HAMSTRING NEUROMECHANICAL PROPERTIES AND BIOMECHANICS IN AN ACL INJURED POPULATION

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ABSTRACT

David Robert Bell: Hamstring Neuromechanical Properties and Biomechanics in an ACL Injured Population
(Under the direction of Dr. Darin A. Padua)

In the general population, females are 1.5-4.6 times more likely to tear their ACL than males. Hormonal fluctuations during the menstrual cycle partially explain this elevated injury risk. Most of the previous research has focused on the influence of hormones on ligament in healthy females with no history of ACL injury. Information is limited regarding the influence of hormones on muscle and ligament in females with a history of ACL injury. The purpose of this investigation was to determine if biomechanical and neuromechanical factors change across the menstrual cycle in females with a history of unilateral ACL injury. Twenty-four participants were recruited to participate with twenty subjects (height=168.6±5.3cm, mass=66.2±9.1kg) completing the testing protocol. Participants were tested (1) 3-5 days after the onset of menses and (2) within 3 days following a positive ovulation test. Separate paired t-tests were performed with menstrual cycle phase as the within-subject factor (menses vs. ovulation) for variables of interest. Knee laxity (P=0.03), hamstring musculotendinous stiffness (P=0.03), estradiol-β-17 (P=0.009), and progesterone (P=0.003) increased at ovulation. Hamstring strength, rate of force production, and free testosterone did not change across the menstrual cycle (P>0.05). During the jump landing at initial contact, the following changes occurred at ovulation: the tibia became externally rotated (P=0.01) and external knee valgus moment decreased (P=0.006). During the absorption phase the following occurred at ovulation: the tibia became externally rotated

(*P*=0.05), the femur became internally rotated (*P*=0.05), knee varus moment decreased (*P*=0.03), knee valgus moment decreased (*P*=0.003), knee external rotation moment decreased (*P*=0.007), and peak vertical ground reaction force decreased (*P*=0.04). Females with a history of unilateral non-contact ACL injury demonstrated altered biomechanical and neuromechanical profiles across the menstrual cycle. Knee joint laxity, hamstring musculotendinous stiffness, and jump landing biomechanics appear highly sensitive to changes in hormones across the menstrual cycle in females with previous history of unilateral non-contact ACL injury. The influence of the observed changes on non-contact ACL injury risk requires further study.

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CHAPTER ONE

INTRODUCTION

Anterior Cruciate Ligament (ACL) rupture is a devastating injury that affects an estimated 1 in 3000 people per year[1] with the most recent data concluding that females are 1.5-4.6 times more likely to tear their ACL than males.[2] ACL injuries cost the health care industry approximately $2 billion per year[3] and seventy-nine percent of those who sustained an ACL rupture develop observable degenerative changes on X-rays 13 years post injury.[4] These observable changes in radiographs are related to early onset arthiritis.[4] Given the cost and long term consequences associated with ACL injury, prevention is key.

Once an athlete has sustained an ACL injury, they are 10.4 times more likely to suffer another injury regardless if it is the previously injured or uninjured leg.[5, 6] Given this information, females with a history of ACL injury are at substantially greater risk for rupture compared to the general population. This creates a strong rationale for utilizing them in research given their propensity for injury. Previous research using prospective, case-control study designs, have observed ACL injured populations with predisposing genetic factors to injury,[7] greater generalized joint laxity,[8] less flexibility,[8, 9] and less hamstring strength.[10] The Research Retreat IV: ACL Injuries – The Gender Bias, identified the need to move research beyond direct gender comparisons that dominate current research. Researchers were instead encouraged to focus on comprehensive testing of potential risk factors.[11] This

research project will investigate multiple theoretical risk factor categories and utilize females with a history of unilateral ACL injury.

Absolute levels of reproductive hormones (estrogen, progesterone, and testosterone) are theorized to influence non-contact ACL injury risk.[12, 13] Estrogen receptors have been found on the female ACL[14] and skeletal muscle,[15] and research has demonstrated a positive relationship between hormone concentration and ACL laxity.[16] A common way to assess whether or not the menstrual cycle influences injury is to assess theoretical factors that may influence injury at times corresponding to low and high levels of hormones. The most common time points in which testing are traditionally implemented are at menses and ovulation, respectively. For example, muscle[17] and ligament[16] stiffness have been found to be decreased at ovulation when estrogen levels are elevated. However, this research area is not without controversy with some authors finding no relationship between menstrual cycle phase and tissue stiffness.[18] To our knowledge, all of the research that has assessed the influence of hormonal fluctuations on tissue has used healthy females with no history of ACL injury and normal menstrual cycles. Utilizing a healthy population is a major limitation of previous research. If reproductive hormones influence ACL injury, theoretically, females with a history of ACL injury should be more responsive to hormonal fluctuations when compared to the general population. Hormonal levels may be a major influence on non-contact ACL injury and further investigation is warranted.

The hormonal consensus statement from Research Retreat IV stated one of the areas ACL researchers know very little about is the role that hormones play in skeletal muscle.[11] The hamstrings are believed to influence non-contact ACL injury because they are synergists to the ACL and provide dynamic stabilization against excessive anterior tibial translation.[19]

Gender differences have been observed in hamstring neuromuscular properties[20, 21] with females having less knee flexor musculotendinous stiffness (MTS)[21-23] and a lower rate of force production (RFP) compared to males.[20] These factors are related to the overall force production capacity of the hamstrings but also describe how quickly and efficiently the hamstrings are at producing force. Both factors are relevant to ACL injury prevention and females seem to differ significantly from males in both measures. However, the literature is inconclusive regarding the ability of neuromuscular factors to change based on hormonal levels and menstrual cycle phase with some authors reporting variation across the menstrual cycle,[17, 24, 25] while others do not.[26-28] However, all of these studies utilize healthy populations and none use females with a history of unilateral non-contact ACL injury.

This research project is a logical progression from previous data collected by our research conglomerate. We previously explored the relationship between hamstring strength properties and reproductive hormones in a healthy males and females as a potential explanation for observed gender differences in neuromechanical properites.[29] When males and females were examined as a single group, estrogen was negatively correlated with knee flexor stiffness and RFP (Tables 3 and 4).[29] Free testosterone was positively correlated with knee flexor stiffness and RFP and negatively correlated with T50% (Table 3).[29] These recent results agree with previously reported research that found a decrease in knee flexor stiffness at ovulation.[17] Together, these factors seem to indicate that reproductive hormones influence hamstring neuromechanical properties. However, this area of research has predominately focused on direct gender comparisons and if neuromechanical properties respond to hormonal changes across the menstrual cycle. This research needs to be replicated using

females with a history of unilateral non-contact ACL injury that may be more responsive to hormones if they play a role in non-contact ACL injury.

Biomechanical variables are the final risk factor category that this project will address. These factors are important to investigate because oftentimes injury occurs during activities such as landing from a jump,[30, 31] cutting,[32, 33] and sudden deceleration,[34] tasks which are provocative ACL loading maneuvers.[35-39] Generally, females tend to perform these activities with less sagittal plane motion and greater frontal plane motion.[35, 38, 40] Neuromechanical properties and kinematics and kinetics are interdependent and not mutually exclusive. Altered neuromechanical response could result in abnormal joint loading during provocative ACL loading maneuvers and increased risk of injury (Figure 1). A prospective investigation by Marshall et al[5] found that subjects with reported prior ACL injury had altered biomechanics during a jump landing. Therefore, it is important to collect three-dimensional hip and knee kinematics and kinetics during a high risk activity as many of these variables have been theorized risk factors for non-contact ACL injury.[41] This study addresses a need by determining if biomechanical changes occur across the menstrual cycle in high risk individuals.

In summary, this project addressed several gaps in the ACL literature. A variety of risk factors were examined including absolute hormonal levels, hamstring strength and neuromechanical properties, as well as biomechanical variables during a jump landing. Most importantly, direct gender comparisons were not made and we did not use a healthy female population; instead we utilized females with a previous history of non-contact ACL injury. This population is 10 times more likely to suffer a second non-contact ACL injury when compared to the normal population and theoretically, is more sensitive to hormonal

fluctuations across the menstrual cycle if hormones are a risk factor for ACL injury. The purpose of this research study was to use this population that has not been previously investigated and: (1) determine if relationships exist between reproductive hormones and hamstring neuromechanical properties at menses and ovulation, (2) determine if hamstring neuromechanical properties and knee laxity change across the menstrual cycle, (3) determine if kinematics, and kinetics change across the menstrual cycle during a jump landing, and (4) determine if correlations exist between hamstring neuromechanical properties and jump landing kinematics and kinetics.

1.1 Research Questions

Research Question 1. Are there significant correlations between concentrations of estrogen, progesterone, and free testosterone and hamstring neuromechanics at menses and ovulation?

Hamstring neuromechanical measures:

- Peak concentric and eccentric isokinetic strength at 300 degrees/second
- Peak isometric hamstring strength
- Rate of force production
- Time to 50% peak force
- Hamstring musculotendinous stiffness

Research Question 2: Do hamstring neuromechanical properties and knee laxity change across the menstrual cycle?

Hamstring neuromechanical measures:

- Peak concentric and eccentric isokinetic strength at 300 degrees/second
- Peak isometric hamstring strength
- Rate of force production
- Time to 50% peak force
- Musculotendinous stiffness

Anterior knee laxity measure:

- Displacement (mm) using the KT-1000nknee arthrometer at 133 N of force

Research Question 3: Do biomechanical variables assessed during a jump landing change across the menstrual cycle?

Peak kinematic variables to be assessed at initial ground contact as well as the absorption phase of the jump landing (initial contact to peak knee flexion)

- Knee sagittal plane motion (flexion / extension)
- Knee frontal plane motion (varus / valgus)
- Tibial rotational motion (internal / external)
- Hip sagittal plane motion (flexion / extension)
- Hip frontal plane motion (adduction / abduction)
- Hip rotational motion (internal / external)
- Anterior tibial shear force
- Knee sagittal plane moment (flexion / extension)
- Knee frontal plane moment (varus / valgus)
- Knee rotation moment (internal / external)

Research Question 4. Are there significant correlations between hamstring neuromechanics, knee laxity, and jump landing kinematics and kinetics at menses and ovulation?

1.2 Research Hypotheses

Research Hypothesis 1: Estrogen and progesterone concentrations will be negatively correlated with hamstring neuromechanics while testosterone will have a positive correlation with hamstring neuromechanics. These relationships will become stronger at ovulation especially for estrogen potentially due to larger spread in the data.

- Estrogen will be negatively correlated with:
  - Peak concentric and eccentric isokinetic hamstring strength at 300 degrees/second
  - Peak isometric hamstring strength
  - Rate of force production
  - Hamstring musculotendinous stiffness

- Progesterone will be negatively correlated with:
  - Peak concentric and eccentric isokinetic hamstring strength at 300 degrees/second
  - Peak isometric hamstring strength

    - Rate of force production
    - Hamstring musculotendinous stiffness

- Free testosterone will have a positive correlation with:
    - Peak concentric and eccentric isokinetic hamstring strength at 300 degrees/second
    - Peak isometric hamstring strength
    - Rate of force production
    - Hamstring musculotendinous stiffness

- Time to 50% peak force will have a :
    - Positive correlation with estrogen
    - Positive correlation with progesterone
    - Negative correlation with free testosterone

Research Hypothesis 2. Hamstring neuromechanical properties and knee laxity will be altered to increase the susceptibility to non-contact ACL injury at ovulation when estrogen concentration is greatest.

- Females will experience the following changes in hormones and muscle properties at ovulation:

    - No change in concentration of testosterone and progesterone
    - An increase in the concentration of estrogen
    - Decreased peak concentric and eccentric isokinetic hamstring strength at 300 degrees / second
    - Decreased peak isometric hamstring strength
    - Decreased hamstring rate of force production
    - Decreased hamstring musculotendinous stiffness
    - Slower time to reach 50% peak force
    - Increased knee laxity

Research Hypothesis 3. Peak biomechanical variables assessed during the absorption phase of a jump landing will be altered at ovulation in manners that may increase the susceptibility to non-contact ACL injury at ovulation when estrogen concentration is greatest.

- Females will experience the following changes at ovulation compared to menses for peak biomechanical variables during initial contact and the absorption phase of a jump landing:

    - Increased peak knee valgus during the absorption phase of the jump landing

- Decreased knee flexion during the absorption phase of the jump landing
- Increased tibial rotation during the absorption phase of the jump landing
- Decreased hip flexion during the absorption phase of the jump landing
- Increased hip adduction during the absorption phase of the jump landing
- Increased femoral rotation during the absorption phase of the jump landing
- Increased anterior tibial shear force during the absorption phase of the jump landing
- Increased knee extension moment
- Increased knee valgus moment
- Increased knee rotation moment

Research Hypothesis 4. Correlations will exist between hamstring neuromechanics, knee laxity, and jump landing kinematics and kinetics at menses and ovulation in ways that will increase ACL loading and increase the susceptibility of ACL injury.

1.3 Operational Definitions

ACL Injury: Forces applied to the knee at the time of injury that resulted from the athlete's own movements and did not involve contact with another athlete or object.[2]

Test Limb: The limb with no previous history of ACL injury.

ACLR: Anterior Cruciate Ligament Reconstruction.

Concentric Isokinetic Hamstring Strength: The maximal output measured by the Biodex from seated position during a concentric hamstring contraction.

Eccentric Isokinetic Hamstring Strength: The maximal output measured by the Biodex from seated position during an eccentric hamstring contraction.

Isometric Hamstring Strength: The peak force output recorded by a compression load cell during an isometric hamstring contraction.

Frequency of Oscillation: The frequency of oscillation was defined using the equation $f=1/(t1-t2)$, where t1 and t2 were the timings of peak knee flexion measured in response to a perturbation during the hamstring musculotendinous stiffness assessment (figure 4).

Hamstring Musculotendinous Stiffness (K): $K = 4\pi^2 mr^2 f^2$. In this case, stiffness was quantified as rotational stiffness where, m was the estimated mass of shank and foot segment[42] with an applied load equal to 10% of the subject's body mass, r was the length of the test limb from the lateral joint line to lateral malleolus, and f was the damped frequency of oscillation from the equation above.

Time to 50% Peak Force: Time to 50% peak force was defined as the time interval between the onset of force production and the instant at which 50% of the peak isometric hamstring force is attained.

Rate of Force Production: The ratio of the output of normalized force from the load cell to the time to 50% peak.

Onset of Force Production: The point at which 5% of peak isometric hamstring force was achieved.

Jump Landing: The jump landing was performed from a 30 cm high box set 50% of the participant's height from the edge of the force plate to the leading edge of the box. The participant jumped forward off the box, landing with the foot of the test limb on the force plate. Immediately upon landing, the participant jumped for maximal height (figure 6).

Initial Contact: The data point after the ground reaction force in the vertical direction exceeds 10 Newton's of force.

Absorption Phase: Initial contact to peak knee flexion during the jump landing.

Knee Laxity: The amount of anterior tibial displacement at 133 N using a standard knee arthrometer.

1.4 Assumptions / Limitations

The following assumptions and limitations will be made for this study:

1. All participants gave their best effort in performing all of the testing protocols.
2. Self report was used to determine if the subject had a normal menstrual cycle.
3. We did not include subjects who used oral contraceptive and the exogenous hormones might influence muscle properties.
4. The results of this study only apply to female populations with a non-contact ACL injury.
5. Type of graft was not considered nor other ligament or meniscus structures associated with the injury.

1.5 Delimitations

The following delimitations were made for this study.

1. Participants were female.
2. Participants were between 18-25 years of age.
3. Participants had to have one limb free from injury.
4. Participants must have had a self reported normal menstrual cycle and not use hormone altering oral contraceptive when they experienced their non-contact ACL injury.
5. Participants were free from lower extremity injury for three months prior to testing.

1.6 Independent Variables

The primary independent variable in this study was:

- Menstrual cycle **PHASE** at two levels:
    - Menses
    - Ovulation

Hamstring strength and EMG variables were assessed to reduce the number of paired t-tests used in this investigation. We utilized several different Independent variables to accomplish this:

- Type of muscle contraction at five levels:
    - Isometric, Concentric at 60°/s, Concentric at 300°/s, Eccentric at 60°/s, Eccentric at 300°/s
- Time of muscle activation at two levels:
    - Pre-perturbation – The pre-perturbation time period was 200ms prior to the onset of the perturbation during the knee flexor stiffness assessment
    - Post-perturbation – The post-perturbation time period was defined from the onset of the perturbation to the second oscillatory peak during the knee flexor stiffness assessment
- Site of muscle activation measurement
    - Medial versus lateral musculature

1.7 Dependent Variables

The following dependent variables will be used in this study:

Blood Reproductive Hormones
- Absolute concentration of estradiol-β-17
- Absolute concentration of progesterone
- Absolute concentration of free testosterone

Hamstring Neuromechanical Properties:
- Peak concentric and eccentric isokinetic strength at 60 and 300 degrees/second
- Peak isometric strength
- Time to 50% peak force (T50%)
- Rate of force production

        - Rate of force production over the first 200ms (RFP 200)
        - Rate of force production to T50% (RFP T50%)
    - Musculotendinous stiffness

Kinematic and kinetic variables were assessed at initial contact and also assessed during the absorption phase of a jump landing:

- Knee valgus
- Knee flexion
- Tibial rotation
- Hip flexion
- Hip adduction
- Femoral rotation
- Anterior tibial shear force
- Knee flexion/extension moment
- Knee varus/valgus moment
- Knee rotation moment

1.8 Significance

This research progresses ACL injury research in a variety of ways. If hamstring neuromechanics change across the menstrual cycle in females with a history of unilateral ACL injury then it provides evidence that hormones influence dynamic knee joint stability, which may partially explain the high rate of re-injury in this population. It will also provide evidence to clinicians to increase awareness that hormonal levels are an issue and they can relay this information to individuals participating in injury prevention and rehabilitation programs. This study will also improve our understanding of the role the hamstrings play in ACL injury. We theorize the hamstrings are important because of their anatomical location, but this project will provide insight into factors that influence hamstring neuromechanical properties. If hormones influence muscle in a way that may eventually influence injury risk, muscle is modifiable. Clinicians can use this information and focus on hamstring muscle strength and force producing capability as part of an injury prevention or rehabilitation program.

CHAPTER TWO

REVIEW OF THE LITERATURE

ACL injuries are costly in the short as well as long term. It is well accepted in the literature that ACL injury risk is not equal between genders.[2] While the numbers vary between studies, a recent review of the literature found that females were 1.4 to 4.6 times more likely to suffer a non-contact ACL compared to males.[2] This topic has generated a great deal of related research. For example, we searched the PubMed database maintained by the National Library of Medicine using the search term "Anterior Cruciate Ligament" and this search engine returned 9,768 results. Much of the non-contact ACL injury literature has focused on gender differences between healthy individuals. However, recent research has focused on individuals with a history of non-contact ACL injury. In fact, persons with a history of ACL injury are at 10.4 times more likely to suffer a subsequent ACL sprain and are at even greater risk compared to the general female population.[5] This is alarming because it indicates that either rehabilitation is not effectively addressing risk factors that result in injury, or, risk factors are present and cannot be addressed during the rehabilitation process. Regardless, the next logical step in ACL research is to study high risk versus low risk individuals rather than direct gender comparisons. The purpose of this study is to investigate risk factors in females with a history non-contact ACL injury history (high risk) and determine if neuromuscular and biomechanical properties change across the menstrual cycle. We will examine theorized risk factors including absolute hormonal levels, a series of

hamstring neuromechanical properties, as well as biomechanical variables during a jump landing. This literature review provides a background and rationale for this study that includes an explanation of the epidemiology of ACL injury, etiology of ACL injury, neuromechanical and biomechanical contributions to joint stability, and finally hormonal influences on ACL injury rate and muscle. Previous research and limitations in these areas will be discussed.

2.1 ACL Injury: The Epidemiology of a Public Health Problem

Participation in athletics has many benefits such as leading a healthy active lifestyle, developing leadership, and learning responsibility. All of these areas benefit the individual as well as society and thus makes sport an attractive option for developing these skills. Since passage of Title IX in 1972, female participation in sport has increased significantly. From 1971 to 2006, collegiate female participation rates increased 456% and high school female participation rates increased 904%.[43] One of the unfortunate consequences potentially associated with athletic participation is injury. Increased sports participation by females resulted in the observation that females are at a greater risk of injury compared to males that participate in similar sports.[44] Injury to the Anterior Cruciate Ligament (ACL) is an all too common and devastating injury that afflicts female athletes.

Recent research has found that females are 1.4 to 4.6 times more likely to injure their ACL compared to males that participate in the same sport.[2] Approximately 1 in 3,000 people tear their ACL every year[1] and the sports that have highest ACL injury rates include basketball and soccer because these sports require motions that are commonly thought to load the ACL such as cutting and landing from jumps.

2.2 Consequences of ACL Injury

When an ACL injury does occur there are both short and long-term consequences that result from the injury. In the short-term, there are psychological and emotional stresses that are placed on athletes as a result of being removed from a team atmosphere. Many athletes identify with their position in society via team membership. Additional stress might be related to whether or not the athlete will be able to return to sport. A study of ACL injured soccer players found that nearly one-third did not return to soccer post injury because of lack of function or fear of re-injury.[45]

Beyond the psychological issues induced by injury, significant cost is associated with injury as well. Gottlob et al[3] estimated the average cost of an ACL surgery and rehabilitation at $11,768. This results in ACL injury costing the overall healthcare system over $2 billion per year. One of the most significant results of ACL injury can be the long term consequences of ACL injury. Salmon et al[4] found that 79% of patients who sustained an injury developed observable degenerative changes on X-rays 13 years post injury. Early changes in radiographs have been linked to early onset arthritis.[4] If a patient is not rehabilitated properly then the chance of developing arthritis increases.[46] Although it make take years for osteoarthritis to develop, the national cost estimate of osteoarthritis in the United States in 2003 was $128 billion dollars and $2.55 billion in North Carolina.[47] As the population of the United States ages, the cost of osteoarthritis will become more burdensome on the health care system.

2.3 Mechanism of Injury and Risk Factor Categories

There is no single mechanism of injury for non-contact ACL injury. There is consensus in the literature that the mechanism for non-contact ACL injury is multifactorial

and encompasses a variety of intrinsic as well as extrinsic factors.[48] McNair et al[30] observed that 70% of ACL injuries were non-contact in nature. Boden et al[49] confirmed these findings in a separate study that reported 72% of ACL injuries were non-contact in nature. A video analysis of ACL injuries found that most injuries occurred during landing from a jump or deceleration when the knee was extended.[49] Olsen et al,[50] also found videographic evidence linking non-contact ACL injury mechanisms to extended knee positions and valgus collapse.

Intrinsic risk factors for non-contact ACL injury are commonly divided into one of four categories: anatomical, hormonal, neuromuscular, and biomechanical.[48] It is important to point out that these risk factor categories are theoretical because investigations have identified gender differences but epidemiological risk factor identification has not been performed in most cases. The only theorized risk factor category we will not address in this study will be the anatomical category. Variables investigated or identified as potential risk factors in this category include decreased femoral notch width,[6, 51, 52] excessive Q-angle,[53, 54] foot pronation,[55, 56] navicular drop,[55] and increased generalized joint laxity.[57, 58] Other intrinsic risk factors will be discussed in great detail in the following sections because this project will address each of these areas.

A recent consensus statement of leading ACL researchers indicated that a majority of previous ACL research has been related to only one risk factor category.[11] The authors of this consensus statement identified the need to perform comprehensive analyses and combine risk factor groups. This project will combine hormonal, neuromuscular, and biomechanical risk factor categories. Specifically, we will investigate variables that result in increased ACL load and/or alter joint stability. Joint stability is important in preventing injury and many factors can influence joint stability. While not exhaustive, some of these factors include

neuromuscular, in-vivo movement patterns (kinematics and kinetics), muscle stiffness, and hormonal influences. These categories will be the focus of this review.

2.4 Factors influencing ACL Loading

Anterior Tibial Translation

The ACL is loaded in a variety of directions and the way that it is injured is most likely multifactorial with no single loading direction entirely responsible for non-contact ACL injury.[30] However, the most likely mechanisms for ACL injury is anterior tibial translation (ATT) or anterior tibial shear force (ATSF) as it places the most strain on the ACL.[59-62] Other potential mechanisms include knee valgus and knee rotation.[59] Therefore, a majority of this discussion will focus on how these conditions load the ACL and how the muscular contractions, specifically of the hamstrings, can protect against these provocative loading conditions.

Muscle contractions influence forces acting on the cruciate ligaments within the knee and a majority of the research has focused on muscles that directly cross the knee including the quadriceps, hamstrings, and gastrocnemius.[63] Cadaver research has demonstrated that isolated quadriceps activity increases ACL strain[19, 64, 65] and these results have been replicated in-vivo.[66, 67] Renstrom et al[68] used in-vitro procedures and determined that quadriceps force increased ACL strain from 0-45° of knee flexion. He concluded that the quadriceps function at knee flexion angles greater than 45° but no additional strain is placed on the ACL.[68] These findings agreed with previous research that found that the ACL resists loading significantly at knee flexion angles less than 45°.[62, 69] Previous research has shown that a 2,100 N tensile load is required to tear the ACL in young health knees[70] but the

quadriceps can produce 3000 N of force in the general population and even greater forces in the trained populous.[71]

Females demonstrate a quadriceps dominant muscle activation patterns which may predispose them to non-contact ACL injuries. During partial weight bearing perturbations, elite female athletes responded with a quadriceps and gastrocnemius first and hamstrings second activation pattern.[72] Whereas, males tended to contract their hamstring musculature first, in response to the same perturbation. By contracting the quadriceps and gastrocnemius first, females may be placing strain on the ACL, resulting in abnormal joint loading which may result in increased susceptibility to injury. Similar quadriceps dominant patterns have been observed in functional running and cutting protocols as well. Females were found to have greater quadriceps activation and lower hamstring activation compared to males indicating joint loading that would favor ACL injury.[73] Finally, increased quadriceps activation in females has been observed during a stop-jump, which is considered a high-risk ACL loading maneuver.[35]

This altered muscular relationship is not restricted to anterior versus posterior musculature, it has also been seen in medial versus lateral musculature.[74] Twenty-one recreationally active adults performed a 100-cm forward hop with EMG sensors collecting muscle activity from the lateral and medial quadriceps and hamstrings as well as peak knee abduction moment. The authors took this information and calculated a medial and lateral quadriceps-to-hamstrings (Q:H) ratio. The results demonstrated that females had less muscle activation compared to men and the medial activation was less than the lateral activation in both genders. Interestingly, the authors also performed a regression analysis and were able to conclude that the medial Q:H ratio explained a significant amount of the variance in peak

knee abduction moment. These results indicate that the musculature surrounding the knee is important to performance and unbalanced loading in females may partially explain ACL injury rates.

The hamstrings reduce the load on the ACL and reduce ATT.[75] It is widely believed that the hamstrings can protect the ACL from excessive loading and, thus, are major focus of this study. However, similar to the quadriceps, the hamstrings have a knee range of motion in which they are most efficient at preventing anterior tibial translation. While the knee flexion angle that is most efficient is debatable, it is generally accepted that the hamstrings are not efficient at protecting the ACL at knee flexion angles less than 30°.[65, 68, 75] Weak or less stiff hamstrings may not be able to respond to abnormal loading patterns and prevent ACL injury. To add to this pattern, females were found to perform cutting and landing maneuvers with less hamstring activation compared to males.[73] More et al[19] used cadavers and observed that a 90 N hamstrings load could decrease the load on the ACL by 40% when the knee was between 15-45°. A subsequent study by Li et al[75] found that an 80 N hamstring load decreased the load on the ACL by 30-44% depending on knee flexion angle. These results show that the ACL is capable of preventing excessive ATT which is one of the direct loading mechanisms of the ACL.

Knee Valgus

Videographic evidence has identified valgus collapse of the knee as a common mechanism of non-contact ACL injury.[50] Markolf et al[59] used fresh frozen cadavers to determine how a variety of loading states influenced ALC loading. Knees were loaded at different knee angles in combination with a variety of frontal or axial conditions along with an anterior force. At low knee flexion angles, knee valgus alone loaded the ACL minimally,

however, when combined with anterior tibial force the result was additive in nature. The combination of tibial internal rotation, anterior force, and knee valgus increased the ACL force in knee flexion angles greater than 70°. Interestingly, knee valgus in combination with tibial external rotation did not result in any increase in ACL force at any knee flexion angle. Fukuda et al[76] wanted to determine if the pivot shift test, a orthopedic special test used to determine the intactness of the ACL, actually stressed the ACL. This study observed that with an intact ACL, in general, increases in valgus torque were associated with increases in anterior tibial translation. After the ACL was transected valgus torque significantly increased anterior tibial translation compared to intact knees. Finally, Miyasaka et al[77] observed a significant increase in ACL tension when a 2 Nm valgus torque was applied to cadaver knees. One of the identified risk factors for non-contact ACL injury in females is knee abduction angle and knee abduction moment during a drop landing.[41] Knee abduction contributes to valgus angle.[78] It is also well established that females perform activities with greater knee valgus angle and moment compared to males.[38, 79-85] Together, this evidence suggests that valgus motion and moment have a significant influence on ACL tension. If knee valgus and excessive knee valgus moments can be minimized or avoided, then it may be possible to prevent non-contact ACL injury.

The hamstrings are capable of preventing valgus angulations. The hamstring and quadriceps co-contraction can be used to prevent valgus motion from occurring.[86, 87] Lloyd & Buchannan[86] observed strategies that are used to respond to valgus moments are to contract the quadriceps and hamstrings first and then contract the gracilis and TFL. They also concluded that the sartorius is capable of preventing valgus loading by increasing varus moment. This group expanded this study by using modeling to determine how movements

load or unload knee ligaments during dynamic tasks that included running and sidestep cutting.[87] Knee muscles counteracted knee valgus moments by producing knee extension moments with the quadriceps or knee flexion moments with the hamstrings. Muscle activation was dependent on the task being formed and the requirements of that task. For example, if a knee flexion moment was required during running, then the hamstrings were responsible for preventing valgus collapse. Complete co-contraction was the second option in preventing knee valgus. This evidence demonstrates that knee valgus motion and moment create abnormal loading patterns and are capable of stressing the ACL. However, the hamstrings are capable of limiting knee valgus motion and moment.

Muscular contractions are also capable of preventing knee valgus angulations prior to landing.[88] Similar to the previous study by Palmieri-Smith et al,[74] twenty-one subjects performed a 100 cm jumping task and EMG prior to landing and kinematics were collected during the task. The results indicated that EMG 100 ms prior to landing predicted the amount of peak knee valgus that an individual would obtain during the task. Low levels of EMG in the medial musculature were associated with higher amounts of knee valgus while higher amounts of EMG in the lateral musculature were associated with less knee valgus. These results indicated that that medial and lateral contribution to knee stability are important and can cause as well as prevent knee valgus.

Tibial Internal Rotation

The final motion that loads the ACL is tibial internal rotation.[59, 77] Several different research groups have found that the ACL is loaded when an internal rotation torque was applied to cadaver knees.[59, 77] Interestingly, the forces placed on the ACL via internal tibial rotation are usually greater than those created by valgus torque alone. However, no studies

have prospectively identified internal rotation as a risk factor for non-contact ACL injury. Also, the quadriceps are capable of creating tibial internal rotation during knee extension.[65] Tibial internal rotation in combination with ATT creates a potentially dangerous scenario that is favorable to ACL loading. In-vivo research has found that females usually move into positions of tibial internal rotation when performing high risk ACL loading maneuvers[89] and females perform jump landings with greater knee internal rotation compared to males.[35, 84] Evidence demonstrates that internal rotation increases the load on the ACL and females tend to perform high risk activities with greater internal rotation compared to males.

The hamstrings are capable of limiting knee internal rotation. More et al[19] observed that a 90 N hamstring load decreased tibial internal rotation. These results have been replicated by Li et al[75] that observed reduced tibial internal rotation using an 80N hamstring force.

Other Factors Influencing ACL Loading

In the previous section we discussed mechanisms that load the ACL, however, the gastrocnemius also crosses the knee joint. The gastrocnemius muscle is an ankle plantar flexor and knee flexor. Modeling has demonstrated that a quadriceps and gastrocnemius contraction load the ACL.[90] Subsequent research has demonstrated, like the quadriceps and hamstrings, the gastrocnemius' ability to influence ACL strain is dependent upon knee flexion angle.[91] Specifically, the gastrocnemius could strain the ACL in low knee flexion angles (15-30°) and the addition of a quadriceps contraction dramatically increased ACL tensile stress.

Summary of ACL Loading Mechanisms

The ACL is ruptured when the load on the ACL exceeds the ability of the ligament to elongate. During movement the ACL is loaded at low knee flexion angles and via ATT primarily created by the quadriceps at low knee flexion angles.[69] The ACL is also loaded during knee valgus and tibial internal rotation. These motions are particularly problematic because females perform athletic activity in these knee positions which may partially explain the increased ACL injury rate. The hamstrings are capable of preventing excessive ATT, knee valgus, and tibial internal rotation and are theorized to be important factors in ACL injury prevention. For this project, we will investigate the role of the hamstrings and their isometric force producing capabilities at 30° of knee flexion in a high risk population that has a history of non-contact ACL injury. This design will allow us to determine if hamstring properties change across the menstrual cycle. We will also investigate biomechanics during a high risk ACL loading drop jump which will provide information about movement during high risk activities.

2.5 Individuals with Prior ACL Injury: What Do We Know

Individuals with previous ACL injury are at a greater risk of sustaining a second ACL injury compared to the general population.[5] Two potential explanations exist that may partially explain this discrepancy. Either this group of individuals has not been rehabilitated correctly or risk factors are still present making them more at risk compared to the average female population. The purpose of this study is to investigate neuromuscular, biomechanical, and hormonal risk factors and determine if these change across the menstrual cycle. In order to rationalize our variable selection we first need to understand what is already known about females who have had reconstruction surgery and completed the rehabilitation process.

There have been a variety of research studies that have utilized a female population with a history of anterior cruciate ligament reconstruction (ACLR) that has shown neuromuscular deficits as a result of the injury and subsequent surgery. Injury to the joint including skin, muscle, ligaments, and tendons damages receptors embedded in these structures and influences the somatosensory system.[92] A decrease in electrical activity of the knee has been demonstrated in ACLR patients during balance board activities indicating compensation in muscular control strategy even after surgery.[93] Threshold for detecting passive motion is one of the more common variables for measuring priorioception,[92, 94-96] however, most of the research in this area has been performed on ACL deficient subjects[94, 95, 97] and limited evidence suggest that ACLR patients also suffer from these proprioceptive deficits.[98] Additionally, most patients are symptomatic in that they have instability and/or accompanied pain.

