

The Effect of Oral Contraceptive Use on Muscle Properties Across the Menstrual Cycle

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THE EFFECT OF ORAL CONTRACEPTIVE USE ON MUSCLE PROPERTIES ACROSS THE MENSTRUAL CYCLE

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Objective: To investigate the effects of oral contraceptive use on active hamstring muscle stiffness, vertical leg stiffness, and hamstring electromechanical delay (EMD).

Hypothesis: Non oral contraceptive pill (Non-OCP) users will have altered muscle properties when hormone levels are greatest resulting in decreased muscle stiffness and increased EMD. Oral contraceptive pill (OCP) users will have no difference in muscle properties.

Subjects: 15 physically active females using monophasic oral contraception (OCP; age = 19.87 ± 1.13 years, height = 165.40 ± 7.96 cm, mass = 61.54 ± 13.12 kg) and 15 females not using oral contraception (Non-OCP; age = 20.40 ± 1.59 years, height = 169.93 ± 5.62 cm, mass = 62.75 ± 10.61) with a normal menstrual cycle.

Design and setting: Controlled laboratory setting. Subjects were tested two times across their menstrual cycle (menses and ovulation). Ovulation was detected using urine based ovulation kits. OCP test sessions were matched to non-OCP sessions using pill counting.

Measurements: Active hamstring stiffness, vertical leg stiffness, electromechanical delay.

Results: There was no significant effect for group or time, nor a group x time interaction for active hamstring stiffness, vertical leg stiffness, and EMD of the hamstrings.

Conclusion: This study does not indicate that taking the oral contraceptive pill would alter muscle properties in manners which are suggested to reduce ACL injury risk.

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Chapter 1 Introduction

Introduction

Physically active females suffer anterior cruciate ligament (ACL) injuries at an alarming rate. It is estimated that 70% of all ACL injuries are sports related, and each year approximately 38,000 young female athletes injure their ACL. Female athletes' risk of injuring their ACL is 2-8 times higher than males who participate in the same activities (Arendt and Dick 1995). Of these injuries, 80% arise from a non-contact mechanism such as jumping, cutting, and decelerating movements (McNair et al. 1990). The cause of females' elevated injury rate is currently unknown. Possible risk factors include anatomical differences, neuromuscular control, muscular strength, and sex hormone.

As of late, researchers have begun studying the influence of hormones on ACL injury risk. Female sex hormone blood concentrations (e.g. estrogen and progesterone) fluctuate rapidly across the menstrual cycle. Estrogen and progesterone levels are lowest at menses and reach their cyclic peak at or very soon after ovulation (Vollman 1977). Estrogen and progesterone receptors have been identified on the female ACL (Liu et al. 1996). Studies using animals have shown a decrease in tensile stress and linear stiffness of the ACL in a high estrogen hormone group when compared to the lower estrogen hormone group (Komatsuda et al. 2006). Wojtys et al.(2002) found in a study of 69 ACL injured females that a greater than expected number of injuries occurred near the ovulatory phase when estrogen concentration is at its peak. This difference may be due to the effects of sex hormone

concentrations on ligament laxity. There is much debate on this issue, but several authors have found an increase in ligament laxity across the various phases of the menstrual cycle (Heitz et al. 1999; Shultz et al. 2004). To date, the effects of female sex hormones on ligament properties and injury risk is still unclear.

Many different structures add to and create the dynamic stability of the knee. The hamstrings have been theorized to prevent anterior displacement of the tibia on the femur and tibial internal rotation. These mechanisms are associated with loading and injury to the ACL and result in lengthening of the hamstring muscles. The hamstrings respond to these motions with a gradual increase in tensile force to resist further lengthening. Active stiffness from various muscles adds to the biomechanical stability to the knee joint. Stiffness refers to the ratio of change of force to change in length ($k = \Delta \text{force} / \Delta \text{length}$). As such, a stiffer hamstring muscle group would provide a greater increase in tensile force per unit of length change compared to a more compliant, less stiff hamstring group. This heightened hamstring stiffness likely provides a greater resistance to lengthening, potentially enhancing joint stability. Interestingly, a positive relationship has been shown between active knee flexor stiffness and functional ability in ACL deficient subjects (McNair et al. 1990). Vertical leg stiffness and active hamstring stiffness of the leg have been found to be lower in females when compared to their male counterparts (Granata et al. 2002). The lesser the active hamstring stiffness and vertical leg stiffness in females may contribute to the higher rate of ACL injuries in this population.

Electromechanical delay is defined as the time interval between initiation of neural activity and force production during skeletal muscle contraction. It is hypothesized that the greater the stiffness of a given muscle is, the more efficient and timely the transfer of force to

the bony insertion will be due to a greater tension being developed per unit of length change. A less stiff muscle may prolong this delay, thus delaying the dynamic stability provided by muscle contraction. These notions support the theory that the stiffness of the knee flexors may serve a protective role for the ACL.

