Changes in Neuromuscular Performance Throughout the Menstrual Cycle in Physically Active Females

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CHANGES IN NEUROMUSCULAR PERFORMANCE THROUGHOUT THE
MENSTRUAL CYCLE IN PHYSICALLY ACTIVE FEMALES

BY

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This thesis is approved as a credible and independent investigation by a candidate for the Master of Science in Sport and Recreation Studies and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>LIST OF FIGURES</th>
<th>vii</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ix</td>
</tr>
</tbody>
</table>

Chapter

1. Introduction .................................................................................................................. 1
   Delimitations/Limitations ......................................................................................... 3
   Definition of terms ................................................................................................. 3
   Assumptions ............................................................................................................. 4
   Hypothesis .............................................................................................................. 4

2. Review of Literature .................................................................................................... 5
   Anatomy .................................................................................................................. 5
   Etiology ................................................................................................................. 14
   Epidemiology ......................................................................................................... 17
   Knee Joint Laxity Assessment ............................................................................... 18
   Isokinetic Muscle Training .................................................................................... 21
   Female Menstrual Cycle Hormones ........................................................................ 22

3. Methods ..................................................................................................................... 26
   Recruitment and Subject Description ..................................................................... 26
   Study Protocol ....................................................................................................... 28
   Protection of Human Subjects and Treatment ...................................................... 31

4. Results ....................................................................................................................... 34
5. Discussion .................................................................................................................. 38

Muscle Strength ........................................................................................................... 38

Knee Joint Laxity ......................................................................................................... 39

Hormone Levels .......................................................................................................... 41

Limitations .................................................................................................................. 43

Future Research .......................................................................................................... 44

Conclusion ................................................................................................................... 45

References ................................................................................................................... 46

Appendixes .................................................................................................................. 49

A. Consent to Participate in a Research Project ......................................................... 49

B. Enrollment Form ..................................................................................................... 53

C. Medical History Form ............................................................................................ 55

D. Menstrual Symptom Questionnaire ........................................................................ 56

E. Data Collection Form ............................................................................................. 57

F. Using Ovulation Test Kits ...................................................................................... 58

G. International Review Board ................................................................................... 59
LIST OF FIGURES

1. Medical Anatomy and Illustrations.................................................................7

2. Normal Anatomy. ................................................................................................9

3. Knee Anatomy ................................................................................................11

4. Fascia, Bones and Muscles ...............................................................................13

5. Diagnosis - Powell Orthopedics.....................................................................16

6. MLH Physio KT-1000 ....................................................................................20

7. Movement lab – NeXt Move – Faculty..............................................................21

8. Hormone Levels During Menstrual Cycle.........................................................24

9. Isokinetic Testing 60°/sec .............................................................................36

10. Isokinetic Testing 180°/sec ..........................................................................36

11. Isokinetic Testing 300°/sec ............................................................................37
LIST OF TABLES

Table

1. Outline of the study visits ................................................................. 26
2. Outcome Measures.............................................................................. 31
3. Participant Characteristics ................................................................. 34
4. Outcome Measures and Hormonal Concentrations by Visit and Contraception Group ... 35
ABSTRACT

CHANGES IN NEUROMUSCULAR PERFORMANCE THROUGHOUT THE MENSTRUAL CYCLE IN PHYSICALLY ACTIVE FEMALES

JOSEPH D. ALBERT

2016

Context: In the United States, an excess of $1 billion is spent annually on anterior cruciate ligament (ACL) repair surgeries/rehabilitation programs in varsity female sports. Research has indicated that female athletes may be 2 to 10 times more likely to sustain an ACL tear than their male counterparts. Objective: The purpose of this study was to analyze the neuromuscular changes in females during different phases of the menstrual cycle. Design: Observational Cohort Setting: Laboratory Participants: Fifty (50) physically active college aged females (25 on oral contraception) were recruited to participate in three separate visits throughout one menstrual cycle. The groups were similar at baseline. Intervention: Visits coincided with follicular, ovulatory, and the luteal phase of the cycle. At each visit, participants had their blood drawn to assess for estradiol, progesterone, and relaxin levels. Along with blood measurements, isokinetic quadriceps strength at 60°/sec, 180°/sec, and 300°/sec, and knee joint laxity were measured at each visit. Main Outcome Measures: Isokinetic quadriceps strength, KT-1000 measurements, blood assays. Results: Isokinetic peak torque at 60°/sec was significantly lower during the follicular (151.6 ± 26.8 NM) than during the ovulatory phases (157.5 ± 27.1 NM, p<0.05). Isokinetic peak torque at 180°/sec was significantly lower during the follicular phase (98.7 ± 17.9 NM) than the ovulatory (107.1 ± 19.5 NM, p<0.05) and luteal phases (111.2 ± 19.5 NM, p<0.05). Results were similar for the
300°/sec isokinetic testing with significantly lower peak torque during follicular (79.0 ± 16.0 NM) than the ovulatory (85.4 ± 16.8 NM, p<0.05) or luteal phases (85.7 ± 16.0 NM, p<0.05). No differences were observed for knee joint laxity among any of the visits.

**Conclusion:** Results show that muscle strength is lowest during the follicular phase of the menstrual cycle and muscle strength and knee joint laxity are independent of hormone level fluctuations.
CHAPTER 1

Introduction

Female athletes are at greater risk of anterior cruciate ligament (ACL) injuries than males at the same competitive level. It has been indicated that adolescent females may be 2 to 10 times more likely to sustain an ACL tear than their male counterparts. In the United States, an excess of $1 billion is spent annually on over 125,000 ACL repair surgeries and rehabilitation programs in varsity female sports.

A possible mechanism for the increased rate of serious knee injuries in female athletes could be related to neuromuscular differences. Some research suggests that this difference in injury rate could be related to differences in neuromuscular training between males and females. Other studies have reported that factors such as female sex hormones may also play a role. Since females go through a monthly menstrual cycle and are known to have an increased risk for ACL injury, it should be determined if hormonal fluctuations have an impact on neuromuscular factors.

Estrogen, progesterone, and relaxin are some female sex hormones that fluctuate throughout the normal menstrual cycle. Hormonal fluctuations throughout the menstrual cycle have been reported to have adverse effects on athletic performance and some of these effects may place them at a greater risk of injury. The fluctuations in female sex hormones concentrations throughout the menstrual cycle combined with the possible relationship of hormone levels to neuromuscular risk factors, indicate the possibility that injury rates may vary based on hormonal changes throughout the menstrual cycle.

While controversial in the effect they have on knee injury rates, female sex hormones have been reported to change the tensile properties of ligaments and tendons.
Some research has reported an increase in knee joint laxity during certain phases of the menstrual cycle.\textsuperscript{13-16} These reports indicate that hormone changes could lead to an increase in joint laxity, thereby potentially increasing the risk of ACL injury in females. In contrast, there are other reports indicating that knee joint laxity is not affected by hormonal changes during the menstrual cycle.\textsuperscript{17-20}

Female sex hormones have been reported to cause changes in quadriceps strength and variations in muscle relaxation rates throughout the menstrual cycle.\textsuperscript{7,15} Athletes with increased quadriceps muscle strength combined with decreased hamstring strength have been linked to an increased risk of ACL injuries.\textsuperscript{21} It has been reported that females have an increased risk of non-contact knee injuries because they have lower hamstring-to-quadriceps strength ratios than males.\textsuperscript{6} In studies using isokinetic testing, females have been reported to have more than twice the muscle strength in their quadriceps compared to their hamstrings.\textsuperscript{6} Having quadriceps strength that is more than double of that of the hamstrings has been reported to assist in anterior tibial forces at the knee and therefore may be linked to an increase in the occurrence of ACL injuries.\textsuperscript{22}

Since the problem with ACL injuries is so significant, special attention should be paid to the extrinsic risk factors associated with the mechanisms of ACL injury. The primary purpose of this study is to analyze the neuromuscular changes in females during different phases of the menstrual cycle. The secondary aims of this study are to analyze:

1. The muscle strength of the quadriceps in females throughout the three phases of the menstrual cycle,

2. The knee joint laxity in females throughout the three phases of the menstrual cycle, and
3. Hormonal changes in estrogen, progesterone, and relaxin, throughout the menstrual cycle, in the oral contraceptive group and non-contraceptive group.

Delimitations/Limitations

The delimitations of this study are the strong methodology, the blinding of researcher completing KT 1000 testing, and use of an only female population. The limitations are no monitoring of oral contraceptive use and the potential for participant drop out.

Definition of terms

Anterior Cruciate Ligament (ACL): Comprises three twisted bands. This ligament prevents the femur from moving posteriorly during weight bearing and limits anterior translation of the tibia in non-weight bearing. Isokinetic: Movement that occurs at a constant velocity.

Monophasic oral contraceptive: A birth control pill that contains the same amount of estrogen and progesterone in each pill.

Estrogen: The primary female sex hormone that is secreted primarily by luteal cells. Progesterone: The main regulator of the menstrual cycle, it helps prepare the female body for conception and inhibits the production of luteinizing hormone.