Landing adaptations have also been reported as a result of ALCR. Decker et al[99] compared eleven ACLR athletes with hamstring grafts to controls. Subjects were instructed to side-step off a 60 cm high box onto a force plate and ground reaction forces and joint powers were calculated to determine if the injured limb behaved similar to healthy controls. ACLR demonstrated a more erect landing posture at initial contact and absorbed more forces through the ankle and less through the hip. The authors attributed the reduced hip loading by ACLR patients to avoid the hamstrings. A limitation of this study is that only the ACLR leg was tested and not both legs so it is impossible to determine if both legs in the ACLR group performed in similar capacities.

Swanik et al,[100] investigated muscle activation firing in ACLR, ACL deficient, and control females. They assessed EMG during downhill walking, level running, hopping and

landing from a 20.3 cm jump. They found no muscle activation differences between ACLR and control groups and no side-to-side differences were present.

Most recently, Ortiz et al.[101] compared healthy and ACLR populations during a 40 cm drop jump and up-down jump. They investigated kinematic, kinetic, and EMG variables between populations as well as side-to-side differences. For the drop jump, no differences were seen between legs for the ACLR group nor between hip and knee kinematics, but, females with ACLR did have altered EMG compared to controls. For our project we are using the healthy limb. These findings support the notion that both limbs should be similar even though there might be differences between ACLR and healthy populations.

Quadriceps strength deficiency is a muscular deficit often observed after ACL injury and repair and even include those who have undergone extensive rehabilitation.[102] This strength deficit can continue for months or years in patients whom are ACL deficient.[102-104] Quadriceps strength prior to surgery is a strong predictor of knee outcomes. An important consideration is that many clinicians and physicians use the uninjured leg as the gold standard to evaluate return to play criteria. However, recent reviews of literature suggest that ACL surgery and injury result in strength deficits in the uninvolved limb as well, making it a poor reference for comparison.[105-107] Interestingly, very little is mentioned about hamstring strength deficits following surgery. This apparent gap in the literature will be addressed by our study.

Hamstring strength and activation levels do not seem to decrease after ALCR and are not as important for patient outcomes as quadriceps strength.[108] However, after ACLR, hamstring strength deficits are not as dramatic as the quadriceps.[109-111] A majority of the findings related to the hamstrings are found in the section entitled “Factors Influencing ACL

Loading". Also, since our study design is a within subjects design, deficits in hamstring or quadriceps strength as a result of rehabilitation will not influence our results.

2.6 Muscle Strength and ACL Injury

Muscle strength is an integral component of injury prevention[112] and females with isokinetic strength imbalances are at greater risk for injury.[113] Previously, we discussed how the quadriceps and hamstrings can influence forces on the ACL, however, a certain amount of strength is required to complete and compete in athletic performance. The high demands of athletics easily surpass the passive ligament restraints and require muscular stabilization in order to protect the ACL from excessive loading during landing and pivoting.[32, 75, 114] Females tend to have weaker thigh strength than males when strength values are normalized to body mass which may play a role in non-contact ACL injury.[115, 116]

Several researchers have found that females have weaker isokinetic hamstring strength compared to males. Hewett et al[117] found females had weaker isokinetic hamstring strength and worse quadriceps-to-hamstring ratio in females compared to males. Plyometric training was able improve the quadriceps-to-hamstring ratios similar to males but not increase hamstring strength significantly. Huston and Wojtys[72] found mixed results when they compared male and female athletes to general population males and females. Quadriceps and hamstring torque was less in female controls compared to both male groups while female athletes had less hamstring and quadriceps strength compared to both male controls but not male athletes. These findings suggest that the general population may have more exacerbated muscle weakness. Finally, Pincivero et al[118] also independently confirmed that males are able to produce more normalized force compared to females. These studies

indicate that there are strength discrepancies between genders and females are not capable of producing the same relative force as males in both the hamstrings and quadriceps.

Myer et al,[10] demonstrated the relationship between strength and injury. He utilized a matched case control study design to track high school and collegiate basketball and soccer players and follow them until injury. Isokinetic concentric quadriceps and hamstring strength were tested at 300 degrees/s. Twenty-two athletes went on to tear their ACL and were matched to female controls and male controls. Female athletes who tore their ACL had decreased hamstring strength and increased quadriceps strength compared to males. Interestingly females who did not tear their ACL had the opposite relationship (decreased quadriceps and increased hamstring strength). This altered relationship between antagonistic muscles may contribute to increased ACL injury by placing abnormal loads on the knee and supporting passive structures during dynamic activity. Interestingly, isokinetic strength (60°/s, 180°/s, 300°/s) has been shown to be a poor predictor of anterior tibial shear force[119] and greater isometric strength is not related to an increased ability to change neuromuscular output post feedback.[120] Strength is obviously an important factor for completing dynamic activity but the overall but non-contact ACL injury is most likely multifactorial in nature.

Gender differences are magnified at higher isokinetic speeds. Hewett et al[121] examined 22 research studies from 1967 to 2004 that used gravity corrected isokinetic strength of the quadriceps and hamstrings. Gender differences were not observed at slow isokinetic speeds (30°/s) but were at higher levels of isokinetic speeds (60°/s, 120°/s, 300°/s, 360°/s) with males always being capable of producing greater force. They also concluded that males are able to maintain proper quadriceps to hamstrings torque ratios at higher speeds

while females were not.[121] The authors concluded that females have a decreased ability to preserve dynamic joint stability especially at higher isokinetic speeds.

In summary, muscle strength imbalances place females at risk of injury[10, 113] and females have less hamstring and quadriceps strength compared to males. Males are capable of keeping similar quadriceps and hamstring ratios at high isokinetic speeds but females cannot.[121] This research demonstrates that females may not be as capable of responding to loads during high risk dynamic activity or the way they respond to dynamic activity increases ACL injury risk. Adequate strength is important to injury prevention along with the appropriate quadriceps to hamstring strength ratio.

In this research study we will examine isokinetic eccentric (300°/s), isokinetic concentric (300°/s), and isometric hamstring strength in females with ACL injury and determine if strength changes across the menstrual cycle in this unique group. We hypothesize that the ovulation testing session will result in decreased hamstring strength compared to the menses testing session and this might be partially responsible for the increased injury rate in ACLI females.

2.7 Literature Review of Additional Strength Properties

Rate of force production is also a theorized risk factor for non-contact ACL injury. In order for a skeletal muscle to act on bone a signal has to be sent from the brain to the skeletal muscle with the longer the time period representing a longer delay. Delays are often measured relative to an external stimulus (light) until electrical activity is detected within a muscle via EMG or between muscle activation and force production.[122] The theoretical premise is that a certain amount of slack is present in a muscle and this slack must be removed before muscle can act on bone. Tendon slack has been shown to be important when

considering EMD.[123] Bell and Jacobs[122] defined EMD as the time between the onset of electrical activity and the onset of force in response to a visual stimulus of the biceps brachii. In a sample consisting of 40 females and 46 males (weak females, strong females, weak males and strong males), they found that both male groups were stronger and had shorter EMD compared to female groups. Stronger groups were also capable of producing greater amounts of force but had equivalent EMD compared to weak groups. Blackburn et al[124] found no difference in EMD between genders and the lateral hamstring muscle when the knee was in 30° of flexion.

Rate of force production (RFP) (also rate of force development) is a variable used in this study and it determines how quickly force can be produced. This variable might be even more important than strength because injuries typically occur quickly after landing. So, the ability to produce a large amount of force in a short period of time may prevent injury from occurring. Gender differences in RFP have been observed in the knee flexors of the recreationally active,[124] the biceps,[122] and in cross-country skiiers.[125] However, EMD and RFP are correlated with longer EMD associated with lower RFP.[122] RFP could partially explain non-contact ACL injury rates between genders.

Time to 50% peak force (T50%) is another representation of how quickly muscle can produce force. Winter and Brooks[126] examined elastic charge time which was defined as the time interval between the onset of force and movement of the heel.[126] Blackburn et al[124] equated T50% as a variable similar to elastic charge time. In both instances, males were faster than females with males being 15.2% faster in elastic charge time and 43% faster in T50%.

We will use similar methodology as Blackburn et al[124] and determine if females with a history of ACL injury have different force production ability across the menstrual cycle and if these variables are correlated with reproductive hormone levels. We hypothesize that T50% will increase at ovulation.

2.8 Hormonal Factors Associated with ACL Injury

Some of the most basic but important differences between genders is the presence of the menstrual cycle in females and the different levels of hormones present in the body. This information, coupled with the fact that female athletes have higher ACL incident rates compared to males, led scientists to theorize that reproductive hormones may influence non-contact ACL injury. The menstrual cycle results in a somewhat predictable pattern of cyclical rising and falling hormonal concentration. The menstrual cycle is generally divided into three phases that correspond with differing hormonal profiles with the first day of menses being day 1. The average menstrual cycle is 28 days in duration however previous research has reported females with normal menstrual cycles ranging from 24-36 days.[16] The first phase of the menstrual cycle is the follicular phase which has an average length of 9 days but is the most variable in length (days 1-9). This phase of the menstrual cycle is associated with low concentrations of estrogen, progesterone, and testosterone. The second phase of the menstrual cycle is the ovulatory phase and is approximately 5 days in length (days 10-14). Some researchers prefer to think of ovulation as an event rather than a phase of the menstrual cycle. Previous research has reported ovulation occurring from day 9 of the menstrual cycle to day 20.[16] Regardless, ovulation is associated with a spike in luteinizing hormone followed by a spike in estrogen. In the average female this spike in estrogen is the largest concentration of estrogen during the menstrual cycle. Progesterone concentration is

low during this time period. The final phase of the menstrual cycle is the luteal phase (day 15-28) which lasts approximately 14 days. The Luteal phase of the menstrual cycle is associated with prolonged elevated estrogen levels that are not as high as the ovulatory period. Progesterone increases significantly during this phase. From this information it is important to understand that menstrual cycles vary between individuals but unique time points exist that make comparisons possible.

Reproductive hormones influence the mechanical properties of ligament. Tissue remodeling is a normal process that occurs continually in healthy and injured tissue.[127-131] The human ACL has matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) which are cells that have opposite function but are important to the remodeling process. MMPs breakdown collagen and TIMPs prevent the breakdown of collagen which is important to ligament structure.[132] Estrogen is partly responsible for the regulation of some MMPs and TIMPs within the ACL.[132-135] The mechanisms that are most likely influenced by estrogen are the collagen matrix that creates the ligament or the metabolism of the tissue itself during the remodeling process.[14, 136-141]

Research has found estrogen receptors on the female ACL[14, 142] and estrogen has a negative influence on the tensile strength of the ACL[138, 140, 143] while others have not.[144, 145] However, a major limitation of all of this research is they have utilized non-injured populations and theoretically, individuals with a history of ACL injury should be responsive to hormonal fluctuations. Slauterbeck et al[138] observed that estrogen decreased the load to failure in rabbit ACL. A subsequent and more complex study used various levels of estrogen and concluded similar results using rabbit ACL's (ACL load to failure decreases with greater levels of estrogen).[143] Lee et al[146] examined the combined effects of mechanical cyclic

loading and estrogen on the ACL. This study found that mechanical loading alone enhanced collagen Type I synthesis but did not change collagen Type III synthesis. Estrogen alone with no mechanical loading applied to the ligaments resulted in increased expression of collagen I and III. However, the combined influences of mechanical loading and estrogen exposure resulted in decreases in Type I collagen and Type III collagen. These results indicate that estrogen combined with mechanical loading has a negative influence on collagen that comprises the ACL. One limitation of this research is that human models cannot be used.

Reproductive hormones seem to influence ACL laxity in humans with normal menstrual cycles and physiological levels of estrogen and progesterone.[16, 147-152] Numerous authors have concluded that anterior laxity differs between gender with males having less laxity compared to females.[55, 56, 72, 151, 153-155] Laxity has not been observed in children although younger children have greater laxity.[156] Negative correlations have been observed between ACL stiffness and estrogen concentration in active females indicating an increase in estrogen is associated with a decreased ligament stiffness.[147] The methodology for assessing ACL laxity across the menstrual cycle uses an instrumented arthrometer that assesses anterior tibial translation. An investigator applies an anterior force that displaces the knee and places stress of the ACL. The laxity is measured via instrumented arthrometer in health females ranges from 3.3 mm[16] to 9.1 mm.[157]

Again, this area is also not without controversy as several authors have concluded that anterior knee laxity does not change across the menstrual.[155, 157-162] However, one of the limitations of all of these studies is they assess specific time points of the menstrual cycle using day counting methods or individualizing to easily observable time points (onset of

menses or ovulation). An assumption is that a specific time point will represent a peak in hormonal concentration (ovulation) or a nadir (menses) for each individual when in actuality it is an educated guess at best. Investigators can have more confidence using the menses test session as opposed to ovulation. Shultz et al[16] completed the most comprehensive investigation that examined the influence of hormone concentration across the menstrual cycle. This study tested 22 females and took daily blood and knee laxity (133 N) measures across one complete menstrual cycle. Individual multiple regression was performed on each subject with hormone concentration as the predictor variable and knee laxity as the criterion variable. The findings revealed two unique groups, one that represented a positive relationship between knee laxity and hormone concentration and the other, opposite. Pooled, 8% of the total variance was explained in knee laxity by the three hormones. When a time delay was added 63.3% of the variance in knee laxity was explained by the three hormones. This illustrates the complexity of the problem when dealing with hormonal profiles. While measuring one time point is not ideal, it is the most cost effective and time efficient manner of addressing such a complicated question.

Evidence suggests that ACL laxity may influence muscular response during dynamic activity.[25, 163, 164] Females with above average knee laxity (7-14mm) had delayed biceps femoris response and higher activation levels after a perturbation compared to a group of females with low knee laxity (3-5mm).[25] The authors concluded that females with increased knee laxity might be less sensitive to loading. Positive correlations have also been found between knee laxity and knee adduction impulse, internal moment, and internal rotation impulse during cutting.[164] Park et al[163] used interesting methodology and collected data at three points across the menstrual cycle but then reorganized the data into low laxity, medium

laxity, and high laxity test sessions based on knee arthrometer readings. This study found no relationship in kinematic and kinetic variables across the menstrual cycle but when organized by laxity found that the high laxity group had a 30% increase in adduction impulse, 20% increase in adduction moment, and 45% increase in external rotation compared to the medium and low laxity groups. This information demonstrates that knee laxity has the ability to influence joint loading and potentially influence non-contact ACL injury via muscular imbalance.

Reproductive hormones influence passive structures that are important to joint stability (ligament).[16, 150] However, reproductive hormones might also influence dynamic stabilizers (muscle and tendon) which might be even more important given their role in responding to dynamic loading conditions.[17, 24] Far less research is available that investigates the mechanisms of how hormones might influence muscle.[165] Estrogen receptors are present in muscle[15] and tendon.[166] Collagen is also present in muscle and it is possible that muscle and tendon respond to estrogen in similar ways to ligament. Much of estrogen's role within muscle has been linked to limiting or preventing muscle damage and most likely acts on cellular membranes.[167, 168]

Most of the information we understand about how estrogen influences muscle is learned from hormone replacement therapy (HRT) literature[169-174] Skelton et al[170] performed a randomized-control trial of post-menopausal subjects of the thumb adductor. Subjects were randomized into a control or HRT group and followed for 1 year. The HRT group had greater estrogen levels and had a 15% increase in thumb adductor strength after 52 weeks compared to a 5% decrease in thumb strength in the control group. Taaffe et al[173] expanded the model used by Skelton but investigated the role of exercise along with HRT over a 1 year

period. Postmenopausal women were randomly divided into one of four groups: (1) HRT, (2) exercise, (3) HRT + exercise, or (4) control. HRT and/ or exercise were able to attenuate muscle loss of the thigh musculature compared to the control group. These findings have been supported by other investigations[169, 171] while refuted by others.[175, 176] This research demonstrates that estrogen seems to have strength preserving capacity in muscle. A limitation of this research is that HRT takes place over weeks or years in duration and is not what is seen during monthly cyclic changes in hormone concentration over the course of the menstrual cycle. If strength does change across the menstrual cycle then theoretically strength would be greatest at ovulation when estrogen has the greatest absolute concentration.

2.9 Non-Contact ACL Injury Risk Across the Menstrual Cycle

If hormones influence ACL injury risk then is stands to reason that the risk of sustaining a non-contact ACL injury would not be equal across the menstrual cycle. From our previous section we know that ACL laxity and stiffness are correlated with estrogen concentration[16, 147] and that increased knee laxity is correlated with greater knee loading.[25, 163, 164] Using this information ACL injury risk should be greater when estrogen is greatest at ovulation.

Currently, ten out of ten research articles have examined the periodicity of non-contact ACL injury across the menstrual cycle and all ten have concluded that non-contact ACL injury is not equal across the menstrual cycle.[12, 13, 177-184] All of these projects used females free from oral contraceptive (OC) use. Summaries of the results from these studies can be found in Table 2. Six of these articles identified the pre-ovulatory phase of the menstrual cycle as the most likely to sustain an injury. While four articles[178, 179, 182, 184]

observed that ovulation was the most likely phase to sustain a non-contact ACL injury. Five of the nine studies included days 3-5 of the menstrual cycle as a time when ACL injury is likely. Interestingly, none of the studies found any association between ACL injury risk and a specific phase of the menstrual cycle. All articles that concluded ovulation was the most likely phase all used similar methodology using recall and survey methodology. All of the studies reported that the ACL injuries occurred during athletic activity which could indicate that dynamic stabilization is important and might influence injury rate. Hewett et al[139] performed a systematic review of this topic and reached similar conclusions. He also concluded that dynamic stability may have more of an influence on ACL injury rate than hormones influencing laxity.[139]

Several authors have investigated the role of OC on injury risk.[178, 184-186] OC use levels out hormonal fluctuations during the menstrual cycle.[137, 185] However, limited evidence is available from which to draw conclusions. Most recently, Ruedl et al,[184] completed a study on 93 recreational alpine skiers with matched controls that were free from OC as well as OC users. This study found that OC use had no protective effect on ACL injury risk and that females not on OC were more likely injured in the preovulatory phase of the menstrual cycle (57%). This study had one of the larger sample sizes but used a questionnaire to determine menstrual cycle phase and did not determine types of OC used by injured individuals, both of which are limitations.

2.10 Muscular Strength and the Menstrual Cycle: A Systematic Review

As part of this literature review, we have decided to include a systematic review of studies that have researched muscle strength across the menstrual cycle.

Introduction

Females are 1.4 to 4.6 times more likely to sustain a non-contact ACL injury compared to males.[2] There is general consensus among researchers that no single mechanism exists for non-contact ACL rupture and that the etiology is most likely multifactorial in nature. There are several risk factor categories that have been identified as different between genders including anatomical, biomechanical, neuromuscular, and hormonal.[48]

A preponderance of the evidence suggest that the risk of sustaining a non-contact ACL injury is not equal across the menstrual cycle with the preovulatory phase identified as the riskiest phase of the menstrual cycle.[12, 13, 139, 180, 181, 185] Reproductive hormones seem to influence ACL laxity in humans with normal menstrual cycles and physiological levels of estrogen and progesterone.[16, 147-152] Numerous authors have concluded that anterior laxity differs between gender with males having less laxity compared to females.[55, 56, 72, 151, 153-155] Negative correlations have been observed between ACL stiffness and estrogen concentration in active females indicating an increase in estrogen is associated with a decreased ligament stiffness.[147] Dynamic stability might be more important that static stability since dynamic stabilizers are more important to preventing injury during athletic activity.

Much of what we know about how hormones influence dynamic stability (muscle) is found in the hormone replacement therapy (HRT) literature. Post menopausal females have a decrease in the levels of estrogen. However, by taking HRT most studies observe an increase in maximal voluntary force after therapy while muscle cross sectional area stays constant.[170, 173] Additionally, research has shown that HRT improves the response to training.[170, 171] The methodology associated with these studies, in most instances, has the participants taking

HRT for 6 months to a year in older populations. While most ACL injuries occur in young active populations that are pre-menopausal.

A great deal of research has investigated the relationship between muscular strength and the reproductive hormones across menstrual cycle.[187-190] Currently, it is unclear if reproductive hormones influence strength and if strength changes with fluctuations in reproductive hormones across the menstrual cycle. The purpose of this paper is to systematically review the available published literature that investigated if muscular strength changed across the menstrual cycle and to provide a comparison of results.

Literature-Review Methods

We searched the literature to identify all articles that met our search criteria. Search criteria included: English language, published in peer-reviewed journal, investigated muscular strength as an outcome measure, and menstrual cycle phase was used as an independent variable. Electronic and manual reviews of the literature were employed.

Electronic Literature Search

For the electronic literature search, we searched the PubMed database maintained by the National Library of Medicine including MEDLINE (1966-2009) and CINAHL (1982-2009). Databases were searched using the terms of "menstrual cycle" and "muscle" and "strength". Abstracts and titles were reviewed to determine eligibility.

Manual Literature Search

We reviewed reference lists associated with each article to determine if any additional articles might have met the inclusion criteria. Titles and abstracts were reviewed to determine eligibility.

Search Results and Data Abstraction

We found 7 articles that met our inclusion criteria and thus included in the study. A brief summary of each article is found below.

Reviewed Study 1: Sawar et al 1996 [188]

Sawar et al investigated the influence of hormonal fluctuations on a variety of strength measures. Ten females with no OC use and ten females with OC use were enrolled in the study. Testing consisted over 2 consecutive menstrual cycles. Testing sessions were early follicular (days 1-7), mid follicular (7-12), mid-cycle (12-18), mid luteal (18-21), and late Luteal (21-32). Hormone values were not assessed. Isometric quadriceps muscle strength with twitch activation was assessed along with contractile properties, fatigability, and grip strength. The authors concluded that for females with NO-OC, quadriceps force was greatest in the mid-cycle phase and that this was greater than all other phases. No differences were seen in the females with OC use for quadriceps strength. Hand grip strength was also the greatest at mid-cycle in the no OC group. Quadriceps contractile properties were also significant. Quadriceps relaxation time was slowest at MC but the quadriceps was most fatigable at MC compared to all other phases. At mid-cycle the muscle was stronger, slower, and more fatigable in females not taking OC. These changes were not seen in females taking OC.

Reviewed Study 2: Phillips et al 1996 [191]

Phillips et al wanted to determine if strength of the adductor pollicis changed across the menstrual cycle in three groups of women. The groups were trained females with menstrual cycles, untrained females with normal menstrual cycles, and OC users. Muscle force of the adductor pollicis was used and each subject and 6-9 repetitions were performed

over an average of 8 individual visits in females and 5 in males. Regression analysis indicated that estrogen increased muscle strength in both trained and untrained females free from OC use. The authors concluded that maximal voluntary of the adductor pollicis was greatest in the follicular phase of the menstrual cycle and decreased at ovulation.

Reviewed Study 3: Friden et al 2003 [190]

Friden et al investigated the influence of hormonal fluctuations on hand grip strength in the dominant hand, one leg hop test on right leg, and isokinetic muscle strength and endurance. Fifteen females with no OC use were enrolled in the study, however, only 10 completed the entire testing protocol. Testing consisted of a familiarization session and then they were tested 3 times over 2 consecutive menstrual cycles. Testing sessions were menses (days 3-5), ovulation, and mid-luteal phases (7 days following positive ovulation test). Estrogen, Progesterone, FSH, and LH were measured at each time point. Isokinetic muscle strength and endurance were measured at 120 deg/sec (5 contractions) for strength and 50 concentric contracts for endurance. Endurance was measured by comparing the percentage of the first 5 contractions with the last 5 contractions. The authors concluded that the variables measured are not influenced by estradiol and progesterone fluctuations. Interestingly, the p-value associated with isokinetic peak torque for MC phase was P=0.056. The menses test session had the lowest average value compared to the other two phases in this non-significant finding.

Reviewed Study 4: Hertel et al 2006 [187]

Hertel et al investigated the influence of the menstrual cycle and hormones on quadriceps and hamstring isokinetic peak force. They also examined other measures including joint position sense, postural control, and knee joint laxity. Fourteen healthy

collegiate athletes with normal menstrual cycles were included in the study. In this study the general test protocol was to monitor the length of menstrual cycle and confirm and predict ovulation using urine based ovulation kit during the first month. In the second cycle, subjects collected daily urine samples. Also, subjects were tested at three time points including the mid-follicular (4-7 days before predicted ovulation), ovulatory (±2 days of predicted ovulation), mid-luteal (7-10 days after predicted ovulation) phases. Estrogen and progesterone changed across the menstrual cycle. However, no differences were observed across the menstrual cycle in any of the other variables. No correlations were significant between neuromuscular or laxity measures and hormones. The authors concluded that they could not detect substantial changes in the neuromuscular properties.

Reviewed Study 5: Bambaeichi et al 2004 [192]

Bambaeichi et al investigated the influence of the menstrual cycle muscle strength. Eight females with no OC use were enrolled in the study. Testing consisted of 2 familiarization sessions followed by testing during 5 phases of the MC. Testing sessions were menses (days 1-4), mid-follicular (7-9), ovulation (detected by LH surge), mid-luteal (19-21), and late-luteal (25-27). Hormone values were not assessed. Dependent strength measures assessed were isokinetic peak torque of the knee extensors and flexors at 60deg/s, 180 deg/s. Additionally, knee flexion and extension MVIC with the knee at 60 deg of flexion were assessed with and without percutaneous electrical twitches. The results of this study found that strength was greatest at ovulation for isokinetic knee flexion (60 deg/s), isokinetic knee extension (180 deg/s), and MVIC knee flexion without electrical stimulation. The authors concluded that the menstrual cycle influenced muscle strength and ovulation was the point when muscle strength was greatest and this may be linked to increased performance.

Reviewed Study 6: Abt et al 2007 [27]

Abt et al investigated the influence of hormonal fluctuations on neuromuscular and biomechanical variables. Ten physically active females were tested 3 times over a 2 month period. The first month was used to familiarize subjects and testing was performed in the second month. The testing time points were day 3 after the onset of menses, 24-36 hours following positive ovulation test, and the mid-luteal phase which was 7 days following positive ovulation test. Estrogen and progesterone concentration was calculated via blood samples. Dependent variables assessed were fine motor coordination, postural stability, hamstrings to quad strength ratio (60 deg/sec), hamstrings to quad strength ratio (180 deg/sec), knee flexion excursion, knee valgus excursion, peak anterior tibia shear force (PATSF), flexion moment at PATSF, valgus moment at PATSF. The authors found the hormonal changes with estrogen concentration greater in the post-ovulatory phase and mid-luteal phases compared to menses. Progesterone was greatest at the mid-luteal phase compared to menses and post-ovulatory phases. None of the biomechanical and neuromuscular variables changed across the three phases.

Reviewed Study 7: Elliott et al 2003 [189]

Elliot et al examined the influence of menstrual cycle phase on muscle strength and the bioavailability of testosterone and estrogen. They examined seven healthy female subjects with self reported normal menstrual cycles. Testing was performed in the early follicular (EF) 2 days after menses and the mid-luteal (ML) which was seven days after ovulation. The order of testing session was randomized. They assessed progesterone (total), estrogen (total and bioavailable) and testosterone (total and bioavailable) along with strength of the first dorsal interossi muscle with percutaneous electrical stimulation. The results of

the study found that total concentration of progesterone increased from EF to ML and total concentration and Estrogen increased from EF to ML. Testosterone and the ratio of estrogen and progesterone did not change across the menstrual cycle. There were no differences in bioavailable testosterone, estrogen between phases. Also, muscle strength did not change between EF and ML and concentrations of hormones did not correlate with muscle strength. The authors concluded that menstrual cycle phase had no effect on bioavailability of estrogen, testosterone or MVIF of the FDI muscle.

Discussion

This systematic review of the literature indicates that muscle strength does not change across the menstrual cycle. The preponderance of the evidence (4 studies) could not detect discernable changes in the muscle strength across the menstrual cycle. Of the remaining 3 research studies, 2 indicated that muscle strength increased at ovulation[188, 192] while 1 indicated the opposite.[191] Two more of these studies concluded that no difference was found across the menstrual cycle but reported a trend for phase ($P<0.10$) indicating that the menses testing session tended to have lower levels of strength. A summary comparison of each article can be found in Table 5.

The methodology used is not consistent across research papers. All of the studies use time points that are chosen to represent, theoretically, differing hormonal profiles. While this is a common procedure used to investigate this phenomenon, the frequency and selection of menstrual cycle points varies. Most studies tested 3 time points (n=3) within the menstrual cycle, however, one article tested twice, one tested 4 times, and another tested 5 times. Also, the range surrounding a specific testing session varies considerably. The average range to complete a testing session is approximately 4 days, but some authors test only on one day

while others allow for a range of 7 days. Without knowledge of a particular testing time, comparison of testing phases within a menstrual cycle and between studies becomes quite difficult. Future research should try and standardize testing points within the menstrual cycle and use individual testing markers (ovulation detection) rather than day counting methods.

A limitation of all of these studies that they use discrete time points within a menstrual cycle. One of the assumptions made using this type of testing is that testing on similar days will yield similar hormonal profiles. Shutlz et al[16] found some females are highly responsive to hormonal fluctuations while others are not. It is possible that many of these studies had some females that are just not responsive to hormonal fluctuations, masking the true relationships. No study to date has attempted to comprehensively examine muscle strength and hormone concentration by assessing strength and hormones daily over the menstrual cycle. Previous work has also identified that knee laxity influences knee joint loading.[152, 164] It might be that knee laxity indicates if a female is more responsive to hormonal changes and this variable might influence strength more so than pure hormonal fluctuations.

In conclusion, a preponderance of the evidence suggests that muscular strength does not change across the menstrual cycle. However, the methodology used in many of these studies is varied and less than ideal, even the studies using more advanced methodology reached similar conclusions. One of the interesting observations is that most studies used individuals that were healthy and free from injury and it is questionable if these individuals were all considered responsive to hormones.

2.11 The Menstrual Cycle and Other Muscle Properties

Our systematic literature review indicated that muscle strength did not change across the menstrual cycle. However, there have recently been investigations into other muscle properties that might influence force producing capability. If these properties change across the menstrual cycle they could potentially have more of an influence in knee and ACL loading than overall strength. These properties include joint loading, muscle stiffness, muscle reaction time, and muscle activation patterns.

Dedrick el al[24] examined neuromuscular characteristics during a drop jump and landing in a female population. This study found that the semitendinosus muscle had delayed onset during the luteal phase compared to the early and late follicular phases. The authors concluded that hormones may influence neuromuscular timing when estrogen is high, delaying the onset of the hamstrings which could negatively influence joint load.

Musculotendinous Stiffness (MTS) is another muscle property that has been investigated across the menstrual cycle.[17, 28, 193] The first study performed in this area was by Eiling et al[17] who assessed vertical leg stiffness in eleven teenage netball players. Vertical leg stiffness was assessed by having the subjects hop in time with a metronome on a force plate. This study found that vertical leg stiffness was decreased at ovulation. Stiffness is one of the basic mechanisms of joint stability so decreased stiffness would indicate that females might not have the muscular stability to protect themselves during high-load dynamic tasks. Bell et al[194] investigated knee flexor stiffness and hamstring extensibility in a group of college aged females free of OC. They were tested at two points of the menstrual cycle corresponding with low (3-5 days post onset of menses) and high estrogen (within 3 days of a positive ovulation test). They found that hamstring extensibility increased from menses to

ovulation. However, knee flexor stiffness did not change between the two time points but there was a trend with a large effect size and low p-value indicating that further research needs to be performed before conclusions can be drawn. This study had a small sample size (n=8) but post-hoc sample size calculation indicated that 19 subjects could make their findings significant. These findings agree with Kubo et al[193] assessed muscle activation and tendon stiffness and found that none of these properties differ across the menstrual cycle.

2.12 Methodology Review: Musculotendinous Stiffness

One of the dependent variables in this project is musculotendinous stiffness of the knee flexors.[21, 23, 29] Stiffness is defined as the ratio of the change in force to the change in length (Δ Force / Δ Length).[21] This makes it different that extensibility or flexibility which is defined as the amount of range of motion available at a joint regardless of the force applied.[195] We have already discussed the importance of the hamstrings in preventing motions that may stress the ACL. As such, increased stiffness is another factor that can result in an improved ability to prevent joint distraction that prevent injuries.[196] Whereas, increased tissue compliance potentially means a greater change of injury. Therefore, there is a need to determine if hamstring stiffness varies in females with previous history of ACL injury.

Stiffness can be derived from a variety of techniques. Some of the most popular methodology for calculating stiffness comes from either passive,[22] oscillatory,[21, 23, 28, 29] or functional.[197-199] In this study we will use the oscillatory method of assessing knee flexor stiffness. During this task, participants will have an accelerometer attached to their foot that will capture the frequency of oscillation of the foot and shank segment following a perturbation which will allow us to calculate stiffness.

Previous research has demonstrated decreased muscle stiffness compared to males of the hamstrings[21-23, 200] and quadriceps.[23] Granata et al[23] using the oscillatory method and measured stiffness using 0kg, 6kg, and 20% maximal voluntary exertion. This study found that females had 53-76% less stiffness compared to males at the different loading levels. Additionally, stiffness was proportional to the knee moment but when non-standardized weight was used (0kg and 6kg) females responded by using greater muscle activation levels. Finally, the authors concluded that gender differences in stiffness are amplified at larger joint loads. This result is somewhat comparative to Hewett et al[121], who found that females were not able to increase hamstring torque at high velocities compared to males. These results agree with previous authors that have found that stiffness increases with increased muscle activation.[196, 201-203] Therefore, it is important to standardize the load placed on the ankle during the active stiffness assessment. Monitoring EMG of the muscles surrounding the knee is also important as this will give an indication of the activation level of the muscles responding to the perturbation.

2.13 Previous Research

In a recent study we observed gender differences in hamstring properties.[20] Twenty males and 20 females were positioned prone on a custom table that maintained their hip and knee at 30 degrees of flexion. The right leg was tested in all cases regardless of leg dominance due to equipment limitations. The foot was attached to a load cell (Honeywell) that prevented movement during a hamstring contraction. When presented with the light stimulus, participants were instructed to contract their hamstrings as quickly and forcefully as possible for the duration of the light stimulus (approximately 4 seconds). Dependent variables, calculated from the load cell included hamstring rate of force production (RFP)

and time to 50% peak force (T50%). Females had lower hamstring RFP and slower T50% compared to males.[20] The authors concluded that neuromechanical hamstring function may limit dynamic knee joint stability in females.[20] These hamstring neuromuscular properties may change across the menstrual cycle in an ACLR population (**Research Question 2**).