In addition to the ACL, estrogen receptors have been identified on human skeletal muscle. Previous literature has found no changes in isometric hamstring strength, fatigability, and electrically stimulated contractile properties across the menstrual cycle (Janse de Jonge et al. 2001). Contradictory to these results, fluctuations in hormonal concentrations across the menstrual cycle may alter the mechanical properties of muscle similar to the effects noted in isolated ligament specimens. These changes in the mechanical properties of the muscle may limit the dynamic stability provided by muscle contraction. Consequently, Eiling et al.(2007) reported a significant decrease in vertical leg stiffness has been found between ovulation and menses.

Statement of Problem

No single risk factor has been identified that explains why female athletes have an increased risk for ACL injuries. To date there is minimal research that focuses on hormone influences on muscle properties. Previous literature has found no changes in muscle contractile properties across the menstrual cycle. Eiling et al.(2007) found a decrease in muscle stiffness from menses to ovulation in normally menstruating females. However a potential limitation to the results reported by Eiling et al.(2007) is that the onset of ovulation was estimated rather than measured directly. Estimation of ovulation rather than direct measurement potentially limits the ability to make assumptions regarding hormonal

fluctuations and associated peaks. Hormonal influences may affect muscle stiffness, which may in turn decrease dynamic stability of the knee.

Additionally, if hormonal fluctuations across the menstrual cycle increase female athletes' risk for ACL injuries, one potential clinical intervention lies in the use of oral contraceptives. These pharmaceuticals limit hormonal fluctuations across the menstrual cycle and prevent ovulation. With estrogen and progesterone surges limited, any effect of estrogen on muscle properties will be reduced, providing an increase in dynamic stability to the knee.

Purpose Statement

The purpose of this study was to investigate the changes in vertical leg stiffness, active hamstring stiffness, and electromechanical delay of the hamstrings across the menstrual cycle in order to characterize the influence of hormonal fluctuations on properties of skeletal muscle which are thought to contribute to joint stability. The secondary purpose of this study was to investigate the effect of oral contraceptive use on the magnitudes of the changes noted in these muscle properties across the menstrual cycle.

Dependent Variables

1. Vertical leg stiffness
2. Active hamstring muscle stiffness
3. Electromechanical delay of the hamstrings

Independent variables

1. Group: oral contraceptive pill users, oral contraceptive pill non-users.
2. Time: menses, ovulation

Research Questions

1. Will the following variables differ between the menstrual and ovulatory phases of

the menstrual cycle in normally menstruating females?

-Vertical Leg Stiffness

H_A: Vertical leg stiffness will be significantly greater at menses compared to ovulation.

-Active Hamstring Stiffness

H_A: Active hamstring stiffness will be significantly greater at menses compared to ovulation.

-Electromechanical Delay

H_A: Electromechanical delay will be significantly longer at ovulation compared to menses.

2. Will there be differences in the following variables between females using monophasic oral contraceptives and females with a normal menstrual cycle?

-Vertical leg stiffness

H_A: Vertical leg stiffness will be significantly greater in females on oral contraceptives when compared to normally menstruating females.

-Active Hamstring Stiffness

H_A: Active hamstring stiffness will be greater in females on oral contraceptives when compared to normally menstruating females.

-Electromechanical Delay

H_A: Electromechanical delay will be shorter in females on oral contraceptives when compared to normally menstruating females.

3. Do group assignment (OPP, non-OCP) and menstrual cycle phase interact to influence the following variables?

-Vertical leg stiffness

H_A: At menses vertical leg stiffness will not differ between the two groups. At ovulation females using OCP will have increased vertical leg stiffness when compared to normally menstruating females.

-Active Hamstring Stiffness

H_A: At menses active hamstring stiffness will not differ between the two groups. At ovulation females using OCP will have increased active hamstring stiffness when compared to normally menstruating females.

-Electromechanical Delay

H_A: At menses electromechanical delay will not differ between the two groups. At ovulation females using OCP will have decreases electromechanical delay when compared to normally menstruating females.

Operational Definitions

1. Oral contraceptive pill: monophasic oral contraceptive pill.
2. Normal menstrual cycle: Females who currently have a 25-32 day menstrual cycle.
3. Oral contraceptive pill user: Females who have continuously taken an oral contraceptive pill for six months.
4. Oral contraceptive pill non-user: Females who have not taken oral contraceptive pills within the last six months..
5. Physically active: Individuals who exercise three days per week for a

minimum of 20 minutes per day.

6. Menses:

-Non oral contraceptive group- Blood hormone levels taken three to five days post menses.