Follicle Stimulating Hormone (FSH): A hormone released during the menstrual cycle that stimulates the maturation of an ovarian follicle.
Luteinizing Hormone (LH): A hormone released at the mid-cycle of menstruation that stimulates the development of luteum and the endocrine structure that secretes progesterone and estrogens. ¹²,²³

Relxin: A hormone that is believed to be responsible for connective tissue changes during late-pregnancy that helps to accommodate childbirth. ²⁶

Assumptions

This study assumes that the participants are either in the contraceptive group or non-contraceptive group will continue with the protocol in order to stay in that particular group.

Hypothesis

Hypothesis 1: We hypothesize that the quadriceps strength will decrease as estrogen increases during the menstrual cycle.

Hypothesis 2: Our hypothesis is that knee joint laxity will increase during the follicular phase of the menstrual cycle.

Hypothesis 3: Our hypothesis is that participants in the non-oral contraceptive group will show changes in quadriceps strength and joint laxity compared to the oral contraceptive group.
CHAPTER 2
Review of Literature

In order to fully understand the effects that hormones have on ACL joint laxity, it is important to gain an understanding of knee anatomy, mechanism of injury, female sex hormones and assessment strategies. This review of literature will address the following topics: 1) Anatomy, 2) Etiology, 3) Epidemiology, 4) Knee Laxity Assessment, 5) Isokinetic Muscle Training, 6) Female Menstrual Cycle Hormones.

Anatomy

The knee joint complex is formed by the tibiofemoral, tibiofibular, and patellofibular joints. It has little bony support which means it has to rely on more soft tissues to transmit force through the joint. The bony anatomy involves 4 major bones: the femur, tibia, fibula and patella. The femur is the longest and strongest bone in the body and accounts for approximately a quarter of the body’s height. The medial and lateral condyles are located on the inferior portion of the femur. The condyles are convex structures that are covered in articular hyaline cartilage in order to freely articulate with the menisci of the tibia. The articular surfaces of the condyles are separated by the deep intercondylar notch of the femur on the posterior side while remaining adjoined on the anterior side. On the anterior side, the two condyles form the femoral trochlea which allows articulation with the patella. The proximal end of the tibia contains the tibial plateau, which is slightly concave shaped and articulates with the femoral condyles.

The patella is the largest sesamoid bone in the body and is located in the patellar tendon and glides over the femoral trochlea. The patella serves a mechanical and
protective purpose by the way it dissipates forces from the extensor muscles and protects the anterior side of the knee.\textsuperscript{27}

The knee is classified as a double condyloid articulation that is capable of three degrees of freedom: (1) flexion and extension, (2) internal and external rotation, (3) abduction and adduction.\textsuperscript{27}

The menisci serve to make up the anatomical differences between the articular surfaces of the tibia and femur.\textsuperscript{27} There are two oval shaped fibrocartilage rings or menisci, which deepen the facets of the tibia, cushion stress placed on the knee joint and also maintain spacing between the tibial plateau and femoral condyles.\textsuperscript{23} The two menisci are named based on their anatomical location. The medial meniscus is on the medial portion of the tibial plateau and is “C” shaped.\textsuperscript{23} It is wider on the posterior side than the anterior side and offers more knee stabilization than the lateral meniscus.\textsuperscript{23} The lateral meniscus is both smaller and more mobile than the medial meniscus and is “O” shaped.\textsuperscript{23,27} It attaches to several different muscles and ligaments which distort its shape to aid in stabilizing the knee while moving through the entire range of motion.\textsuperscript{27} There are three main portions of the menisci. The outer portion is known as the “red zone” and has the most vascularization, which means it is most likely to heal if torn.\textsuperscript{27} The middle portion is known as the “pink zone” and is less vascularized than the “red zone”.\textsuperscript{27} The “white zone” is the most inner portion of the menisci and is avascular which means it has no direct vascularization.\textsuperscript{27}
Ligaments

The knee is held together almost entirely by four ligaments: the medial collateral ligament (MCL), the lateral collateral ligament (LCL), the anterior cruciate ligament (ACL), and the posterior cruciate ligament (PCL). The MCL is the primary medial stabilizer of the knee and contains both a deep and superficial layer. The anterior fibers of the MCL are taut in the midrange of knee flexion and both the anterior and posterior fibers are taut in complete extension. The LCL is the primary lateral stabilizer of the knee and also limits external and internal tibial rotation.
structure and helps the lateral knee to withstand increased stress from varus forces and also the initial contact phase of gait.\textsuperscript{23,27} The anterior cruciate ligament (ACL) protects against a number of different knee movements but it is most known for its protection of anterior translation of the tibia on the femur.\textsuperscript{27} Other movements that the ACL helps protect against are internal and external rotation of the tibia on the femur and also hyperextension of the tibiofemoral joint.\textsuperscript{23,27} There are two discrete segments of the ACL, the anteromedial bundle and the posterolateral bundle. When the knee is fully extended the posterolateral bundle is taut and when the knee is fully flexed the anteromedial bundle is taut.\textsuperscript{27} This allows for the ACL to provide support throughout a large range of motion.\textsuperscript{27} The PCL is another primary stabilizer of the knee and prevents internal rotation of the tibia, hyperextension of the knee, anterior translation of the femur during weight bearing, and posterior translation of the tibia in non-weight bearing.\textsuperscript{23} It has two main components: the anterolateral and posteromedial bundles. The anterolateral bundle is taut when the knee is flexed and the posteromedial bundle is taut when the knee is extended. Together, the ACL and PCL work with each other in what is known as the “screw home mechanism”.\textsuperscript{27} In this mechanism, the two ligaments encircle each other in flexion and unwind in extension.\textsuperscript{27}
Muscles

There are many muscles that play a role in the static and dynamic stabilization of the knee joint. They are located on both the anterior and posterior aspect of the thigh and leg and assist with flexion, extension, and internal and external tibial rotation.\textsuperscript{23,27}

The main muscles that assist the knee with flexion are the following: biceps femoris, semitendinosus, semimembranosus, gracilis, sartorius, gastrocnemius, and popliteus muscles.\textsuperscript{23} The origin of the biceps femoris is quite complex. The long head originates at the ischial tuberosity and sarcotuberous ligament while the short head originates at the lateral lip of the linea aspera and upper two-thirds of the supracondylar line.\textsuperscript{27} The insertion of the biceps femoris is to the lateral fibular head and the lateral tibial condyle.\textsuperscript{27} The semitendinosus originates at the ischial tuberosity and inserts at the
medial portion of the tibial flare. The semimembranosus originates at the ischial tuberosity and inserts at the posteromedial portion of the tibia’s medial condyle. The origin of the gracilis is the symphysis pubis and the inferior ramus of the pubic bone while the insertion at the proximal portion of the antero-medial tibial flare. The sartorius muscle originates at the anterior superior iliac spine and also inserts at the proximal portion of the antero-medial tibial flare. The gastrocnemius is another muscle that has a complex origination. The medial head originates at the posterior surface of the medial femoral condyle and the adjacent portion of the femur and knee capsule. The lateral head originates at the posterior surface of the lateral femoral condyle and the adjacent portion of the femur and knee capsule. The gastrocnemius inserts to the calcaneus via the Achilles tendon. The origination of the popliteus muscle is the lateral femoral condyle and the oblique popliteal ligament. The insertion of the popliteus is to the posterior tibia superior to the soleal line and to the fascia covering the soleus muscle.
The semitendinosus, semimembranosus, gracilis, sartorius, and popliteus muscles all assist in both knee flexion and internal tibial rotation. The biceps femoris is responsible for both knee flexion and external rotation of the tibia.²³
Knee extension is accomplished with the help of the following muscles: the vastus lateralis, vastus medialis, vastus intermedius and rectus femoris, all of which combine to form the quadriceps femoris muscle group. The vastus lateralis’ origination is quite complex. It originates at the proximal intertrochanteric line, greater trochanter of the femur, gluteal tuberosity and the upper one half of the linea aspera. The insertion of the vastus lateralis is to the patellar tendon which attaches to the tibial tubercle. The origination of the vastus medialis is split into two main portions. The longus portion originates at the distal half of the intertrochanteric line and the medial portion of the linea aspera. The oblique portion originates from tendons attached to the adductor longus and adductor magnus. The insertion of the vastus medialis is the patellar tendon which attaches at the tibial tubercle. Another muscle that is part of the quadriceps femoris muscle group is the vastus intermedius. It originates at the anterolateral portion of the upper two thirds of the femur and also from the lower one half of the linea aspera. Like the other quadriceps muscles it inserts at the tibial tubercle via the patella and patellar tendon. The rectus femoris is the last of the quadriceps femoris muscle group. It originates at the anterior inferior iliac spine and the groove located superior to the acetabulum. The insertion of this muscle is also the tibial tubercle via the patella and the patellar ligament.
Figure 4: Fascia, Bones and Muscles


https://www.google.com/search?q=anatomy+of+the+knee&es_sm=93&source=lnms&tbnm=isch&sa=X&ved=0CAcQ_AUoAWoVChMIgpX5gOi1yAIVAnc-Ch1k1gKu&biw=1517&bih=714&dpr=0.9#tbm=isch&tbs=rimg%3ACVUasO08WR4FfJiuWJ21hFmyPf8ibQDBfw_1RLH9gH_1kK2J7m2L9E673Z2dXCwCkI1q0pRyCvdw_f93tFRFr-aiCSoSCa5YnbWEWb19Ee7OYkBw7cniKhIJ_1yJtAMF_1D9ERKwE6T3sXpWYqEgksf2Af-QrYnhGVC5-NUc1q2SoSCebYv0TrvdnZEYEZ1jltgsUVKhIJ1cLAKQjWrSgR0ahd-MTuKKQqEgkpHIK923B_1xFJD_1CpOEF1mSoSCXe0VEWv5qIJESNkEOLVC9Rb&q=anatomy%20of%20the%20knee%20muscles&imgrc=Afez-4vCuz-D8M%3A. Accessed October 9, 2015.
Etiology