In a subsequent study, as a potential explanation for previously observed gender differences, we explored the relationship between hamstring properties and reproductive hormones.[29] Males (n=15) and females (n=15) were tested and when genders were combined, estrogen concentration (mean = 46.0 ± 28.2 pg/ml) was negatively correlated with knee flexor stiffness (mean = 12.8 ± 2.6 N/cm) and RFP (mean = 758.77 ± 507.6 $N/kg \cdot sec^{-1}$). Free testosterone (mean = 13.17 ± 13.0 pg/ml) was positively correlated with knee flexor stiffness and RFP and negatively correlated with T50% (mean = 114.73 ± 38.88 ms) (Table 1). These results indicate that hormones may influence muscle properties and that greater amounts of testosterone tend to equate to the ability to produce force more quickly. Estrogen also seems to influence hamstring neuromuscular function in a negative manner creating a condition more likely to limit dynamic joint stability. This research provided us with new information about how hormones may influence muscle properties and help explain gender differences. Correlations might be greater when considering high risk individuals (**Research Question 1**). ACL injured populations might be more sensitive to hormonal influences.

In summary, previous research has identified gender differences in hamstring muscle properties. Females have lower RFP and slower T50% which may influence dynamic knee support. Additionally, we observed positive correlations between hamstring properties and testosterone and negative correlations between hamstring properties and estrogen. The proposed research project will attempt to address the next step in this research by trying to

determine if ACLI individuals will have altered neuromuscular hamstring properties compared to controls. Additionally, ACLI females may have stronger correlations between hamstrings properties and reproductive hormones.

2.14 Summary of Literature Review

The purpose of this study is to determine if relationships exist between reproductive hormones and muscle properties at menses and ovulation and to determine if these variables change across the menstrual cycle. We will assess muscle properties 3-5 days post menses when hormones are theorized to be low and within three days of ovulation. These time periods were selected for several reasons. Based on previous research muscle properties are correlated with absolute hormone concentration during this time period so further investigation is warranted. Also, non-contact ACL injury risk is not equal across the menstrual cycle these are the time periods when injury is theorized to be the greatest, when hormones are at their peaks or at their nadirs.

The variables selected for this project were based off pilot work using healthy females and males with no history of non-contact ACL injury. The hamstrings are of particular interest because they are capable of preventing ACL loading by reducing ATT, knee valgus, and tibial internal rotation. Muscular contractions are in general are capable of reducing provocative knee positions that may load the ACL and subsequently result in ACL failure.

CHAPTER THREE

METHODOLOGY

3.1 Experimental Design

This study utilized correlation and repeated measures study designs. The purposes were to (1) investigate the relationships between reproductive hormones and neuromuscular hamstring properties, (2) determine if neuromuscular properties and biomechanics change across the menstrual cycle, and (3) determine the relationship between hamstring neuromechanical properties and three dimensional lower extremity kinematics and kinetics. A schematic diagram of testing procedures is located in Figure 2. Females were recruited for this study if they suffered a non-contact ACL injury and were free from oral contraceptive use. Participants were tested twice across their menstrual cycle at points that corresponded with low levels of estrogen and progesterone (menses) and high levels of estrogen and low levels of progesterone (ovulation). Subjects were tested (1) 3-5 days after the onset of menses and (2) within 3 days following a positive ovulation test. The primary independent variable was menstrual cycle phase (menses vs. ovulation).

3.2 Participants

Twenty-four participants were recruited to participate in this research study. Based on previously collected data from our laboratory and previous research we performed a priori power analysis for each dependent variable (Table 6). This analysis revealed power greater than 0.80 to find differences between menstrual cycle phases for most variables of interest

assuming a moderate to large effect size (0.70-0.80) was present using an n of 20. In some instances the effect size calculated using previous research was low because we estimated 10% change would be significant, and in combination with high variability, increased the estimated sample size. Overall, we had sufficient power for our primary variables of interest (stiffness and biomechanics) but we did not have sufficient power for our secondary variables of interest (neuromechanical factors). Recruiting 24 subjects allowed for drop out to occur based on either voluntary withdrawal or withdrawal based on an anovulatory menstrual cycle.

Participants were recruited using a variety of techniques including chart review from several local orthopedic physicians, mass emails, flyers, and class recruitment. All subjects read and signed an informed consent agreement approved by the University's Institutional Review Board. To be included in the study, participants had to satisfy the following criteria: 18-25 years of age, no history of pregnancy or neurological disorder, self reported normal menstrual cycle, no oral contraception use six months prior to testing, no oral contraception use at the time of non-contact ACL injury, sustained a unilateral non-contact ACL defined as, "forces applied to the knee at the time of injury resulted from the athlete's own movements and did not involve contact with another athlete or object,"[2] and had been cleared by a physician to return to sport participation. Participants described their injury history, mechanism, sport, circumstance, and surgical repair method to the primary investigator (DRB). All dependent variables were assessed on the test limb which was defined as the limb with no ACL injury.

3.3 Procedures

Once it was determined that a female met the inclusion criteria, she tracked her menstrual cycle and contacted an investigator after the onset on menses or after a positive ovulation test using a urine based ovulation predication kit. The menses data collection session was scheduled 3-5 days after the onset of menses.[29] To identify this time period, participants counted from the onset of menses (Day 1). For the ovulation test session, participants contacted an investigator when a positive ovulation test was detected using urine based ovulation detection kit (Earth's Magic, Cary, NC). The two test phases and dependent variables were counterbalanced to avoid an order effect. Subjects were tested during the same time of day for each test session and were instructed to not eat 2 hours prior to testing or exercise the day of testing. The primary investigator was blinded to the menstrual cycle phase for each subject.

Testing sessions were identical except descriptive information and the IDKC health form were collected during the first test session. Descriptive information included height, weight, current age, age when ACL injury occurred, dominant leg, involved leg, healthy limb, type of graft used to repair ACL, other structures involved during injury, and current activity level. Part of the IKDC health form was completed by the participant during the first visit. The portions of the IKDC form completed included the Current Health Assessment Form, the Subjective Knee Evaluation Form, and the Knee History Form. This form provided information about the level of function of each participant. This form has not been directly included in our statistical analyses.

Blood Sampling

Each subject had a venous blood specimen analyzed for select reproductive hormone levels. Specimens were obtained by veni-puncture from a vein located in the cubital fossa using a 3 cc syringe with a 23 gauge needle (1 inch length). The veni-puncture procedure was performed by a nationally certified phlebotomist (ACH) using standard clinical procedures.

Blood hormone levels (estrogen, progesterone, free testosterone) were assessed using radioimmunoassay procedures by an experienced researcher. Assay quality control steps and procedures were instituted.[204] Blood was immediately transferred to a Vacutainer tube® containing EDTA as an anti-coagulant and immediately placed on ice. Blood sample tubes were centrifuged at 3000g at 4^{o}C until plasma was separated, which was then stored at -80^{o}C until hormonal analysis was performed. Plasma specimens were analyzed for estradiol-β-17, progesterone, and free testosterone concentration using a solid-phase, single antibody radioimmunoassay procedures (Siemens Medical, Los Angeles, CA). All assay samples were processed in duplicate and quality control procedures as recommended in the literature were utilized.[204] Total estrogens in adult women are comprised primarily of estrone, estriol, estradiol-β-17 and their conjugates, with estradiol-β-17 being the major component, which is why it was analyzed in this study.[205] Along with concentrations of each hormone, an estrogen to progesterone (E:P) ratio was also calculated by dividing progesterone into estrogen values. The ACL is an estrogen receptor dependent tissue and is influenced by both estrogen and progesterone because of their antagonistic relationship.[206-208] E:P ratio provides a basic and general assessment of how these hormones are interacting with one another.[206]

Knee Laxity Assessment

Knee laxity was defined as the amount of anterior tibial displacement at 133 N, measured by a KT-1000 knee arthrometer (MEDmetric Corp, San Diego, CA).[16] Subjects were positioned supine with the knee supported the knee in 25 degrees of flexion. The subject's ankles were then placed in a cradle and a thigh strap was added to control rotation of the thighs. After subject positioning was complete the KT 1000 was placed on the anterior shank, aligned with the joint line, and secured to the lower limb using Velcro straps. A force was applied by the primary investigator that resulted in anterior displacement of the tibia. Two practice trials were used to ensure that the subject was relaxed and the KT 1000 was secured properly. Then 5 trials were recorded and averaged. The primary investigator demonstrated excellent reliability prior to data collection (intrasession: ICC [2,k] = 0.98, SEM = 0.43 mm; intersession: ICC [2,k] = 0.88, SEM = 0.40 mm).

Hamstring Musculotendinous Stiffness Assessment

knee flexor stiffness was assessed by measuring the damping effect caused by the knee flexors after a perturbation.[23] Electromyography (EMG) sensors were used to monitor potential changes in muscle activation levels between sessions as well as before and after the perturbation. Knee flexor stiffness is proportional to muscle activation level,[209] so EMG verifies that changes in muscle stiffness are not due to changes in muscle amplitude levels. EMG sensors (Ag/AgCl) were placed on the test leg over the medial and lateral hamstrings and quadriceps in parallel with the muscle fibers. Medial and lateral hamstring EMG sensors were placed 50% of the distance between the greater trochanter and knee joint line. The medial and lateral quadriceps EMG sensors were applied over the bellies of the vastus lateralis (VL) and vastus medialis (VM). The electrodes for the quadriceps were placed over the VL, approximately 10 cm superior and 7 cm lateral to the superior border of the patella

oriented at 10 degrees to the vertical.[210] For the VMO the electrode was placed approximately 4 cm superior and 3 cm medial to the superomedial border of the patella oriented at a 55 degree angle.[210] A reference electrode was placed on the lateral malleolus. Participants were positioned prone on a plinth with an extension affixed to the end. The extension supported the thighs but permitted knee motion by allowing the shanks to hang freely (Figure 3). An electromagnetic motion capture sensor (Flock-of-birds, Ascension Technologies, Inc., Burlington, VT) was placed on the participant's lateral thigh and proximal tibia to measure oscillatory motion of knee flexion and extension following an applied perturbation. A load equal to 10% body mass was secured at the ankle (cuff weights) and subjects were instructed to support the shank parallel to the floor via isometric hamstring contraction. The investigator applied a perturbation to the calcaneous, forcing the knee into slight extension and initiating oscillatory knee flexion/extension. This oscillatory motion was captured by measuring the knee flexion and extension motion about the Y-axis. Knee flexor stiffness was estimated from the damped frequency of oscillation.[22] Three successful trials were recorded at each weight with success defined as a clear oscillatory pattern in the knee flexion motion pattern position (Figure 4).[22] Knee flexor stiffness was calculated using the formula $k = 4\pi^2 mr^2 f^2$, where k was rotational stiffness, m was the summed mass of the foot and shank segment[42] and the applied load, r was the distance from the lateral joint line to the lateral malleolus (m), and f is the damped frequency of oscillation calculated as $f = (1/t_2 - t_1)$ (Figure 4). Knee flexor stiffness values were normalized to body mass and this procedure has been shown to have moderate-to-high intra-session reliability ($ICC_{2,1} = 0.70$, SEM = 28.83 N·m/rad).[22]

Hamstring Neuromechanical Assessment

The next series of tests assessed hamstring neuromechanical / force properties.[20] The following variables were calculated from a maximal hamstring isometric contraction: 1) time to produce 50% peak force (T50%), 2) rate of force production (RFP) over the first 200 ms after onset of muscle force contraction (RFP 200), 3) RFP to the T50% time point (RFP T50%), and 4) isometric peak force. Participants were positioned in a seated position with the knee and hip flexed to 90°. The foot was fixed to a load cell (Honeywell Sensotec, Columbus, OH) (Figure 4) and participants were instructed to relax and wait for a visual light stimulus. When the light stimulus was presented, the participant contracted the hamstrings as forcefully and quickly as possible against a load cell for 3-5 seconds, and five trials were recorded.

T50% was measured in milliseconds and calculated as the time between the onset of force and the instant at which 50% peak force was achieved. Force onset was determined using computer algorithms that have been previously established.[20] The threshold to determine the onset of force was 5% of the peak hamstring force. Peak force was defined as the largest force output measured by the load cell during the test period. RFP 200 was calculated during the first 200 ms after the onset of force and was defined as the slope of the force-time curve line created by the load cell output. The equation used was: m (N/s) = (X2-X1)/(Y2-Y1), where X1 was equal to the load cell 200 ms after the onset of perturbation, X1 was the load cell value at 5% peak hamstring force, and (Y2-Y1) was equal to .2s. This procedure was repeated for the RFP T50% time point except the time value used in the equation was T50%. The slope equation assumes a linear relationship between the onset of force production and the second time point and R^2 values were calculated to quantify the linearity of this relationship.

Peak concentric and eccentric isokinetic hamstring force were assessed using previously described methods that demonstrated high reliability.[10, 211] Participants were seated on an isokinetic dynamometer (Biodex System 3 Pro isokinetic dynamometer, Biodex Medical Systems, Shirley, NY) with their hip flexed to 85° and knee flexed to 90°. Testing was performed at 60 and 300 °/s through 90° of motion (0-90° of knee flexion). A warm-up, consisting of 5 submaximal knee flexion/extensions was performed. Testing for the 60°/s condition consisted of 5 concentric and 5 eccentric isokinetic hamstring contractions with peak torque measured in Newton-meters (Nm). Testing for the 300 °/s consisted of 10 concentric and 10 eccentric isokinetic hamstring contractions with peak torque measured in Newton-meters (Nm). Sampling of the Biodex was the highest sampling rate allowed by the commercial software (60 Hz). Biodex data were corrected for gravity correction.

Biomechanical Analysis

Lower extremity kinematics and kinetics were collected using the Flock of Birds electromagnetic motion analysis system (Ascension Technologies, Inc., Burlington, VT) during a jump landing task. Measurements were recorded by the Motion Monitor software system (Innovative Sports Training, Inc., Chicago, IL) with a kinematic sampling rate of 144 Hz. A transmitter was affixed to a stationary stand, 0.773 meters in height, to establish a global reference system with the positive x-axis pointing anterior, positive y-axis to the left, and positive z-axis directed vertically.

Electromagnetic tracking sensors were placed on each subject over the apex of the sacrum, midpoint of the lateral thigh, and shank. Sensors were placed on the test limb in areas consisting of the least amount of muscle mass to minimize potential artifact introduced by muscle contraction. Sensors were affixed using double-sided tape, pre-wrap, and athletic

tape. After the electromagnetic sensors were attached, subjects stood in a neutral posture with their arms relaxed at their sides. Bony landmarks were digitized, in the following order, using a mobile electromagnetic sensor attached to a stylus: medial femoral condyle, lateral femoral condyle, medial malleolus, lateral malleolus, left anterior superior iliac spine, and right anterior superior iliac spine. Digitization of bony landmarks defined the segment end-points and joint centers of the lower extremity segments. The ankle joint center was defined as the midpoint between the medial and lateral malleoli. Knee joint center was defined as the midpoint between the medial and lateral femoral condyles. The hip joint center was determined by the Bell method.[212] This method consists of estimating the location of the hip joint center using the left and right anterior superior iliac spine as landmarks to mathematically estimate the hip joint center. A static trial was recorded prior to data collection with the feet shoulder width apart and toes straight ahead.

Subjects completed five successful trials of a jump landing task from a 30cm high box positioned 50% of the subject's height from the edge of a force plate. Subjects jumped forward and landed on both feet, with the test limb on the force plate. Immediately after landing, subjects jumped as high as possible. A nonconductive force plate (Bertec Corporation, Columbus, OH) was used to record ground reaction forces with a sampling rate of 1,440 Hz and was synchronized with the kinematic data.

3.4 Data Sampling and Reduction

Knee Flexor Stiffness and Neuromechanical Data Reduction

EMG and load cell data were collected at 1,440 Hz and electromagnetic sensor data were collected at 144 Hz using the Motion Monitor motion capture software (Innovative Sports Training, Chicago, IL). Knee flexion data were filtered using a 4th order, zero-phase-

lag Butterworth low-pass filter at 14.5 Hz.[213] EMG data were corrected for DC bias, bandpass (20-350 Hz), and notch (59.5-60.5 Hz) filtered (4^{th} order, zero-phase-lag, Butterworth), and smoothed using a 20 ms root-mean-square sliding window function. Load cell and Biodex data were low pass filtered at 10 Hz (4^{th} order, zero-phase-lag Butterworth filter). Add data were filtered and reduced using customized MATLAB software (Mathworks, Natick, MA, v7.0).

Biomechanical and Jump Landing Data Reduction

Embedded right-hand Cartesian coordinate systems were defined for the shank, thigh, and hip to describe the three-dimensional position and orientation of these segments and were aligned with the global reference system. Euler angles were used to calculate the knee joint angle between the shank and thigh and the hip joint angle between the thigh and pelvis in an order of rotations of (1) flexion-extension about the Y-axis, (2) valgus-varus about the X-axis, and (3) internal and external rotation about the Z-axis. Kinematic and kinetic data were exported into customized MATLAB software programs (Mathworks, Natick, MA, Version 7.0) for data reduction. Kinematic data were filtered using a 4^{th} order zero-phase-lag Butterworth low-pass filter at 14.5 Hz.[213] Each subject's kinematic neutral stance was subtracted out from each trial. Ground reaction forces were normalized to body weight (N) and moment data were analyzed in raw form and normalized to the product of body weight and height (BW*BH). The Vertical ground reaction force defined the landing phase of the jump landing task. Initial contact was defined as the point at which the vertical ground reaction force exceeded 10N, and toe-off was defined as the point at which the vertical ground reaction force dropped below 10N. Peak kinematic and kinetic variables were assessed at initial contact and during the absorption phase of the jump landing which was

defined as the point between initial contact and peak knee flexion. The following variables were collected during these time points: knee flexion angle, knee valgus angle, tibial rotation angle, hip flexion angle, hip adduction angle, hip rotation angle, knee extension moment (internal), knee rotation moment (max and min), knee valgus moment (max and min), anterior tibial shear force, and peak vertical ground reaction force (peak during the absorption phase). Joint moments were calculated as internal moments. Joint moments and anterior tibial shear force were calculated using standard inverse dynamic procedures.[214]

3.5 Statistical Analysis

Statistical significance was set *a priori* at $P \leq 0.05$. SPSS (version 17.0) was used for all analyses. The primary independent variable was menstrual cycle phase with two levels: menses versus ovulation.

Research Question 1: Are hormone levels correlated with muscle properties at menses and ovulation?

Statistical Procedure: Bivariate correlation coefficients were calculated between reproductive hormone levels and each hamstring property within each time point (menses and ovulation) as well as the change scores. Change scores were calculated but subtracting the menses testing session value from the ovulation testing session values (ovulation – menses).

Research Hypothesis: Generally, estrogen and progesterone will be negatively correlated with muscle properties while testosterone will have a positive correlation with muscle properties. These correlations will increase at ovulation especially for estrogen.

Research Question 2: Do knee flexor stiffness, hamstring neuromechanical properties, quadriceps and hamstrings EMG, and knee laxity change across the menstrual cycle?

Statistical Procedure: Paired samples t-tests were used to determine if knee laxity, knee flexor stiffness, RFP 200, RFP T50% changed from menses to ovulation. Muscle strength was examined using a repeated measures ANOVA with type of contraction (isometric, concentric 60°/s, concentric 300°/s, eccentric 60°/s, and eccentric 300°/s) and phase (menses vs. ovulation) used as within subjects factors. Separate repeated measures ANOVAs were used to examine hamstring and quadriceps EMG muscle activity with phase (menses vs. ovulation), time (pre or post perturbation, and side (medial vs. lateral) used as within subject variables. A Tukey's post-hoc analysis was performed when necessary.

Research Hypothesis: Hamstring neuromechanical properties and knee laxity will change in ways that increase the susceptibility of non-contact ACL injury at ovulation when estrogen is greatest.

Research Question 3: Do biomechanical variables assessed during a jump landing change across the menstrual cycle?

Statistical Procedure: Individual paired t-tests were performed for each dependent variable and the within-subject factor was menstrual cycle phase with 2 levels (menses vs. ovulation).

Research Hypothesis: Kinematics and kinetics assessed during a jump landing will change across the menstrual cycle in ways that increase the susceptibility to non-contact ACL injury at ovulation when estrogen is greatest at ovulation.

Research Question 4. Are there significant correlations between hamstring neuromechanics, knee laxity, and jump landing kinematics and kinetics at menses and ovulation?

Statistical Procedure: Bivariate correlation coefficients were calculated between hamstring neuromechanical variables (predictor) and kinematics and kinetics during a jump landing (criterion) at menses and ovulation.

Research Hypothesis: Generally, hamstring neuromechanical properties and kinematics will be correlated in directions and magnitudes associated with non-contact ACL injury.

CHAPTER FOUR

SUMMARY OF RESULTS

4.1 Introduction

This chapter will provide a summary of the results for each research question. In depth discussion for research questions one and two are located in manuscript one, while discussion for research question three is found in manuscript two. Manuscripts one and two are included as appendices in this document and are primary research questions. Research question four and the exploratory analyses will be discussed in depth in chapter five and are secondary analyses.

4.2 Overview and Subject Demographics

Twenty-four participants were recruited to participate in this research study with twenty subjects (height = 168.6 ± 5.3 cm, mass = 66.2 ± 9.1 kg, age = 19.6 ± 1.31 years) successfully completing the testing protocol. Recruiting twenty-four participants allowed for drop out to occur based on voluntary withdrawal (n=1) or anovulatory menstrual cycles (n=3). Participants were recruited through a variety of techniques including chart review from several local orthopedic physicians, mass emails, flyers, and class recruitment. To be included in the study, participants had to satisfy the following criteria: 18-25 years of age, no history of pregnancy or neurological disorder, self reported normal menstrual cycle, no oral contraception use six months prior to testing, no oral contraception use at the time of non-

contact ACL injury, sustained a unilateral non-contact ACL defined as, "forces applied to the knee at the time of injury resulted from the athlete's own movements and did not involve contact with another athlete or object",[2] and had been cleared by a physician to return to sport participation. Participants described their injury history, mechanism, sport, circumstance, and surgical repair method to the primary investigator (DRB). All dependent variables were assessed on the test limb which was defined as the limb with no ACL injury. All of the other participants completed the study. We identified 59 females with a history of ACL injury (figure 7). Approximately 34% of the identified subjects with previous ACL injury met our inclusion criteria and were enrolled in the study.

There were no changes in body mass between the two testing sessions (n = 20, Menses: 66.2 ± 9.1 kg, Ovulation: 66.1 ± 9.1 kg, $t_{(19)}$=0.32, P = 0.70). A summary of each subject's ACL injury history can be found in table 7 including which limb was injured, the number of months from ACL injury to the first testing session, the mechanism of injury, the sport they were participating in when ACL injured occurred, as well as the type of graft used to repair the injury. A summary of the significant findings and phase of the menstrual cycle in which the variable was greatest can be found in table 8.

4.3 Results

4.3.1 Research Question 1

Our first analysis examined the relationship between hormone levels and hamstring neuromechanics including knee flexor stiffness, isometric peak strength, isokinetic concentric and eccentric peak torque (60°/s and 300°/s), rate of force production (RFP 200ms and RFP T50%), and time to 50% peak force (T50%). Bivariate correlation coefficients were calculated between reproductive hormone levels and each hamstring neuromechanical

property within each time point (menses and ovulation) as well as the change scores. Change scores were calculated by subtracting the menses testing session value from the ovulation testing session values (ovulation – menses). Means and standard deviations for each variable can be found in Tables 9, 10, 11, and 12. Correlation coefficients and *P*-values for the menses testing session can be found in Table 13, ovulation in Table 14, and change score correlations can be found in Table 15.

At the menses test session, no significant correlations were observed between estrogen or free testosterone and hamstring neuromechanical properties ($P > 0.05$, Table 13). A significant correlation was observed between progesterone and T50% at the menses test session ($r = 0.75$, $P < 0.001$). This finding supports our hypothesis that greater amounts of progesterone are associated with slower time to reach a standardized time point which could have implications related to knee joint stability. Similar relationships were seen between progesterone and RFP 200 ($r = -0.31$, $P = 0.19$) and RFP T50% ($r = -0.31$, $P = 0.22$) although not statistically significant. Interestingly, progesterone had the largest negative correlations and was the most consistent in regards to the expected direction of the three hormones assessed in this study. The correlation coefficients related to estrogen and free testosterone at menses were not consistent with regards to the expected relationships and the magnitudes of the coefficients were much smaller than those associated with progesterone. These findings do not support our hypothesis. Finally, we revealed significant relationships between the E:P ratio and T50% ($r = -0.49$, $P = 0.04$, Table 13), RFP 200 ($r = 0.58$, $P = 0.01$, Table 13), and RFP T50% ($r = 0.64$, $P = 0.005$, Table 13).

At ovulation a negative correlation was found between estrogen and knee flexor stiffness ($r = -0.45$, $P = 0.05$, Table 14). Higher concentrations of estrogen were associated

with lower levels of knee flexor stiffness which supports our hypothesis. A significant correlation was also observed between progesterone and T50% (r = -0.50, *P* = 0.04, Table 14). This finding was contrary to our hypothesis and indicated that higher levels of progesterone were associated with shorter T50%. A shorter T50% indicates less time needed to reach 50% of peak knee flexion force production, which would theoretically be advantageous for knee stability. We observed no significant relationships when change scores were analyzed (*P* > 0.05). Finally we observed a significant correlation between T50% and the E:P ratio (r = 0.55, *P* = 0.02, Table 14).

4.3.2 Research Question 2

The first analysis examined changes in knee laxity as well as several hamstring neuromechanical variables across the menstrual cycle including knee flexor stiffness, isometric peak strength, isokinetic concentric and eccentric peak torque, rate of force production (RFP), and time to 50% peak force (T50%). Means, standard deviations, 95% confidence intervals, and p-values for each variable can be found in Tables 9, 10, 11 and 12.

Knee Laxity and Hamstring Musculotendinous Stiffness Assessments

Knee laxity increased at ovulation compared to menses ($t_{(19)}$ = -2.33, *P* = 0.03, effect size = 0.53). During the knee flexor stiffness assessment, 4 trials were determined to be unusable in the menses test session data due to equipment error. The value of each trial was more than 4 standard deviations from the mean of the remaining two trials and was attributed to equipment error. Means were calculated from the two usable trials in individuals identified as having bad data. A significant increase in knee flexor stiffness was observed at ovulation ($t_{(19)}$ = -2.31, *P* = 0.03, effect size = 0.54). This relationship was still significant after stiffness values were normalized to body mass ($t_{(19)}$ = -2.25, *P* = 0.03, effect size =

0.65). To verify that this change was not due to muscle activation, the average EMG amplitude was examined from the medial and lateral quadriceps and hamstrings. The average EMG amplitude was calculated 200 ms prior to the onset of the perturbation (pre-perturbation) and from the onset of the perturbation to the second peak in the oscillatory flexion/extension motion (post-perturbation). We examined the average EMG amplitude using a variety of different methods including: the average of each muscle individually, average group (medial and lateral musculature averaged together), as well as co-activation ratio (average hamstring activity divided into average quadriceps activity). Means and standard deviations for each of these variables can be found in table 16.

For the quadriceps, a main effect for test was observed indicating that quadriceps activation increased from pre to post perturbation but this increase was only 1% MVIC (Pre: 3.71 ± 1.32 , Post: 4.77 ± 2.44, $F_{(1,16)} = 54.18$, $P < 0.001$) and no main effect was observed for phase ($F_{(1,16)} = 0.409$, $P = 0.532$) and no test by side by phase interaction was observed ($F_{(1,16)} = 1.890$, $P = 0.188$). For the hamstrings, a main effect for test was observed indicating that hamstring activation increased from pre to post perturbation (Pre: 37.22 ± 16.01, Post: 44.06 ± 18.46, $F_{(1,16)} = 63.87$, $P < 0.001$) but no main effect was observed for phase ($F_{(1,16)} = 0.736$, $P = 0.404$) nor a test by side by phase interaction was observed ($F_{(1,16)} = 1.46$, $P = 0.248$). We observed no change in any of the muscle activation levels across the menstrual cycle for pre perturbation average quadriceps ($t_{(19)} = 0.74$, $P = 0.47$), average hamstrings ($t_{(19)}=0.94$, $P = 0.36$), co-contraction ratio ($t_{(19)}=-0.21$, $P = 0.84$) or post-perturbation average quadriceps ($t_{(19)} = 0.53$, $P = 0.60$), average hamstrings ($t_{(19)} = 0.76$, $P = 0.46$), co-contraction ratio ($t_{(19)} = -0.19$, $P = 0.85$). Finally, we quantified the perturbation applied by the primary investigator to the posterior aspect of the heel by assessing the degree

change in knee flexion. Perturbation amplitude was not different between sessions ($t_{(19)}$ = 0.28, P = 0.78).

Hamstring Strength and Force Production Assessments

Two subjects had unusable load cell data and were removed from the isometric hamstring contraction as well as the RFP and T50% measures. The RM ANOVA for strength revealed no type by phase interaction ($F_{(4,14)}$ = 0.439, P = 0.778) or main effect for phase ($F_{(1,17)}$ = 0.004, P = 0.95) but it did reveal a significant main effect for contraction type ($F_{(4,14)}$ = 3.419, P = 0.038). The Tukey post hoc revealed the isometric contraction was greater than the concentric contraction at 60°/s and 300°/s and the eccentric contraction at 300°/s. However the eccentric contractions tended to be greater than the concentric contractions (table 12).

Force production capabilities did not change across the menstrual cycle. RFP 200ms tended to decrease during the ovulation testing phase ($t_{(17)}$=1.84, P = 0.08, effect size = 0.24) but these findings did not reach statistical significance. There were no significant changes observed in RFP T50% ($t_{(17)}$=0.87, P = 0.40) nor in T50% peak force ($t_{(17)}$=-0.72, P = 0.48). Thus, there were no significant changes in hamstrings force production capabilities between the menses and ovulation phases (table 5).

Concentration of Reproductive Hormones Across the Menstrual Cycle

We examined concentrations of the reproductive hormones estradiol-β-17 (E), progesterone (P), and free testosterone (FT) across the menstrual cycle. We were unable to successfully obtain a blood sample at ovulation from one subject. At ovulation there was an increase in E ($t_{(18)}$ = -2.93, P = 0.009, effect size = 0.72) and P ($t_{(18)}$ = -3.44, P = 0.003, effect size = 0.80). However, there was no change in the concentration of FT ($t_{(18)}$ = -0.836, P =

0.414) between menses and ovulation. The increase in E agrees with our hypothesis, however, the increase in P was somewhat unexpected. Ideally, our study design would have allowed us to only capture increases in E with P and T remaining unchanged at ovulation. Any changes observed across the menstrual cycle may be due in part to either E or P or the interaction between the two hormones. Our values of the three hormones were similar to previously reported values.[17, 27]

4.3.3 Research Question 3

Our third analysis was focused on lower extremity kinematic and kinetic variables at menses and ovulation. These variables were assessed during a jump landing task at initial contact and also over the absorption phase of the jump landing task (initial contact to peak knee flexion).

At initial contact, there were significant differences in select kinematic and kinetic variables between menses and ovulation. Subjects displayed the following at ovulation in comparison to menses: increased tibial external rotation angle ($t_{(19)} = 2.80$, $P = 0.01$, effect size = 0.63) and decreased internal knee varus moment ($t_{(18)} = 3.09$, $P = 0.006$) even after normalization ($t_{(19)} = 3.37$, $P = 0.003$). All other comparisons at initial contact were not significant ($P > 0.05$). These values suggest that subjects were in a more "toe out" position at ovulation during initial contact; however, there was less frontal plane knee loading at this same time point. Means and standard deviations can be found in Tables 17 and 18.

During the absorption phase there were several significant differences in kinematics and kinetics between menses and ovulation. Specifically, in comparison to menses the subjects displayed tibial external rotation angle, ($t_{(19)} = 2.15$, $P = 0.05$), increased femoral internal rotation angle ($t_{(19)} = -2.18$, $P = 0.05$), increased internal knee valgus moment ($t_{(18)} =$

2.43, $P = 0.03$), decreased internal knee varus moment ($t_{(18)} = 3.37$, $P = 0.003$), increased internal tibial internal rotation moment ($t_{(18)} = -3.07$, $P = 0.007$) and decreased peak vertical ground reaction force ($t_{(19)} = 2.20$, $P = 0.04$) at ovulation (Figure 18). Means and standard deviations can be found in Tables 19 and 20. These values suggest that subjects were in a more "toe out" position combined with increased hip internal rotation at ovulation during the absorption phase of the jump landing. The body posture displayed at ovulation is similar to the position of no return, which is frequently described to occur during non-contact ACL injury mechanisms.[215] However, during the menses phase the subjects appear to experience greater overall loading as evidenced by increased vertical ground reaction force and internal knee varus moment at menses compared to ovulation. All other variables assessed during the absorption phase of the jump landing were not significant ($P > 0.05$).

4.3.4 Research Question 4

Our fourth analysis examined the relationship between hamstring neuromechanical properties and kinematic and kinetic variables assessed during the jump landing. Correlation coefficients and p-values can be found in Tables 21-28. Our general hypothesis stated that hamstring strength and movement would be correlated with movement in ways that would increase the susceptibility to non-contact ACL injury. Sixteen out of 360 correlations reached statistical significance. This section will describe the relationship between each of the significant findings. Discussion and interpretation of these findings are found in chapter 5. This research question was of secondary interest.

The most interesting finding was the negative relationship between knee laxity and knee extension moment ($r = -0.46$, $P = 0.05$, Table 25). Greater amounts of knee laxity were associated with greater knee extension moment. The second interesting finding was the

positive correlation between isokinetic eccentric hamstring strength at 60°/s and knee extension moment during the absorption phase of the jump landing (r = 0.55, *P* = 0.02, Table 26). Increased hamstring strength was associated with lesser knee extension moment since knee extension is a negative internal moment.

The second pattern that emerged from the data was the relationship between hamstring strength and knee rotation moment as seven of the significant correlations involved these data. During the menses testing session, knee rotation moment at initial contact was correlated with isokinetic concentric hamstring strength at 60°/s (r = 0.52, *P* = 0.02, Table 22), isokinetic concentric hamstring strength at 300°/s (r = 0.59, *P* = 0.007, Table 22), RFP 200 (r = 0.51, *P* = 0.03, Table 23) and during the absorption phase, knee rotation moment was correlated with isokinetic eccentric hamstring strength at 60°/s (r = 0.46, *P* = 0.05, Table 26). During the ovulation testing session, knee rotation moment at initial contact was correlated with isokinetic concentric hamstring strength at 60°/s (r = 0.49, *P* = 0.03, Table 24), isokinetic concentric hamstring strength at 300°/s (r = 0.49, *P* = 0.03, Table 24), and isokinetic eccentric hamstring strength at 300°/s (r = 0.48, *P* = 0.04, Table 24). These findings suggest that greater levels of hamstring strength are associated with internal knee rotation moment and weaker hamstring strength are associated with knee external rotation moment. Interestingly, all of these findings are opposite to our original hypothesis that greater amounts of hamstring strength would be associated with conditions that would not load the ACL.