-Contraceptive group- Oral contraceptive pill numbers 3 to 5 of the monthly cycle using the pill counting method.

7. Ovulation:

-Non oral contraceptive group - Blood hormone levels taken 1-3 days post ovulation, verified by positive urine based ovulation kits.

-Contraceptive group - Oral contraceptive pill number 15 to 17 of the monthly cycle using the pill counting method.

8. Vertical leg stiffness: The ratio of the change in the vertical ground reaction force to the downward displacement of the total body center of mass during continuous double leg hopping.

9. Active hamstring muscle stiffness: The active change in force for a given change of length measured by modeling the lower extremity as a single degree of freedom mass spring system.

10. Electromechanical delay: the time from the onset of EMG activity to the onset of force production during skeletal muscle contraction.

Assumptions

1. Subjects will accurately report to examiner the onset of menses.
2. Subjects will use home based ovulation kits correctly and report for measurement 1 to 3 days post ovulation.

3. All subjects currently taking the oral contraceptive pill will have done so routinely for the past six months.
4. Differences in type of activity and level of activity between test sessions will not affect muscle properties.
5. Muscle stiffness can be accurately measured using existing vertical leg stiffness and active stiffness protocols.
6. Sex hormone levels will be at their highest at the ovulatory phase of the menstrual cycle.
7. Monophasic oral contraceptive pill estrogen levels will be constant over the monthly cycle.
8. Instruments used in data collection are reliable.

Delimitations

1. Subjects will consist of physically active females between the ages of 18 and 25 years.
2. All subjects will have a normal menstrual cycle of 25-32 days for the past 6 months.
3. All subjects will be injury free in the lower extremity for the past six months.
4. Subjects will have no history of serious knee injury or surgery to the lower extremity.
5. Subjects will have no prior history of pregnancy.
6. Subjects will have normal neurological function of the lower extremity.
7. The primary examiner will be blinded to which subjects are currently taking oral contraceptive pills.

Limitations

1. Subjects for this study will be volunteers and not randomly assigned to groups.
2. Muscle stiffness measurements will only be taken on two separate days of the menstrual cycle.
3. Females taking oral contraceptive pills will be limited to monophasic doses.
4. Only subjects with a normal menstrual cycle will be accepted for this study.
5. Using subject recall of current menstrual cycle instead of objective measurement for menses testing sessions may lead to inaccuracies.

Significance of the Study

Active females are suffering ACL injuries at alarming rates when compared to their male counterparts. The role of female sex hormones on ligamentous structures has been studied with conflicting results. The hamstrings have been theorized to limit anterior translation of the tibia on the femur which is a common injury mechanism for the ACL. Estrogen receptors have recently been identified on human skeletal muscle. Throughout the female menstrual cycle there are a variety of hormones including estrogen, progesterone, luteinizing hormone, and follicle stimulating hormone, that peak at different times throughout the three phases. These surges of hormones may bind to the receptor sites and cause decreased muscle stiffness which may increase a female athlete's risk of a serious knee injury. Monophasic oral contraceptive pills release estrogen and progesterone hormones into the system at a constant concentration across the menstrual cycle. Identifying the influence of hormonal fluctuations on muscle properties thought to contribute to knee joint stability may provide insight into the derivation of the higher female ACL injury rate. Additionally,

knowledge of how oral contraceptive use influences these changes may elucidate the prophylactic potential for oral contraceptive with regard to ACL injury

Chapter 2 Review of the Literature

Introduction

With the passing of Title IX, female participation in athletics has increased dramatically. Past research has concluded that female athletes demonstrate a greater risk of suffering a serious knee injury, including ACL injuries, than their male counterparts who compete in the same sport. Of these female athletic injuries, 80% are estimated to arise from a non-contact mechanism of injury such as jumping, cutting, and decelerating movements (McNair et al. 1990). ACL injury surgical and rehabilitation costs are estimated at \$650 million in the United States alone (Myer et al. 2004). Long term effects of knee injury can be devastating to an athlete in many ways including loss of participation, increased risk for re-injury, and degenerative osteoarthritis of the knee joint.

Anatomy

The knee joint is composed of the bony articulations of the femur, patella, and tibia. The femur and tibia articulate at the condyles on the medial and lateral side of the knee. The patella articulates with the femur between the medial and lateral condyles. Due to the lack of congruency of the bony articulations of the knee, it is mechanically weak. The bony stability of the knee is greatest in full extension due to the increase in congruency of the tibia and femur.