The primary purpose of the ACL is to prevent anterior translation of the tibia on the femur.\textsuperscript{27} It also helps with the “screw home mechanism” and limits hyperextension.\textsuperscript{23} The most common mechanism of injury to the ACL occurs when the tibia is externally rotated and the knee is in a valgus position.\textsuperscript{23,24,27} This position puts the most strain on the ACL and often times when an extreme valgus force is applied the MCL and medial meniscus can also be injured.\textsuperscript{23,27} Other mechanisms of injury include forced hyperextension, non-contact hyperextension, and non-contact deceleration.\textsuperscript{27} Studies have shown that as many as 78\% of ACL injuries occur from non-contact rotational mechanisms.\textsuperscript{24}

There are also other anatomical structures that assist the ACL and prevent injuries to it. The hamstrings are known to help prevent both hyperextension of the knee and anterior tibial translation.\textsuperscript{24,28} Females have been reported to have decreased dynamic knee control compared to males.\textsuperscript{3} It has been found that muscle strength and coordination are known to have a large role in injury prevention.\textsuperscript{4} Internal rotation is another knee movement that benefits from muscle stabilizers, the biceps femoris, tensor facial latae and the iliotibial band combine to limit internal rotation.\textsuperscript{28} Relating to the meniscus, it is found that the medial meniscus offers more aid to the ACL than the lateral meniscus because it helps reduce anterior translation of the tibia on the femur.\textsuperscript{28}

In regards to ACL injuries one must look at the many potential factors that could predispose an individual to ACL injuries. There are a number of different risk factors that are generally classified as intrinsic or extrinsic. Intrinsic risk factors are generally more difficult to control and involve individual physiological and psychological factors.\textsuperscript{23}
Some intrinsic risk factors of the knee include joint laxity, limb alignment (Q-Angle), narrow intercondylar notch width, small ACL size, and genu recurvatum. Extrinsic risk factors are more manageable and are seen as something that could be part of an injury prevention plan. Some examples of extrinsic risk factors include muscle strength and endurance, muscle coordination and activation, playing style, playing environment, navicular drop, anterior pelvic tilt, and a wider pelvis to femoral length ratio.

The increased ACL injury rate in female athletes when compared to male athletes is explained through several theories. One suggested mechanism for the increase in injuries is the increased Q-angle (quadriceps angle) in females. Q-angle refers to the angle of a line from the anterior-superior-iliac-spine to the middle of the patella and from the middle of the patella to the tibial tubercle. Females typically have a greater Q-angle than males because of the anatomical differences in the hip to accommodate for child birth. While some clinicians and researchers readily accept this theory, other research has indicated that no relationship between Q-angle and ACL injuries exists.
Another anatomical difference between males and females is the size of the intercondylar notch in the femur. Females typically have a more narrow intercondylar notch than males relative to their body size. Some researchers believe that the narrow notch itself causes the increase in ACL injuries, while others believe that the narrow notch results in the ACL being more narrow thus decreasing the strength of the ligament. Research shows that a narrower intercondylar notch increases the likelihood of an ACL injury. However, research involving the intercondylar notch is inconclusive in determining the correlation between intercondylar notch width and ACL size.

Neuromuscular differences have been suggested as another possible mechanism for the increase in the rate of serious knee injuries in female athletes. After maturation,
it has been found that female athletes tend to land with greater medial motion of the knees and also a greater lower extremity valgus angle than male athletes. These factors are considered to be contributors of the increased likelihood of injury in females. Some research suggests that differences in training between males and females may lead to differences in injury rates. It has been found by Hewitt et al. that by implementing a program which incorporates flexibility, plyometrics, and weight training to increase muscle strength and decrease landing forces can decrease injury rates. That same study found that the incidence rate of injury for untrained females was 3.6 times higher compared to trained females and 4.8 times higher than that of the male control group. That was then compared to the trained female group which was only 1.3 times higher than the untrained male control group. Other factors such as female sex hormones also may be a factor.

Epidemiology

Female college aged athletes, specifically soccer and basketball players have been found to be more susceptible to ACL injuries in several cohort studies. Ireland stated that from 1990-1998, female soccer and basketball players experienced 2.29 to 2.89 times more ACL injuries than males in those same sports. The same study looked at noncontact ACL basketball injuries at 29 different colleges over 2 seasons of play and found that female athletes were 6.1 times more likely to sustain an ACL injury than males. A study by Hootman et al, reported that the four sports with the highest rates of ACL injury in college athletics were women’s gymnastics, basketball, and soccer, along with men’s spring football. Overall, rates of noncontact ACL tears range anywhere
from 2 to 10 times greater in female basketball and soccer athletes than males playing the same sports.\textsuperscript{3,33}

Knee Joint Laxity Assessment

In order to determine the amount of laxity in a ligament, special tests that are both precise and accurate need to be used. Several manual as well as mechanical ligamentous tests can be used for on field and clinical evaluations determining ACL laxity and also provide objective measurements. The most widely used manual tests for anterior knee laxity are the Lachman’s test, anterior drawer test and the lateral pivot shift test while the KT-1000 is one of the most common mechanical tests.

The Lachman’s test is performed with the patient laying in a supine position with their knee flexed at 20-25°.\textsuperscript{27} The examiner is positioned so that they have one hand grasping the tibia at the level of the tibial tuberosity and the other hand grasping the femur just above the condyles. As the examiner is supporting the weight of the leg and the knee is flexed at the correct angel, the tibia is pulled anteriorly while a posterior pressure is put on the femur in order to stabilize. A positive test is noted when there is an increase in anterior tibial translation compared with the opposite limb or if there is a lack of end feel. The Lachman’s test has been found to be effective in 91% of acute and 100% of chronic ACL injuries preoperatively (n=50).\textsuperscript{36}

The anterior drawer test is performed with the patient laying supine with the hip flexed at 45° and knee flexed at 45°.\textsuperscript{27} The examiner then sits on the end of the examination table in front of the involved knee while grasping the tibia just distal to the joint line with thumbs placed along the joint line on each side of the patellar tendon. The tibia is then pulled anteriorly and a positive test is noted when there is an increased
amount of anterior tibial translation compared to opposite limb or if there is a lack of end feel. It is important to remember that in order to get accurate results the hamstrings must be relaxed. The anterior drawer test has been found to be effective in 20% of acute and 60% of chronic ACL injuries preoperatively (n=50).36

The lateral pivot shift test is performed with the patient laying supine with the hip flexed to 30°.27 The examiner is positioned standing laterally to the patient with the distal lower leg/ankle grasped while maintaining 20° of internal tibial rotation. The opposite hand of the examiner is then placed on the lateral portion of the leg superior to the joint line while increasing the force of internal rotation. While the test is being performed, internal rotation is maintained while also applying a valgus force to the knee and slowing flexing it. A positive test is noted when then tibia’s position on the femur is reduced as the knee flexed in the range of 30°-40° or if during extension there is anterior subluxion of the tibia. The pivot shift test has been found to have a specificity of 0.96 and a sensitivity of 0.60.27 All three of the manual tests have modifications to compensate for differences in size ratios between examiner and patient.

Clinical assessments and manual ligamentous tests are the most common form of identifying ACL deficiencies. However, sometimes subtle changes are more easily noted with the use of mechanical devices because of their ability to provide objective results. The KT-1000 is one of the mechanical devices used to assess knee joint laxity. In order to use the KT-1000 it is applied to the anterior aspect of the tibia, parallel to the line of the tibial tuberosity while the patient is laying supine.28 The patient’s knees are placed into 20-30° of flexion by putting a thigh support under the distal femur. Tibial rotation is placed at neutral with the help of a foot support which prevents external tibial rotation.
There are two Velcro straps that wrap around the proximal and distal ends of the tibia at points where the arthrometer is able to have proper positioning. The position puts the patellar sensor over the patella and joint line indicator directly in line with the joint line. Pressure is then applied to the sensor pad and is given in either an anterior or posterior direction with forces of 15, 20 and 30 lbs. Each different level of force is indicated by audible tones. Displacement is then recorded in millimeters as indicated by a dial on the arthrometer. According to a meta-analysis by van Eck, the KT-1000 was found to have a sensitivity of .93, a specificity of .93, accuracy of .93 and positive predictive value of 6.9 for diagnosing ACL rupture.