Hamstring strength is related to knee valgus moment. Concentric isokinetic peak hamstring force at 300°/s was negatively correlated with knee varus moment at initial contact (r = -0.56, *P* = 0.01, Table 22). This relationship indicates that as strength increases, internal

knee varus moment decreases. This finding agrees with our hypothesis that increased strength would put less stress on the moments surrounding the knee. Internal knee varus is similar to external knee valgus moment that has been identified as a risk factor for ACL injury.[41] Longer T50% was associated with greater internal knee valgus (r = -0.49, *P* = 0.04, Table 25) and knee varus (r = 0.54, *P* = 0.02, Table 25) moments. This finding agrees with our original hypothesis that delayed ability to reach force would be associated with greater amounts of knee loading. Finally, concentric hamstring strength at 60°/s was associated with hip external rotation during the absorption phase of the jump landing task during the menses test session (r = -0.46, *P* = 0.04, Table 26). Our hypothesis was not supported since hip external rotation will increase ACL loading. RFP 200 has a relationship with knee internal rotation angle during the absorption phase of the jump landing in the menses test session (r = -0.48, *P* = 0.05, Table 25). This finding supports our hypothesis that greater RFP 200 is associated with less knee internal rotation angle.

4.3.5 Exploratory Analyses

We observed significant differences in several variables collected in this project. Knee laxity and how it changes across the menstrual cycle is a variable that has received significant amount of research in this field. Therefore, we wanted to determine if baseline values of knee laxity influenced any of the variables assessed in this project. The average knee laxity value at menses (mean = 6.64 mm) was used to divide the collected population into high knee laxity and low knee laxity groups. Subjects with knee laxity average below the mean were grouped into the low knee laxity (n = 11) group and subjects with knee laxity above the mean were grouped in the high knee laxity group (n = 9). We then used a group (low laxity vs. high laxity) by phase (menses vs. ovulation) repeated measures ANOVA to

determine if knee laxity influenced changes across the menstrual cycle. This analysis was only performed on variables that were found to change across the menstrual cycle in previous analyses (Table 8). It is important to note that our study was not powered for this analysis. Statistical significance was set a-priori at $P \leq 0.05$. SPSS (version 17.0) was used for all analyses.

When this analysis was used to examine knee laxity, we observed a non-significant group by phase interaction (low laxity: menses = 5.60 ± 0.60 mm, ovulation = 6.75 ± 0.86 mm; high laxity: menses = 8.05 ± 0.83 mm, ovulation = 8.18 ± 1.54 mm; $F_{(1,17)} = 2.83$, $P = 0.11$). A significant group main effect was found with the high laxity group having greater laxity compared to the low laxity group (low laxity = 6.12 ± 0.93 mm, high laxity = 8.12 ± 1.19 mm, $F_{(1,17)} = 33.13$, $P < 0.001$). These findings are of interest to our previous analysis because females with low knee laxity were the ones that changed across the menstrual cycle. Thus, a ceiling effect may be associated with hormonal influence on ACL laxity.

We performed this analysis on knee flexor stiffness and found no significant interaction ($F_{(1,18)} = 0.001$, $P = 0.98$), a main effect for phase ($F_{(1,18)} = 5.01$, $P = 0.038$), and no main effect for group (low laxity = 178.56 ± 35.68 Nm/rad; high laxity = 199.05 ± 37.94 Nm/rad, $F_{(1,17)} = 1.15$, $P = 0.29$). When this analysis was performed on E and P concentrations, no group by phase interaction was observed (E: $F_{(1,17)} = 0.03$, p = 0.87; P: $F_{(1,17)} = 0.05$, $P = 0.82$) nor were differences present between groups (E: $F_{(1,16)} = 0.04$, $P = 0.84$, P: $F_{(1,17)} = 0.03$, $P = 0.87$). No group by phase interaction was observed ($F_{(1,18)} = 0.05$, $P = 0.83$) when VGRF was stratified by laxity group nor was VGRF different between groups ($F_{(1,18)} = 0.05$, $P = 0.82$).

At initial contact our original analysis found that tibial rotation angle and knee valgus moment changed across the menstrual cycle. When these variables were examined using the RM ANOVA no group by phase interaction was observed between knee rotation angle at initial contact and knee laxity (low laxity = menses: -0.24 ± 8.45°, ovulation: -2.89 ± 8.78°; high laxity = menses: 3.79 ± 5.11°, ovulation = -3.82 ± 7.73°; $F_{(1,18)} = 2.14$, $P = 0.16$). These findings indicate that the high laxity group tended to change more across the menstrual cycle by approximately 7° of external rotation. However, no group main effect ($F_{(1,17)} = 0.24$, $P = 0.62$) was observed between laxity groups. Knee laxity did not influence knee valgus moment at initial contact. No group by phase interaction was observed ($F_{(1,17)} = 0.38$, $P = 0.54$) nor was knee valgus moment different between low laxity groups and high laxity groups ($F_{(1,17)} = 0.54$, $P = 0.47$). A significant interaction was observed for phase ($F_{(1,17)} = 10.64$, $P = 0.006$) which agrees with our original findings related to menstrual cycle phase.

In our original analysis we observed that menstrual cycle phase influenced knee rotation angle, hip rotation angle, knee valgus moment, knee varus moment, and knee rotation moment during the absorption phase of the jump landing. When knee rotation angle was stratified by laxity groups we observed a significant group by phase interaction for knee rotation angle (low laxity = menses: -5.60 ± 8.59°, ovulation: -5.96 ± 10.05°; high laxity = menses: -0.63 ± 3.94°, ovulation = -9.75 ± 7.33°; $F_{(1,18)} = 6.00$, $P = 0.03$) with the high laxity group externally rotating the tibia by approximately 8° at ovulation. A main effect for phase was ($F_{(1,18)} = 7.02$, $P = 0.02$) observed which agrees with our previous analysis but no group main effect was observed ($F_{(1,18)} = 0.04$, $P = 0.85$). We observed no hip rotation angle (max) group by phase interaction ($F_{(1,18)} = 0.36$, $P = 0.56$), however, a main effect for phase ($F_{(1,18)}$

= 4.58, $P = 0.05$) was observed which agrees with our previous findings. No group main effect was found between high laxity and low laxity groups ($F_{(1,18)} = 0.52$, $P = 0.48$).

Peak moments calculated during the absorption phase of the jump landing were also investigated during this exploratory analysis. No group by phase interaction was observed for knee valgus moment (min) ($F_{(1,17)} = 0.38$, $P = 0.55$) nor was a main effect for group ($F_{(1,17)} = 0.00$, $P = 0.99$), however, we did observe a main effect for phase ($F_{(1,17)} = 6.01$, $P = 0.03$). No group by phase interaction was observed for knee valgus moment (max) ($F_{(1,17)} = 1.08$, $P = 0.31$), nor was a main effect for group ($F_{(1,17)} = 0.01$, $P = 0.92$), however, we did observe a main effect for phase ($F_{(1,17)} = 10.06$, $P = 0.006$). Finally, no group by phase interaction was observed for knee rotation moment (min) ($F_{(1,17)} = 0.18$, $P = 0.68$), nor was a main effect for group (low laxity: -65.43±49.77, high laxity: -44.16±27.58, $F_{(1,18)} = 2.95$, $P = 0.10$). A main effect for phase ($F_{(1,17)} = 8.36$, $P = 0.01$) was observed.

CHAPTER FIVE

DISCUSSION OF RESULTS

5.1 Introduction

This chapter will provide a discussion of results not included in either manuscript. Manuscript one will discuss research questions 1 and 2 while manuscript 2 will address research question 3. The following will address research question 4 and the exploratory analyses.

5.2 Research Question 4

The aim of this investigation was to examine potential relationships between hamstring neuromechanical properties and movement during a jump landing task. Our findings demonstrate that hamstring strength and neuromechanical characteristics do not have a significant relationship with three dimensional motions during the jump landing task. We identified 16 significant correlations out of 360 and no consistent pattern emerged from the data. That is, correlations were sporadic and no specific contraction type, speed, or menstrual cycle phase consistently predicted jump landing movement.

Our findings agree with previous research that demonstrate a disconnect between muscle strength and movement.[116, 216-219] Shultz et al[116] utilized quadriceps and hamstring strength and EMG amplitude to predict movement during a jump landing. While some of the variance in jump landing kinematics was explained by strength and amplitude, overall, it seemed to be a poor predictor of movement. Neural strategies may be important factors in

predicting how a person moves. Therefore, future research should investigate motor programs or muscle timing during athletic activity to better determine how muscles influence movement.

The most interesting finding was the significant relationship between knee extension moment and knee laxity. Greater amounts of knee laxity are associated with greater knee extension moment. This correlation was found during the menses test session and only during the absorption phase of the jump landing. This relationship was not found at ovulation and no significant correlations were observed at initial contact. Shultz et al[220] observed that higher knee laxity was associated with greater work absorption at the knee. Increased knee laxity is a risk factor for ACL injury[57] and anterior tibial shear force can increase the load on the ACL. [59-62] The muscle group directly responsible for knee extension moment is the quadriceps muscle group. In fact, a powerful quadriceps contraction can load and rupture the ACL.[221] Increased knee laxity combined with large levels of quadriceps loading could create a condition that significantly loads the ACL.

Hamstring strength was also correlated with internal knee varus moment at initial contact. This relationship indicates that weak hamstrings are associated with greater knee varus moment. Internal knee varus moment is similar to external knee valgus moment which has been demonstrated as a risk factor for ACL injury.[41] This finding also agrees with previous literature that demonstrates that hamstrings are capable of preventing knee loading.[88] However, the only significant predictor or knee varus moment was concentric isokinetic hamstring strength at 300°/s at menses. This relationship was not observed at ovulation or during the absorption phase of the jump landing. The relationship between hamstring strength and knee varus moment was moderate.

Our data demonstrate that hamstring muscle strength does not predict how people move during a jump landing task. Future research should investigate the relationship between muscle activation levels and biomechanical variables to determine if they better predict movement patterns during a jump landing task. Also, one of the predictors of strength and non-contact ACL injury is the quadriceps to hamstring ratio.[10] Therefore, future research should examine how this relationship changes across the menstrual cycle and if this ratio better predicts movement during jump landing and cutting tasks.

5.3 Exploratory Analyses

The aim of this exploratory analysis was to determine the influence of knee laxity on variables that changed across the menstrual cycle in our original investigation. Our findings demonstrated that knee laxity influenced several variables, specifically, knee laxity, knee flexor stiffness, and tibial rotation angle. It is important to note that we did not power our study to answer this question.

Our data agree with previous research that knee laxity influences tibial rotation angle. Shultz et al[222] performed a similar stratification and found that the high laxity group had less control over transverse plane knee motion during a jump landing task. They concluded that persons that exhibited a high laxity profile had movements that were associated with non-contact ACL injury. A major difference compared to our study was we used healthy females with a history of ACL injury while they used a healthy population. Our data support the idea that high laxity is associated with greater rotation values. Research is limited in persons with a history of non-contact ACL injury no research has stratified their population by knee laxity and examined these changes across the menstrual cycle.

Our data do not agree with previous research with regard to moments and knee loading. Park et al[163] stratified groups by knee laxity measures and found that a 1.3mm increase in knee laxity was associated with a 30% increase in internal knee adduction impulse, and a 20% increase in internal knee adduction moment. We found that knee laxity changes did not seem to alter internal knee varus or valgus moments. Again, a major difference was the population, however, knee laxity seemed to change across the menstrual cycle and was not specific to high or low laxity groups.

When our data were stratified into groups, we found that groups were statistically different with the high laxity group having greater knee laxity than the low laxity group (effect size = 1.68). We also observed a trend in the group by phase interaction. This interaction was driven by the low laxity group who had increased knee laxity at ovulation compared to the menses test session. Mean knee laxity values in the high laxity group were elevated at menses and did not change across the menstrual cycle. These findings may indicate that there is a ceiling effect associated with knee laxity and that once the ACL reaches a certain laxity level, no further hormonally driven increases are possible. No group differences were observed between estradiol-β-17 or progesterone. However, the low laxity group had lower levels of estradiol-β-17 and higher levels of progesterone at the early follicular phase of the menstrual cycle. Shultz et al[223] found that this relationship was one of the best predictors in determining the magnitude change of knee laxity across the menstrual cycle. Our data support this notion since our low laxity females increased knee laxity across the menstrual cycle. It is important to note that this interaction was not statistically significant. Future research should increase the sample size to determine if statistical significance can be reached.

Another interesting finding using this group stratification was related to tibial internal rotation angle at initial contact. Again, the interaction for this analysis was not statistically significant, which is a result of our being underpowered, but we did identify it as a trend in the data. When our means and standard deviations were examined, the high laxity group had more changes in tibial rotation angle compared to the low laxity group. At the menses test session the high laxity group presented with tibial internal rotation angle and moved toward tibial external rotation at ovulation by approximately 8°. The low laxity group changed knee rotation angle to a smaller degree but externally rotated at ovulation by 2.65° compared to menses. Previous research[163, 220] has stratified individuals by laxity group but we are the first to examine tibial rotation using this technique across the menstrual cycle. We also saw changes in tibial rotation using this technique during the absorption phase of the jump landing. A significant group by phase interactions was observed for knee rotation with the high laxity group externally rotating the tibia at the ovulation testing session by approximately 8°. No changes were observed across the menstrual cycle in the low laxity group. The high laxity group did not appear to be sensitive to hormonal changes across the menstrual cycle when we assessed knee laxity. These changes in knee rotation could be explained by increased laxity in another plane that was not assessed in this study. Knee laxity influences tibial rotation during the jump landing.

When knee flexor stiffness was examined using the group stratification we observed no significant group by phase interaction. However, groups tended ($P = 0.24$) to be different with greater knee flexor stiffness in the high laxity group compared to the low laxity group. Group differences were associated with a moderate effect size (effect size = 0.45). We are the first to examine knee flexor stiffness across the menstrual cycle and stratify by knee

laxity groups. To further explain this relationship we examined isometric hamstring strength using this technique and found that isometric hamstring strength tended to be different between laxity groups as well ($P = 0.16$) with the high laxity group tending to have greater isometric hamstring strength compared to the low laxity group (high laxity: 128.46 ± 37.77 N, low laxity: 102.46 ± 41.37 N, effect size = 0.63). However, our data demonstrate that stiffness and strength are not correlated. Knee flexor stiffness was assessed from an isometric contraction with an activation level of approximately 35% MVIC which is the same as previously reported literature.[22] These changes in hamstring strength and stiffness are most likely a compensation mechanism to increased knee laxity. Our subject sample was on average 2 years removed from initial ACL injury (mean = 25 ± 12.57 months). Most subjects had surgery with 1-2 months post injury. Two years is enough time for muscle strength and stiffness to adapt to increased changes in knee laxity. Increased strength and stiffness would be needed to increase knee joint stability during dynamic tasks. Another possibility is that rehabilitation was different between groups. Unfortunately, we did not collect information about individual rehabilitation programs but only required individuals to be cleared by their physician for return to sporting activity. Future research should determine if rehabilitation plays a role in altering stiffness of the hamstrings and also determine if graft choice influences hamstring stiffness. A hamstring graft is a popular choice for reconstructing the ACL and hamstring properties may be influenced by after harvesting the hamstring tendon.

We also examined knee rotation moment stratified by high and low laxity groups. While we observed no group by phase interaction, we did observe a trend in the main effect for group. Interestingly, the low laxity group tended to have greater internal tibial external

rotation moment compared to the high laxity group, however, both groups had a tibial external rotation moment. Decreased external rotation moment loading may be a compensatory mechanism by the high laxity group to limit knee loading. High ligament laxity may be able to attenuate rotational forces on the knee better than the low laxity group. Hewett et al[224] described females as tending to be more ligament dominant while males tend to be more muscle dominant. While our population is all females, it is possible that the high laxity group tends to absorb forces utilizing a ligament strategy. Future research should attempt to determine the role of knee laxity on knee rotational moment and determine if knee rotational loading is related to ACL injury.

We observed no other significant group by phase interactions or group main effects. Knee laxity did not influence VGRF, knee valgus moment at initial contact, hip rotation (absorption phase), knee valgus moment (absorption), knee varus moment (absorption), Findings related to hip rotation are in contrast to our findings related to the tibia. This data indicate that the tibia is more sensitive to changes in static stabilizers of the knee including the ACL, than the femur caused by the hormonally driven changes in knee laxity across the menstrual cycle. This conclusion makes sense given the proximal muscular control that exists on the femur. Our findings also disagree with previous research related to knee valgus moment.[163] Our results indicate that knee laxity does not influence the internal knee valgus moments. These findings disagree with Park et al that observed a 1.3mm increased in knee laxity was associated with increased internal adduction impulse and moment.[163] Our knee laxity measures were greater than the values reported by Park et al[163] but they used healthy individuals with no history of ACL injury.

These data have implications for both future research as well as clinical practice. Knee laxity tends to have the most influence tibial rotation angle and muscle stiffness. Both of these factors are important for knee stability and have implications for ACL injury prevention research as well as rehabilitation. Clinicians should be aware that the biomechanical profile of females with unilateral ACL injury change across the menstrual cycle and could be a potential explanation as to why they are at greater risk of subsequent ACL injury.

The major limitation of this exploratory analysis is that our study was not powered to support the analysis. However, it provides valuable pilot data to research in females with unilateral ACL injury. Future research should increase our sample size to further elucidate the role of ligament laxity and how it influences biomechanics across the menstrual cycle. Additionally, research should investigate the role that hamstring graft reconstruction has on knee flexor stiffness and if this influences knee stability.

Table 1. Data from previous research demonstrating the relationships between hamstring neuromuscular properties and reproductive hormones for males and females.

		MTS	***T50%***	***RFP***
Estradiol-β-17	r	-0.43	0.15	-0.39
	p	*0.02	0.41	0.03
Progesterone	r	0.27	0.09	0.03
	p	0.16	0.62	0.87
Free Testosterone	r	0.46	-0.43	0.56
	p	*0.01	*0.02	0.001

*$P<.05$

Table 2. ACL injury risk across the menstrual cycle. Darkened areas represent the period of the menstrual cycle that the authors concluded had the greatest risk of injury. Overall, the preovulatory phase of the menstrual cycle seems the most at risk for injury.

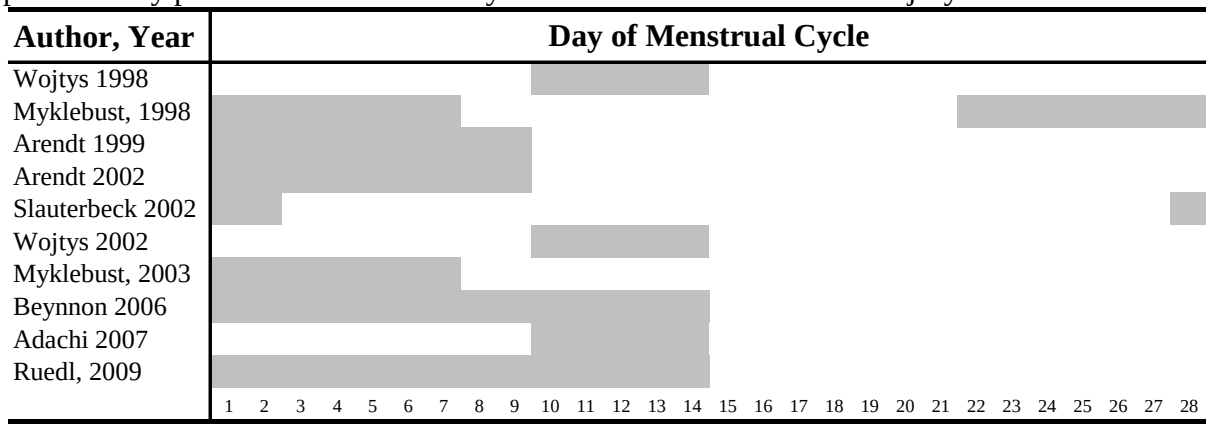

Table 3. Data from previous research demonstrating the relationships between hamstring neuromuscular properties and reproductive hormones in males.

		MTS	***T50%***	***RFP***
Estradiol-β-17	r	0.19	-0.15	0.18
	p	0.50	0.60	0.51
Progesterone	r	0.36	-0.28	0.22
	p	0.18	0.32	0.42
Free Testosterone	r	0.24	-0.25	0.22
	p	0.39	0.37	0.42

*Represents $P<.05$

Table 4. Data from previous research demonstrating the relationships between hamstring neuromuscular properties and reproductive hormones in females.

		MTS	***T50%***	***RFP***
Estradiol-β-17	r	-0.53	-0.38	0.01
	p	*0.04	*0.05	0.96
Progesterone	r	0.42	0.32	-0.05
	p	0.12	0.24	0.88
Free Testosterone	r	-0.52	-0.44	0.20
	p	*0.04	0.10	0.53

*$P<.05$

Table 5. Summary of articles used in systematic review.

Author, Year	*Population*	*Hormones*	*Muscle*	*Testing Times*
Sawar, 1996	10 healthy	N/A	Isometric quadriceps Grip strength	EF (days 1-7) Mid-follicular (days 7-12) Mid-cycle (12-18) Mid-luteal (18-21) Late-luteal (21-32)
Phillips, 1996	10 trained 10 untrained 5 Oral contraception	Oestradio-β-17	Adductor pollicis	3 times per week for six months
Friden, 2003	10 general population	Estrogen Progesterone, FSH LH	Grip Strength, Isokinetic muscle strength, Isokinetic muscle endurance	Menses (days 3-5) Ovulation (kit) Mid-luteal (7 days post ovulation)
Hertel, 2006	14 healthy collegiate athletes	Urinary analysis of: Estrone-3-glucuronide Pregnanediol-3-glucoronide	Isokinetic quadriceps and hamstring (concentric) at 120°/s	Mid-follicular (4-7 days prior to ovulation), Ovulation, Mid-luteal (7-10 days post ovulation)
Bambaeichi, 2004	8 sedentary	N/A	Isokinetic and isometric quadriceps and hamstring 60°/s, 180°/s	Days 1-4 7-9 Ovulation 19-21 25-27
Abt, 2007	10 physically active	Estrogen Progesterone	Hamstring to quadriceps strength ratio (60°/s, 180°/s)	Day 3 after menses 24-26 hours post ovulation 7 days post ovulation
Elliot, 2003	7 healthy	Progesterone Estrogen* Testosterone* *Total and Bioavailable	1st Dorsal interosseus	2 days after menses Mid-luteal (7 days post ovulation)
Lebrun, 1995	16 recreationally active	Estradiol Progesterone	Isokinetic knee flexion and extension at 30°/s	EF (days 3-8) ML (4-9 post ovulation)

Table 6. A-priori estimated power calculations for each dependent variable.

Variable	*Article*	*Calculated Effect Size*	*n for Power 80*
Linear Stiffness	Bell 2009	1.97	6
Time to 50% Peak Force	Blackburn 2009	0.56	>1000
Rate of Force Production	Blackburn 2009	0.22	>200
ATSF	Sell 2007	0.64	20
Knee Valgus Angle	McLean 2005	1.00	36
Knee Flexion Angle	Cruz (In review)	2.69	15
Tibial Rotation Angle	McLean 2005	1.05	100
Hip Flexion Angle	McLean 2005	2.93	9
Femoral Rotation Angle	McLean 2005	1.03	95
Knee Extension Moment	Cruz (In review)	2.20	<10
Knee Valgus Moment	Cruz (In review)	0.76	<10
Knee Rotation Moment	Cruz (In review)	1.81	<10
Hamstrings Isokinetic Peak Torque	Kaminski 1998	1.70	<10

Power calculations were performed assuming a 10% change in the dependent variable at ovulation and a 50% decrease in the standard deviation compared to the control condition. Effect sizes were corrected for correlations between multiple test sessions.

Bell 2009[29]
Blackburn 2009[124]
Sell 2007[225]
McLean 2005[85]
Cruz (In review)[226]
Kaminski 1998[227]

Table 7. Subject ACL injury history.

Subject Number	*ACL Injured Limb*	*Months From ACL Injury to Testing*	*Mechanism of Injury*	*Sport Participation during Injury*	*Type of Graft*
1	R	18	Landing	Gymnastics	Hamstring
2	R	52	Pivoting/Throwing	Softball	Patellar Tendon
3	L	42	Pivoting	Soccer	Patellar Tendon
4	R	16	Landing	Basketball	Hamstring
5	R	7	Cutting	Soccer	Patellar Tendon
6	R	34	Cutting	Soccer	Allograft
7	R	33	Cutting	Soccer	Allograft
8	L	12	Cutting	Flag Football	Patellar Tendon
9	R	45	Landing	Basketball	Patellar Tendon
10	L	17	Landing	Long Jumping	Patellar Tendon
11	L	29	Cutting	Soccer	Hamstring
12	L	14	Cutting	Field Hockey	N/A
13	L	24	Cutting	Flag Football	Allograft
14	R	26	Landing	Volleyball	Allograft
15	R	38	Cutting	Soccer	Patellar Tendon
16	L	17	Cutting	Soccer	Patellar Tendon
17	R	36	Tumbling/Landing	Gymnastics	Hamstring
18	R	13	Cutting	Handball	Hamstring
19	R	14	Cutting	Soccer	Hamstring
20	R	26	Cutting	Flag Football	Patellar Tendon

Subject 12 did not have ACL reconstruction surgery in ACL injured limb.

Table 8. Summary of significant findings across the menstrual cycle.

	P
Variables that Increased at Ovulation	
Estradiol-β-17 (pg/ml)	0.009
Progesterone (ng/ml)	0.003
Knee Laxity (mm)	0.03
Knee Flexor Stiffness (Nm/rad)	0.03
Stiffness Normalized to Body Mass	0.03
Hip Rotation Angle (Max) (°)	0.05
Tibial Rotation Moment (Min) (Nm)	0.007
Normalized Tibial Rotation Moment (Min)	0.007
Variables that Decreased at Ovulation	
Peak Vertical GRF (N)	0.04
Tibial External Rotation Angle at IC (°)	0.01
Varus Moment at IC (Nm)	0.006
Normalized Varus Moment at IC	0.003
Tibial Rotation Angle (ABS) (Min) (°)	0.05
Knee Valgus Moment (ABS) (Nm)	0.03
Normalized Knee Varus Moment (ABS)	0.03
Knee Varus Moment (Max) (Nm)	0.003
Normalized Knee Varus Moment (Max)	0.003

Arrows represent the change in the variable from menses to ovulation.
IC = initial contact
ABS = Absorption phase of the jump landing task
GRF = Ground Reaction Force

Table 9. Variables collected during the knee flexor stiffness assessment and knee laxity assessment. Values represent Mean ± Standard Deviation. Values are taken from each subject's healthy limb.

	Menses	**Ovulation**	**Menses 95% CI**		**Ovulation 95% CI**		**P**
			Upper	Lower	Upper	Lower	
Knee Flexor Stiffness (Nm/rad)	178.56±35.68	199.05±37.94	161.86	195.25	181.29	216.81	*0.03
Stiffness Normalized to Body Mass	2.71±0.46	3.01±0.43	2.49	2.92	2.81	3.22	*0.03
Perturbation Amplitude (°)	9.53±1.53	9.42±1.29	8.81	10.25	8.82	10.03	0.78
Knee Laxity (mm)	6.64±1.38	7.34±1.32	5.99	7.29	6.72	7.96	*0.03

*$P \leq 0.05$

Table 10. Concentrations of reproductive hormones from the selected time points. Values are mean ± standard deviation.

	Menses	Ovulation	Menses 95% CI		Ovulation 95% CI		P
Estradiol-β-17 (pg/ml)	31.12 ± 13.72	70.35 ± 54.66	24.49	37.72	44.01	96.70	*0.009
Progesterone (ng/ml)	0.51 ± 0.25	3.92 ± 4.24	0.39	0.63	1.87	5.96	*0.003
Free Testosterone (ng/ml)	0.80 ± 0.26	0.86 ± 0.22	0.67	0.92	0.75	0.97	0.414

*$P \leq 0.05$

Table 11. Hamstring force production variables. Values are mean ± standard deviation and are reported in N/s. The slope was calculated over the first 200ms after the onset of force production and to the time point of when 50% peak force was attained (T50%). R2 represents the linear relationship between the time points of interest. Slope was calculated using the equation m=(Y2-Y1)/(X2-X1), where Y was the force applied in Newtons and X was the defined time period in seconds.

	Menses	**Ovulation**	**Menses 95% CI**		**Ovulation 95% CI**		**P**
T50% (ms)	115.55±55.89	121.20±58.22	87.75	143.34	92.24	150.15	0.40
Slope							
200ms	255.59±143.57	221.61±101.99	184.20	326.99	170.89	272.33	0.08
T50%	333.28±221.29	304.22±174.05	223.24	443.33	217.66	390.77	0.39
R^2							
200ms	0.96±0.04	0.93±0.11					
T50%	0.99±0.01	0.98±0.03					

Table 12. Peak hamstring strength values. Values are mean ± standard deviation.

	Menses	Ovulation	Menses 95% CI		Ovulation 95% CI	
Isometric	115.37±47.05	112.83±36.11	91.97	138.77	94.87	130.79
Isokinetic, 60°/s						
Concentric	95.51±21.53	94.18±17.17	84.59	104.59	87.15	103.55
Eccentric	101.98±16.25	100.51±20.17	93.98	108.94	91.92	110.56
Isokinetic, 300°/s						
Concentric	93.66±23.82	91.38±18.98	83.15	104.91	83.91	100.91
Eccentric	100.67±16.43	96.98±14.81	93.39	108.39	90.66	104.34

Contraction by phase interaction, $P = 0.78$
Main effect for contraction, $P = 0.03$*
Main effect for phase, $P = 0.95$
*The Tukey Post Hoc revealed the isometric hamstring contraction was greater than the concentric contraction at 60°/s and 300°/s and eccentric 300°/s

Table 13. Correlation coefficients between the hamstring muscle properties and reproductive hormone levels at menses**.**

		E	P	FT	E:P Ratio
Knee Laxity	r	0.228	-0.122	0.073	0.192
	p	0.333	0.609	0.761	0.418
Knee Flexor Stiffness	r	-0.081	-0.242	0.267	-0.037
	p	0.733	0.303	0.255	0.877
Normalized Stiffness	r	-0.053	-0.177	0.398	-0.110
	p	0.825	0.455	0.082	0.643
T50%	r	0.117	0.749	0.315	-0.487
	p	0.482	*<0.001	0.203	*0.040
RFP 200	r	0.053	-0.314	-0.082	0.575
	p	0.829	0.190	0.737	*0.010
RFP T50%	r	0.135	-0.313	0.026	0.636
	p	0.593	0.222	0.918	*0.005
Isometric HS	r	0.150	-0.026	-0.286	0.430
	p	0.539	0.916	0.222	0.066
Isokinetic at 60°/s					
Concentric	r	0.252	0.308	-0.286	-0.181
	p	0.284	0.187	0.222	0.444
Eccentric	r	0.189	0.288	0.028	-0.324
	p	0.424	0.219	0.908	0.164
Isokinetic at 300°/s					
Concentric	r	0.227	0.235	-0.318	-0.070
	p	0.336	0.318	0.172	0.770
Eccentric	r	0.217	0.350	-0.361	0.289
	p	0.358	0.130	0.118	0.216

*$P \leq 0.05$

MTS – Musculotendinous stiffness

T50 – Time to 50% peak force

RFP – Rate of force production

Table 14. Correlation coefficients between the hamstring muscle properties and reproductive hormone levels at ovulation.

		E	P	FT	E:P Ratio
Knee Laxity	r	-0.200	-0.250	0.198	-0.026
	p	0.397	0.287	0.416	0.914
Knee Flexor Stiffness	r	-0.455	0.053	0.071	-0.262
	p	*0.050	0.830	0.773	0.279
Normalized Stiffness	r	-0.312	-0.048	0.071	-0.155
	p	0.194	0.845	0.773	0.527
T50%	r	0.049	-0.500	0.263	0.554
	p	0.851	*0.041	0.308	*0.021
RFP 200	r	0.148	0.127	-0.298	-0.177
	p	0.545	0.627	0.229	0.469
RFP T50%	r	0.086	0.077	-0.368	-0.183
	p	0.743	0.770	0.146	0.483
Isometric HS	r	0.053	-0.262	0.235	-0.074
	p	0.835	0.311	0.333	0.769
Isokinetic at 60°/s					
Concentric	r	0.310	0.024	0.069	0.200
	p	0.743	0.923	0.780	0.411
Eccentric	r	0.160	-0.111	0.070	0.193
	p	0.512	0.652	0.777	0.429
Isokinetic at 300°/s					
Concentric	r	0.160	0.249	-0.064	-0.211
	p	0.512	0.302	0.795	0.385
Eccentric	r	0.340	0.385	0.011	-0.179
	p	0.154	0.103	0.964	0.464

*$P \leq 0.05$
MTS – Musculotendinous stiffness
T50 – Time to 50% peak force
RFP – Rate of force production

Table 15. Correlation coefficients between change scores of the hamstring muscle properties and reproductive hormone levels.

		E	P	FT	E:P Ratio
Knee Laxity	r	-0.150	-0.305	-0.103	0.128
	p	0.540	0.205	0.675	0.601
Knee Flexor Stiffness	r	0.331	0.241	-0.015	0.152
	p	0.166	0.320	0.951	0.535
Normalized MTS	r	-0.345	-0.194	0.071	-0.218
	p	0.148	0.426	0.772	0.369
T50%	r	0.265	-0.058	-0.047	0.309
	p	0.304	0.824	0.858	0.228
RFP 200	r	-0.074	-0.182	0.182	0.195
	p	0.777	0.485	0.485	0.454
RFP T50%	r	-0.058	-0.107	0.024	0.140
	p	0.826	0.681	0.928	0.591
Isometric HS	r	-0.175	-0.398	0.103	0.363
	p	0.501	0.113	0.695	0.152
Isokinetic at 60°/s					
Concentric	r	0.242	-0.024	-0.019	0.163
	p	0.319	0.922	0.939	0.504
Eccentric	r	0.065	-0.086	0.164	-0.025
	p	0.793	0.728	0.503	0.920
Isokinetic at 300°/s					
Concentric	r	0.279	0.004	-0.046	0.003
	p	0.247	0.988	0.850	0.989
Eccentric	r	0.082	0.321	0.347	-0.382
	p	0.739	0.180	0.146	0.106

MTS – Musculotendinous stiffness
T50 – Time to 50% peak force
RFP – Rate of force production

Table 16. Average EMG for the medial and lateral quadriceps and hamstrings before and after the perturbation during the active stiffness protocol. Average quadriceps and hamstring values were calculated by averaging the medial and lateral muscle EMG values. Co-contraction ratios were calculated by dividing the hamstring average values into the quadriceps average values. Values are mean %MVIC ± standard deviation.