To provide added stability, the knee joint depends on surrounding muscles, tendons, and ligaments that attach to the femur and tibia. There are four main ligaments of the knee, with two located extracapsular (fibular collateral ligament and tibial collateral ligament) and

two intracapsular (anterior cruciate ligament and posterior cruciate ligament). The fibular collateral ligament, otherwise known as the lateral collateral ligament, extends from the lateral epicondyle of the femur to the head of the fibula. The tibial collateral ligament, otherwise known as the medial collateral ligament, extends from the medial epicondyle of the femur to the medial condyle and superior medial surface of the tibia with attachments to the medial meniscus. The collateral ligaments are both taut when the knee is in full extension, and become less taut as the knee is moved into flexion. The cruciate ligaments of the knee get their name from the X shape they make as they cross inside the middle of the knee joint. The anterior cruciate ligament attaches at the posterior part of the medial side of the lateral condyle of the femur and travels distally to the anterior intercondylar area of the tibia. The ACL has been shown to provide up to 86% of the restraint to anterior translation of the tibia on the femur (Butler et al. 1980). The posterior cruciate ligament attaches to the anterior part of the lateral surface of the medial condyle and inserts distally in the posterior intercondylar area of the tibia. The PCL prevents anterior translation of the femur on the tibial plateau. The PCL is the stronger and least injured of the two cruciate ligaments.

The two menisci of the knee deepen the articular surfaces of the tibiofemoral joint and aid in shock absorption. The medial meniscus is C-shaped and is attached to the anterior intercondylar area of the tibia and to the posterior intercondylar area of the tibia. The medial meniscus is wider posteriorly than anteriorly and is the less mobile on the tibial plateau of the two menisci. The lateral meniscus is the smaller and more mobile of the two menisci, and is circular in shape.

There are numerous muscles in the lower extremity that cross the knee joint and aid in movement and stability. The quadriceps femoris is located on the anterior thigh, and is

composed of four separate muscles: rectus femoris, vastus lateralis, vastus intermedius, and the vastus medialis. The main action of the quadriceps femoris is extension of the leg at the knee joint. The quadriceps femoris is the most essential muscle for stabilization of the knee joint. The tendons of all four quadriceps muscles unite distally to form a single quadriceps tendon. The quadriceps tendon becomes the patellar tendon which holds the patella within it. The patella acts as a lever at the knee allowing for a greater mechanical advantage. A relatively weak muscle of the anterior thigh is the sartorius muscle. It originates on the anterior superior iliac spine and attaches distally on the superior part of the medial surface of the tibia at the pes anserinus. The main function of the sartorius at the knee is to flex the leg at the knee joint, and it provides stability to the medial side of the knee in conjunction with the other muscles that share its distal attachment. The gracilis is another muscle that attaches at the pes anserinus and originates on the body and inferior ramus of the pubis. The gracilis, like the sartorius, provides stability to the medial aspect of the knee and flexes the leg at the knee. On the posterior aspect of the lower leg are three muscles, the hamstrings, that all share a proximal attachment at the ischial tuberosity. These muscles all work together to flex the leg at the knee joint. The semitendinosus is located on the medial side of the posterior leg, and is the last muscle that shares the common distal attachment at the pes anserinus along with the gracilis and sartorius. The semitendinosus, like the other pes anserinus muscles, acts to stabilize the medial aspect of the knee. The semimembranosus is another muscle on the medial side of the posterior upper leg. It attaches distally on the posterior part of the medial condyle of the tibia and provides added stability to the posterior medial aspect of the knee. The semitendinosus and semimembranosus together can medially rotate the tibia on the femur 10 degrees when the knee is at 90 degrees of flexion. The biceps femoris is located on the

lateral aspect of the posterior thigh and has a long head and short head. The long head, like the other hamstring muscles is attached proximally at the ischial tuberosity. The short head proximally attaches to the linea aspera and the lateral supracondylar line of the femur. Both heads of the biceps femoris insert distally into the lateral side of the head of the fibula. When the knee is flexed to 90 degrees, the biceps femoris and the iliotibial tract can produce 40 degrees of lateral rotation of the tibia on the femur. The biceps femoris also aids in stability of the posterior lateral aspect of the knee. The hamstrings have been theorized to prevent anterior displacement of the tibia on the femur and tibial internal rotation. The gastrocnemius is the single major muscle of the lower leg that crosses the knee joint. The gastrocnemius has a lateral and medial head at the proximal portion of the muscle. The lateral head attaches at the lateral aspect of the lateral condyle of the femur, while the medial head attaches at the popliteal surface of the femur just superior to the medial condyle. The gastrocnemius aids in flexing the leg at the knee joint and with stability on the posterior aspect of the knee.

