time Exercise Questionnaire, the individual is asked to complete a self-explanatory, brief four-item query of usual leisure-time exercise habits.

CALCULATIONS

For the first question, weekly frequencies of strenuous, moderate, and light activities are multiplied by nine, five, and three, respectively. Total weekly leisure activity is calculated in arbitrary units by summing the products of the separate components, as shown in the following formula:

Weekly leisure activity score = (9 x Strenuous) + (5 x Moderate) + (3x Light)

The second question is used to calculate the frequency of weekly leisure-time activities pursued "long enough to work up a sweat" (see questionnaire).

EXAMPLE

Strenuous = 3 times/wk

Moderate = 6 times/wk

Light = 14 times/wk

Total leisure activity score = (9x3) + (5x6) + (3x14) = 27 + 30 + 42 = 99

1. During a typical 7-Day period (a week), how many times on the average do you do the following kinds of exercise for more than 15 minutes during your free time (write on each line the appropriate number).

a. STRENUOUS EXERCISE (HEART BEATS RAPIDLY)

(e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)

Times per week: _____

b. MODERATE EXERCISE (NOT EXHAUSTING)

(e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)

Times per week: _____

c. MILD EXERCISE (MINIMAL EFFORT)

(e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)

Times per week: _____

2. During a typical 7-Day period (a week), in your leisure time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

OFTEN SOMETIMES NEVER

APPENDIX E

TESTING FORMS

Subject #: _____

Birthday: _____

Testing session # _____

Phase: _____

Height: _____

Weight: _____

Isometric/Strength Testing:

Trial 1: _____
Trial 2: _____
Trial 3: _____

EMG Testing:

Neurocom Balance Mat:

T- Test Drill:

Time 1: _____

Time 2: _____

Time 3: _____

APPENDIX F

IRB APPROVAL LETTER

February 22, 2019

Dear Douglas Wajda,

RE: IRB-FY2019-64
Menstrual Cycle Effects on Knee Injury Mechanisms

The IRB has reviewed and approved your application for the above named project under the category noted below.
Application renewal is not necessary unless indicated below.

Approval Category: Expedited Category 2a, 4
Approval Date: February 21, 2019
Expiration Date: --

By accepting this decision, you agree to notify the IRB of: (1) any additions to or changes in procedures for your study that modify the subjects' risk in any way; and (2) any events that affect that safety or well-being of subjects. Notify the IRB of any revisions to the protocol, including the addition of researchers, prior to implementation.

Thank you for your efforts to maintain compliance with the federal regulations for the protection of human subjects. Please let me know if you have any questions.

DO NOT REPLY TO THIS EMAIL. IF YOU WISH TO CONTACT US, PLEASE SEND AN EMAIL MESSAGE TO cayuseirb@csuohio.edu.

Sincerely,

Mary Jane Karpinski
IRB Analyst
Cleveland State University
Sponsored Programs and Research Services
(216) 687-3624
m.karpinski2@csuohio.edu