	Menses	**Ovulation**	**Menses 95% CI**		**Ovulation 95% CI**	
Pre-Perturbation			Lower	Upper	Lower	Upper
Medial Quadricep	2.30±1.32	2.42±1.44	1.62	2.98	1.67	3.16
Lateral Quadricep	5.57±4.74	4.50±2.26	3.14	8.01	3.37	5.70
Medial Hamstring	37.90±16.18	35.54±14.20	29.76	46.22	28.24	42.85
Lateral Hamstring	39.40±15.74	35.43±17.49	31.91	48.09	26.43	44.43
Average Quadriceps	3.94±2.99	3.47±1.77	2.40	5.48	2.57	4.39
Average Hamstrings	38.94±13.94	35.87±14.25	31.78	46.12	28.16	42.82
Co-activation Ratio	0.10±0.04	0.10±0.04	0.08	0.12	0.08	0.12
Post-Perturbation						
Medial Quadricep	3.15±1.41	3.22±1.94	2.42	3.87	2.22	4.22
Lateral Quadricep	6.74±4.65	5.96±2.96	4.35	9.13	4.44	7.49
Medial Hamstring	43.49±18.41	40.21±15.83	34.16	52.82	32.08	48.35
Lateral Hamstring	47.76±17.02	44.77±21.35	39.02	56.52	33.79	55.75
Average Quadriceps	4.94±2.93	4.59±2.26	3.44	6.45	3.43	5.76
Average Hamstrings	45.62±14.13	42.49±13.81	38.85	52.13	33.85	51.13
Co-activation Ratio	0.11±0.03	0.11±0.03	0.09	0.13	0.09	0.13

Table 17. Kinematic variables at *initial contact*. Values are in degrees and represent: mean ± standard deviation.

	Menses	Ovulation	Menses 95% CI		Ovulation 95% CI		P
Knee Flexion	21.13±5.45	21.02±7.41	18.58	23.68	17.55	24.50	0.94
Knee Valgus	-1.29±3.94	-0.98±5.27	-3.14	0.56	-3.45	1.49	0.77
Knee Rotation	1.57±7.27	-3.31±8.12	-1.83	4.97	-7.11	0.49	*0.01
Hip Flexion	-41.42±8.95	-43.46±6.64	-41.62	-37.24	-46.58	-40.36	0.34
Hip Adduction	-3.40±4.91	-3.74±5.45	-5.70	-1.11	-6.30	-1.20	0.79
Hip Rotation	-2.16±8.62	-0.56±7.82	-6.20	1.88	-4.22	3.10	0.33

*$P \leq 0.05$

Table 18. Kinetic and force variables. Vertical ground reaction force was calculated as peak during the jump landing. All other variables are calculated at *initial contact*. Values are mean ± standard deviation. Moments are reported raw and normalized (BW*BH). One subject was determined to have unusable kinetic data and was removed from the analyses (n=19).

	Menses	**Ovulation**	**Menses 95% CI**		**Ovulation 95% CI**		**P**
VGRF (N)	1968.31±623.82	1775.80±642.94	1676.36	2260.27	1474.90	2076.71	*0.04
ATSF (N)	1.03±69.34	6.10±89.53	-31.43	33.48	-35.80	48.00	0.73
Knee Valgus Moment	10.69±13.56	3.72±11.95	4.16	17.23	-2.04	9.49	*0.006
Normalized Knee Valgus Moment	0.01±0.01	0.003±0.01	0.004	0.016	-0.002	0.009	*0.003
Knee Extension Moment	-3.68±28.69	-1.37±24.59	-17.52	10.15	-13.23	10.48	0.59
Normalized Knee Extension Moment	-0.005±0.02	-0.003±0.02	-0.018	0.008	-0.014	0.008	0.59
Knee Rotation Moment	-2.19±4.02	-2.23±3.29	-4.14	-0.26	-3.83	-0.65	0.96
Normalized Knee Rotation Moment	-0.002±0.004	-0.002±0.003	-0.004	0.005	-0.004	0.005	0.99

VGRF – Vertical Ground Reaction Force
ATSF – Anterior Tibial Shear Force

Table 19. Peak kinematic variables calculated during the *absorption phase* of the jump landing. Values are in degrees and represent: mean ± standard deviation.

	Menses	**Ovulation**	**Menses 95% CI**		**Ovulation 95% CI**		**P**
Knee Flexion	93.66±8.86	92.68±12.40	89.51	97.81	86.87	98.48	0.49
Knee Valgus	-13.24±10.62	-12.39±7.92	-18.21	-8.27	-16.11	-8.69	0.67
Knee Rotation (Max)	9.19±7.50	5.27±8.73	5.68	12.70	1.19	9.36	0.07
Knee Rotation (Min)	-3.36±7.20	-7.66±8.92	-6.74	0.007	-11.84	-3.49	*0.05
Hip Flexion	-77.74±12.31	-80.77±16.76	-83.50	-71.98	-88.62	-72.92	0.25
Hip Adduction	0.86±5.86	0.90±6.29	-1.88	3.61	-2.04	3.84	0.98
Hip Rotation (Max)	2.89±8.70	6.23±6.83	-1.18	6.97	3.03	9.43	*0.05
Hip Rotation (Min)	-8.09±7.98	-5.63±6.97	-11.82	-4.35	-8.89	-2.37	0.15

*$P \leq 0.05$

Table 20. Kinetic and force variables calculated at peak during the *absorption phase* of the jump landing. Values are mean ± standard deviation. Moments are reported raw and normalized (BW*BH). One subject was determined to have unusable kinetic data and was removed from the analyses (n=19). Moments are calculated as internal moments.

	Menses	**Ovulation**	**Menses 95% CI**		**Ovulation 95% CI**		**P**
ATSF (N)	132.24±74.61	130.61±119.78	93.36	163.29	72.96	182.70	0.95
Normalized ATSF	0.21±00.13	0.21±0.19	0.14	0.27	0.12	0.29	0.87
Knee Valgus Moment	-17.24±35.57	-60.65±71.60	-34.39	-0.09	-95.16	-26.14	*0.03
Normalized Knee Valgus Moment	-0.02±0.03	-0.06±0.06	-0.04	-0.002	-0.08	-0.03	*0.03
Knee Varus Moment	89.77±44.85	57.78±29.74	68.15	111.29	43.44	72.11	*0.003
Normalized Knee Varus Moment	0.14±0.08	0.09±0.05	0.11	0.18	0.07	0.11	*0.003
Knee Extension Moment	-235.32±77.66	-230.90±80.34	-272.76	-197.90	-269.62	-192.17	0.83
Normalized Knee Extension Moment	-0.21±0.06	-0.21±0.07	-0.24	-0.18	-0.24	-0.18	0.91
Knee ER Moment	-75.31±50.55	-37.64±21.27	-99.68	-50.95	-47.89	-27.39	*0.007
Normalized Knee ER Moment	-0.07±0.05	-0.03±0.02	-0.10	-0.05	-0.05	-0.03	*0.007
Knee IR Rotation Moment	28.53±22.98	40.79±35.48	17.45	39.60	23.69	57.89	0.23
Normalized Knee IR Moment	0.03±0.03	0.03±0.03	0.02	0.04	0.02	0.05	0.30

*$P \leq 0.05$

ER – External Rotation

IR – Internal Rotation

Table 21. Correlation coefficients between hamstring neuromechanical variables and jump landing kinematics and kinetics. Variables were calculated at initial contact during the menses test session. Moments are calculated as internal moments.

		Knee Laxity	Active Hamstring MTS	T50%	RFP 200	RFP T50%
Knee Flexion	r	0.286	0.245	-0.009	0.269	0.277
	p	0.222	0.298	0.972	0.265	0.143
Knee Valgus	r	-0.216	0.224	0.252	0.000	-0.158
	p	0.361	0.342	0.314	0.999	0.532
Knee Rotation	r	0.258	0.085	-0.046	-0.224	-0.017
	p	0.273	0.721	0.220	0.358	0.945
Hip Flexion	r	-0.325	-0.041	-0.180	-0.170	0.187
	p	-0.162	0.863	0.475	0.488	0.457
Hip Abd/Add	r	0.118	-0.135	-0.180	0.189	0.268
	p	0.620	0.569	0.476	0.439	0.283
Hip Rotation	r	-0.195	0.194	0.010	0.119	-0.049
	p	0.409	0.414	0.970	0.627	0.847
Kn Ext. Mom	r	0.144	-0.156	0.214	0.100	0.058
	p	0.557	0.524	0.394	0.159	0.820
Kn. Varus Mom	r	-0.272	0.215	-0.005	-0.337	-0.221
	p	0.260	0.376	0.983	0.159	0.378
Kn. Rot. Mom	r	0.034	-0.413	-0.206	0.025	0.027
	p	0.890	0.079	0.413	0.920	0.915

Table 22. Correlation coefficients between hamstring neuromechanical variables and jump landing kinematics and kinetics. Variables were calculated at *initial contact* during the menses test session.

		HS Isometric	Concentric 60°/s	Eccentric 60°/s	Concentric 300°/s	Eccentric 300°/s
Knee Flexion	r	0.378	-0.246	-0.346	-0.286	-0.163
	p	0.111	0.295	0.135	0.222	0.492
Knee Valgus	r	-0.050	-0.089	0.062	0.093	0.171
	p	0.838	0.708	0.794	0.695	0.471
Knee Rotation	r	-0.215	0.238	0.239	-0.027	-0.077
	p	0.838	0.313	0.311	0.911	0.748
Hip Flexion	r	-0.143	0.114	0.350	0.001	0.003
	p	0.558	0.633	0.130	0.996	0.991
Hip Abd/Add	r	0.174	0.061	0.051	0.080	-0.042
	p	0.477	0.800	0.830	0.738	0.861
Hip Rotation	r	0.074	-0.375	-0.357	0.123	0.234
	p	0.762	0.103	0.122	0.604	0.321
Kn. Ext. Mom	r	0.010	0.318	-0.008	0.299	-0.055
	p	0.968	0.185	0.973	0.214	0.824
Kn. Valgus Mom	r	-0.162	-0.310	-0.125	-0.557	-0.200
	p	0.507	0.196	0.610	*0.013	0.411
Kn. Rot. Mom	r	0.001	0.519	0.041	0.594	0.151
	p	0.996	*0.023	0.867	*0.007	0.537

*$P \leq 0.05$

Table 23. Correlation coefficients between hamstring neuromechanical variables and jump landing kinematics and kinetics. Values were calculated at *initial contact* during the ovulation test session.

		Knee Laxity	Active Hamstring MTS	T50%	RFP 200	RFP T50%
Knee Flexion	r	0.231	0.143	0.185	0.408	0.060
	p	0.326	0.548	0.461	0.092	0.813
Knee Valgus	r	-0.130	0.045	0.197	0.330	0.015
	p	0.584	0.850	0.433	0.180	0.951
Knee Rotation	r	0.092	0.206	0.239	0.112	-0.181
	p	0.701	0.384	0.340	0.659	0.472
Hip Flexion	r	-0.322	-0.407	0.337	0.129	-0.322
	p	0.166	0.075	0.172	0.609	0.193
Hip Abd/Add	r	-0.139	-0.333	-0.454	-0.227	0.007
	p	0.558	0.152	0.058	0.364	0.978
Hip Rotation	r	-0.190	-0.170	-0.291	-0.254	0.066
	p	0.423	0.486	0.242	0.309	0.795
Kn Ext. Mom	r	0.244	0.319	0.068	0.141	0.156
	p	0.313	0.184	0.790	0.578	0.537
Kn. Valgus Mom	r	0.135	0.204	0.174	-0.216	-0.090
	p	0.581	0.402	0.490	0.389	0.724
Kn. Rot. Mom	r	0.062	-0.051	-0.462	0.509	0.414
	p	0.801	0.837	0.054	*0.031	0.088

Ovulation: Knee laxity and stiffness (r=0.499, p=.03)*
Menses: Knee laxity and stiffness (r=0.272, p=.260)

Table 24. Correlation coefficients between hamstring neuromechanical variables and peak jump landing kinematics and kinetics. Values were calculated during *initial contact* at the ovulation test session.

		HS Isometric	Concentric 60°/s	Eccentric 60°/s	Concentric 300°/s	Eccentric 300°/s
Knee Flexion	r	0.142	0.127	0.184	0.221	0.079
	p	0.573	0.595	0.437	0.349	0.742
Knee Valgus	r	0.284	0.100	0.089	-0.002	-0.131
	p	0.254	0.675	0.709	0.992	0.583
Knee Rotation	r	-0.081	-0.364	-0.345	-0.249	-0.181
	p	0.750	0.115	0.136	0.290	0.445
Hip Flexion	r	-0.161	0.068	0.017	0.004	0.258
	p	0.524	0.777	0.944	0.986	0.272
Hip Abd/Add	r	-0.323	-0.049	-0.157	-0.003	-0.025
	p	0.191	0.839	0.510	0.989	0.918
Hip Rotation	r	-0.040	0.130	0.077	-0.003	-0.166
	p	0.874	0.584	0.747	0.989	0.485
Kn Ext. Mom	r	0.269	0.024	-0.106	0.103	0.154
	p	0.281	0.921	0.664	0.675	0.529
Kn. Valgus Mom	r	-0.240	-0.194	-0.165	-0.224	-0.217
	p	0.338	0.427	0.499	0.257	0.373
Kn. Rot. Mom	r	0.416	0.493	0.424	0.492	0.477
	p	0.086	*0.032	0.070	*0.032	*0.039

*$P \leq 0.05$

Table 25. Correlation coefficients between hamstring neuromechanical variables and jump landing kinematics and kinetics during the absorption phase of the menses test session.

		Knee Laxity	Active Hamstring MTS	T50%	RFP 200	RFP T50%
Knee Flexion (max)	r	0.250	0.146	0.357	-0.005	-0.128
	p	0.288	0.540	0.145	0.984	0.612
Knee Valgus (min)	r	-0.326	-0.091	0.201	0.129	-0.242
	p	0.160	0.703	0.423	0.611	0.333
Knee Rotation (min)	r	0.292	0.063	0.124	-0.247	-0.227
	p	0.211	0.793	0.624	0.323	0.366
Knee Rotation (max)	r	0.146	0.129	0.149	-0.478	-0.060
	p	0.539	0.587	0.624	*0.045	0.813
Hip Flexion	r	-0.404	-0.108	-0.199	-0.120	0.042
	p	0.078	0.652	0.428	0.635	0.867
Hip Abd/Add (max)	r	0.076	-0.020	0.034	-0.006	0.190
	p	0.751	0.934	0.894	0.981	0.449
Hip Rotation (max)	r	-0.067	0.376	0.002	-0.015	-0.231
	p	0.781	0.103	0.993	0.954	0.357
Hip Rotation (min)	r	-0.360	0.306	0.246	0.146	-0.398
	p	0.118	0.190	0.324	0.564	0.102
Kn Ext Mom	r	-0.464	-0.071	0.413	-0.336	-0.415
	p	*0.045	0.771	0.089	0.159	0.087
Kn. Varus Mom (max)	r	-0.083	-0.029	-0.484	0.014	0.242
	p	0.737	0.905	*0.042	0.956	0.334
Kn. Valgus Mom (min)	r	0.208	-0.008	-0.540	0.367	0.416
	p	0.392	0.973	*0.021	0.122	0.086
Kn. Rot. Mom (min)	r	0.204	0.217	0.392	-0.175	-0.271
	p	0.401	0.373	0.107	0.475	0.276
Kn. Rot. Mom (max)	r	-0.287	-0.175	0.172	-0.171	-0.190
	p	0.233	0.475	0.494	0.483	0.450

Table 26. Correlation coefficients between hamstring strength variables and jump landing kinematics and kinetics. Values were calculated during the absorption phase of the menses test session.

		HS Isometric	Concentric 60°/s	Eccentric 60°/s	Concentric 300°/s	Eccentric 300°/s
Knee Flexion (max)	r	-0.170	0.176	0.274	-0.212	-0.071
	p	0.486	0.458	0.242	0.369	0.767
Knee Valgus (min)	r	-0.138	-0.094	-0.191	-0.150	-0.232
	p	0.573	0.695	0.420	0.529	0.326
Knee Rotation (min)	r	-0.382	0.216	0.190	0.166	0.060
	p	0.106	0.360	0.420	0.485	0.803
Knee Rotation (max)	r	-0.261	0.006	-0.035	-0.006	-0.219
	p	0.280	0.979	0.882	0.981	0.354
Hip Flexion	r	-0.133	-0.030	-0.145	0.313	0.124
	p	0.586	0.899	0.542	0.179	0.601
Hip Abd/Add (max)	r	0.215	-0.012	0.142	-0.085	-0.082
	p	0.377	0.960	0.551	0.721	0.730
Hip Rotation (max)	r	-0.117	-0.462	-0.440	-0.092	0.001
	p	0.632	*0.040	0.052	0.699	0.997
Hip Rotation (min)	r	-0.159	-0.402	-0.329	-0.115	0.074
	p	0.514	0.079	0.156	0.628	0.756
Kn Ext Mom	r	-0.259	0.378	0.551	0.169	0.254
	p	0.284	0.111	*0.015	0.488	0.294
Kn. Varus Mom (max)	r	-0.057	-0.220	-0.328	-0.309	-0.449
	p	0.815	0.365	0.170	0.198	0.054
Kn. Valgus Mom (min)	r	0.284	-0.202	-0.649	0.026	-0.374
	p	0.239	0.365	0.003	0.915	0.114
Kn. Rot. Mom (min)	r	-0.011	0.258	0.247	0.133	0.281
	p	0.965	0.286	0.309	0.586	0.244
Kn. Rot. Mom (max)	r	-0.209	0.032	0.456	-0.324	0.033
	p	0.389	0.895	*0.050	0.176	0.892

*$P \leq 0.05$

Table 27. Correlation coefficients between hamstring neuromechanical variables and jump landing kinematics and kinetics. Values were calculated during the absorption phase of the ovulation test session.

		Knee Laxity	Active Hamstring MTS	T50%	RFP 200	RFP T50%
Knee Flexion (max)	r	0.034	0.281	0.330	-0.044	0.110
	p	0.887	0.230	0.181	0.860	0.663
Knee Valgus (min)	r	-0.024	0.012	0.072	0.411	0.189
	p	0.921	0.960	0.777	0.081	0.452
Knee Rotation (min)	r	0.130	0.059	0.262	-0.147	-0.261
	p	0.585	0.806	0.293	0.549	0.295
Knee Rotation (max)	r	0.111	0.237	0.215	-0.021	-0.194
	p	0.641	0.315	0.392	0.549	0.442
Hip Flexion	r	-0.122	-0.430	-0.102	0.081	-0.251
	p	0.609	0.058	0.686	0.741	0.315
Hip Abd/Add (max)	r	-0.324	-0.378	-0.294	-0.185	-0.158
	p	0.163	0.100	0.236	0.449	0.531
Hip Rotation (max)	r	-0.160	-0.130	-0.125	-0.319	-0.079
	p	0.502	0.586	0.622	0.183	0.754
Hip Rotation (min)	r	-0.139	0.144	0.073	-0.133	-0.193
	p	0.559	0.544	0.775	0.588	0.443
Kn Ext Mom	r	0.125	-0.002	0.271	-0.600	-0.532
	p	0.611	0.995	0.276	*0.008	0.023
Kn. Varus Mom (max)	r	0.142	-0.052	-0.296	0.283	0.355
	p	0.563	0.831	0.233	0.255	0.148
Kn. Valgus Mom (min)	r	0.254	0.197	-0.035	0.345	0.381
	p	0.295	0.420	0.891	0.161	0.119
Kn. Rot. Mom (min)	r	-0.284	-0.144	-0.228	0.157	0.150
	p	0.238	0.557	0.363	0.534	0.553
Kn. Rot. Mom (max)	r	-0.304	-0.301	-0.123	0.030	-0.091
	p	0.205	0.210	0.627	0.905	0.718

Table 28. Correlation coefficients between hamstring strength variables and jump landing kinematics and kinetics. Values were calculated during the absorption phase of the ovulation test session.

		HS Isometric	Concentric 60°/s	Eccentric 60°/s	Concentric 300°/s	Eccentric 300°/s
Knee Flexion (max)	r	0.264	-0.028	0.079	-0.192	-0.194
	p	0.290	0.907	0.740	0.417	0.412
Knee Valgus (min)	r	0.354	0.165	0.107	0.310	0.279
	p	0.150	0.488	0.653	0.183	0.233
Knee Rotation (min)	r	-0.201	-0.164	-0.207	-0.103	0.021
	p	0.424	0.489	0.380	0.666	0.930
Knee Rotation (max)	r	-0.066	-0.189	-0.233	-0.167	-0.087
	p	0.794	0.426	0.322	0.481	0.716
Hip Flexion	r	-0.214	0.218	0.114	0.398	0.475
	p	0.395	0.356	0.632	0.082	*0.035
Hip Abd/Add (max)	r	-0.483	0.069	0.015	-0.05	-0.024
	p	0.042	0.771	0.951	0.819	0.920
Hip Rotation (max)	r	-0.236	-0.166	-0.318	-0.029	-0.220
	p	0.346	0.486	0.172	0.903	0.351
Hip Rotation (min)	r	-0.219	-0.114	-0.124	-0.224	-0.328
	p	0.382	0.632	0.603	0.342	0.158
Kn Ext Mom	r	-0.381	-0.138	-0.217	0.092	0.199
	p	0.118	0.573	0.372	0.709	0.415
Kn. Varus Mom (max)	r	0.102	0.118	0.182	-0.046	0.045
	p	0.686	0.630	0.457	0.851	0.856
Kn. Valgus Mom (min)	r	0.318	0.321	0.297	0.030	0.134
	p	0.199	0.180	0.216	0.901	0.585
Kn. Rot. Mom (min)	r	0.056	-0.440	-0.389	-0.418	-0.468
	p	0.825	0.060	0.100	0.075	*0.043
Kn. Rot. Mom (max)	r	-0.036	-0.021	0.023	0.147	0.008
	p	0.886	0.931	0.925	0.547	0.973

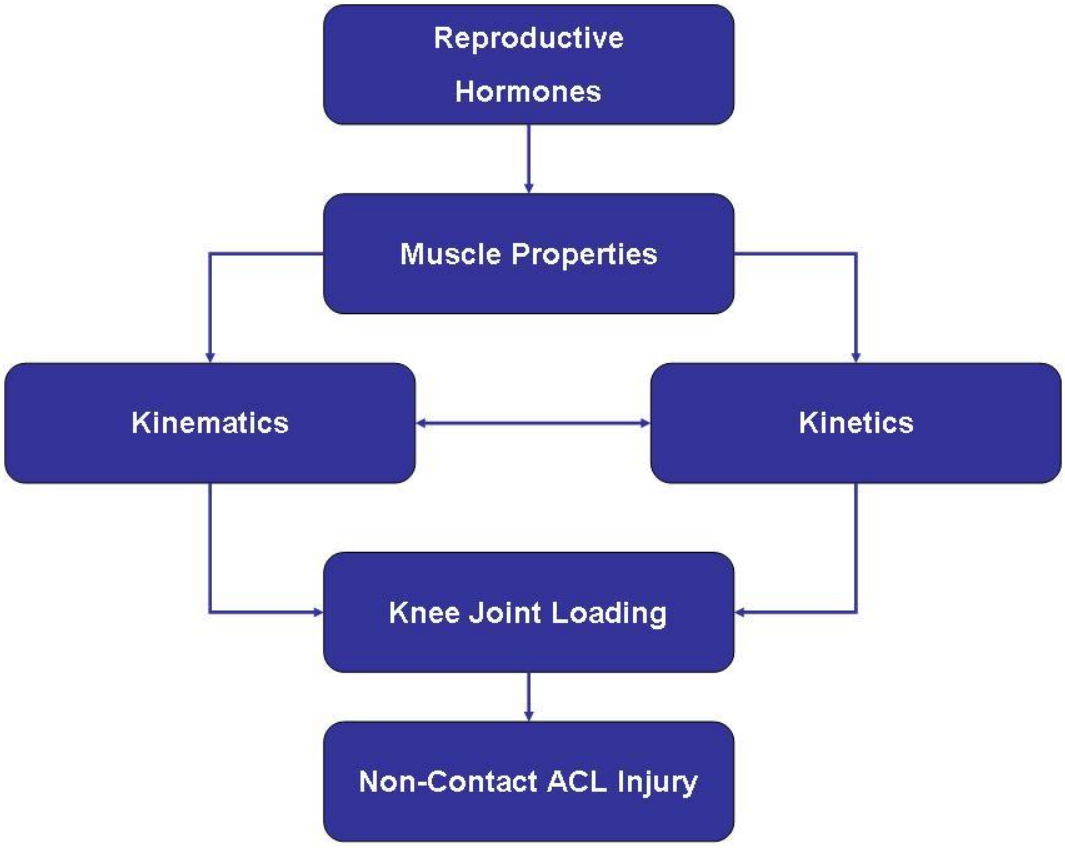

Figure 1. Theoretical Model between reproductive hormones and non-contact ACL injury.

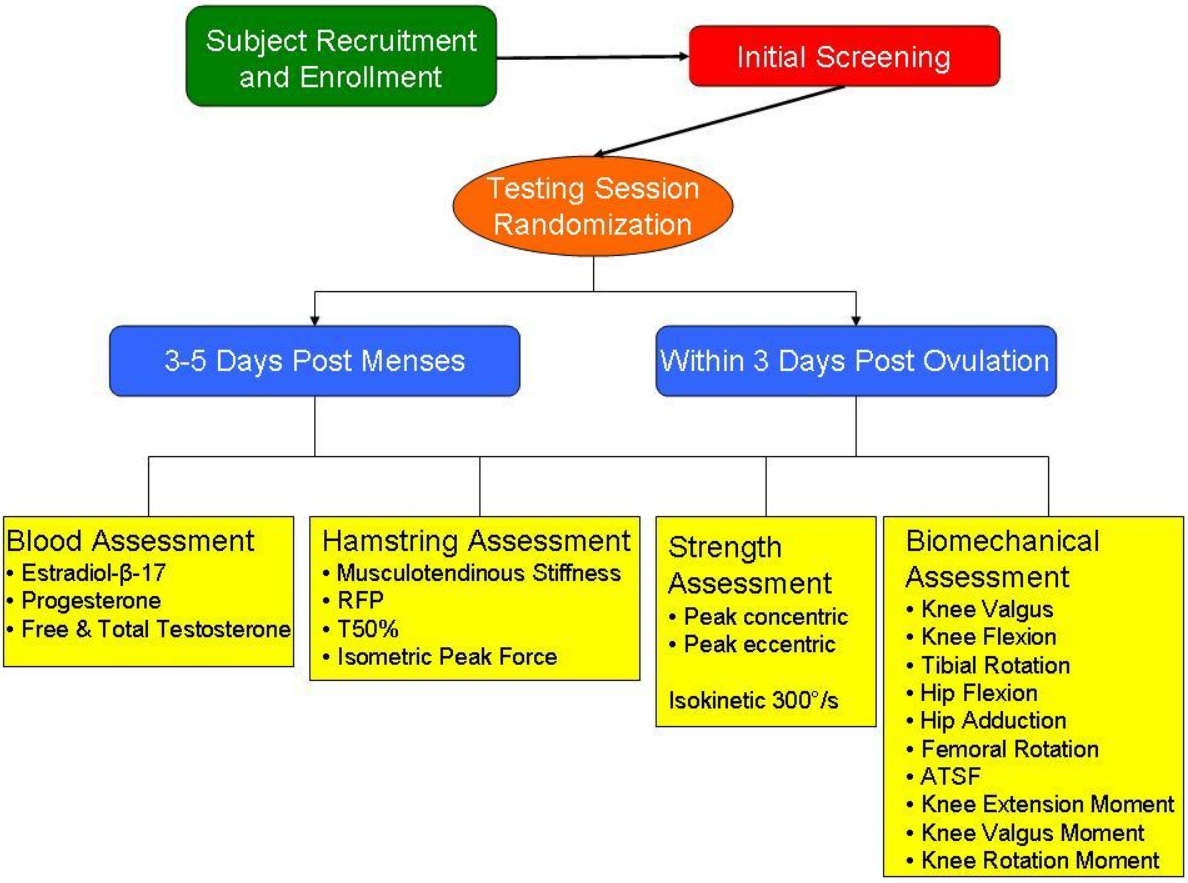

Figure 2. Flow chart depicting the testing procedures.

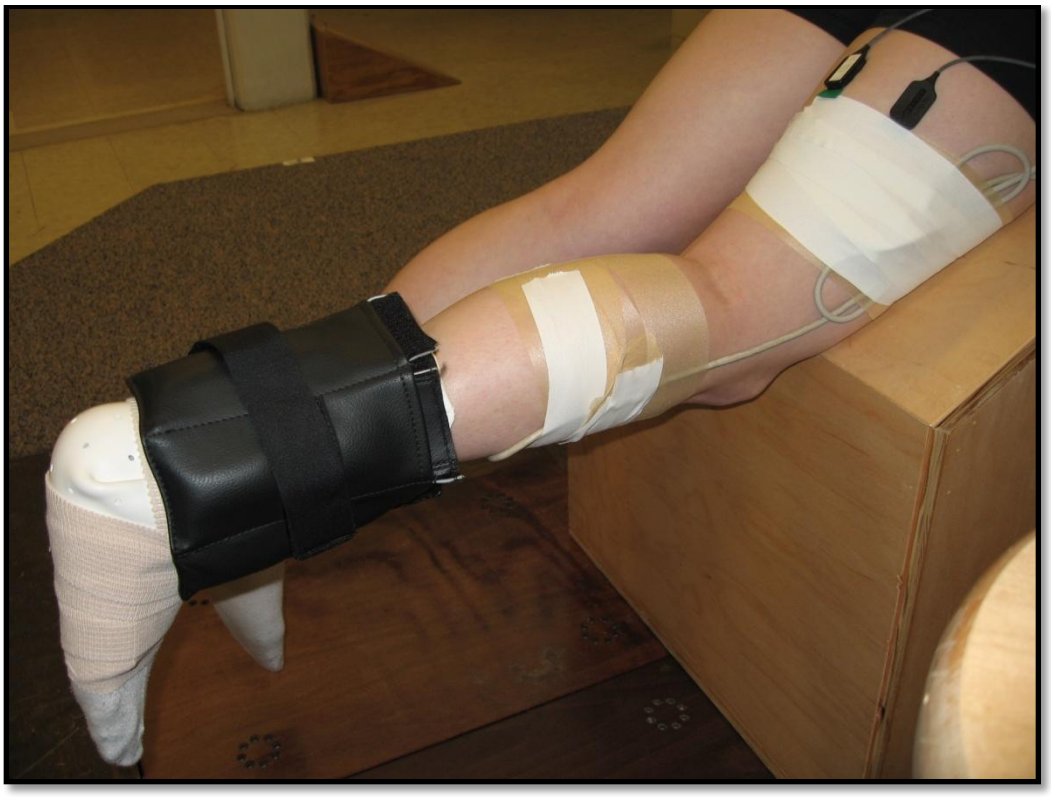

Figure 3. Subject positioning during the hamstring musculotendinous stiffness assessment.

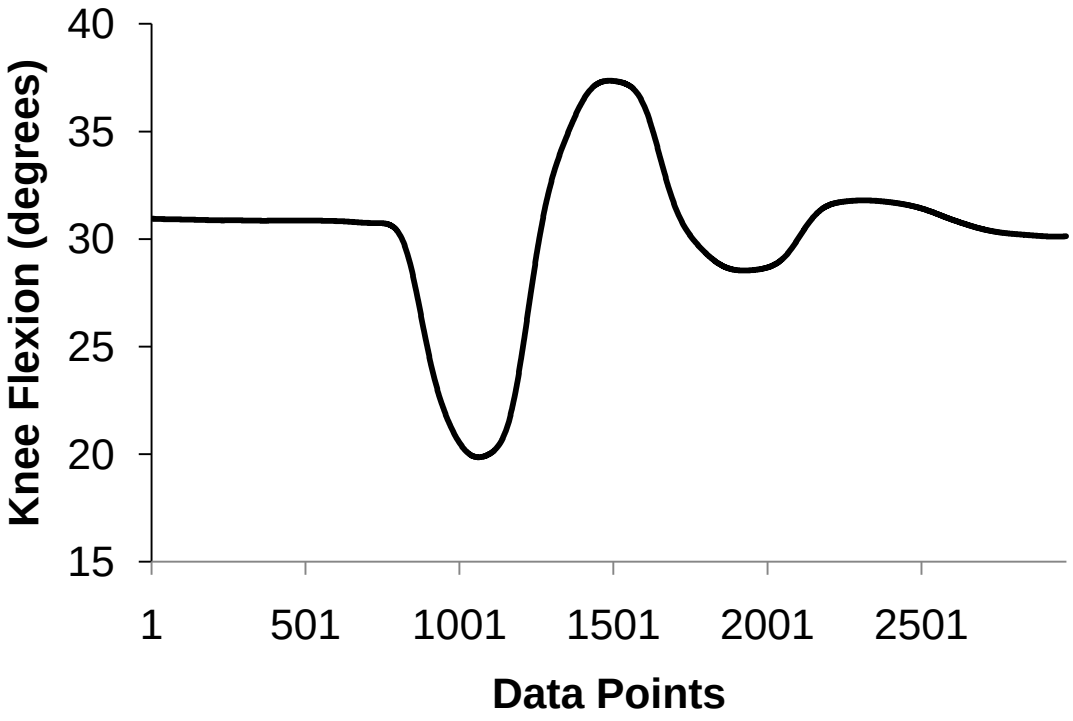

Figure 4. Knee Flexion trace following perturbation.