**Figure 6: MLH Physio KT-1000**


Isokinetic Muscle Training

In isokinetic testing, females have been reported to have a mean hamstring-to-quadriceps ratio of less than 0.5. Hamstring-to-quadriceps ratios of 0.5 or less have been reported to possibly assist in anterior tibial translation and therefore may increase the occurrence of ACL injuries. An example of an Isokinetic testing machine is the Biodex System 4 (Biodex Medical Systems, Shirley, NY). The Biodex has been reported to be a reliable tool for measuring isokinetic torque production up to 300 degrees/second (ICC=0.99, CV < 2%).

Figure 7: Movement lab – NeXt Move – Faculty

Movement lab – NeXt Move – Faculty. Google Image.

The Biodex measures peak torque by computing the amount of pressure generated through the entire range of motion at the selected speed (degrees/sec). Isokinetic machines do this by using a combination of hydraulic, pneumatic, and mechanical
pressure systems to produce a constant velocity of motion.\textsuperscript{23,38} That information is then used to compute force or power.\textsuperscript{35} Isokinetic testing has high clinical value because it allows clinicians to allow for passive motion, isometric and isotonic strengthening, eccentric and concentric muscle action, and open chain exercise.\textsuperscript{35}

**Female Menstrual Cycle Hormones**

The main hormones associated with a naturally occurring menstrual cycle are progesterone, estradiol, Luteinizing Hormone (LH), and, Follicle Stimulating Hormone (FSH). Estradiol and progesterone are primarily produced by the ovaries and corpus luteum, while LH and FSH are produced by the hypothalamus and pituitary gland.\textsuperscript{39}

These hormone levels vary across the menstrual cycle based on the menstrual phase. There are three phases associated with a normal menstrual cycle which are listed as follows: the follicular phase, the ovulatory phase, and the luteal phase. In this study blood assays were used to determine hormone levels in each of the three menstrual cycle phases. The progesterone assays have intra and inter assay CVs of 2-12\% and 5-12\%, respectively and a lower limit of detection of 0.11ng/mL. The estradiol-17\textbeta\ assays have intra and inter assay CVs of 5-11\% and 6-12\%, respectively and a lower limit of detection of 7.2pg/mL. Relaxin RIA kits were obtained from Phoenix Pharmaceuticals, Burlingame, California. The Relaxin assays have intra and inter assay CVs of 5-7\% and 12-15\%, respectively and a lower limit detection of 36-53 pg/assay.

During the follicular phase, most hormone levels begin to increase in preparation for ovulation. Normal estradiol levels range from 25-75 pg/mL in this phase while normal progesterone levels are <1.5ng/mL. Progesterone is the only hormone that does not spike immediately before or at ovulation. LH and FSH have normal levels of 3-20 mlU/mL.
during this phase with the highest levels occurring at ovulation. LH and FSH are the main gonadotropin hormones that lead to the release of the egg from the mature follicle.\textsuperscript{39}

Once the ovulatory phase occurs, levels in estradiol peak and then dramatically drop with a normal peak of >200 pg/mL. The drop in estradiol levels occurs with the start of the luteal phase when progesterone levels begin to increase. Progesterone levels are normally >5 ng/mL during the ovulatory phase but begin to rise because of the release of progesterone from the corpus luteum. The corpus luteum is a small body that develops within a ruptured ovarian follicle following ovulation.\textsuperscript{39} Normal levels of FSH during this phase peak at >25 mlU/mL and then drop at the start of the luteal phase. LH follows a similar pattern as it peaks at >20 mlU/mL and then drops once the luteal phase begins.

The final phase of the menstrual cycle is the luteal phase. During this phase estradiol levels return to a normal level of 25-75 pg/mL. Progesterone levels are highest during this phase because of the production that comes from the corpus luteum and are normally >15 ng/mL. These high levels inhibit the production of LH and FSH until the end of this phase when FSH begins to rise again in order to initiate the maturation of new follicles for the next cycle.\textsuperscript{39} Normal values for LH are 3-20 mlU/mL while normal values for FSH are <25 mlU/mL. The figure below illustrates the fluctuation of hormones across the entire menstrual cycle.
In summary, there are a number of different anatomical and physiological factors that can affect a person’s likelihood of sustaining an ACL injury, especially in the female population. It is important to remember that female athletes are up to 10 times more likely to injure their ACL and therefore it is necessary for additional research to be
completed to determine which risk factors can be modified to decrease ACL injury incidence.\textsuperscript{2,3,33}
CHAPTER 3

Methods

Participants (n=50) were asked to participate in three separate study visits over the course of one menstrual cycle. Each of the three visits coincided with different phases of their menstrual cycle. The first visit was between days 1 and 5 of the menstrual cycle in order to coincide with the follicular phase when estrogen and progesterone are low. Visit number two was performed within 48 hours of the onset of menses. The final visit was 7 days after the ovulation visit and coincided with the luteal phase. Blood tests were performed at all of the visits to confirm the menstrual cycle phase participants were in.

All study visits are outlined in Table 1.

Table 1. Outline of the study visits

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Enrollment</th>
<th>Follicular</th>
<th>Ovulation</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed Consent</td>
<td>Signed informed consent</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Questionnaires</td>
<td>Medical History</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthropometrics</td>
<td>Height, Weight</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Tests</td>
<td>Estrogen, Progesterone, Relaxin</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Muscle Strength</td>
<td>Uni-lateral Isokinetic Protocol (Biodex)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Laxity Testing</td>
<td>Passive displacement and maximal displacement tests</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Recruitment and Subject Description

College-aged, physically active females (n=50) were recruited from South Dakota State University. Recruitment was completed through on-campus advertising and through meetings with classes in the Department of Health and Nutritional Sciences.
Twenty-five females that were taking oral contraceptives were recruited and twenty-five females that were not taking oral contraceptives were recruited.

Inclusion and Exclusion Criteria

Inclusion Criteria for Potential Participants

An initial questionnaire was administered at the enrollment visit to determine whether the menstrual status was regular, and the participant was physically active at least 3 times per week.

Inclusion Criteria for Non-contraceptive Group

Participants were active College aged females with regular menstrual cycles.

Inclusion Criteria for Contraceptive Group

Participants were active College aged females with regular menstrual cycles taking a monophasic oral contraceptive.

Exclusion Criteria for Non-contraceptive and Contraceptive Group

Participants were excluded if they were men, women with amenorrhea, college aged females who are not taking oral contraceptives as directed, college aged females not taking monophasic contraceptives, history of knee injury within last 6 months or prior ACL reconstruction. Participants with irregular menstrual cycles were excluded as to eliminate hormonal variability that women with irregular cycles may exhibit.

Methods to Increase Participation

Participants were given the torque curve data from their Biodex protocol which could help them to identify functional deficits that could be improved with training. In addition to the information from Biodex testing, participants received twenty dollars per
experimental visit for a total of sixty dollars at the conclusion of experiment.

Participant data was kept confidential and stored in a secure location.

**Study Protocol**

**Enrollment Visit**

On the day of the initial visit, an informed consent (Appendix A) was obtained along with a medical history questionnaire that asked about personal and family history of diseases and chronic injury as well as information regarding the participant’s menstrual cycle (Appendix B). From this information, potential participants were screened for conditions that may exclude them from participation.

**Menstrual Cycle Monitoring**

All participants were asked to contact the study coordinator on the first day of their menstrual cycle to schedule an appointment for their first testing session within 72 hours of the onset of menses (Appendix C,D,E). The participants who were taking oral contraceptives had their subsequent visits on cycle day 14 and cycle day 21. Participants who were not taking oral contraceptives were asked to take a home ovulation test every day beginning at day 10. When a positive test occurred, the participants were asked to contact the study coordinator and set up a visit within 48 hours (Appendix F). A reminder telephone call was made to each participant on day 10 of their cycle to improve compliance. This helped to determine the day that ovulation occurred in each of the women to aid us in determining an individualized testing schedule for each of the participants. The final visit took place 7 days after the ovulation visit. During the testing period, participants had their menstrual cycle phase confirmed through blood testing.
Experimental Period

Participants were asked to complete testing over the course of one menstrual cycle. Participants were asked to complete a DEXA scan, Biodex isokinetic test, a knee joint laxity exam, and provide blood samples. Specific details and rationale regarding these tests are given below. (Appendix A)

Anthropometrics

Anthropometric measurements were taken during the first experimental session. Height was measured to the nearest 0.5cm using a portable stadiometer (Seca Model 225) and weight was measured to the nearest 0.1kg using a digital scale (Seca Model 770).

Blood Draws

A venipuncture was performed by a trained phlebotomist. The blood was analyzed for estradiol, progesterone, and relaxin using radioimmunoassay (RIA). Estradiol-17β and progesterone double antibody RIA kits were obtained from MP Biomedical in Solon, OH. All the blood assays were analyzed using radioimmunoassays in the Animal Science lab on campus at South Dakota State University.