Menstrual Cycle

One characteristic that separates females and males is the periodic cycle of fluctuating hormones within the body and menstruation. This cycle is commonly called the menstrual cycle. In literature, the menstrual cycle is calculated to last an average of 28 days (Vollman 1977). Menses is defined as periodic cyclic bleeding from the uterus. This bleeding is brought about by fluctuating hormone levels circulating within the blood. One study calculated that 55% of girls have their first menstruation between the ages of 13-14 years (Vollman 1977). The most common hormones found throughout the cycle are estrogen, progesterone, follicle stimulating hormone, relaxin, and luteinizing hormone (LH). The spikes and declines in the circulating hormone levels along with physical characteristics help

define the three different phases of the menstrual cycle. The first phase of the menstrual cycle is the follicular phase which is characterized by the first day of menses. The follicular phase is the phase of the menstrual cycle which can have the greatest fluctuation in length. During the early follicular phase estrogen and progesterone are at their lowest levels, with estrogen peaking during the latter part of the stage. The ovulatory phase is the second phase of the menstrual cycle and occurs on average 14 days after the onset of menses (Vollman 1977). The ovulatory phase will typically only account for one day of the menstrual cycle, and is commonly called ovulation. Immediately following ovulation, the luteal phase will begin. The luteal phase on average is a constant 14 days in length prior to subsequent menstruation, and is characterized by a sharp rise in progesterone.

Estrogen and progesterone are two of the many hormone concentrations that are circulating in the bloodstream that differ between females and males. Estrogen levels increase at around day 7 of the follicular phase, with the peak being characterized as just prior to ovulation (Carcia et al. 2004; Eiling et al. 2007). Estrogen levels return to near normal levels and have a second lesser peak in the early luteal phase, and slowly decrease back to baseline throughout the luteal phase. Progesterone blood concentration levels are near zero during the follicular and ovulatory phases of the menstrual cycle. Just after the ovulatory phase, progesterone levels increase abruptly to a maximum level for the duration of the luteal phase and decrease sharply near menses. Luteinizing blood hormone levels are relatively constant across the menstrual cycle except for a surge just prior to ovulation (Eiling et al. 2007). This surge can help predict the exact day of ovulation for a particular female's menstrual cycle. Ovulation detection strips monitor this surge in hormone levels and predict ovulation when the particular strip's LH concentration threshold is reached. Relaxin levels

are fairly stable across the menstrual cycle and reach their highest point during the luteal phase (Dragoo et al. 2003). Follicle stimulating hormone levels are fairly steady across the menstrual cycle with a small peak occurring just prior to ovulation.

Epidemiology

An injury to the knee of a physically active person can be potentially devastating. The worst of these injuries of the knee is commonly thought to be an injury to the anterior cruciate ligament. These injuries result from a wide variety of mechanisms that range from contact with another object or player to a non-contact injury from running and cutting. A non-contact ACL injury refers to any injury that is the result of a non-contact mechanism of injury and documented by clinical or imaging exam (Agel et al. 2006). One study found that the most common mechanism of injury recorded for female athletes was planting and pivoting (Arendt et al. 1999).

Seventy percent of all ACL injuries are typically related to sporting activities (Arendt and Dick 1995). Female athletes have a 2-8 times increased risk of injuring their ACL when compared to male athletes competing in the same sports (Arendt and Dick 1995). These injuries have been studied, and there is a variance in rate between the sexes for sports activities and time of competition when the injury occurs. Incidence of ACL is 30 times higher during competition than training for females when compared to males (Myklebust et al. 1998). When looking across all sports, the number of females injured has been found to be statistically higher than males for non contact ACL injuries regardless of the sport being played (Agel et al. 2006). Women's ACL injury rate for soccer has been found to be more than double that of males, and 63% of all female ACL injuries had a non-contact mechanism

of injury compared to 48% for males. For basketball, female athletes were found to have a non-contact injury rate of 80%, and their overall injury rate was four times more than males.

When an athlete suffers a knee injury, many different factors affect the athlete's life such as time lost to injury, monetary cost, and long term medical problems. A typical ACL injury requires surgery, and six to twelve months of physical rehabilitation to recover. The total financial burden of serious knee injuries in female athletes may reach \$100 million at the high school and collegiate levels combined (Hewett et al. 1999). Total cost of ACL injuries in the United States alone is estimated to be \$650 million every year (Myer et al. 2004). When the athlete returns to athletic activity, it does not mean this is the end of the recovery journey. Long term tendonitis, degenerative changes, increased injury risk, and osteoarthritis may affect the injured athlete the rest of their lives. One study found that by 13 years after surgery, clinical signs of degenerative changes were present in three of four patients with ACL reconstruction with patellar tendon autograft (Salmon et al. 2006).

Risk Factors for ACL Injury

Researchers have studied a variety of risk factors that may increase an athlete's risk for an ACL injury. No one risk factor has been identified that significantly increases a female athlete's risk for injury. There are both extrinsic factors that influence the level of loads within the joint and intrinsic risk factors that influence the anatomy and physiology of the knee directly. Some extrinsic factors that have been researched lately are body movement patterns, muscular strength, shoe-surface interface, and skill level. Intrinsic risk factors that have been studied are joint laxity, Q-angle, intercondylar notch dimensions, ligament size, and hormonal influences (Griffin et al. 2006). Conflicting results have been published on these risk factors, and future research needs to be conducted in order to achieve a consensus.