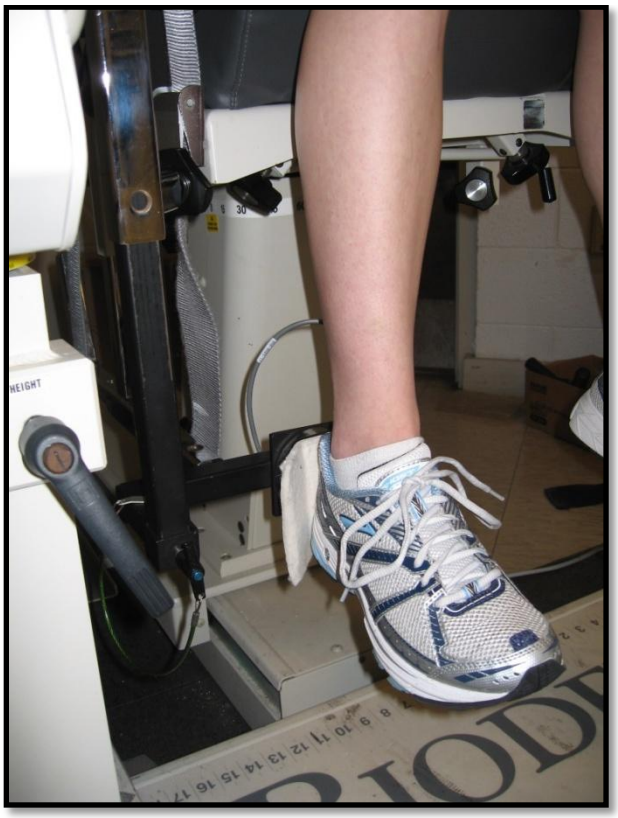

Figure 5. Subject set-up for hamstring neuromechanical assessment.

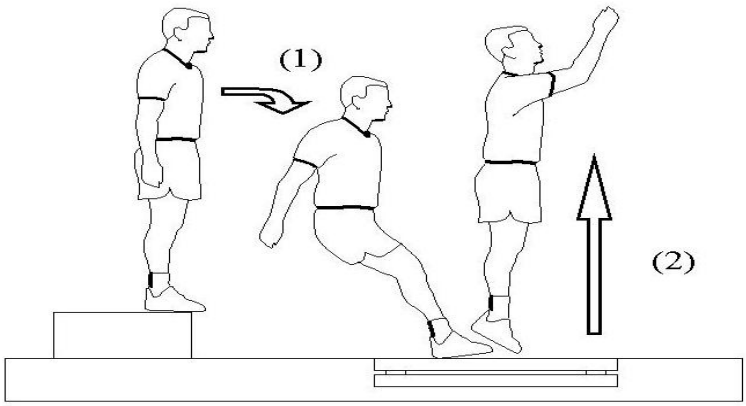

Figure 6. Diagram of the jump landing task.

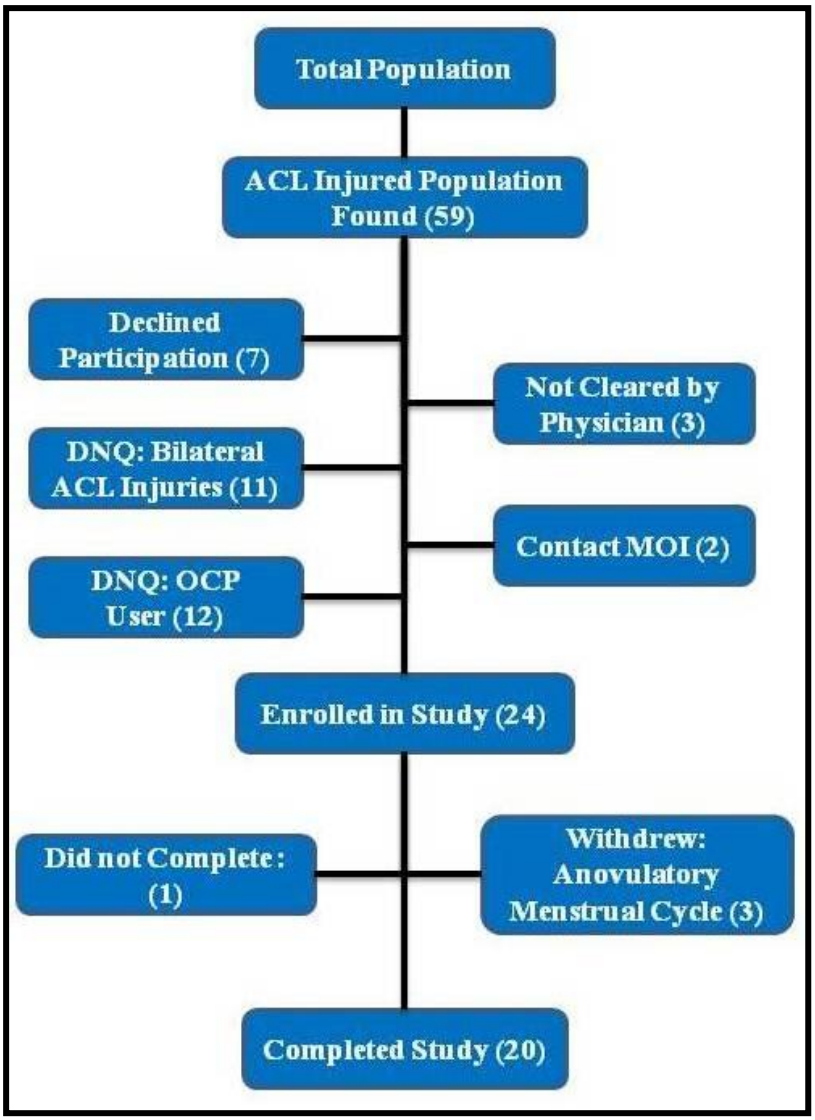

Figure 7. Flow chart depicting the subject enrollment and screening process

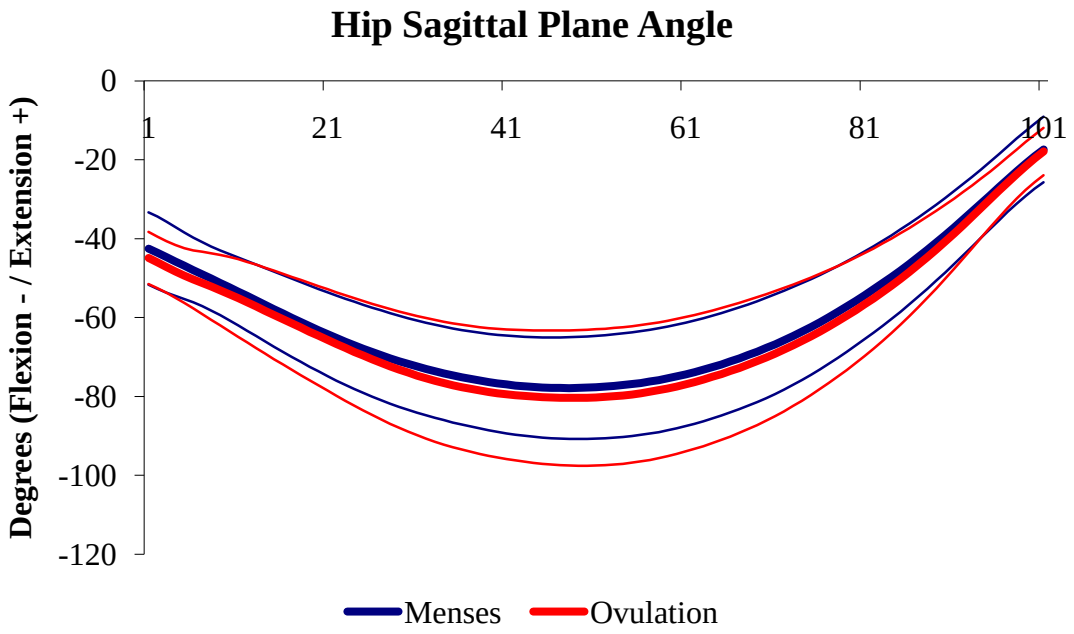

Figure 8. Hip sagittal plane angle normalized over the landing phase of the jump landing. The landing phase of the jump was from initial contact to toe off.

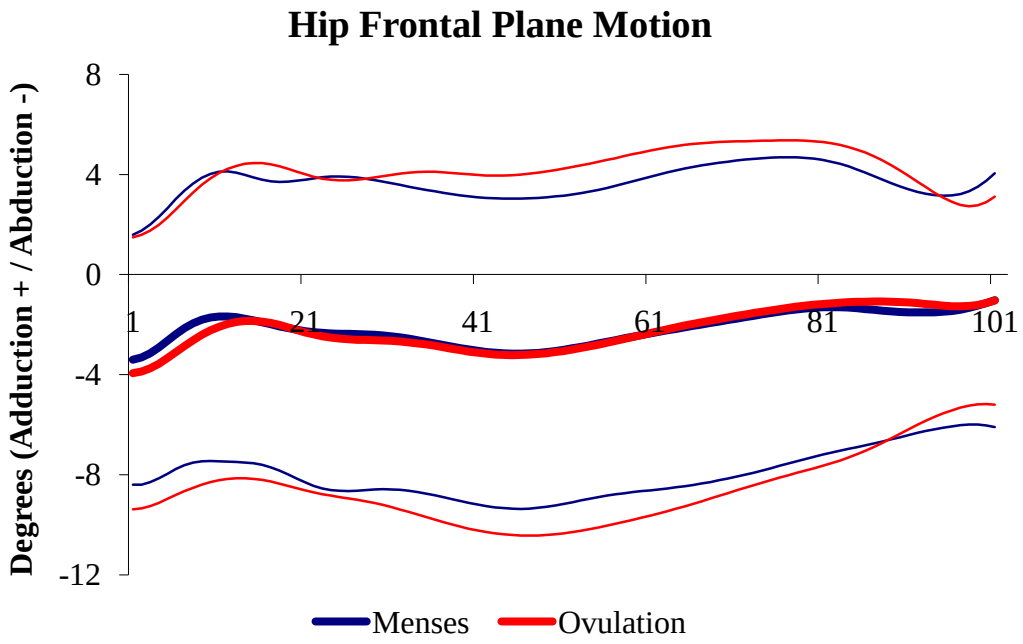

Figure 9. Hip frontal plane angle normalized over the landing phase of the jump landing. The landing phase of the jump was from initial contact to toe off.

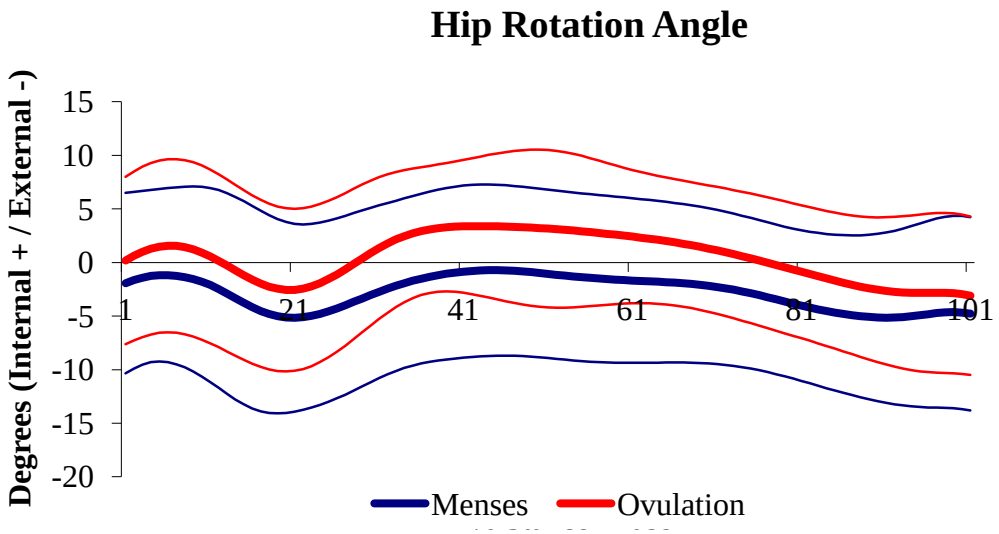

Figure 10. Hip rotation angle normalized over the landing phase of the jump landing. The landing phase of the jump was from initial contact to toe off.

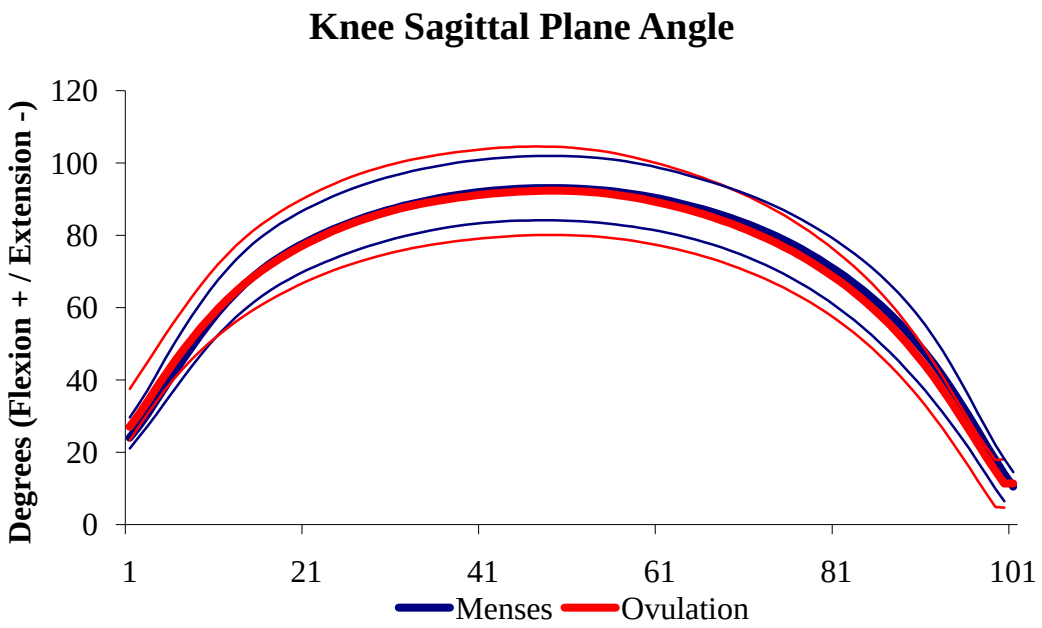

Figure 11. Knee sagittal plane angle normalized over the landing phase of the jump landing. The landing phase of the jump was from initial contact to toe off.

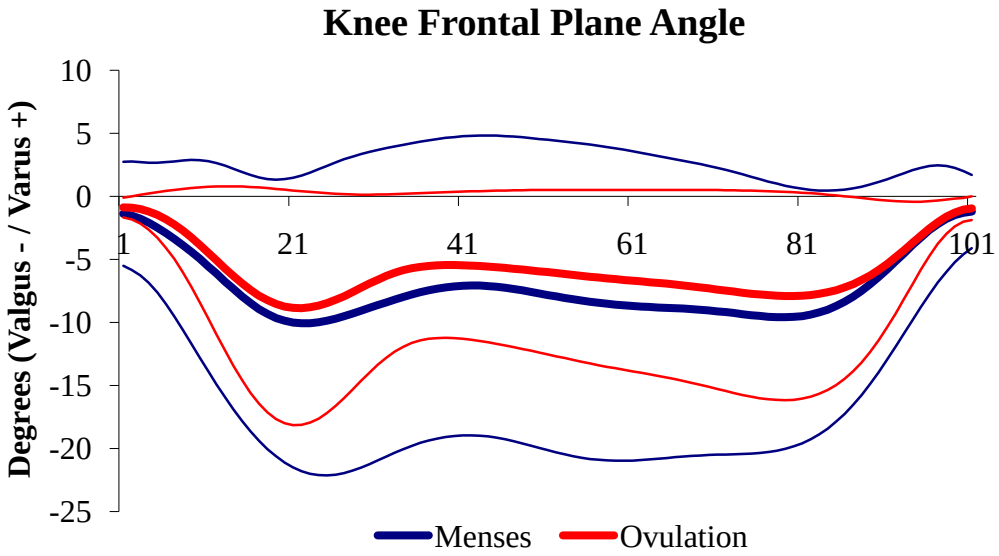

Figure 12. Knee frontal plane angle normalized over the landing phase of the jump landing. The landing phase of the jump was from initial contact to toe off.

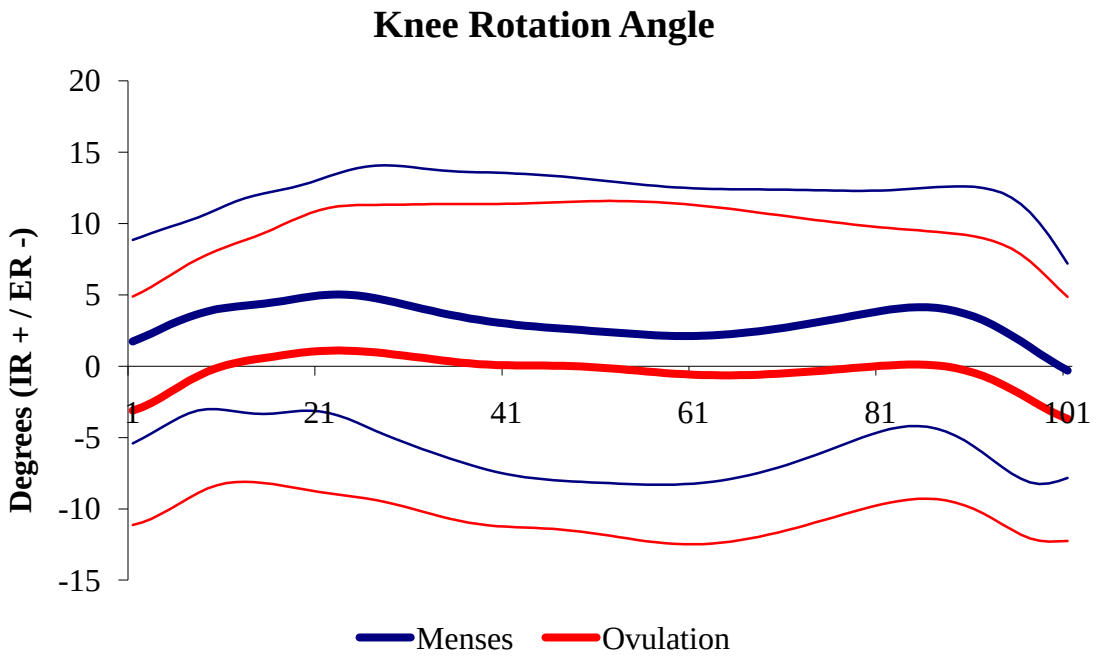

Figure 13. Knee rotational plane angle normalized over the landing phase of the jump landing. The landing phase of the jump was from initial contact to toe off.

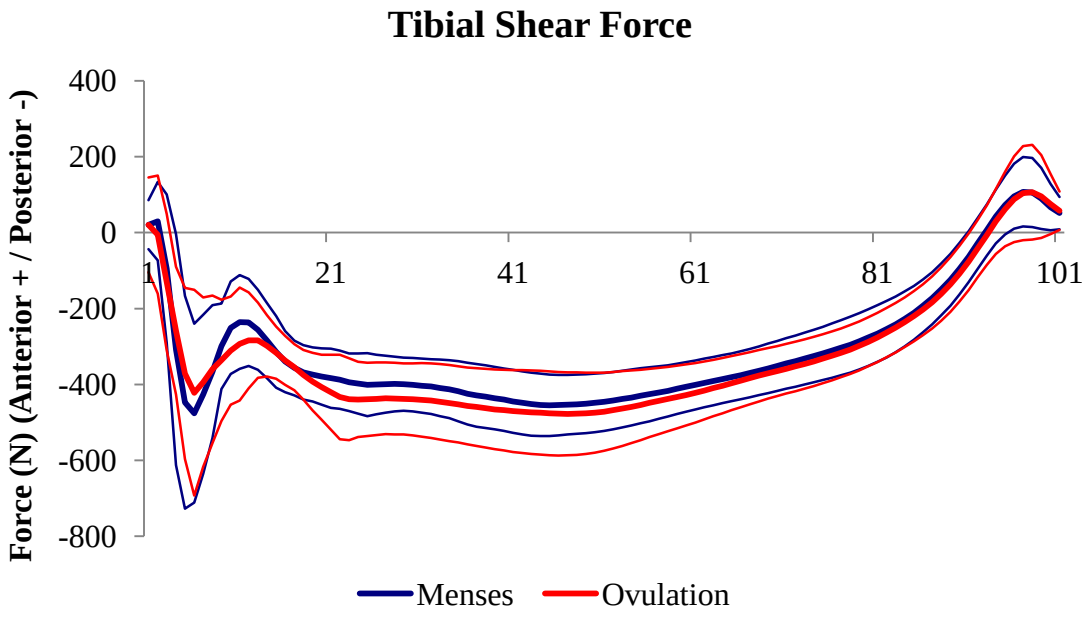

Figure 14. Tibial shear force normalized over the landing phase of the jump landing. The landing phase of the jump was from initial contact to toe off.

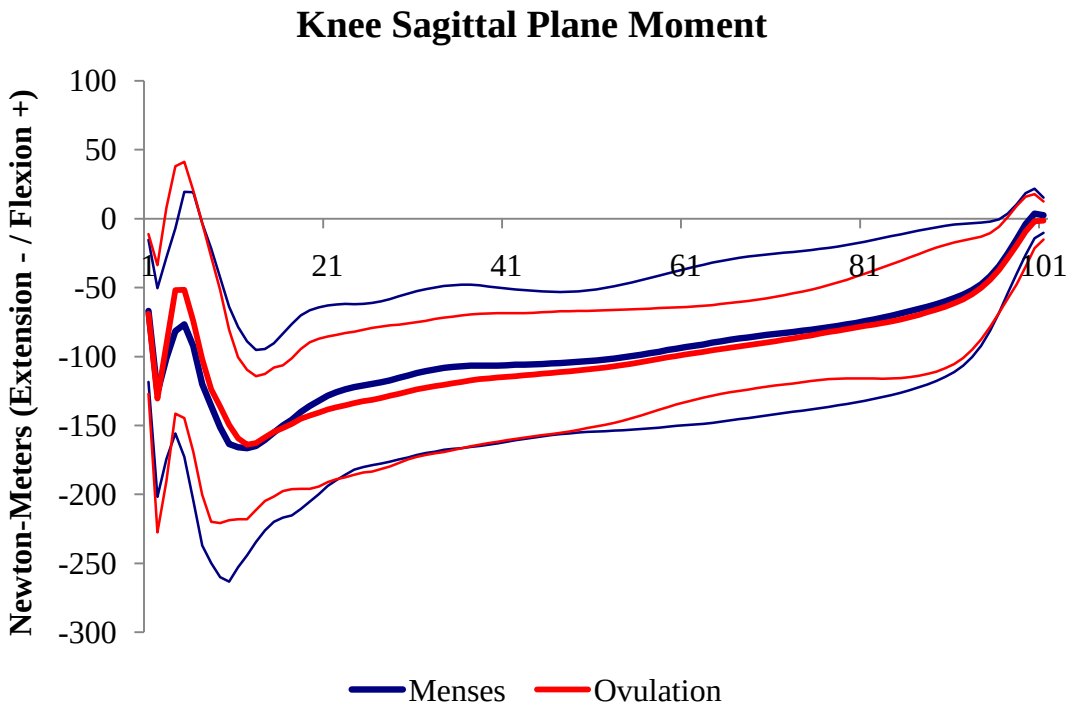

Figure 15. Knee sagittal plane moment normalized over the landing phase of the jump landing. The landing phase of the jump was from initial contact to toe off. Moments were assessed as internal moments.

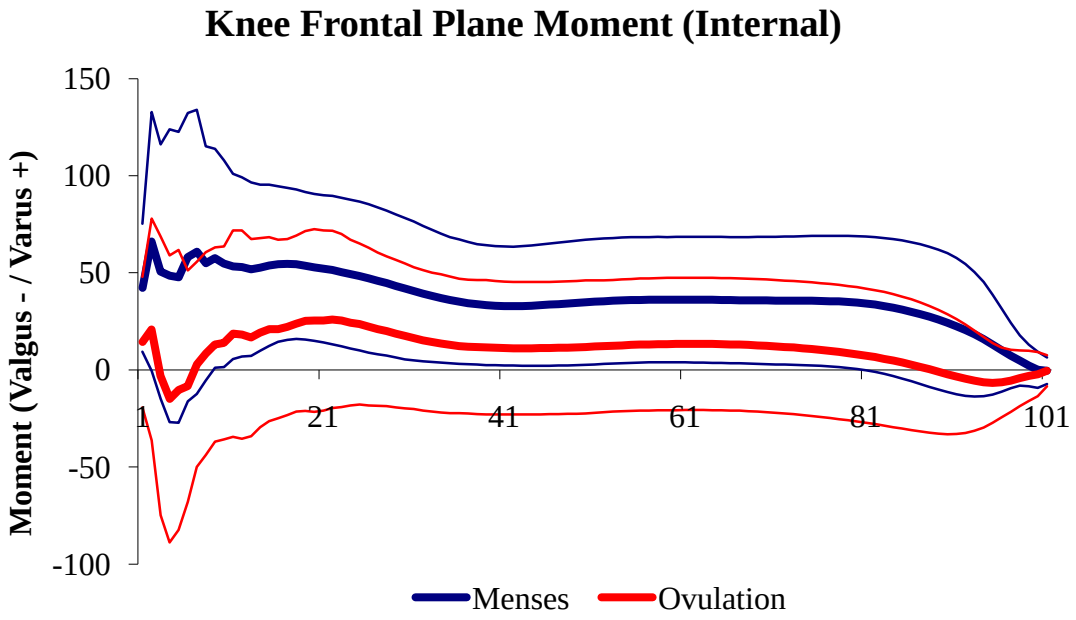

Figure 16. Knee frontal plane moment normalized over the landing phase of the jump landing. The landing phase of the jump was from initial contact to toe off. Moments were assessed as internal moments.

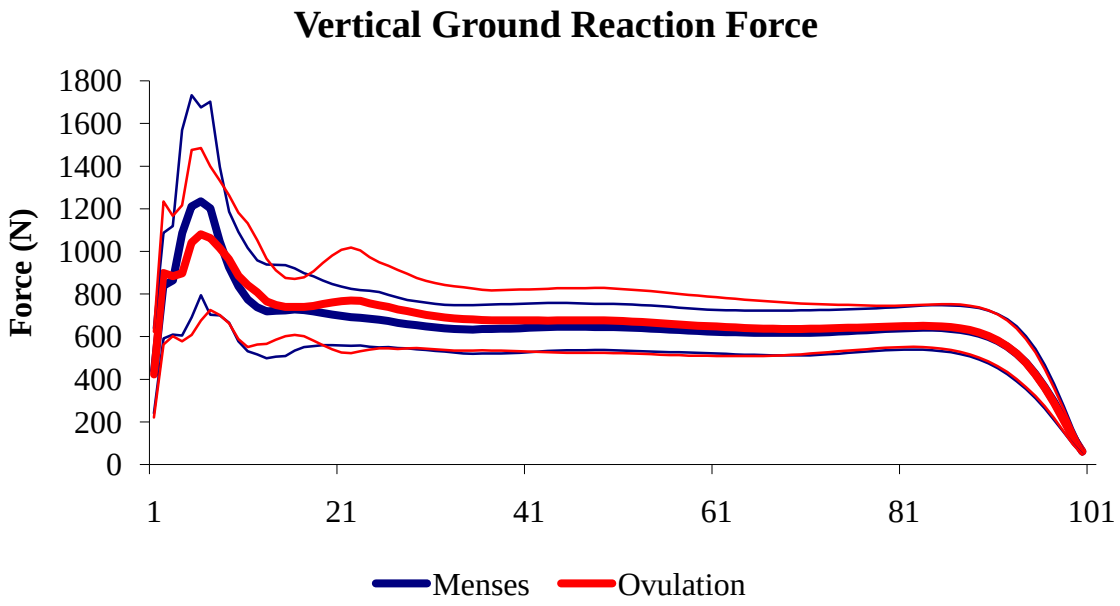

Figure 17. Vertical ground reaction force normalized over the landing phase of the jump landing. The landing phase of the jump was from initial contact to toe off.

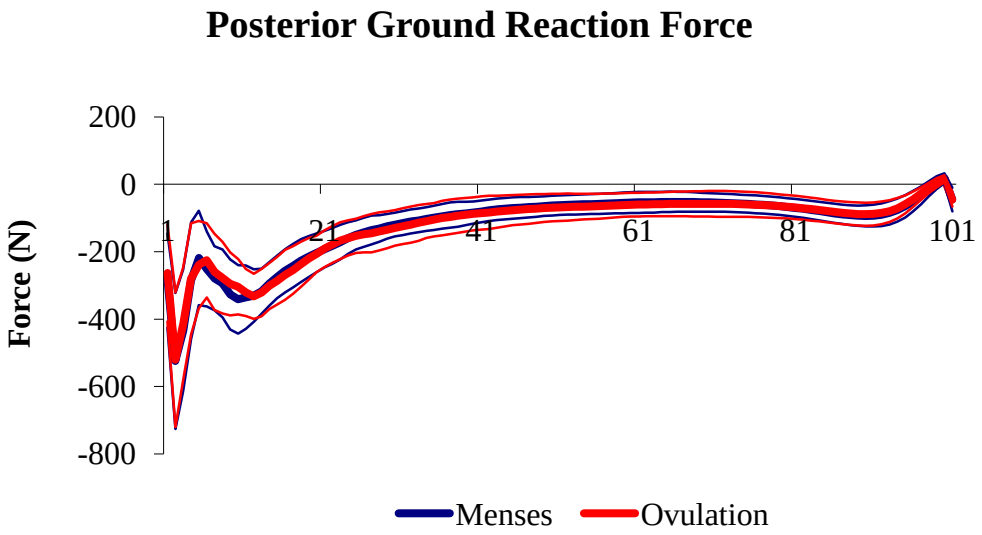

Figure 18. Anterior / Posterior ground reaction force normalized over the landing phase of the jump landing. The landing phase of the jump was from initial contact to toe off.

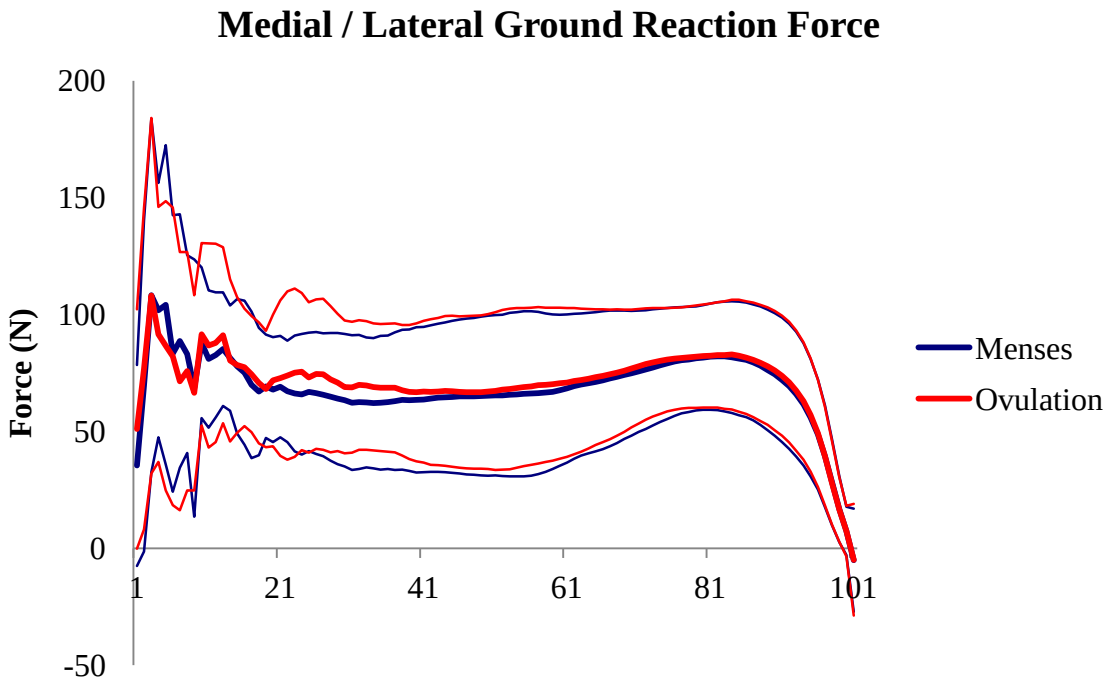

Figure 19. Medial / Lateral ground reaction force normalized over the landing phase of the jump landing. The landing phase of the jump was from initial contact to toe off.

APPENDIX A. MANUSCRIPT I

Manuscript I

Hamstring Neuromechanics Change Across the Menstrual Cycle in Females with a History of Anterior Cruciate Ligament Injury

(Journal of Athletic Training)

ABSTRACT

***Context*:** Previous research has been inconclusive in determining if hormonal fluctuations during the menstrual cycle influence neuromuscular properties. If hormones influence ACL injury risk by altering the properties of muscle tissue, then females that have suffered a non-contact ACL injury may be more sensitive to hormonal fluctuations than females who have not suffered an ACL injury.

***Objective*:** To determine if hamstring neuromuscular properties and knee laxity changed across the menstrual cycle in females with a previous history of unilateral non-contact ACL injury. A secondary purpose was to determine if there was a relationship between hamstring neuromuscular properties and reproductive hormone levels.

***Design*:** Cross-Sectional

***Setting*:** Research Laboratory

***Patients or Other Participants*:** Twenty females with a previous history of unilateral non-contact ACL injury (Height = 168.6 ± 5.3 cm, Mass = 66.2 ± 9.1 kg, Age = 19.6 ± 1.31 years) completed the study. The previously uninjured limb was used for testing and females were tested twice: 3-5 days after the onset of menses and within 3 days after a positive ovulation test using urine based ovulation predication kits. The testing session order was randomly assigned and the primary investigator was blind to menstrual cycle phase of the subjects at the time of testing.

***Intervention(s)*:** N/A

***Main Outcome Measures*:** Knee laxity (mm) assessed by a knee arthrometer and hamstring neuromuscular properties including knee flexor stiffness (Nm/rad), time to 50% peak force (ms), rate of force production (N/s), and isometric, concentric, and eccentric hamstring strength. Concentrations of estradiol-β-17, progesterone, and free testosterone.

***Results*:** Knee laxity (M: 6.64±1.38 mm, O: 7.34±1.32 mm, $P = 0.03$), knee flexor stiffness (M: 179.17±35.98 Nm/rad, O: 198.36±37.65 Nm/rad, $P = 0.04$), estradiol-β-17 (M: 31.12±13.72 pg/ml, O: 70.35±54.66 pg/ml, $P = 0.009$), and progesterone (M: 0.51±0.25 ng/ml, O: 3.92±4.24 ng/ml, $P = 0.003$) increased at ovulation. Hamstring strength, rate of force production, and free testosterone did not change across the menstrual cycle ($P > 0.05$).

***Conclusions*:** Increased knee flexor stiffness at ovulation may be a compensatory mechanism to offset increased anterior knee laxity in an effort to increase knee joint stability. Neuromuscular variability across the menstrual cycle may increase the risk of non-contact ACL injury.