Biodex

To perform the Biodex isokinetic testing, the subject was seated with their shoulders and waist strapped to the chair. The participant’s ankle was secured to the leg holder with a Velcro strap. Once the participant was properly positioned, they went through a series of familiarization repetitions to get accustomed to the machine. The performance trial consisted of 5 isokinetic quadriceps extension and hamstring curl repetitions at 60 degrees per second, 5 repetitions at 180 degrees per second, and 5 repetitions at 300 degrees per second. Each series of repetitions was separated by a 30
second rest period. This testing was used to determine quadriceps strength. After the performance trials, a fatigue protocol was prescribed to each participant. The fatigue protocol was done at 180 degrees per second and required the participants to perform knee flexion and extension as many times as they could until they dropped below 50 percent of their max torque for three consecutive repetitions based on information gained from the performance test.

**KT 1000**

To perform laxity testing using the KT 1000 knee arthrometer, the subject was placed in a comfortable supine position. An adjustable thigh support platform was placed under both legs just proximal to the popliteal space. A foot support platform was then placed under both feet with the patient’s feet located in a position that allowed the knee to be in a neutral position. Next, the knee was placed in a neutral quadriceps or 30 degree flexion position using the adjustable thigh support. The thigh was then secured using the Velcro strap and the arthrometer was placed on the anterior aspect of the tibia with the joint line arrow bisecting the joint line. Three separate testing protocols were used to test specific characteristics of the ACL. A maximal displacement test was performed to quantify that amount of laxity in the joint and to determine the endpoint with the knee in 30 degrees of flexion. KT-1000 measurements were done prior to and immediately following the Biodex testing to assess the effect of muscle fatigue on knee joint laxity. All measurements were performed by the same certified athletic trainer using the KT-1000 knee arthrometer.
Protection of Human Subjects and Treatment

This protocol was reviewed and approved by the Institutional Review Board (Appendix G) at South Dakota State University. All records were double entered into a database on a computer that is not connected to the internet. All data and computers were stored in a card swipe protected facility.

Table 2. Outcome Measures

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Modality/Unit</th>
<th>Follicular Phase Norms</th>
<th>Ovulatory Phase Norms</th>
<th>Luteal Phase Norms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torque</td>
<td>Newton-Meters</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Laxity</td>
<td>Millimeters</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Estradiol</td>
<td>Radioimmunoassay</td>
<td>25-75pg/mL</td>
<td>&gt;200pg/mL</td>
<td>25-75pg/mL</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Radioimmunoassay</td>
<td>&lt;1.5ng/mL</td>
<td>&gt;2ng/mL</td>
<td>&gt;4.5ng/mL</td>
</tr>
<tr>
<td>Relaxin</td>
<td>Radioimmunoassay</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = not available

Sample Size and Power

The sample size calculations were based on the number of subjects per group for the contraception and non-contraception groups to test for differences in isokinetic quadriceps strength. (Hypothesis 1); and knee joint laxity, (Hypothesis 2). In order to take into account differences between the oral contraception and non-oral contraception groups (Hypothesis 3), we could use the calculated sample size per group and include contraception group as a covariate in the analysis, or we could choose to enroll the number of participants per group for contraception. We chose to use the more conservative approach of enrolling based on contraception group size because of the large hormonal differences between the two groups. This sample size also will allow us to test for the hormonal group-by-menstrual phase interaction on the neuromuscular function (Hypothesis 3).
Hypothesis 1: The sample size estimate for hypothesis 1 is based on the number of participants required to detect differences in isokinetic quadriceps strength among menstrual cycle phases. This measurement has been implicated as a potential risk factor for ACL injuries.\textsuperscript{22} In order to calculate this sample size, we used data from Hewett et al.\textsuperscript{6} Assuming a 20% withdrawal rate, we will recruit 50 women for the project. This is a conservative based on detecting differences between two means calculated on independent observations and does not consider that we will be taking repeated measures on the same participants.

Hypothesis 2: The sample size for hypothesis 2 is based on the number of participants required to detect differences in mean anterior knee joint laxity, joint stiffness, and joint moments among different phases of the menstrual cycle. In order to calculate the sample size for knee joint laxity, we used data from Deie et al. 2002.\textsuperscript{13} The mean knee joint laxity was 4.7 mm (SD=0.8). A sample size of 40 will be necessary to detect an 11 percent difference between means. Similarly to hypothesis 1, we will recruit 50 women to test hypothesis 2. This is a conservative estimate based on detecting differences between two means calculated on independent observations and does not consider that we will be taking repeated measures on the same participants.

Hypothesis 3: The sample size for hypothesis 3 is based on the number of participants in the oral contraception and non-oral contraception groups required to detect differences in hamstring-to-quadriceps ratio, and joint laxity. Sample sizes for each of the variables were calculated using the same data used in hypotheses 1 and 2. Given the large hormonal variation that exists between individuals who are and are not on oral contraception, we expect these to be physiologically reasonable differences.
The sample size per group needed to detect a difference between two means was calculated using the equation below.

\[ N = \frac{[2(SD)^2 \ (Z_{\alpha/2} + Z_{\beta})^2]}{(\mu_1 - \mu_2)^2} \]

SD = the standard deviation of the outcome variable

\( \mu_1 \) = the mean of one of the contraception groups

\( \mu_2 \) = the mean of the other contraception group

\( (Z_{\alpha/2} + Z_{\beta})^2 \) = reflects the coordinates on the normal distribution that correspond to the specified type 1 and type 2 errors. Our hypotheses will be tested using a 2-sided p value at a significance level of 0.05 and 80% power.
CHAPTER 4

Results

Fifty participants were enrolled in the study and signed informed consents. Of those, 25 were using oral contraception and 25 were not. In the contraception group, one participant did not complete all three visits due to problems with blood sample acquisition and therefore was excluded from the analysis. Additionally, in the non-contraceptive group, 3 participants did not ovulate and therefore were excluded from the analysis. There was no significant difference in baseline characteristics between groups (Table 3).

Table 3. Participant Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Non-contraception (n=22)</th>
<th>Contraception (n=24)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>20.39 [18.6-25.1]</td>
<td>20.81 [19.4-23.2]</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.09 ± 6.45</td>
<td>169.87 ± 6.91</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.82 ± 8.84</td>
<td>65.70 ± 11.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

*P-Value for the difference between contraception and non-contraception groups

Height and Weight are given as mean ± SD

Age is given as mean [range]

Isokinetic tests showed that peak torque levels were significantly lower at 60, 180 and 300°/sec during the follicular phase as compared to the ovulatory and luteal phase (Table 4) (Figure 9). This finding was consistent between groups and within group.
<table>
<thead>
<tr>
<th>Visit 1: Follicular Phase</th>
<th>Non-Contraception (n=22)</th>
<th>Contraception (n=24)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rested Laxity</td>
<td>6.4 ± 2.6</td>
<td>7.0 ± 2.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Fatigued Laxity</td>
<td>6.9 ± 2.0</td>
<td>7.0 ± 2.4</td>
<td>0.9</td>
</tr>
<tr>
<td>60 Peak Torque**</td>
<td>148 ± 30</td>
<td>155 ± 24</td>
<td>0.4</td>
</tr>
<tr>
<td>180 Peak Torque**</td>
<td>95 ± 18</td>
<td>102 ± 18</td>
<td>0.2</td>
</tr>
<tr>
<td>300 Peak Torque**</td>
<td>76 ± 18</td>
<td>81 ± 14</td>
<td>0.2</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>67.6 ± 20.2</td>
<td>45.8 ± 20.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>0.06 ± 0.03</td>
<td>0.04 ± 0.04</td>
<td>0.3</td>
</tr>
<tr>
<td>Relaxin (pg/ml)</td>
<td>29.8 ± 19.4</td>
<td>27.2 ± 15.7</td>
<td>0.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Visit 2: Ovulation Phase</th>
<th>Non-Contraception (n=22)</th>
<th>Contraception (n=24)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rested Laxity</td>
<td>6.1 ± 2.1</td>
<td>6.6 ± 2.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Fatigued Laxity</td>
<td>6.4 ± 2.2</td>
<td>6.3 ± 2.4</td>
<td>0.9</td>
</tr>
<tr>
<td>60 Peak Torque**</td>
<td>156 ± 28</td>
<td>159 ± 24</td>
<td>0.6</td>
</tr>
<tr>
<td>180 Peak Torque**</td>
<td>102 ± 20</td>
<td>110 ± 18</td>
<td>0.1</td>
</tr>
<tr>
<td>300 Peak Torque**</td>
<td>81 ± 16</td>
<td>88 ± 15</td>
<td>0.1</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>134.5 ± 64.5</td>
<td>36.0 ± 18.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>1.2 ± 2.6</td>
<td>0.05 ± 0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Relaxin (pg/ml)</td>
<td>26.7 ± 18.4</td>
<td>27.6 ± 16.5</td>
<td>0.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Visit 3: Luteal Phase</th>
<th>Non-Contraception (n=22)</th>
<th>Contraception (n=24)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rested Laxity</td>
<td>6.2 ± 2.2</td>
<td>6.1 ± 2.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Fatigued Laxity</td>
<td>6.3 ± 1.7</td>
<td>6.5 ± 2.1</td>
<td>0.6</td>
</tr>
<tr>
<td>60 Peak Torque**</td>
<td>153 ± 30</td>
<td>155 ± 22</td>
<td>0.6</td>
</tr>
<tr>
<td>180 Peak Torque**</td>
<td>107 ± 20</td>
<td>113 ± 18</td>
<td>0.3</td>
</tr>
<tr>
<td>300 Peak Torque**</td>
<td>83 ± 16</td>
<td>87 ± 14</td>
<td>0.4</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>154.1 ± 62.1</td>
<td>33.7 ± 15.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>3.4 ± 3.5</td>
<td>0.05 ± 0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Relaxin (pg/ml)</td>
<td>31.3 ± 21.3</td>
<td>27.0 ± 14.6</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*P-Value for the difference between contraception and non-contraception groups
All values are given as mean ± SD
**Torque values are reported in Newton-Meters (NM)
**Figure 9:** Isokinetic Testing 60°/sec