Biomechanics

A small change in biomechanics in the lower extremity can be felt throughout the entire kinetic chain. In normal gait, at or just after foot strike, the knee is positioned at an angle that will allow the quadriceps muscle to strain the ACL (Colby et al. 2000). Two risk factors that have been researched heavily in the past are Q-angle and knee abduction angles. Knee abduction angles are significantly different between ACL injured and uninjured groups both at initial contact and at maximal displacement (Hewett et al. 2005). Also, female's exhibit increased normalized peak valgus moments during the stance phase of sidestepping when compared to males (McLean et al. 2005). The female athletes are having their body mechanics put their knee in a position that may increase their chances of ACL injury and may account for the sex differences in ACL injury risk. Female athletes not only have an increased risk for injuring their ACL, but a greater percentage of these injuries are coming from a non contact mechanism of injury. Female athletes on average have knee motion patterns in selected athletic tasks that frequently bring them close to body positions in which non contact ACL injuries may occur (Malinzak et al. 2001).

Neuromuscular control

Neuromuscular control has a significant effect on how well a person is able to control and sense their body segments in space. Many factors can influence neuromuscular control such as training level and skill level. Untrained female athletes were 4.8 to 5.8 times more likely than untrained male athletes to suffer a knee injury and trained females were 1.3 to 2.4 times more likely than their male counterparts to suffer a knee injury (Hewett et al. 1999). These findings provide one factor that may play a role in increased ACL risk. Neuromuscular training limited injury risk but did not do away with it all together. Differences in

neuromuscular control between the sexes seem to diverge after maturation. Neuromuscular control indices in the female athlete decreased with the onset of puberty, and decreases continued into the late or post pubertal stage (Hewett et al. 2004). After maturation, adolescent boys regained neuromuscular control, whereas girls did not make a similar neuromuscular adaptation (Hewett et al. 2004). The life stage that separates the males from the females is the change in sex hormones and the start of the female menstrual cycle. No significant fluctuations in knee joint position sense and postural control were noticed across the menstrual cycle (Hertel et al. 2006). Female athletes though may commonly demonstrate one or more neuromuscular imbalances of ligament dominance, quadriceps dominance, and leg dominance (Myer et al. 2004). .

Hormonal Factors

When looking at the increased rate of ACL injuries in female athletes, researchers have identified the sex hormone blood concentrations as one possible explanation. This idea has come to the forefront because many of these sex hormones are not seen at equal levels in both sexes, and blood concentrations fluctuate across the female menstrual cycle. Much of this research has come about with conflicting results. A variety of sex hormone receptors have been identified on the female and male anterior cruciate ligament. Specific binding was seen in female ACL tissues incubated in biotitrated relaxin, suggesting a possibility of relaxin receptors on the female ACL with no binding of relaxin to male ACL tissues (Dragoo et al. 2003). Along with relaxin, estrogen receptors have also been identified on the ACL.

The identification of sex hormone receptors on the ACL gives further rational for investigating the relationship between sex hormone concentrations and injury risk. The ACL has been shown in the animal model to have a lower load to failure in rabbits given estrogen

for 30 days when compared to a control group (Slauterbeck et al. 1999). Yu et al. (1999) found a dose dependent decrease in type 1 collagen synthesis in the human ACL. One study observed an equal number of ligament sprains in girls and boys prior to adolescence, yet girls were found to have a higher rate of injury following their growth spurt and into maturity (Tursz and Crost 1986). The significant event that happens between adolescence and maturity in the female athlete is the beginning of the menstrual cycle and fluctuating blood sex hormone levels. Conflicting research has been published lately on the changes in knee laxity across the menstrual cycle. While no current research has directly correlated increased knee laxity with increased ACL injury risk, greater joint laxity has been suggested as an important injury risk factor. Previous studies have measured blood hormone levels on a selection of days throughout the different phases of the female menstrual cycle and compared them to KT-2000 knee arthrometer measurements of knee ligament laxity. Numerous authors have reported that knee laxity does not differ across the menstrual cycle (Belanger et al. 2004; Eiling et al. 2007; Hertel et al. 2006; Van Lunen et al. 2003). Additional studies found no direct effect of estrogen on the mechanical and material properties of the ACL in the primate model (Warden et al. 2006; Wentorf et al. 2006). However, Shultz et al.(2004) found that estradiol, progesterone, and testosterone each contributed to changes in knee laxity across the menstrual cycle. The most significant finding of this study was that the relationship between the changes in sex hormone concentrations and knee laxity became stronger three to four days after the estrogen peak. This raises the question of whether there is a delayed effect of sex hormones on the ACL. The delayed effect of hormonal fluctuations on knee joint laxity may partially explain the lack of agreement in previous literature.