***Key Words*:** Muscle, estrogen, stiffness, progesterone

INTRODUCTION

Anterior Cruciate Ligament (ACL) rupture is a devastating injury that affects an estimated 1 in 3000 people per year[1] with the most recent data concluding that females are 1.5-4.6 times more likely to tear their ACL than males.[2] Once an athlete has sustained an ACL injury, they are 10.4 times more likely to suffer another injury regardless if it is the previously injured or uninjured limb.[3-5] Either rehabilitation is not successfully returning individuals to pre-injury levels or powerful intrinsic risk factors exist and elevate the risk of injury in some individuals. Given this information, there is a strong rationale for utilizing females with a history of non-contact ACL injury to research risk factors of subsequent injury. Previous research using this population has compared reconstructed limbs to healthy limbs in uninjured subjects in order to further our understanding of rehabilitation protocols. However, using subjects with a history of unilateral ACL injury and testing their healthy limb may provide insight into pre-injury status of the injured limb.

ACL injury risk is not equal across the menstrual cycle with the preovulatory phase of the menstrual cycle identified as the time point when non-contact ACL injury is most likely to occur.[6] Therefore, the absolute levels of reproductive hormones (estrogen, progesterone, and testosterone) are theorized intrinsic risk factors for non-contact ACL injury. Reproductive hormones influence the mechanical properties of the ACL.[7-9] Estrogen receptors have been found on the female ACL[10] and skeletal muscle,[11] and research has demonstrated a positive relationship between hormone concentration and ACL laxity.[12] Greater generalized joint laxity has been linked to increased ACL injury[13] and increases in knee laxity have been linked to increases in joint loading during activities associated with non-contact ACL injury.[14]

Variables related to knee joint stability are of great significance to ACL injury prevention. Muscle stiffness is one of those variables and is defined as the ratio of change in muscular force to its change in length.[15, 16] Greater muscle stiffness equates to decreased joint distraction, thus muscle stiffness is important to joint stability by limiting joint distraction which is necessary to prevent injuries.[17] Muscle and tendon both have estrogen receptors [11, 18] and thus could be sensitive to hormonal fluctuations and change across the menstrual cycle.[19] Hormonal driven decreases in hamstring stiffness could influence dynamic joint stability. Previous research investigating the influence of sex hormones on muscle stiffness is limited to investigating females without prior history of ACL injury.[19, 20] We hypothesize that females with a prior history of ACL injury may be sensitive to changes in sex hormones, thus stiffness in these individuals may be modified over the menstrual cycle.

Previous research has identified gender differences in hamstring strength as well as how quickly the hamstrings can produce force. Females produce force at lower rates (rate of force production) and take longer to reach a standardized force which may influence dynamic knee support (time to 50% peak force).[21] Additionally, previous work has observed positive relationships between these properties and testosterone and negative correlations with estrogen.[22] However, hamstring neuromechanical properties were found to not change across the menstrual cycle.[23] This study has the same limitation previously mentioned in that all subjects were healthy females. Reproductive hormones influence on hamstring neuromechanical and strength properties are still unclear.

Therefore, the purpose of this investigation was to determine if knee flexor stiffness, neuromechanical properties, hamstring and quadriceps EMG, and knee laxity change across the menstrual cycle. A secondary purpose of this investigation was to determine the

relationship between hamstring neuromechanical properties, knee laxity, and absolute hormonal levels at each phase of the menstrual cycle. Our general hypotheses were that hamstring neuromechanical properties would change in ways that would increase ACL injury risk at the ovulation testing session when estrogen concentration is greatest. Based on previous studies, we also hypothesized that estrogen and progesterone would be negatively correlated with muscle properties while testosterone would have a positive correlation with muscle properties.

METHODOLOGY

Experimental Design

A repeated measures study design was used to determine if hamstring neuromechanical and strength properties changed across the menstrual cycle. Subjects were tested (1) 3-5 days after the onset of menses and (2) within 3 days following a positive ovulation test. These time points were selected as they correspond with low levels of estrogen and progesterone (menses) and high levels of estrogen and low levels of progesterone (ovulation).

Participants

Twenty-four participants were recruited to participate in this research study with twenty subjects (height = 168.6 ± 5.3 cm, mass = 66.2 ± 9.1 kg, age = 19.6 ± 1.31 years) successfully completing the testing protocol. All subjects read and signed an informed consent agreement approved by the University's Institutional Review Board prior to participation. To be included in the study, participants had to satisfy the following criteria: 18-25 years of age, no history of pregnancy or neurological disorder, self reported normal menstrual cycle, no oral contraception use six months prior to testing, no oral contraception

use at the time of non-contact ACL injury, sustained a unilateral classic non-contact ACL defined as "forces applied to the knee at the time of injury resulted from the athlete's own movements and did not involve contact with another athlete or object",[2] and had been cleared by a physician to return to sport participation. Participants described their injury history, mechanism, sport, and surgical repair method to the primary investigator (DRB). All dependent variables were assessed on the limb with no ACL injury.

Procedures

Once it was determined that a participant met the inclusion criteria, she tracked her menstrual cycle and contacted a member of research team after the onset of menses or after a positive ovulation test. The menses data collection session was scheduled 3-5 days after the onset of menses[22] with the onset of menses being Day 1. For the ovulation test session, participants contacted a member of research team when a positive ovulation test was detected using a urine based ovulation detection kit (Earth's Magic, Cary, NC). Test phases (menses and ovulation) were counterbalanced to avoid an order effect with the first test session being at menses in 11 participants and at ovulation in 9 participants. Subjects were tested during the same time of day for each session and were instructed not to eat 2 hours prior to testing or exercise on the day of testing. The primary investigator was blinded to the menstrual cycle phase of each subject at the time of testing. Testing sessions were identical except background information was collected during the first test session including: height, age, month and year when ACL injury occurred, dominant leg, ACL injured leg, type of graft used to repair ACL, and identification of other knee structures damaged during injury (e.g. meniscus, MCL, etc).

Blood Sampling

Each subject had a venous blood specimen analyzed for select reproductive hormone levels. Specimens were obtained by veni-puncture from a vein located in the cubital fossa using a 3 cc syringe with a 23 gauge needle (1 inch length). The veni-puncture procedure was performed by a nationally certified phlebotomist (ACH) using standard clinical procedures.

Blood hormone levels (estrogen, progesterone, free testosterone) were assessed using radioimmunoassay procedures by an experienced researcher. Assay quality control steps and procedures were instituted.[24] Blood was immediately transferred to a Vacutainer tube® containing EDTA as an anti-coagulant and immediately placed on ice. Blood sample tubes were centrifuged at 3000 x g 4^{o}C until plasma was separated, which was stored at -80^{o}C until hormonal analysis was performed. Plasma specimens were analyzed for estradiol-β-17, progesterone, and free testosterone concentration using a solid-phase, single antibody radioimmunoassay procedures (Siemens Medical, Los Angeles, CA). All assay samples were processed in duplicate and quality control procedures as recommended in the literature were utilized.[24] Total estrogens in adult women are comprised primarily of estrone, estriol, estradiol-β-17 and their conjugates, with estradiol-β-17 being the major component, which is why it was analyzed in this study.[25] Along with concentrations of each hormone, an estrogen to progesterone ratio was calculated.

Knee Laxity Assessment

Knee laxity was defined as the amount of anterior tibial displacement resulting from an anterior drawer force of 133 N, using a KT-1000 knee arthrometer (MEDmetric Corp, San Diego, CA).[12] Subjects were positioned supine with the knee in 25 degrees of flexion. The subject's ankles were then placed in a cradle and a thigh strap was added to control rotation of the thighs. The KT-1000 was placed on the anterior shank, aligned with the joint line, and

secured to the lower limb using Velcro straps. Two practice trials were used to ensure that the subject was relaxed and the KT-1000 was secured properly. Then 5 trials were recorded and averaged. Good intra-rater reliability was established prior to data collection (intrasession: ICC [2,k] = 0.98, SEM = 0.43 mm; intersession: ICC [2,k] = 0.88, SEM = 0.40 mm).

Knee Flexor Stiffness Assessment

Knee flexor stiffness was assessed by measuring the damping effect caused by the knee flexors after a perturbation.[16] Electromyography (EMG) sensors were used to monitor potential changes in muscle activation levels between sessions as well as before and after the perturbation. Stiffness is correlated with muscle activation level, so EMG verified that changes in muscle stiffness were not due to changing muscle amplitude levels. EMG sensors (Ag/AgCl) were placed on the test leg over the medial and lateral hamstrings and quadriceps in parallel to the muscle fibers. The hamstring electrodes were placed 50% of the distance between the greater trochanter and knee joint line. The medial and lateral quadriceps EMG sensors were placed over the bellies of the vastus lateralis (VL) and vastus medialis (VM). The electrodes for the quadriceps were placed over the VL, approximately 10 cm superior and 7 cm lateral to the superior border of the patella oriented at 10 degrees to the vertical.[26] For the VMO the electrode was placed approximately 4 cm superior and 3 cm medial to the superomedial border of the patella oriented at a 55 degree angle.[26] A reference electrode was placed on the lateral malleolus. Participants were positioned prone on a plinth with an extension affixed to the end. The extension supported the thighs but permitted knee motion by allowing the shanks to hang freely. Electromagnetic sensors (Flock-of-birds, Ascension Technologies, Inc., Burlington, VT) were placed on the participant's proximal tibia and

lateral thigh to measure oscillatory motion of the knee following an applied perturbation. The medial and lateral epicondyles were digitized to define the knee joint center. A load equal to 10% body mass was secured at the ankle (cuff weights) and subjects were instructed to support the shank parallel to the floor via isometric hamstring contraction. The investigator applied a perturbation to the calcaneous, forcing the knee into slight extension and initiating oscillatory knee flexion/extension. This oscillatory motion was captured by measuring knee flexion and extension about the Y-axis using the electromagnetic tracking system. Knee flexor stiffness was estimated from the damped frequency of oscillation.[27] Three successful trials were recorded at each weight with success defined as a clear oscillatory pattern in the knee motion.[27] Knee flexor stiffness was calculated using the formula $k = 4\pi^2 mr^2 f^2$, where k was rotational stiffness, m was the summed mass of the foot and shank segment [28] and the applied load, r was the distance from the lateral joint line to the lateral malleolus (m), and f was the damped frequency of oscillation calculated as $f = (1/t_2\text{-}t_1)$. Knee flexor stiffness values were normalized to body mass and this procedure has been shown to have adequate intra-session reliability ($ICC_{2,1}$ = 0.70, SEM = 28.83 N·m/rad).[27]

Hamstring Neuromechanical Assessment

A series of tests assessed hamstring neuromechanical / force properties.[29] The following variables were calculated from a maximal hamstring isometric contraction: 1) time to produce 50% peak force (T50%), 2) rate of force production (RFP) over the first 200 ms after onset of contraction (RFP 200), 3) RFP to the T50% time point (RFP T50%), and 4) isometric peak force. Participants were positioned in a seated position with the knee and hip flexed to 90°. The foot was fixed to a load cell (Honeywell Sensotec, Columbus, OH) and participants were instructed to relax and wait for a visual light stimulus. When the light

stimulus was presented, the participant contracted the hamstrings as forcefully and quickly as possible against the load cell. Participants maintained the contraction for 3-5 seconds and five trials were recorded.

T50% was measured in ms and calculated as the time between the onset of force and the instant at which 50% peak force was achieved. Force onset was determined using computer algorithms that have been previously established.[29] The threshold to determine the onset of force was 5% of the peak hamstring force. Peak force was defined as the largest force output measured by the load cell during the test period. RFP 200 was calculated during the first 200 ms after the onset of force using the load cell and was calculated as the slope of the line created by the load cell output. The equation used was: m (N/s) = (X2-X1)/(Y2-Y1), where X1 was equal to the load cell 200 ms after the onset of perturbation, X1 was the load cell value at 5% peak hamstring force, and (Y2-Y1) was equal to .2s. This procedure was repeated for the RFP T50% time point except the time value used in the equation was T50%. The slope equation assumes a linear relationship between the onset of force production and the second time point and R^2 values were calculated quantify this relationship.

Peak concentric and eccentric isokinetic hamstring force was assessed using previously described methods that have demonstrated high reliability.[30, 31] Participants were seated on an isokinetic dynamometer (Biodex System 3 Pro isokinetic dynamometer, Biodex Medical Systems, Shirley, NY) with their hip flexed to 85° and knee flexed to 90°. Testing was performed at 60 and 300 °/s through 90° of motion (0-90° of knee flexion). A warm-up, consisting of 5 submaximal knee flexion/extensions was performed. Testing for the 60°/s condition consisted of 5 concentric and 5 eccentric isokinetic hamstring contractions with peak torque measured in Newton-meters (Nm). Testing for the 300 °/s consisted of 10

concentric and 10 eccentric isokinetic hamstring contractions with peak torque measured in Newton-meters (Nm). Sampling of the Biodex was the highest sampling rate allowed by the commercial software (60 Hz). Biodex data were corrected for gravity correction.

Data Sampling and Reduction

EMG and load cell data were collected at 1,440 Hz and electromagnetic sensor data was collected at 144 Hz using the Motion Monitor motion capture software (Innovative Sports Training, Chicago, IL). Knee flexion data were filtered using a 4th order, zero-phase-lag Butterworth low-pass filter at 14.5 Hz.[32] EMG data were corrected for DC bias, bandpass (20-350 Hz) and notch (59.5-60.5 Hz) filtered (4th order, zero-phase-lag, Butterworth), and smoothed using a 20 ms root-mean-square sliding window function. Load cell and Biodex data were low pass filtered at 10 Hz (4th order, zero-phase-lag Butterworth filter). Add data were filtered and reduced using customized MATLAB software (Mathworks, Natick, MA, v7.0)

Statistical Analysis

All data analyses were performed using SPSS version 17.0 (SPSS, Inc., Chicago, IL) and statistical significance was set with an *a priori* alpha level of 0.05. Separate paired t-tests were performed with menstrual cycle phase as the within-subject factor (menses vs. ovulation). Muscle strength was examined using a repeated measures ANOVA with type of contraction (isometric, concentric 60°/s, concentric 300°/s, eccentric 60°/s, and eccentric 300°/s) and phase (menses vs. ovulation) as within subjects factors. Separate repeated measures ANOVAs were used to examine hamstring and quadriceps EMG activity with phase (menses vs. ovulation), time (pre or post perturbation, and side (medial vs. lateral) used as within subject variables. A Tukey's post-hoc analysis was performed when necessary.

Bivariate correlation coefficients were calculated between reproductive hormone levels and each hamstring property within each time point (menses and ovulation) as well as the change scores. Change scores were calculated but subtracting the menses testing session value from the ovulation testing session values (ovulation – menses).

RESULTS

Three of the twenty four participants did not complete the study because of anovulatory cycles and one of the twenty four voluntarily withdrew because of scheduling conflicts. Twenty females completed the study (Height = 168.6 ± 5.3 cm, Mass = 66.2 ± 9.1 kg, Age = 19.6 ± 1.31 years). Body mass did not change between the two testing sessions (Menses: 66.2±9.1 kg, Ovulation: 66.1±9.1 kg, $t_{(19)}$=0.32, P = 0.70). Concentrations of estradiol-β-17 (M: 31.12±13.72 pg/ml, O: 70.35±54.66 pg/ml, P = 0.009) and progesterone (M: 0.51±0.25 ng/ml, O: 3.92±4.24 ng/ml, P = 0.003) increased at ovulation but free testosterone did not change across the menstrual cycle (M: 0.80±0.26 ng/ml, O: 0.86±0.22 ng/ml, P =0.41).

Knee Laxity and Hamstring Musculotendinous Stiffness Assessments

Knee laxity increased at ovulation compared to menses ($t_{(19)}$ = -2.33, P = 0.03, effect size = 0.51). During the knee flexor stiffness assessment, 4 trials were identified as outliers in the menses test session data due to equipment malfunction. For the individuals identified as having bad data, means were calculated from the two usable trials. An increase in knee flexor stiffness was observed at ovulation ($t_{(19)}$ = -2.31, P = 0.03, effect size = 0.54). This relationship remained after stiffness values were normalized to body mass ($t_{(19)}$ = -2.25, P = 0.03, effect size = 0.65). To verify that this change was not due to muscle activation, the average EMG amplitude was examined from the medial and lateral quadriceps and

hamstrings. The average EMG amplitude was calculated 200 ms prior to the onset of the perturbation (pre-perturbation) and from the onset of the perturbation to the second peak in the oscillatory flexion/extension motion (post-perturbation). We examined the average EMG amplitude using a variety of different methods including: the average of each muscle individually, average group (medial and lateral musculature averaged together), as well as co-activation ratio (average hamstring activity divided into average quadriceps activity). Means and standard deviations for each of these variables were similar at menses and ovulation (table 3).

For the quadriceps, a main effect for test was observed indicating that quadriceps activation increased from pre to post perturbation but this increase was only 1% MVIC (Pre: 3.71 ± 1.32 , Post: 4.77 ± 2.44, $F_{(1,16)} = 54.18$, $P < 0.001$) and no main effect was observed for menstrual phase ($F_{(1,16)} = 0.409$, $P = 0.532$) and no test by side by phase interaction was observed ($F_{(1,16)} = 1.890$, $P = 0.188$). For the hamstrings, a main effect for test was observed indicating that hamstring activation increased from pre to post perturbation (Pre: 37.22 ± 16.01, Post: 44.06 ± 18.46, $F_{(1,16)} = 63.87$, $P < 0.001$) but no main effect was observed for menstrual phase ($F_{(1,16)} = 0.736$, $P = 0.404$) nor a test by side by phase interaction was observed ($F_{(1,16)} = 1.46$, $P = 0.248$). We observed no change in any of the muscle activation levels across the menstrual phase for pre perturbation average quadriceps ($t_{(19)} = 0.74$, $P = 0.47$), average hamstrings ($t_{(19)}=0.94$, $P = 0.36$), co-activation ratio ($t_{(19)}=-0.21$, $P = 0.84$) or post-perturbation average quadriceps ($t_{(19)} = 0.53$, $P = 0.60$), average hamstrings ($t_{(19)} = 0.76$, $P = 0.46$), co-activation ratio ($t_{(19)} = -0.19$, $P = 0.85$). The perturbation applied by the primary investigator to the posterior aspect of the heel was not different between sessions ($t_{(19)} = 0.28$, $P = 0.78$).

Hamstring Strength and Force Production Assessments

Two subjects had unusable load cell data due to operator error and were removed from the isometric hamstring contraction as well as the RFP and T50% measures. The RM ANOVA for strength revealed no contraction type by menstrual phase interaction ($F_{(4,14)}$ = 0.439, P = 0.778) or main effect for menstrual phase ($F_{(1,17)}$ = 0.004, P = 0.95) but it did reveal a significant main effect for contraction type ($F_{(4,14)}$ = 3.419, P = 0.038). The Tukey post hoc revealed the isometric hamstring contraction was greater than the concentric contraction at 60°/s and 300°/s and eccentric 300°/s (table 4).

Force production capabilities did not change across menstrual phase (table 5). RFP over the first 200ms tended to be different between phases ($t_{(17)}$=1.84, P = 0.08, effect size = 0.24) with a decrease in RFP during the ovulation testing phase. However, no trend was observed in RFP to T50% ($t_{(17)}$=0.87, P = 0.40) nor in T50% peak force ($t_{(17)}$=-0.72, P = 0.48).

Bivariate Correlations

Correlation coefficients and p-values can be found in tables 7, 8, and 9. At the menses test session, no significant correlations were observed between estrogen or free testosterone and hamstring neuromechanical properties (P > 0.05). A significant correlation was observed between progesterone and T50% at the menses test session (r = 0.75, P < .001). This finding supports our hypothesis that greater amounts of progesterone are associated with slower time to reach a standardized force which could have implications related to knee joint stability. Similar relationships were seen between progesterone and RFP 200 (r = -0.31, P = 0.19) and RFP T50% (r = -0.31, P = 0.22) although not statistically significant. Interestingly, progesterone tended to have the largest negative correlations and was the most consistent in

regards to the expected direction of the three hormones assessed in this study. The correlation coefficients related to estrogen and free testosterone at menses were not consistent. In regards to the expected relationships and the magnitudes of the coefficients were much smaller than expected and do not support our hypothesis. Finally, we revealed significant relationships between the E:P ratio and T50% (r = -0.49, *P* = 0.04), RFP 200 (r = 0.58, *P* = 0.01), and RFP T50% (r = 0.64, *P* = 0.005).

At ovulation a negative correlation was found between estrogen and knee flexor stiffness (r = -0.46, *P* = 0.05). Higher concentrations of estrogen were associated with lower levels of knee flexor stiffness which supports our hypothesis. A significant correlation was also observed between progesterone and T50% (r = -0.50, *P* = 0.04). This finding was contrary to our hypothesis and indicated that higher levels of progesterone were associated with shorter T50%. A shorter T50% would theoretically be advantageous for knee stability. We observed no significant relationships when change scores were analyzed (*P* > 0.05). Finally we observed a significant correlation between T50% and the E:P ratio (r = 0.55, *P* = 0.02).

DISCUSSION

The aim of this investigation was to test females with a history of non-contact ACL injury and examine knee laxity and hamstring neuromechanical properties across the menstrual cycle while simultaneously measuring blood hormone levels. Our findings demonstrate that knee laxity and knee flexor stiffness increase at ovulation. Previous research examining the changes in knee laxity across the menstrual cycle in healthy females have found mixed results with some researchers concluding that knee laxity changes across

the menstrual cycle while others do not.[12, 33, 34] Muscle stiffness has also been investigated in this population with similar equivocal results.[19, 20]

However, previous research has utilized healthy females with no history of non-contact ACL injury. Our study is unique in that it uses a population of females that have suffered a non-contact ACL injury. Utilizing an injured population may increase effect sizes associated with these findings and increase the likelihood of finding changes across the menstrual cycle. Theoretically, females who suffer an ACL injury may be highly susceptible to hormonal fluctuations across the menstrual cycle. The most significant finding of this study is that this group of unilaterally ACL injured females demonstrated increased knee laxity as measured by a knee arthrometer as well as increased knee flexor stiffness at ovulation.

Knee Laxity

Knee laxity increased at ovulation which corresponded to an increase in estrodiol-β-17. This finding supports our hypothesis and agrees with previous literature demonstrating an increase in knee laxity values when estrogen levels were greater.[12, 34-38] Hormonal levels of estrogen, progesterone, and testosterone have been shown to predict the amount of knee laxity in a non-injured, female population.[38] Shultz et al[38] observed that the minimal level of estrogen and progesterone as well as the peak concentrations of estrogen and testosterone predicted 57.6% of the variance in knee laxity change scores. This demonstrates the complex relationship between hormones and tissue. Explanations as to how reproductive hormones influence knee laxity are becoming more clear with a variety of studies demonstrating that estrogen reduces ACL stiffness[39] as well as load to failure in animal models.[9] The mechanisms that are most likely influenced by estrogen are either direct or indirect

mechanisms.[7, 9, 10, 40] Youshida et al[40] reported that estrogen could directly influence the biological properties of the proximal and middle portions of the ACL. Specifically, estrogen influenced proteins in the extra cellular matrix which consisted of type 1 collagen fibers and collagen has been correlated with mechanical strength.[40, 41] However, estrogen can also influence fibroblast metabolism which could influence ACL strength through the remodeling process.[40] The values of estrogen, progesterone, and testosterone were similar to previously reported values.[12, 19, 38, 42]

Our knee laxity values were approximately 1.5 mm greater than previously reported values.[37] This is an interesting finding considering the population utilized in this study has a history of ACL injury. Greater generalized joint laxity has previously been noted in an ACL injured population.[43] Uhorchak et al[13] compared 24 ACL injured military cadets and found that the cadets that went on to suffer an ACL injury had greater generalized joint laxity and also had knee laxity measures more than 1 standard deviation greater than cadets with no ACL injury. Increased joint laxity may be one of the potential explanations for the high injury rate in this population. This evidence suggests that increased joint laxity is present in our population and is exacerbated at ovulation.

The average magnitude of change in knee laxity across the menstrual cycle was 0.7mm of displacement which is a 10% increase in knee laxity. This change in knee laxity is similar to values reported in previous investigations.[12] Shultz et al[12] reported the average magnitude of knee laxity change across the menstrual cycle was 3.2 mm. Our values are well within this reported range and are associated with a moderate effect size of 0.51 which indicates that these findings are clinically relevant. We were able to remove a threat of internal validity by blinding of the PI to menstrual cycle phase. Future research should

including blinding of the PI and include a control group to determine if these changes are of equal magnitude with similar methodology.

Knee Flexor Stiffness

Knee flexor stiffness increased at ovulation which did not support our hypothesis. This hypothesis was based on previous research that observed decreases in vertical leg stiffness at ovulation.[19] Vertical leg stiffness is an assessment of the stiffness behavior of the entire lower extremity during functional tasks. Although it can be debated whether these measurements of leg stiffness truly record mechanical stiffness,[44, 45] research illustrates that leg stiffness is attributed to the active muscle stiffness of the controlling joints thereby influencing biomechanical stability. We measured knee flexor stiffness, which has been shown to have no relationship with leg stiffness measured during functional tasks.[46] Thus, the discrepancy between our findings of increased knee flexor stiffness at ovulation and previous research may be due to differences in stiffness measurement.

Our findings also disagree with previous investigations using a similar technique to assess active knee flexor stiffness.[20] Bell et al[20] used a similar oscillatory methodology and found no significant change in knee flexor stiffness across the menstrual cycle in healthy females. They had a small sample size (n=8), did not blind their investigators, and all females entered the study in the menses phase of the menstrual cycle. A learning effect or unintentional bias could have influenced their results and potentially explains the differences between our findings. However, our inclusion criteria of females with a history of unilateral ACL injury is still the most compelling reason for the differences between these similar studies. Future research is needed in both populations (healthy and unilateral injured) before definitive conclusions can be reached.

We hypothesize increased knee flexor stiffness at ovulation may be a mechanism to maintain knee joint stability in response to increased anterior knee laxity. Increased knee flexor stiffness may be possible through a neurologic pathway that exists between the ACL and the hamstrings.[47] The reflexive arc described by Solomonow et al[47] was initiated by placing traction on a load wire attached to a cat ACL. They observed increased EMG activity of the hamstrings after creating tensile stress on the ACL. It is plausible that this reflexive arc increased knee flexor stiffness via muscle activation during the ovulation testing phase, however, there are several problems with this explanation. We assessed hamstring and quadriceps EMG and observed no change across the menstrual cycle. This relationship could have been masked by averaging EMG activity over a large period of time. Also, we did not assess ACL loading in this study but it seems unlikely that the ACL would have been loaded tremendously under our testing conditions. It is not clear if our testing paradigm would have resulted in similar ACL loading. Future research is needed to determine if increases in knee flexor stiffness are caused by these reflexive factors or hormonal fluctuations.

Hamstring Strength

Hamstring strength properties did not change across the menstrual cycle. We hypothesized that muscle strength would decrease at ovulation. Our strength findings were consistent with previous investigations examining muscle strength across the menstrual cycle.[42, 48, 49] Friden et al[48] assessed isokinetic quadriceps strength at 120°/s and observed no change in quadriceps strength across the menstrual cycle. These findings were supported by several other authors that examined quadriceps-to-hamstring ratio[42] or peak quadriceps or hamstring strength at 120°/s.[49] Our findings add to existing knowledge because we

examined hamstring strength in an ACL injured population at 60°/s and 300°/s. Our results indicate that hamstring force production did not change across the menstrual cycle at either speed. While hamstring strength may be important, as evidenced by the findings of Myer et al,[30] our testing protocol was very different as we assessed only hamstring force production and not the quadriceps to hamstring ratio. Future research should try and determine if the quadriceps to hamstring ratio changes across the menstrual cycle as this may be a more important risk factor for non-contact ACL injury that hamstring strength alone. Future research should utilize an electrically stimulated muscle contraction to ensure muscle is fully contracted.

Hamstring Force Production

We observed no difference in the hamstrings ability to produce force across the menstrual cycle. Hamstring RFP (200 and T50%) and T50% were not different across the menstrual cycle. These findings agree with previous research in healthy females that found similar force producing capabilities did not change across the menstrual cycle.[23] Our study adds to current literature by investigating a population with a history of unilateral ACL injury that may be more susceptible to hormonal fluctuations. It seems unlikely that absolute levels of reproductive hormones influence these variables to a great degree and do not change across the menstrual cycle.

Correlations

Estrogen and knee flexor stiffness had a negative correlation at ovulation. This relationship is most likely caused by the effect of estrogen on collagen synthesis within the muscle.[8, 50] Interestingly, this relationship disappeared when correlated with the E:P ratio. Histological examination of muscle is limited but we may be able to infer some information

from this research conducted in ligament. The ACL is an estrogen receptor dependent tissue, but progesterone may have a mitigating effect on estrogen.[40] This relationship also changes based on collagen type (1 vs. 3) and expression changes based on location within the ACL. Previous research by Yoshida et al[40] demonstrated that in the proximal ACL, estrogen negatively reduced the expression type 1 collagen while progesterone reduced expression of type 3 collagen. Thus, the E:P relationship or interaction is important to examine along with estrogen and progesterone individually in order to gain a full appreciation for the complex relationship between reproductive hormones and tissue. We observed this similar relationship when observing the E:P ratio which suggests that progesterone is mitigating the effect of estrogen and decreasing the influence on knee flexor stiffness.[40] We also observed a significant relationship between the E:P ratio and RFP 200 and RFP T50% at menses. However, these findings were coupled with non-significant findings when estrogen and progesterone were examined alone. The significant findings associated with the increased magnitude seem to indicate that muscle may be influenced differently by the interaction between estrogen and progesterone, with estrogen mitigating the role of progesterone in contractile variables (RFP). We can only extrapolate the ligamentous findings to the muscle findings. Future research should attempt to determine the interaction between estrogen and progesterone by testing more time points across the menstrual cycle and determining the histological function of these hormones as they relate to acute injury.

Finally, we observed no relationship between hamstring neuromechanical properties and free testosterone. Our data agree with previous research that indicates free testosterone is not a good predictor of hamstring neuromuscular properties.[23] In a previous investigation from our research laboratory, we observed no relationship between testosterone and T50% or

RFP in healthy females. However, we did observe a negative relationship between knee flexor stiffness and free testosterone but could not explain this finding because of the lack of histological research in the area.[22] We found no relationship between knee flexor stiffness and free testosterone indicating that, at least in these females, muscle may not be as sensitive to testosterone. This finding agrees with previous research that found female skeletal muscle to not be as sensitive to sex hormone status, specifically testosterone, as male skeletal muscle.[51]

Limitations

Several limitations exist in this study. The primary limitation is that we only tested two time points of the menstrual cycle. Ideally, we would test as many time points as possible, however, each testing session lasted one and a half hours in duration and testing more than a select few time points was not feasible. However, these methodological limitations are acceptable since this is a new population. The results of this study may not be generalizable to the larger population, but only applicable to females who meet the inclusion criteria. Additionally, our findings may have limited application to initial ACL injury due to potential changes after surgery and rehabilitation protocols. During the course of the investigation we found 21 of 59 potential subjects with a history of ACL injury qualified for enrollment (36%). Common reasons for study disqualification included oral contraception use (n=12, 20%) and bilateral ACL injury (n=11, 18%). While this may limit generalizability, we feel these inclusion criteria are a strength of this study because of increased homogeneity of our sample.

Summary

Our findings may help elucidate why females may be more at risk of non-contact ACL in the pre-ovulatory phase of the menstrual cycle.[6] Research examining the periodicity of non-contact ACL injury across the menstrual cycle tends to point to the pre-ovulatory phase of the menstrual cycle (menses through ovulation) as the time when risk of sustaining an injury is greatest.[52-56] We revealed females with a previous history of ACL injury have increased knee laxity at ovulation and decreased muscle stiffness at menses. Both of these time points are in the pre-ovulatory phase of the menstrual cycle[6] but have drastically different hormonal profiles and risk might change based on ligamentous or muscular contributions to joint stability.

Clinical Application

Clinicians should be aware of the high rate of secondary ACL injury in females. The changes observed in knee laxity and knee flexor stiffness might be a major underlying risk factor of non-contact ACL injury and partially explain the high rate of second injury. Both of these factors are related knee stability, thus, knee stability could be influenced at either menses or ovulation based on either ligamentous or knee flexor stiffness profile. While ligamentous laxity is not modifiable, knee flexor stiffness is via muscle activation.[57] These individuals should participate in injury prevention programs during and after the rehabilitation process and efforts should be undertaken to enhance knee flexor stiffness and increase knee stability as much as possible.

Table 1. Subject ACL history.

Subject Number	*ACL Injured Limb*	*Months From ACL Injury to Testing*	*Mechanism of Injury*	*Sport Participation during Injury*	*Type of Graft*
1	R	18	Landing	Gymnastics	Hamstring
2	R	52	Pivoting/Throwing	Softball	Patellar Tendon
3	L	42	Pivoting	Soccer	Patellar Tendon
4	R	16	Landing	Basketball	Hamstring
5	R	7	Cutting	Soccer	Patellar Tendon
6	R	34	Cutting	Soccer	Allograft
7	R	33	Cutting	Soccer	Allograft
8	L	12	Cutting	Flag Football	Patellar Tendon
9	R	45	Landing	Basketball	Patellar Tendon
10	L	17	Landing	Long Jumping	Patellar Tendon
11	L	29	Cutting	Soccer	Hamstring
12	L	14	Cutting	Field Hockey	N/A
13	L	24	Cutting	Flag Football	Allograft
14	R	26	Landing	Volleyball	Allograft
15	R	38	Cutting	Soccer	Patellar Tendon
16	L	17	Cutting	Soccer	Patellar Tendon
17	R	36	Tumbling/Landing	Gymnastics	Hamstring
18	R	13	Cutting	Handball	Hamstring
19	R	14	Cutting	Soccer	Hamstring
20	R	26	Cutting	Flag Football	Patellar Tendon

Table 2. Variables collected during the knee flexor stiffness assessment and knee laxity assessment. Values represent Mean ± Standard Deviation. Values are taken from each subject's healthy limb.

	Menses	**Ovulation**	**Menses 95% CI**		**Ovulation 95% CI**		**P**
			Upper	Lower	Upper	Lower	
Knee Flexor Stiffness (Nm/rad)	178.56±35.68	199.05±37.94	161.86	195.25	181.29	216.81	*0.03
Stiffness Normalized to Body Mass	2.71±0.46	3.01±0.43	2.49	2.92	2.81	3.22	*0.03
Perturbation Amplitude (°)	9.53±1.53	9.42±1.29	8.81	10.25	8.82	10.03	0.78
Knee Laxity (mm)	6.64±1.38	7.34±1.32	5.99	7.29	6.72	7.96	*0.03

Table 3. Average EMG for the medial and lateral quadriceps and hamstrings before and after the perturbation during the active stiffness protocol. Average quadriceps and hamstring values were calculated by averaging the medial and lateral muscle EMG values. Co-contraction ratios were calculated by dividing the hamstring average values into the quadriceps average values. Values are mean %MVIC ± standard deviation.