Bars with similar letters are different at p<0.05

**Figure 10:** Isokinetic Testing 180°/sec

Bars with similar letters are different at p<0.05
Figure 11: Isokinetic Testing 300°/sec

Bars with similar letters are different at p<0.05

Our findings indicate that there was no significant difference in knee joint laxity in either the non-contraceptive group or oral contraceptive group across the menstrual cycle. (Table 4)

Results of blood assays showed that there was no significant difference between groups for relaxin concentration. (Table 4) There were significant differences with estrodial and progesterone concentration between groups. (Table 4) We found that estrodiol (p<0.001 and p<0.001) and progesterone (p<0.04 and p<0.001) concentrations were lower during the ovulatory and luteal phases in the oral contraceptive group. The inclusion of estrodiol, progesterone, and relaxin in the statistical models had no significant affect on muscle strength or knee joint laxity for either group. (Table 4)
CHAPTER 5
Discussion

The primary purpose of this study was to analyze the neuromuscular changes in females during different phases of the menstrual cycle.

Muscle Strength

The first aim of this study was to analyze the muscle strength of the quadriceps in females throughout the three phases of the menstrual cycle. It was hypothesised that quadriceps strength would decrease as estrogen increased during the menstrual cycle. Upon analysis of our results it was found that isokinetic peak torque at 60°/sec, 180°/sec, and 300°/sec was lowest during follicular phase. Testing at the same parameters during the ovulation and luteal phases showed significant increases in peak torque compared to the follicular phase. We rejected our hypothesis because during the follicular phase, which has the lowest estradiol concentrations, peak torque was also lowest.

Strength Differences throughout the Menstrual Cycle

Sarwar et al reported that the greatest muscle strength was evident during the mid-cycle or ovulation phase and the lowest muscle strength was found in the luteal phase. This contradicts what was found in the current study. We found that the greatest muscle strength was in the luteal phase. Our results at 60°/sec, are similar to Sarwar et al because they show an insignificant peak muscle torque decrease from the ovulatory to luteal phase. Phillips et al, found a correlation between menstrual cycle phase and measured muscular force. The study reported a significant increase in maximum voluntary force during the follicular phase while estrogen levels were rising. However, this study used a non-weight bearing muscle (adductor pollicus).
In a systematic review by Constantini et al, it was reported that muscle strength does not appear to fluctuate significantly during the menstrual cycle. Constantini et al researched the connection between strength differences and menstrual cycle phase finding that a majority (5) of studies found an insignificant correlation. While only three studies reported a significant change between muscle strength and menstrual cycle phase.\(^\text{12}\) Constantini attributed the differences to methodological quality and individual participant variability. Another study by Janse de Jong et al\(^\text{42}\) also demonstrated no correlation between muscle strength and menstrual cycle phase.

**Knee Joint Laxity**

The second aim of this study was to analyze knee joint laxity in females throughout the three phases of the menstrual cycle. Our hypothesis was that knee joint laxity would increase during the follicular phase of the menstrual cycle. Our results did not show a significant change between phases of the menstrual cycle. However, it should be noted that our findings did show an insignificant trend that supported our hypothesis. In our results (Table 4) knee joint laxity measurements changed slightly from the follicular phase compared to the ovulatory and luteal phases. In studies completed by Karageanes et al,\(^\text{18}\) Van Lunen et al,\(^\text{17}\) Beynnon et al,\(^\text{19}\) Eiling et al,\(^\text{43}\) and Belanger et al \(^\text{44}\) it was concluded that there were no significant changes in knee joint laxity across the menstrual cycle.

However, there are also studies that did report significant changes in knee joint laxity across the menstrual cycle. In one study done by Heitz et al.\(^\text{14}\) it was concluded that; “female ACL laxity significantly increases in conjunction with surging levels of estrogen and progesterone during the normal menstrual cycle.” However this could be
due to the timing of the blood draws and joint laxity testing. In the Heitz study, blood draws and laxity tests were done more frequently compared to our study. Heitz et al\textsuperscript{14} conducted joint laxity tests and blood draws on all seven subjects on nine different days throughout the menstrual cycle. This was done so that they could more accurately associate peak hormone levels with ACL laxity. While this adds to the methodological quality, the amount of visits that were performed in the Heitz study would not have been feasible for our current study since we used 50 participants. With this in mind, further research should be done looking at larger sample sizes while using a method involving more than three blood draws.

Another study that found significant changes in knee joint laxity was completed by Deie et al.\textsuperscript{13} The researchers concluded that there is “a significant difference in the anterior displacement of the knee between the follicular phase and the ovulatory and luteal phases.” However, they also concluded that “there was no statistically significant relation between ACL laxity and the concentrations of female hormones.” The reason that they were unable to correlate the significant changes in joint laxity with concentrations of hormones was thought to be because they were unable to complete a sufficient amount of blood draws. Hormone levels were measured once per week (4 times) so they concluded that they did not have sufficient evidence to properly monitor hormone fluctuations and thus have significant findings. This same argument could be used in our study as well since we only measured hormone levels three times and instead relied on self reported ovulation tests to direct the monitoring of each participant’s cycle. Another point worth noting is that in Deie et al \textsuperscript{13} they used a KT-2000 arthrometer while we used a KT-1000. Both have 95\% confidence intervals to support their use but the KT-
2000 is newer and provides computer plotted X-Y graphs while measurements are taken. For this reason, small variations in measurements could occur.

Another study which found differences in knee joint laxity across the menstrual cycle was completed by Shultz et al. Their study concluded that: “estradiol, progesterone, and testosterone each contribute to changes in knee laxity across the cycle, and that this relationship is stronger when changes in hormone concentrations are compared with changes in knee laxity occurring approximately 3–4 days later.” It was also noted that since each subject’s time delay varies, it is difficult to make a correlation between one sex hormone and all subjects. Our study did not consider a time delay in hormone response and thus we did not make adjustments based on that notion.

**Hormone Levels**

The third aim of this study was to analyze hormonal changes in estrogen, progesterone, and relaxin, throughout the menstrual cycle. Our hypothesis was that participants in the non-oral contraceptive group would show changes in quadriceps muscle strength and knee joint laxity compared to the oral contraceptive group. Our findings show that there are no significant differences between the oral contraceptive and non-contraceptive groups in regards to quadriceps muscle strength or knee joint laxity at any stage in the menstrual cycle.

**Strength Differences Between Groups**

In reports by Sarwar et al and Hewitt et al, it was reported that there are between group significant changes in contractile and relaxing properties in skeletal muscle during the ovulatory phase. Both studies attribute these changes to rising estrogen levels. Hewitt et al found that hamstring to quadriceps peak torque ratios are lower in the non
contraceptive population because of higher hormone levels. Sarwar et al 7 also reported a significant difference in muscle strength between contraceptive and non-contraceptive groups. Our results show an insignificant trend that peak torque was higher for the contraceptive group throughout the menstrual cycle but it wasn’t enough to show significance. The reason for inconsistent results could be due to different strength testing methods. In this study we used a Biodex with a built in fatigue protocol and Sarwar et al used a conventional strength testing chair with electrical stimulation. Differences could also be attributed to patient effort and conditioning.

The results of the current study showed an insignificant trend in a relationship between increased estradiol concentrations and increased muscle strength in the non contraceptive group. This trend was also noted in a Sarwar et al study 7 in which they found that the highest estrogen levels occur just prior to ovulation and that the surge may be responsible for an increase in muscle strength at that time. More research needs to be completed to determine if muscle strength increases can be attributed to an increase in estrogen levels or an increase in progesterone levels.

Hormone Fluctuations

There were significant differences found in estradiol and progesterone levels throughout the menstrual cycle with estradiol and progesterone levels being lower in the ovulatory and luteal phases in the oral contraceptive group. These results support other studies that have been mentioned previously.4,7,12 Relaxin is one female sex hormones that is not extensively researched. One of the goals of this study was to see if we could correlate increased relaxin levels with increased joint laxity findings. The results of our study suggest that there were no significant correlations with relaxin and knee joint
laxity. A study by Dragoo et al. researched the differences in female sex hormone levels between two groups, oral contraceptive and non-contraceptive and how they correlated with serum relaxin concentration. It was found that there is a positive correlation between progesterone levels and serum relaxin concentration. They concluded that the non contraceptive group had higher serum relaxin concentration levels compared to the contraceptive group. This contradicts what was found in our study because we found significant differences between progesterone levels between the two groups and significant differences in relaxin levels were not noted. The reason for the difference in findings could be due to the variance in the number of participants. The Dragoo study reported having 169 participants compared to the 50 that were involved in the current study.