The three phases of the menstrual cycle are characterized by changes in hormone levels and physical changes. Much research has gone into ACL injury risk and phase of the menstrual cycle. However, the results with respect to ACL injury are conflicting. A significant difference has been found in anterior displacement of the knee between the follicular phase, ovulatory phase, and luteal phase (Deie et al. 2002). Slauterbeck et al.(2002) found that 26 of 37 athletes in their study who tore their ACL did so in the follicular phase of the menstrual cycle. It is during this phase that estrogen, luteinizing hormone, follicle stimulating hormone, and relaxin all have their peak levels. A greater than expected amount of ACL injuries were found during ovulation based on the length of phase, whereas fewer injuries than expected were found during the luteal phase (Wojtys et al. 2002). In the same study, women currently taking oral contraceptives had an injury rate much closer to the expected values (Wojtys et al. 2002). A combination of studies looking and ACL injury risk found that most injuries occurred either just before or just after menses (Agel et al. 2006; Arendt et al. 1999; Slauterbeck and Hardy 2001). A study looking into injury rates in female alpine skiers found that there was a raised injury occurrence during the pre-ovulatory phase of the menstrual cycle (Beynnon et al. 2006).

Stability of the knee is comprised of both ligamentous and muscular support. The hamstrings respond to anterior tibial translation with a gradual increase in tensile force to resist further lengthening. Stiffness refers to the ratio of change of force to change in length ($k = \Delta \text{force} / \Delta \text{length}$). As such a stiffer hamstring muscle group would provide a greater increase in tensile force per unit of length change compared to a more compliant, less stiff hamstring group. This heightened hamstring stiffness likely provides a greater resistance to lengthening, potentially enhancing joint stability. With the distal attachments of the

hamstrings medially on the tibia and laterally on the fibula, internal/external rotation of the knee and anterior translation of the tibia on the femur would produce a lengthening of the hamstring muscles. ACL injuries with a non-contact mechanism of injury, commonly seen in female athletes, typically occur with hip adduction, knee valgus, and an externally rotated tibia. In this position the hamstrings and gluteus maximus are unable to protect the ACL (Ireland 1999). Several studies have identified estrogen and progesterone receptors on human and animal model skeletal muscles. The presence of estrogen receptor type alpha mRNA has been found in both male and female deltoid muscle, and also in only the female pectoral muscle (Lemoine et al. 2003). A later study found both estrogen receptor type alpha and type beta in skeletal muscle in pigs (Kalbe et al. 2007). The existence of hormone receptors on skeletal muscle suggests the potential for changes in muscle properties across the menstrual cycle, and potential changes in joint stability and injury risk.

Several studies have been published investigating the effects of estrogen and menstrual cycle on musculotendinous stiffness and muscular strength. Eiling et al.(2007) found a significant effect of estrogen on musculotendinous stiffness throughout the menstrual cycle with a significant decrease in stiffness at ovulation. Just prior to ovulation, estrogen levels are found to be at their highest concentration throughout the menstrual cycle. Many studies that have investigated muscular strength have found no significant differences across the menstrual cycle (Elliott et al. 2005; Hertel et al. 2006; Janse de Jonge et al. 2001). Contradictory evidence has come forward showing significant changes in quadriceps strength and fatigability and a discrepancy in quadriceps to hamstring strength ratios occurring shortly after menarche (Ahmad et al. 2006; Sarwar et al. 1996). While many researchers have focused on the effects of estrogen on strength and muscle stiffness, experimental evidence

has come about suggesting that estrogen plays a role in mitigating muscle damage and in inflammation-related leucocyte infiltration in skeletal muscle, primarily in the animal model (Tiidus 2005). This raises the question of whether estrogen's role is more on the mechanical properties of muscle or more on the healing process of an injured muscle.

Oral Contraceptives

More and more women are being prescribed contraceptives today for a variety of reasons. Oral contraceptives are just one form of contraceptive that is currently being used by females today. These pills are produced in varying estrogen levels that may have high hormonal content (50 ug) or low hormonal content (20, 30, or 35 ug). Most prescriptions for oral contraceptives today are for 35 ug estradiol doses. There are around 40 different brands of low dose oral contraceptives in the United States that physicians have available to prescribe. Oral contraceptives also vary by how hormone concentrations change across the different phases of a menstrual cycle. There are currently monophasic, biphasic, and triphasic formulations of oral contraceptives. Monophasic oral contraceptives have constant doses of both estrogen and progestin across the entire cycle. Biphasic oral contraceptives are rarely used in the United States and keep estrogen constant across the cycle while containing one dose of progestin for 10 days and then 11 days of another dose. Triphasic concentrations vary the dose of progestin or estrogen across the three phases of the menstrual cycle. The purpose of OC pills is to control the estrogen surge during the late follicular phase, thus preventing ovulation and pregnancy. A series of sugar pills end each cycle's regimen of pills, allowing the body to menstruate.