	Menses	**Ovulation**	**Menses 95% CI**		**Ovulation 95% CI**	
Pre-Perturbation			Lower	Upper	Lower	Upper
Medial Quadricep	2.30±1.32	2.42±1.44	1.62	2.98	1.67	3.16
Lateral Quadricep	5.57±4.74	4.50±2.26	3.14	8.01	3.37	5.70
Medial Hamstring	37.90±16.18	35.54±14.20	29.76	46.22	28.24	42.85
Lateral Hamstring	39.40±15.74	35.43±17.49	31.91	48.09	26.43	44.43
Average Quadriceps	3.94±2.99	3.47±1.77	2.40	5.48	2.57	4.39
Average Hamstrings	38.94±13.94	35.87±14.25	31.78	46.12	28.16	42.82
Co-activation Ratio	0.10±0.04	0.10±0.04	0.08	0.12	0.08	0.12
Post-Perturbation						
Medial Quadricep	3.15±1.41	3.22±1.94	2.42	3.87	2.22	4.22
Lateral Quadricep	6.74±4.65	5.96±2.96	4.35	9.13	4.44	7.49
Medial Hamstring	43.49±18.41	40.21±15.83	34.16	52.82	32.08	48.35
Lateral Hamstring	47.76±17.02	44.77±21.35	39.02	56.52	33.79	55.75
Average Quadriceps	4.94±2.93	4.59±2.26	3.44	6.45	3.43	5.76
Average Hamstrings	45.62±14.13	42.49±13.81	38.85	52.13	33.85	51.13
Co-activation Ratio	0.11±0.03	0.11±0.03	0.09	0.13	0.09	0.13

Table 4. Peak hamstring strength values. Values are mean ± standard deviation.

	Menses	Ovulation	Menses 95% CI		Ovulation 95% CI	
Isometric	115.37±47.05	112.83±36.11	91.97	138.77	94.87	130.79
Isokinetic, 60°/s						
Concentric	95.51±21.53	94.18±17.17	84.59	104.59	87.15	103.55
Eccentric	101.98±16.25	100.51±20.17	93.98	108.94	91.92	110.56
Isokinetic, 300°/s						
Concentric	93.66±23.82	91.38±18.98	83.15	104.91	83.91	100.91
Eccentric	100.67±16.43	96.98±14.81	93.39	108.39	90.66	104.34

Contraction by phase interaction, $P = 0.78$
Main effect for contraction, $P = 0.03$*
Main effect for phase, $P = 0.95$
*The Tukey Post Hoc revealed the isometric hamstring contraction was greater than the concentric contraction at 60°/s and 300°/s and eccentric 300°/s

Table 5. Hamstring force production variables. Values are mean ± standard deviation and are reported in N/s. The slope was calculated over the first 200ms after the onset of force production and to the time point of when 50% peak force was attained (T50%). R2 represents the linear relationship between the time points of interest. Slope was calculated using the equation m=(Y2-Y1)/(X2-X1), where Y was the force applied in Newtons and X was the defined time period in seconds.

	Menses	**Ovulation**	**Menses 95% CI**		**Ovulation 95% CI**		**P**
T50% (ms)	115.55±55.89	121.20±58.22	87.75	143.34	92.24	150.15	0.40
Slope							
200ms	255.59±143.57	221.61±101.99	184.20	326.99	170.89	272.33	0.08
T50%	333.28±221.29	304.22±174.05	223.24	443.33	217.66	390.77	0.39
R^2							
200ms	0.96±0.04	0.93±0.11					
T50%	0.99±0.01	0.98±0.03					

Table 6. Concentrations of reproductive hormones from the selected time points. Values are mean ± standard deviation and are reported in pg/ml.

	Menses	Ovulation	Menses 95% CI		Ovulation 95% CI		P
Estradiol-β-17 (pg/ml)	31.12 ± 13.72	70.35 ± 54.66	24.49	37.72	44.01	96.70	*0.009
Progesterone (ng/ml)	0.51 ± 0.25	3.92 ± 4.24	0.39	0.63	1.87	5.96	*0.003
Free Testosterone (ng/ml)	0.80 ± 0.26	0.86 ± 0.22	0.67	0.92	0.75	0.97	0.414

Table 7. Correlation coefficients between the hamstring muscle properties and reproductive hormone levels at menses.

		E	P	FT	E:P Ratio
Knee Laxity	r	0.228	-0.122	0.073	0.192
	p	0.333	0.609	0.761	0.418
MTS	r	-0.081	-0.242	0.267	-0.037
	p	0.733	0.303	0.255	0.877
MTS Norm	r	-0.053	-0.177	0.398	-0.110
	p	0.825	0.455	0.082	0.643
T50%	r	0.117	0.749	0.315	-0.487
	p	0.482	*<0.001	0.203	*0.040
RFP 200	r	0.053	-0.314	-0.082	0.575
	p	0.829	0.190	0.737	*0.010
RFP T50%	r	0.135	-0.313	0.026	0.636
	p	0.593	0.222	0.918	*0.005
Isometric HS	r	0.150	-0.026	-0.286	0.430
	p	0.539	0.916	0.222	0.066
Isokinetic at 60°/s					
Concentric	r	0.252	0.308	-0.286	-0.181
	p	0.284	0.187	0.222	0.444
Eccentric	r	0.189	0.288	0.028	-0.324
	p	0.424	0.219	0.908	0.164
Isokinetic at 300°/s					
Concentric	r	0.227	0.235	-0.318	-0.070
	p	0.336	0.318	0.172	0.770
Eccentric	r	0.217	0.350	-0.361	0.289
	p	0.358	0.130	0.118	0.216

*$P \leq 0.05$

Table 8. Correlation coefficients between the hamstring muscle properties and reproductive hormone levels at ovulation.

		E	P	FT	E:P Ratio
Knee Laxity	r	-0.200	-0.250	0.198	-0.026
	p	0.397	0.287	0.416	0.914
MTS	r	-0.455	0.053	0.071	-0.262
	p	*0.050	0.830	0.773	0.279
MTS Norm	r	-0.312	-0.048	0.071	-0.155
	p	0.194	0.845	0.773	0.527
T50%	r	0.049	-0.500	0.263	0.554
	p	0.851	*0.041	0.308	*0.021
RFP 200	r	0.148	0.127	-0.298	-0.177
	p	0.545	0.627	0.229	0.469
RFP T50%	r	0.086	0.077	-0.368	-0.183
	p	0.743	0.770	0.146	0.483
Isometric HS	r	0.053	-0.262	0.235	-0.074
	p	0.835	0.311	0.333	0.769
Isokinetic at 60°/s					
Concentric	r	0.310	0.024	0.069	0.200
	p	0.743	0.923	0.780	0.411
Eccentric	r	0.160	-0.111	0.070	0.193
	p	0.512	0.652	0.777	0.429
Isokinetic at 300°/s					
Concentric	r	0.160	0.249	-0.064	-0.211
	p	0.512	0.302	0.795	0.385
Eccentric	r	0.340	0.385	0.011	-0.179
	p	0.154	0.103	0.964	0.464

*$P \leq 0.05$

Table 9. Correlation coefficients between change scores of the hamstring muscle properties and reproductive hormone levels.

		E	P	FT	E:P Ratio
Knee Laxity	r	-0.150	-0.305	-0.103	0.128
	p	0.540	0.205	0.675	0.601
MTS	r	0.331	0.241	-0.015	0.152
	p	0.166	0.320	0.951	0.535
MTS Norm	r	-0.345	-0.194	0.071	-0.218
	p	0.148	0.426	0.772	0.369
T50%	r	0.265	-0.058	-0.047	0.309
	p	0.304	0.824	0.858	0.228
RFP 200	r	-0.074	-0.182	0.182	0.195
	p	0.777	0.485	0.485	0.454
RFP T50%	r	-0.058	-0.107	0.024	0.140
	p	0.826	0.681	0.928	0.591
Isometric HS	r	-0.175	-0.398	0.103	0.363
	p	0.501	0.113	0.695	0.152
Isokinetic at 60°/s					
Concentric	r	0.242	-0.024	-0.019	0.163
	p	0.319	0.922	0.939	0.504
Eccentric	r	0.065	-0.086	0.164	-0.025
	p	0.793	0.728	0.503	0.920
Isokinetic at 300°/s					
Concentric	r	0.279	0.004	-0.046	0.003
	p	0.247	0.988	0.850	0.989
Eccentric	r	0.082	0.321	0.347	-0.382
	p	0.739	0.180	0.146	0.106

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APPENDIX B. MANUSCRIPT II

Manuscript II

Jump Landing Biomechanics Change Across the Menstrual Cycle in Females with Previous ACL Injury

(Medicine & Science in Sports and Exercise)

Abstract (275 word limit)

Purpose: The purpose of this study was to examine three dimensional hip and knee kinematics and kinetics across the menstrual cycle in females with a history of unilateral ACL injury

Methods: Subjects were tested 3-5 days after the onset of menses and within 3 days following a positive ovulation. Subjects completed 5 trials of a jump landing task from a 30cm high box positioned 50% of body height from the edge of a force plate. Three dimensional hip and knee kinematics and kinetics were calculated at initial contact as well as peak during the absorption phase of the jump landing. Moments were calculated as internal moments. Blood hormone levels (Estradiol-β-17, Progesterone, Free Testosterone) were assessed using radioimmunoassay procedures.

Results: Estradiol-β-17 and Progesterone increased at ovulation. At initial contact, the following changes occurred at ovulation: tibia external rotation and decreased knee varus moment. During the absorption phase at ovulation: tibial external rotation increased, femoral internal rotation increased, knee valgus moment increased, knee varus moment decreased, knee internal rotation moment decreased and peak vertical ground reaction force decreased.

Conclusions: The observed changes in biomechanics of the menstrual cycle may explain why ACL risk varies over the menstrual cycle.

Keywords: Hormones, Estrogen, Knee, Vertical Ground Reaction Force, Knee Valgus Moment

The risk of sustaining a non-contact ACL injury is not equal across the menstrual cycle (2, 3, 6, 41). A recent systematic review of research in this area concluded that the greatest risk of non-contact ACL injury was associated with the pre-ovulatory phase of the menstrual cycle is the phase, which includes both the follicular and ovulation phases (26). Risk of sustaining a non-contact ACL injury is theorized to occur due to changes in neuromuscular and biomechanical characteristics in response to hormone fluctuations over different menstrual cycle phases. Previous research has identified changes in variables linked to knee joint stability across the menstrual cycle including knee laxity (38, 44, 45) and muscle stiffness (16). However, this area is not without controversy with other studies observing no changes across the menstrual cycle in similar variables (5, 18, 23, 50). Changes in these variables may influence biomechanical characteristics during tasks associated with ACL injury.

Unfortunately, very little research has examined biomechanical characteristics during tasks associated with ACL injury (e.g. landing from a jump) over the course of the menstrual cycle (1, 11, 23, 39). These studies all concluded that biomechanical profiles do not change across the menstrual cycle. A major limitation of these studies is they all examine biomechanical changes across the menstrual cycle in healthy females with no history of ACL injury. Individuals are 10 times more likely to sustain another ACL injury to the healthy or reconstructed limb if they have a previous history of ACL injury (30, 49). Thus, those with prior non-contact ACL injury history are at high risk for sustaining a future ACL injury event. Subjects with prior ACL injury display altered jump landing biomechanics when compared to healthy uninjured subjects (30) as well as bilateral lower extremity deficiencies (37). These movement patterns may explain re-injury rates in the reconstructed limb but not

the healthy limb. However, other underlying risk factors may be present that could influence re-injury rates in the healthy limb. We theorize that females who have suffered an injury may be sensitive to hormonal fluctuations and show changes in variables associated with ACL injury risk when examined across the menstrual cycle.

Therefore, the purpose of this study was to examine three dimensional hip and knee kinematics and kinetics across the menstrual cycle in a population of females with previous unilateral ACL injury. We hypothesized that biomechanical variables assessed during a jump landing would be altered at ovulation in ways that would increase the susceptibility to non-contact ACL injury.

METHODS

Experimental Design and Subjects

Twenty-four participants were recruited to participate in this research study with twenty subjects (height = 168.6 ± 5.3 cm, mass = 66.2 ± 9.1 kg, age = 19.6 ± 1.31 years) successfully completing the testing protocol. Three of the twenty four participants did not complete the study because of anovulatory cycles and one of the twenty four voluntarily withdrew because of scheduling conflicts. All subjects read and signed an informed consent agreement approved by the University's Internal Review Board prior to participation. To be included in the study, participants had to satisfy the following criteria: 18-25 years of age, no history of pregnancy or neurological disorder, self reported normal menstrual cycle, no oral contraception use six months prior to testing, no oral contraception use at the time of non-contact ACL injury, sustained a unilateral non-contact ACL defined as "forces applied to the knee at the time of injury resulted from the athlete's own movements and did not involve contact with another athlete or object"(31), and had been cleared by a physician to return to

sport participation. Participants described their injury history, mechanism, sport, and surgical repair method to the primary investigator (DRB). All data were collected from the healthy/non-injured limb.

Protocol

A repeated measures study design was used to determine if jump landing biomechanics changed across the menstrual cycle. Participants were tested at two time points during their menstrual cycle which corresponded with low levels of estrogen and progesterone (menses) and high levels of estrogen and low levels of progesterone (ovulation). Participants were tested (1) 3-5 days after the onset of menses and (2) within 3 days following a positive test using a urine based ovulation prediction test (Earth's Magic, Cary, NC). Test phases (menses and ovulation) were counterbalanced to avoid an order effect with the first test session occurring at menses in 11 participants and at ovulation in 9 participants. Subjects were tested during the same time of day for each session and were instructed to not eat 2 hours prior to testing or exercise the day of testing. The primary investigator was blinded to the menstrual cycle phase of each subject.

Blood Sampling. Each subject had a venous blood specimen analyzed for select reproductive hormone levels. Specimens were obtained by veni-puncture from a vein located in the cubital fossa using a 3 cc syringe with a 23 gauge needle (1 inch length). The veni-puncture procedure was performed by a nationally certified phlebotomist (ACH) using standard clinical procedures.

Blood hormone levels (estrogen, progesterone, free testosterone) were assessed using radioimmunoassay procedures by an experienced researcher. Assay quality control steps and procedures were instituted (22). Blood was immediately transferred to a Vacutainer tube®

containing EDTA as an anti-coagulant and immediately placed on ice. Blood sample tubes were centrifuged at 3000g at 4^{o}C until plasma was separated, which was stored at -80^{o}C until hormonal analysis was performed. Plasma specimens were analyzed for estradiol-β-17, progesterone, and free testosterone concentration using a solid-phase, single antibody radioimmunoassay procedures (Siemens Medical, Los Angeles, CA). All assay samples were processed in duplicate and quality control procedures as recommended in the literature were utilized (22). Total estrogens in adult women are comprised primarily of estrone, estriol, estradiol-β-17 and their conjugates, with estradiol-β-17 being the major component, which is why it was analyzed in this study (17).

Biomechanical Analysis. Lower extremity kinematics and kinetics were collected using the Flock of Birds electromagnetic motion analysis system (Ascension Technologies, Inc., Burlington, VT) during a jump landing task. Measurements were recorded by the Motion Monitor software system (Innovative Sports Training, Inc., Chicago, IL) with a kinematic sampling rate of 144 Hz. The global reference system was established with the positive x-axis pointing anterior, positive y-axis to the left, and positive z-axis directed vertically.

Electromagnetic tracking sensors were placed on each subject over the apex of the sacrum, midpoint of the lateral thigh, and medial tibia. Sensors of the thigh and tibia were placed on the test limb in areas consisting of the least amount of muscle mass to minimize potential artifact induced by muscle contraction. Sensors were affixed using double-sided tape, prewrap and athletic tape. After the electromagnetic sensors were attached, subjects stood in a neutral posture with their arms relaxed at their sides. Bony landmarks were digitized in the following order using a mobile electromagnetic sensor attached to a stylus:

medial femoral condyle, lateral femoral condyle, medial malleolus, lateral malleolus, left anterior superior iliac spine, and right anterior superior iliac spine. Digitization of bony landmarks defined the segment end-points and joint centers of the lower extremity segments. The ankle joint center was defined as the midpoint between the medial and lateral malleoli. The knee joint center was defined as the midpoint between the medial and lateral femoral condyles. The hip joint center was determined by the Bell method (4) in which the hip joint center is determined using the left and right anterior superior iliac spine as landmarks to mathematically estimate the hip joint center. A static trial was recorded prior to data collection with the feet shoulder width apart and toes straight ahead.

Subjects completed five successful trials of a jump landing task from a 30cm high box positioned 50% of the subject's height from the edge of a force plate. Subjects jumped forward and landed on both feet, with the test limb on the force plate. Immediately after landing, subjects jumped as high as possible. A nonconductive force plate (Bertec Corporation, Columbus, OH) was used to record ground reaction forces with a sampling rate of 1,440 Hz and was synchronized with the kinematic data.

Data Reduction

Embedded right-hand Cartesian coordinate systems were defined for the tibia, thigh, and hip to describe the three-dimensional position and orientation of these segments and were aligned with the global reference system. Euler angles were used to calculate the knee joint angle between the shank and thigh (reference) and the hip joint angle between the thigh and pelvis (reference) in an order of rotations of (1) flexion-extension about the Y-axis, (2) valgus-varus about the X-axis, and (3) internal and external rotation about the Z-axis. Kinematic and kinetic data were exported into customized MATLAB software programs

(Mathworks, Natick, MA, Version 7.0) for data reduction. Kinematic data were filtered using a 4th order zero-phase-lag Butterworth low-pass filter at 14.5 Hz.(51) Each subject's kinematic neutral stance was subtracted out from each trial. Ground reaction forces were normalized to body weight (N) and moment data were analyzed as raw values and normalized to the product of body weight and height (BW*BH). Ground reaction forces defined the landing phase of the jump landing task. Initial contact was defined as the point at which the vertical ground reaction forces exceeded 10N, and toe-off was defined as the point at which vertical ground reaction forces drop below 10N. Peak kinematic and kinetic variables were assessed at initial contact and during the absorption phase of the jump landing which was defined as the point between initial contact and peak knee flexion for each trial. The following variables were collected during these time points: knee flexion angle, knee valgus angle, tibial rotation angle, hip flexion angle, hip adduction angle, hip rotation angle, knee extension moment (internal), knee rotation moment (max and min), knee valgus moment (max and min), anterior tibial shear force, peak vertical ground reaction force (peak during the absorption phase). Joint moments were calculated as internal moments. Joint moments and anterior tibial shear force were calculated using standard inverse dynamic procedures (21).

Statistical Analysis

SPSS version 17.0 (SPSS, Inc., Chicago, IL) was used to perform all data analyses and statistical significance was set with an *a priori* alpha level of 0.05. Individual paired t-tests were performed to compare each dependent variable between menses and ovulation.

RESULTS

Descriptive statistics for select reproductive hormones are presented in table 1. There were no changes in testosterone between menses and ovulation (P =0.41). Estradiol-β-17 (P = 0.009) and progesterone (P = 0.003) both increased at ovulation. Descriptive statistics for joint kinematics and kinetics at initial contact are presented in tables 2 and 3. There was a significant difference in tibia rotation angle ($t_{(19)}$ = 2.80, P = 0.01, effect size = 0.56) and internal knee varus moment ($t_{(19)}$ = 3.37, P = 0.003, effect size = 0.51). There was significantly greater tibial external rotation (47%) and less internal knee varus moment (65%) during ovulation compared to menses. All other findings at initial contact were not significant (P > 0.05).

Descriptive statistics for peak joint kinematics and kinetics during the absorption phase are presented in tables 4 and 5. There were no changes in frontal and sagittal plane kinematics between menses and ovulation (P > 0.05). However, there were significant changes in femoral ($t_{(19)}$ = -2.18, P = 0.05, effect size = 0.38) and tibial rotation ($t_{(19)}$ = 2.15, P = 0.05, effect size = 0.48). Specifically, there was increased femoral internal rotation (53% increase) and tibial external rotation (56% increase) at ovulation compared to menses. Select joint kinetic data were also significantly altered by menstrual cycle phase as internal knee valgus moment both increased ($t_{(18)}$ = 2.43, P = 0.03, effect size = 0.67) and decreased ($t_{(18)}$ = 3.37, P = 0.003, effect size = 0.63) at ovulation compared to menses. Also the internal tibial internal rotation moment was increased ($t_{(18)}$ = -3.07, P = 0.007, effect size = 0.80) while peak vertical ground reaction force (VGRF) decreased ($t_{(19)}$ = 2.20, P = 0.04, effect size = 0.30, table 3) at ovulation. All other variables assessed during the absorption phase of the jump landing were not significant (P > 0.05).

DISCUSSION

The aim of this study was to examine changes in jump-landing biomechanics across the menstrual cycle in females with a history of unilateral non-contact ACL injury. The most important finding of our study was that select jump-landing biomechanics were sensitive to changes in hormones over the menstrual cycle in females with unilateral non-contact ACL injury. Females performed the jump-landing task with greater tibial external rotation and hip internal rotation during the ovulation phase compared to the menses phase. Thus, altered hip and knee kinematics in the transverse and planes, respectively, were associated with the ovulation phase. During the menses phase the females displayed increased peak vertical ground reaction force, internal varus moment, and internal tibial external rotation moment compared to ovulation. Thus, thus greater overall knee joint loading was associated with the menses phase.

Interestingly, we observed that landing position at ovulation was similar to the "position of no return" (27). During the absorption phase of landing, the hip was internally rotated and the tibia was externally rotated. While hip internal rotation and tibial external rotation do not place direct tensile load on the ACL it is possible that the ACL can become impinged on the intercondylar notch resulting in rupture (20, 28). Thus, these factors cannot be ignored as potential ACL mechanisms of injury (42). The position of no return was described as a common mechanism of injury associated with non-contact ACL injury. Females with unilateral ACL injury had the most biomechanical changes in the frontal and rotational planes. Individuals with unilateral ACL injury have greater rotational motion than the general population (8). These findings disagree with previous authors that investigated similar variables across the menstrual cycle, specifically with regards to rotational kinematics and moments (1, 11, 39). We observed no changes in sagittal plane kinematics or kinetics

across the menstrual cycle which agrees with previous investigations (1, 11, 39). The greatest amount of disagreement in our findings and previous research was observed in rotational kinematics and kinetic findings.

We observed changes in knee frontal plane moment across the menstrual cycle which disagrees with previous research (1, 11, 39). In our sample, at menses, knee varus moment was 65% greater at initial contact (effect size = 0.51) and 35% greater during the absorption phase (effect size = 0.67) of the jump landing. From our previous investigation, knee laxity is approximately 1.5mm greater in this population of females compared to previously reported values in healthy females. Knee laxity has been shown to influence how the body is able to respond to dynamic loading conditions (39, 40, 43, 46, 47) which may have a significant influence on frontal plane moment at the knee. Internal knee varus moment is similar to external knee valgus moment which has been identified as a prospective risk factor for non-contact ACL injury (25). Thus, we observed greater ACL loading mechanisms in the menses phase of the menstrual cycle. Previous research has demonstrated that sagittal knee joint moments alone are not sufficient to rupture the ACL (32). McLean et al (32) used forward dynamic musculoskeletal modeling simulations to conclude that sagittal plane moments can not injure the ACL and that valgus loading was an important injury mechanism, specifically in females. Collectively, these results demonstrate the importance of internal knee varus loading in ACL injury and point to menses as the phase of menstrual cycle where this load is greatest. Our inclusion criteria of prior unilateral ACL injury is perhaps one of the most important reasons why we were able to demonstrate observable changes in these variables across the menstrual cycle but these changes are potentially caused by increased laxity in this population.

These findings contradict our hypothesis which theorized that only the ovulation phase would be associated with a biomechanical profile of increased ACL loading and injury mechanisms. Our hypothesis was based on previous findings that indicated greater amounts of estradiol-β-17 and progesterone would negatively influence ligamentous laxity (14) and muscle stiffness (16) and these factors would alter biomechanical profiles at ovulation. We are not the first group to observe altered performance at menses. In a clinical study, Friden et al (19) observed improved performance via a hopping protocol at ovulation which the authors attributed to improved coordination. While Dedrick et al (13) observed improved co-contraction behavior between the gluteus maximus and semitendinosus at ovulation. Biomechanical profiles at either menses or ovulation may contribute to increased non-contact ACL injury risk.

Peak VGRF decreased at ovulation by approximately 10% (effect size = 0.30). Larger GRF are associated with lower extremity injuries (15, 25) while lower values of VGRF are associated with chronic patellofemoral pain (7). Decreased VGRF at ovulation may be linked with increased joint laxity caused by elevated levels of estrogen and progesterone. Hewett et al. (24) suggested that females are more ligament dominant while males are muscle dominant. Increased ligamentous laxity may allow for altered absorption strategies and attenuation of ground reaction forces during landing, however, this is speculative given that we observed no sagittal plane changes in this study. Further research is needed.

Hormonal fluctuations may influence knee laxity, which in turn can influence rotational angles and moments during landing (39, 46). During menses, the tibia was internally rotated at initial contact (effect size = 0.57). Tibial internal rotation motion and

moment are provocative ACL loading scenarios especially when associated with low knee flexion angles (29, 35). At initial contact the knee flexion angle was approximately 20 degrees and videographic evidence has linked non-contact ACL injury to extended knee positions (36) and tibial internal rotation collapse has been observed using videographic evidence after ACL injury in rugby matches (12). Another interesting finding was that hip external rotation changed across the menstrual cycle with the femur in less external rotation at menses. At menses during the absorption phase of the jump landing, females had less hip internal rotation. Relative hip external rotation increases ACL loading and previous research has shown that females have greater hip external rotation during a jump landing task (10). McLean et al (33) also investigated the role of hip external rotation on ACL loading using computer modeling and found that non-contact ACL injury risk increased with greater hip external rotation velocity. These results demonstrate the importance of tibial and femoral rotation how these factors may change in across the menstrual cycle.

We observed appreciable increases in estradiol-β-17 and progesterone at ovulation. Minimum concentrations of estrogen and progesterone have been found to be important predictors of knee laxity's response to hormonal fluctuations across the menstrual cycle (44). Specifically, low levels of estrogen and higher minimum levels of progesterone during the early follicular phase of the menstrual cycle, had a significant influence on knee laxity (44). The relationship between minimum levels of estrogen and progesterone may be similar for muscle as well which may help explain why we observed a high risk biomechanical profile at menses. However, the role of estrogen and progesterone in ligament and tendon is better understood (9, 34, 48) than how these hormones influence muscle. Future research is needed to better understand the role these hormones play in muscle.

This study has several implications for non-contact ACL injury research. This is the first study to examine jump landing biomechanics across the menstrual cycle in females with a history of non-contact ACL injury. The findings of our research demonstrate that the biomechanical profiles change across the menstrual cycle in a complex manner and exhibit, depending on the variable of interest, high risk profiles at both menses and ovulation. Clinicians working with these individuals should be aware of these findings and enroll these individuals in injury prevention programs to address as many of these issues as possible. While hormonal changes will not directly change biomechanics, these individuals might be more sensitive to hormonal fluctuations causing altered tissue mechanics.

Our subjects had a history of unilateral non-contact ACL injury and thus, represents a population at increased risk of subsequent ACL injury (30). A limitation is that we cannot extrapolate these findings to the original non-contact ACL injury. This population was very specific and limited our ability to control for factors related to rehabilitation and surgery. The numbers of months from ACL injury to testing ranged from 7-52 months but all subjects were cleared to return to sport participation by their physician. The types of grafts used for reconstruction varied with the patellar tendon (n=9) being the most common graft utilized followed by a hamstring graft (n=6), and allograft (n=4). One subject was ACL deficient. To our knowledge, no evidence exists that suggests graft type influences kinematics or kinetics on the contralateral limb but future research should attempt to control for these factors.

Clinical Applications. This study highlights the importance of developing injury prevention programs on populations with previous history of ACL injury. Clinicians should be aware that biomechanical profiles of these individuals change during the preovulatory

phase of the menstrual cycle. These changes in biomechanical profiles may increase the risk of ACL injury.

Table 1. Concentrations of reproductive hormones from the selected time points. Values are mean ± standard deviation. These values were reported in our previous manuscript.

	Menses	**Ovulation**	**Menses 95% CI**		**Ovulation 95% CI**		**P**
Estradiol-β-17 (pg/ml)	31.12 ± 13.72	70.35 ± 54.66	24.49	37.72	44.01	96.70	*0.009
Progesterone (ng/ml)	0.51 ± 0.25	3.92 ± 4.24	0.39	0.63	1.87	5.96	*0.003
Free Testosterone (ng/ml)	0.80 ± 0.26	0.86 ± 0.22	0.67	0.92	0.75	0.97	0.414

*$P \leq 0.05$

Table 2. Kinematic variables at *initial contact*. Values are in degrees and represent: mean ± standard deviation.

	Menses	**Ovulation**	**Menses 95% CI**		**Ovulation 95% CI**		**P**
Knee Flexion	21.13±5.45	21.02±7.41	18.58	23.68	17.55	24.50	0.94
Knee Valgus	-1.29±3.94	-0.98±5.27	-3.14	0.56	-3.45	1.49	0.77
Knee Rotation	1.57±7.27	-3.31±8.12	-1.83	4.97	-7.11	0.49	*0.01
Hip Flexion	-41.42±8.95	-43.46±6.64	-41.62	-37.24	-46.58	-40.36	0.34
Hip Adduction	-3.40±4.91	-3.74±5.45	-5.70	-1.11	-6.30	-1.20	0.79
Hip Rotation	-2.16±8.62	-0.56±7.82	-6.20	1.88	-4.22	3.10	0.33

*$P \leq 0.05$

Table 3. Kinetic and force variables. Vertical ground reaction force was calculated as peak during the jump landing. All other variables are calculated at *initial contact*. Values are mean ± standard deviation. Moments are reported raw and normalized (BW*BH). One subject was determined to have unusable kinetic data and was removed from the analyses (n=19).

	Menses	**Ovulation**	**Menses 95% CI**		**Ovulation 95% CI**		**P**
VGRF (N)	1968.31±623.82	1775.80±642.94	1676.36	2260.27	1474.90	2076.71	*0.04
ATSF (N)	1.03±69.34	6.10±89.53	-31.43	33.48	-35.80	48.00	0.73
Knee Valgus Mom. (min)	10.69±13.56	3.72±11.95	4.16	17.23	-2.04	9.49	*0.006
Normalized Knee Valgus Mom.	0.01±0.01	0.003±0.01	0.004	0.016	-0.002	0.009	*0.003
Knee Extension Mom.	-3.68±28.69	-1.37±24.59	-17.52	10.15	-13.23	10.48	0.59
Normalized Knee Extension Mom.	-0.005±0.02	-0.003±0.02	-0.018	0.008	-0.014	0.008	0.59
Knee Rotation Mom.	-2.19±4.02	-2.23±3.29	-4.14	-0.26	-3.83	-0.65	0.96
Normalized Knee Rotation Mom.	-0.002±0.004	-0.002±0.003	-0.004	0.005	-0.004	0.005	0.99

* $P \leq 0.05$

Table 4. Peak kinematic variables calculated during the *absorption phase* of the jump landing (initial contact to peak knee flexion). Values are in degrees and represent: mean ± standard deviation.

	Menses	**Ovulation**	**Menses 95% CI**		**Ovulation 95% CI**		**P**
Knee Flexion	93.66±8.86	92.68±12.40	89.51	97.81	86.87	98.48	0.49
Knee Valgus	-13.24±10.62	-12.39±7.92	-18.21	-8.27	-16.11	-8.69	0.67
Knee Rotation (Max)	9.19±7.50	5.27±8.73	5.68	12.70	1.19	9.36	0.07
Knee Rotation (Min)	-3.36±7.20	-7.66±8.92	-6.74	0.007	-11.84	-3.49	*0.05
Hip Flexion	-77.74±12.31	-80.77±16.76	-83.50	-71.98	-88.62	-72.92	0.25
Hip Adduction	0.86±5.86	0.90±6.29	-1.88	3.61	-2.04	3.84	0.98
Hip Rotation (Max)	2.89±8.70	6.23±6.83	-1.18	6.97	3.03	9.43	*0.05
Hip Rotation (Min)	-8.09±7.98	-5.63±6.97	-11.82	-4.35	-8.89	-2.37	0.15

*$P \leq 0.05$

Table 5. Kinetic and force variables calculated at peak during the *absorption phase* of the jump landing (initial contact to peak knee flexion angle). Values are mean ± standard deviation. Moments are reported raw and normalized (BW*BH). One subject was determined to have unusable kinetic data and was removed from the analyses (n=19). Moments are calculated as internal moments.

	Menses	**Ovulation**	**Menses 95% CI**		**Ovulation 95% CI**		**P**
ATSF (N)	132.24±74.61	130.61±119.78	93.36	163.29	72.96	182.70	0.95
Norm ATSF	0.21±00.13	0.21±0.19	0.14	0.27	0.12	0.29	0.87
Kn Vlg Mom (Min)	-17.24±35.57	-60.65±71.60	-34.39	-0.09	-95.16	-26.14	*0.03
Norm Kn Vlg Mom (Min)	-0.02±0.03	-0.06±0.06	-0.04	-0.002	-0.08	-0.03	*0.03
Kn Vlg Mom (Max)	89.77±44.85	57.78±29.74	68.15	111.29	43.44	72.11	*0.003
Norm Kn Vlg Mom (Max)	0.14±0.08	0.09±0.05	0.11	0.18	0.07	0.11	*0.003
Kn Ext Mom	-235.32±77.66	-230.90±80.34	-272.76	-197.90	-269.62	-192.17	0.83
Norm. Knee Ext Mom	-0.21±0.06	-0.21±0.07	-0.24	-0.18	-0.24	-0.18	0.91
Kn Rot Mom (Min)	-75.31±50.55	-37.64±21.27	-99.68	-50.95	-47.89	-27.39	*0.007
Norm Kn Rot Mom (Min)	-0.07±0.05	-0.03±0.02	-0.10	-0.05	-0.05	-0.03	*0.007
Kn Rot Mom (Max)	28.53±22.98	40.79±35.48	17.45	39.60	23.69	57.89	0.23
Norm Kn Rot Mom (Max)	0.03±0.03	0.03±0.03	0.02	0.04	0.02	0.05	0.30

$*P \leq 0.05$

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