In a study completed by Arnold et al. it was reported that there were no correlations found between knee joint laxity and serum relaxin concentration. They studied the differences between men and women and found that women had higher serum relaxin levels but did not find significant changes in knee joint laxity between groups. This is similar to results of the current study, although it was insignificant, we did see a small trend which showed the non contraceptive group had higher serum relaxin concentration when compared to the oral contraceptive group but again no changes in knee joint laxity were noted in either group.

Limitations

There are several limitations that should be noted in this study, including: small number of data points, no monitoring of oral contraceptive use, and the potential for participant drop out. It was found in some studies that additional visits, provided more
accurate hormone monitoring which led to more specific findings.\textsuperscript{14} Perhaps scheduling more visits would yield significant findings.

The KT-1000 is a clinically relevant tool with strong research\textsuperscript{46} to support the use. According to a meta-analysis by van Eck et al,\textsuperscript{34} the KT-1000 was found to have a sensitivity of .93, a specificity of .93, accuracy of .93 and positive predictive value of 6.9 for diagnosing ACL rupture. The KT-2000 has been shown to be quite reliable as well with a 95\% confidence interval for reliability estimates.\textsuperscript{45} However, it could be scientifically beneficial to use an even more valid arthrometric device to assess knee joint laxity in the future. There are many variables that could change the outcome of the readings for the KT-1000 from patient to patient. Examples of these variables are: patient positioning, equipment positioning, and test re-test reliability. We attempted to eliminate these variables as much as possible by completing an interrater reliability test with our researchers and used only one tester for every participant, who was blinded to group assignment. However, small errors could still occur.

**Future Research**

Future research should include more scheduled visits and tests for each participant group throughout the menstrual cycle. That change may lead to more significant findings related to muscle strength or knee joint laxity across the menstrual cycle.\textsuperscript{7,15} Using a more scientifically reliable method to assess knee joint laxity, may account for the minute changes that previously went unnoticed. Future studies should also look into the underlying cause of the decrease in muscle strength that was found to occur during menstruation. Also, more studies need to be done to see if hormonal surges lead to delayed knee joint laxity and quadriceps muscle strength.
Conclusion

Our results show that muscle strength is lowest during the follicular phase of the menstrual cycle and muscle strength and knee joint laxity are independent of hormone level fluctuations. Therefore additional research is necessary to understand the decrease in muscle strength during menstruation.
Bibliography

18. Karageanes SJ, Blackburn K, Vangelos ZA. The association of the menstrual cycle with the laxity of the anterior cruciate ligament in adolescent female athletes. *Clinical journal*


Appendix A

Consent to Participate in a Research Project
South Dakota State University
Brookings, SD 57007

Department of: Ethel Austin Martin Program in Human Nutrition
Project Director: Lee Weidauer, PhD Phone Number: (605) 688-4630
E-mail: Lee.Weidauer@sdstate.edu Date _____________________

Please read (listen to) the following information:

1. This is an invitation for you to participate in a research project under the direction of Dr. Lee Weidauer.

2. The project is entitled The Role of the Menstrual Cycle in Neuromuscular Risk Factors for ACL Injuries.

3. The purpose of the project is to determine the change in neuromuscular performance throughout the menstrual cycle in college-aged females and the effect of hormonal contraception on these changes.

4. If you consent to participate, you will be involved in the following process which will take about 3 hours of your time:

Questionnaires

You will be asked to complete a medical history questionnaire. This questionnaire will ask you to list any medications you are taking and also ask you about certain medical conditions you may or may not have. Additionally, you will be asked to complete a questionnaire that will ask you about certain symptoms related to your menstrual cycle that you may or may not be experiencing.

Height and Weight

We will measure your height and weight without shoes and wearing light clothing.
**Grip Strength**

We will measure your grip strength using a device that you will be asked to squeeze as hard as you can for 3 seconds.

**Quadriceps Angle**

Quadriceps angle will be measured to the nearest degree using a goniometer. Quadriceps angle is the angle formed by a line connecting the anterior, superior, iliac spine to the patellar midline and then patellar midline to the midline of the tibial tubercle.

**Body Composition**

Dual-Energy X-Ray absorptiometry (DXA) scans will be completed on the EA Martin Program’s mobile unit. The scan takes approximately 3 minutes to complete and provides a measure of body composition.

**24 Hour Diet Record**

You will be asked to complete a 24 hour diet log. Diet logs will be completed online using ASA24 software. The software uses a system that allows you several different chances to log everything you ate on a given day to ensure high quality dietary data is collected.

**Blood Tests**

A small needle will be used to draw 10ml of blood from a vein in your arm by a trained phlebotomist. The blood will be analyzed for estradiol, progesterone, and relaxin using radioimmunoassay.
**Leg Strength**

You will be asked to complete a series of leg exercises. You will be asked to push as hard as you can against the machine while the speed of your movement is limited to 60, 180, and 280 degrees per second.

**Joint Laxity**

We will measure the amount of movement in your knee using a KT 1000. The KT 1000 is a non-invasive and safe method to measure movement in the knee. During this test you will be asked to lie flat on your back while relaxing the muscles in your leg.

5. Participation in this project is voluntary. You have the right to withdraw at any time without penalty and doing so will not affect you in any way. If you have any questions, you may contact the project director at the number listed above.

6. You may experience lower extremity muscle fatigue and soreness from the isokinetic testing. You may experience discomfort and possible bruising at the site of your blood draw. You may feel uncomfortable providing some information to us and can decide if there is a question you do not want to answer. You will be exposed to a small amount of radiation during the whole body DXA scan. A total body DXA has a radiation dose of 0.35mrem. This is lower than the radiation dose of a transcontinental flight (5mrem).

7. There are no direct benefits to you as a result of participation in this study; however, you will be given data regarding body composition and leg strength that might help you in making positive lifestyle decisions.

8. As a thank you for your participation, you will be given 60 dollars as a thank you for your participation.

9. Your responses are strictly confidential. When the data and analysis are presented, you will not be linked to the data by your name, title or any other identifying item.
As a research participant, I have read the above, have had any questions answered, and agree to participate in the research project and acknowledge that I have received a copy of this form for my information.

Participant's Signature ______________________________ Date __________

Project Director's Signature __________________________ Date __________

If you have any questions regarding this study you may contact the Project Director. If you have questions regarding your rights as a participant, you can contact the SDSU Research Compliance Coordinator at (605) 688-6975 or SDSU.IRB@sdstate.edu.

This project has been approved by the SDSU Institutional Review Board, Approval No.: IRB-1411007-EXP
Appendix B

ENROLLMENT FORM

Name: ___________________ DOB: ____________ Participant ID: ___________________

Telephone Number: ____________ Email Address: __________________________

Does the study staff have permission to send text messages to remind you of upcoming visits? YES NO

Race: White  Black  American Indian/Alaska Native  Asian or Pacific Islander
Unknown

Height: _________ Weight: _________ BMI: _____________

Have you suffered any lower extremity injury in the past 6 months? YES NO

Have you ever suffered an injury to your anterior cruciate ligament (ACL)? YES NO

How many days per week do you perform at least 30 minutes of physical activity? _____

What was the first day of your last menstrual period? ___________________________

Do you have a regular menstrual cycle? (Every 21 to 35 days) YES NO

Are you currently taking oral contraception? YES NO

If yes, what type? ____________________________
**List of monophasic oral contraceptives:** Alesse, Apri, Aviane, Balziva, Brevicon, Demulen 1/35, Demulen 1/50, Femcon, Junel, Kariva, Lessina, Levlen, Levlite, Levora, Lo/Ovral, Loestrin, Lutera, Marvelon, Mercilon, Microgestin, Microgynon, Mircette, Modicon, MonoNessa, Necon, Norinyl, Notrel, Ogestrel, Ortho-cept, Ortho-cyclen, Ortho-Novum, Ovcon, Ovral, Ovranette, Portia, Seasonale, Seasonique, Sprintec, Yaz, Yasmin, Zovia

**PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q)**

<table>
<thead>
<tr>
<th>Questions</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Has your doctor ever said that you have a heart condition and that you should only perform physical activity recommended by a doctor?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Do you feel pain in your chest when you perform physical activity?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 In the past month, have you had chest pain when you were not performing any physical activity?</td>
<td></td>
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</tr>
<tr>
<td>4 Do you lose your balance because of dizziness or do you ever lose consciousness?</td>
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<tr>
<td>5 Do you have a bone or joint problem that could be made worse by a change in your physical activity?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Is your doctor currently prescribing any medication for your blood pressure or for a heart condition?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Do you have ANY other reason why you should not engage in physical activity?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix C

MEDICAL HISTORY QUESTIONNAIRE

Name: ___________________________ Participant ID: ________________ Visit
Date: _______________

Are you currently taking any medications (initial visit only)? YES NO
If Yes, please list below.

<table>
<thead>
<tr>
<th>Medication</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Do you have any of the following conditions (Initial Visit Only)? (Check all that apply)

_____ Acid Reflux (Heartburn)  _____ Chronic Low Back Pain
_____ Alcoholism                _____ Depression
_____ Allergies                 _____ Diabetes Type 1
_____ Anxiety                   _____ Diabetes Type 2
_____ Asthma                     _____ High Blood Pressure
_____ Atrial Fibrillation       _____ Irritable Bowl Syndrome
_____ Cancer                    _____ Migraines
_____ Coagulation (bleeding)    _____ Low Bone Density or Osteoporosis
_____ problem                  _____ Thyroid Problem
_____ High Cholesterol          _____ Kidney Disease
_____ Heart Disease             _____ Osteoarthritis
_____ Blood Clots               _____ Rheumatoid Arthritis
_____ Tuberculosis              _____ Anemia
_____ Emphysema                 _____ Muscular Dystrophy
_____ Hernia                    _____

Do you currently use tobacco (Initial Visit Only)? YES NO

Do you currently use alcohol (Initial Visit Only)? (circle the response that best describes your alcohol use)

NEVER RARELY (1-2 Drinks/month) SOCIALLY (1/week most weeks) FREQUENTLY (>2/week)
Appendix D

MENSTRUAL SYMPTOM QUESTIONNAIRE

Name: ___________________________ Participant ID: __________ Visit Date: ___

Visit Phase:  Follicular   Ovulation   Luteal

Answer the following questions based on how you have felt for the past 24 hours:

1. I feel more irritable than normal.................................YES NO
2. I am experiencing menstrual cramps..............................YES NO
3. I am feeling more depressed than normal ......................YES NO
4. I am experiencing more stomach pain than normal .......YES NO
5. I get tired more easily..................................................YES NO
6. I am taking a prescription for menstrual pain ..........YES NO
7. I am feeling weak or dizzy .........................................YES NO
8. I am feeling tense or nervous ......................................YES NO
9. I am experiencing diarrhea.........................................YES NO
10. I am experiencing backaches or lower back pain .......YES NO
11. I am taking OTC medication for pain .........................YES NO
12. I am experiencing pain in my breasts .........................YES NO
13. I am using heat to treat my abdominal and/or back pain .YES NO
14. I am experiencing constipation ....................................YES NO
15. I am experiencing muscle spasms in my legs ..........YES NO
16. I am feeling more bloated than normal ......................YES NO
17. I am feeling more nauseous than normal .................YES NO
18. I am having more headaches than normal .................YES NO
Appendix E

DATA COLLECTION FORM

Name: ___________________________ Participant ID: _____ Visit Date: _______

Visit Phase:   Follicular   Ovulation   Luteal

Height (cm):__ Weight (kg):__________

Standing Q-Angle:__________ Supine Q-Angle:__________

Grip Hand   R   L   Grip Strength (highest):__________

Rested Joint Laxity: Measure 1:_______Measure 2:_______Measure 3:_______

Fatigued Joint Laxity: Measure 1:_______Measure 2:_______Measure 3:_______

Peak Torque 180:______________ 50% Peak Torque:______________Reps to Fatigue:______________

DXA Completed:   YES   NO   Not Applicable

Lean Mass: _____________ Fat Mass:___________Percent Fat:___________

Blood Draw Success:   YES   NO

Biodex Completed:   YES   NO

Notes:______________________________________________________
____________________________________________________________
____________________________________________________________
Appendix F

Using Ovulation Test Kits

1. Try to take the test the first time you urinate in the morning. If you forget, DO NOT skip the test, but rather take it when you remember.
2. Pass a small amount of urine into the cup provided.
3. While holding the test strip by the blue portion, immerse the strip in urine close to but not past the MAX line for three seconds.
4. Remove the strip from the urine and lay the strip flat for 5 minutes.
5. If a line that matches the control line appears, the test is positive. Call or email Lee to schedule an appointment.

If you have any questions regarding this part of the study, call Lee at 507-829-1631.

Thank you
Appendix G

Consent to Participate in a Research Project
South Dakota State University
Brookings, SD 57007

Department of: Ethel Austin Martin Program in Human Nutrition

Project Director: Lee Weidauer, PhD Phone Number: (605) 688-4630
E-mail: Lee.Weidauer@sdstate.edu Date _____________________

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8. As a thank you for your participation, you will be given 60 dollars as a thank you for your participation.

9. Your responses are strictly confidential. When the data and analysis are presented, you will not be linked to the data by your name, title or any other identifying item.

As a research participant, I have read the above, have had any questions answered, and agree to participate in the research project and acknowledge that I have received a copy of this form for my information.

Participant's Signature _____________________ Date ________________

Project Director's Signature _____________________ Date ________________

If you have any questions regarding this study you may contact the Project Director. If you have questions regarding your rights as a participant, you can contact the SDSU Research Compliance Coordinator at (605) 688-6975 or SDSU.IRB@sdstate.edu.

This project has been approved by the SDSU Institutional Review Board, Approval No.: __________

Human Subjects Committee - Checklist
South Dakota State University
COMPLETE by checking all appropriate items and INCLUDE THIS SHEET IN ALL SUBMISSIONS

Project Director: Lee Weidauer

Project Title: The role of the menstrual cycle in neuromuscular risk factors for ACL injuries

TITLE
1. _X_ Does the title of the study appear and match the title used throughout the proposal?

INVITATION TO PARTICIPATE
2. _X_ Does the consent form begin with a clear invitation to participate?
3. _X_ Is there a description of who participants will be; how they were selected?

PURPOSE
4. _X_ Is there a clear statement of the purpose of the research?
5. _X_ Does it state who is conducting the research?
6. _X_ Does the consent form state that participation is voluntary?
7. _X_ Is it stated that the participant may withdraw without penalty?

PROCEDURES
8. _X_ Is the explanation of procedures adequate?
9. _X_ Are copies of the instruments attached?
10. _X_ Has permission to use instruments been obtained, if was developed by someone else?
11. _X_ Does it state amount of time the participant will be involved?

**BENEFITS**

12. _X_ Is the statement of potential benefits complete?

**COMPENSATION**

13. _X_ Is the availability of compensation stated?
14. __ Is there any cost to the participants?
15. __ Is there compensation in case of injury?
16. __ Is there alternative treatment available?
17. __ Is there a statement on emergency medical treatment (for more than minimal risk studies)?

**RISKS**

18. _X_ Is the description of the potential risks and discomforts complete?
19. _X_ Are methods of risk reduction in place? (i.e., referral in case of upset due to questions asked)
20. _X_ Does it state that the investigator may remove a participant from the study if it is in their best interest?

**CONFIDENTIALITY**

21. __ Is the assurance of confidentiality, when applicable clear and complete?
22. __ Is the FDA access (or other access) to research records statement included, if applicable?
23. _X_ Has the participant had an opportunity to ask questions and they have been provided with contact information should they questions in the future?
24. _X_ Does it state that participants will receive a copy of the consent form?

**SIGNATURES**

25. _X_ Are there dated subject and investigator blanks?

**GENERAL QUESTIONS**

26. _X_ Is the investigator's name and phone number on the form (i.e., signature block)
27. _X_ Is the consent form written in "lay language"?
28. _X_ Is the consent form free of any exculpatory language? (That is, no PI can claim that they are not responsible for anything that happens to a participant do to their participation in their study).
29. __ If children are included as subjects, is provision made for securing the assent of the child and the consent of the parent/guardian?
30. __ Has permission been obtained from schools, agencies involved?
31. _X_ What is the overall risk classification? Minimal? Greater than minimal?

**PROTOCOL QUESTIONS**

32. __ Do you have any major questions pertaining to the protocol (indicate on back with page # and section referenced)?
**Additional Research Staff:**

<table>
<thead>
<tr>
<th>Name</th>
<th>Title/Department</th>
<th>Telephone/Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonny Specker, PhD</td>
<td>Director and Endowed Chair</td>
<td>605-688-1644</td>
</tr>
<tr>
<td>Matt Vukovich, PhD</td>
<td>EA Martin Program Department Head</td>
<td><a href="mailto:Bonny.Specker@sdstate.edu">Bonny.Specker@sdstate.edu</a></td>
</tr>
<tr>
<td>Jeffrey Clapper, PhD</td>
<td>Health and Nutritional Sciences Professor</td>
<td>605-688-6580</td>
</tr>
<tr>
<td>Mary Beth Zwart, EdD</td>
<td>Animal Science Assistant Professor</td>
<td><a href="mailto:Jeffrey.Clapper@sdstate.edu">Jeffrey.Clapper@sdstate.edu</a></td>
</tr>
<tr>
<td>Joseph Albert, ATC</td>
<td>Health and Nutritional Sciences Graduate Student</td>
<td>712-540-4505</td>
</tr>
</tbody>
</table>