Many studies have been performed comparing knee properties in females using oral contraceptives and females not using oral contraceptives. Due to the fact that oral

contraceptives are designed to prevent the estrogen peak during ovulation, use of oral contraceptives may limit the potential influence of estrogen on factors which contribute to knee joint stability. Athletes on oral contraceptives demonstrate lower impact forces and reduced torques at the knee, increased quadriceps to hamstrings strength ratios, increased stability on one leg, and decreased knee laxity relative to non-users (Hewett 2000). Also, a study following a women's soccer league found that women using oral contraceptives have a lower injury rate than women not using the pill (Moller-Nielsen and Hammar 1989). Wojtys et al.(2002) found that women taking oral contraceptives had an injury rate much closer to the expected values than those not using OCP.

With increased interest in research of female hormone levels and the menstrual cycle's effects on ACL injury risk, female oral contraceptive use and its effects on injury should be studied. Oral contraceptives control the hormone levels and phases across the menstrual cycle and limit hormone surges. A two year study researched oral contraceptive use in female soccer and basketball athletes at NCAA institutions. Over this time span, oral contraceptive use among female athletes did not significantly change and 42% of female basketball players and 70% of female soccer players reported using a form of oral contraceptive (Agel et al. 2006).

Dynamic Joint Stability

Stiffness

When looking at the muscle's role in adding to dynamic joint stability we first need to define muscle stiffness. Muscle stiffness is defined as the ratio of change in force in a muscle to its change in length. Muscle stiffness comes from the elastic properties within the components of the muscle the can be generally classified as parallel elastic components

(PEC) or as series elastic components (SEC). The PEC gets its' name due to the fact that the arrangement of the tissues is in parallel with the contractile component. The PEC is primarily made up of connective tissues including the sarcolemma, epimysium, endomysium, and perimysium. As with the PEC, the SEC receives its name due to the arrangement of its components in series with the contractile component, and includes the tendon, Z discs, and cross bridge formations. Muscle stiffness will also receive contributions from the viscoelastic properties within the muscle and muscle activation. The PEC is the main contributor to passive muscle stiffness due to axial loading of the collagen fibers with lengthening of the muscle fiber and isn't active until the muscle passively reaches its' resting length. The SEC provides a major contribution to muscle stiffness within the sarcomere through the crossbridges formed between the actin and myosin filaments. When a muscle is lengthened, the Z disks are pulled away from one another, and the actin and myosin crossbridges that are formed will resist this motion causing tension within the muscle, generating muscle stiffness. Sinkjaer et al(1988) found that as maximal voluntary contraction of the muscle increased, muscle stiffness increased curvilinearly. Other major contributors to muscle stiffness are length tension relationship of the muscle, neural activation, or number of motor units activated, and the stretch reflex via the muscle spindle due to an increase in cross bridge formations

Muscle stiffness is important to joint stability because a less stiff muscle may allow for greater muscle lengthening which in turn could result in a greater amount of joint translation. This increase in joint translation may increase the likelihood for joint and ligamentous injury (Blackburn et al. 2004). The muscular system is in place to serve a protective role in limiting the external forces and moments created through boney motions

that ultimately result in tension of the ACL (Dedrick et al. 2006). The muscular system serves an important role in joint stability for joints lacking bony stability. Not only can a less stiff muscle affect the stability of the knee, but also an inequality in strength ratios between agonists and antagonists may place the joint at an increased risk for injury. When there is minimal hamstring activation coupled with forces generated by the quadriceps muscles at the knee, this interaction could produce significant anterior displacement of the tibia (Colby et al. 2000). Commonly the ACL is injured by anterior translation of the tibia on the femur with internal or external rotation of the knee joint. With the attachments of the hamstrings, proximally on the ischial tuberosity and distally on the tibia and fibula, this common mechanism of injury would produce a lengthening within hamstring muscles. The hamstrings will respond by generating a tensile force resisting this lengthening. This resistance will limit harmful motions at the knee providing a protective effect to the ACL.

If an athlete has reduced active stiffness, this may limit the ability of neuromuscular control system to provide dynamic joint stability (Blackburn et al. 2004). Gender differences have been noted in active joint stiffness between males and females (Granata et al. 2002). Males displayed greater active and passive knee flexor stiffness and lesser extensibility compared to their female counterparts. Gender differences in stiffness are amplified at higher joint loads, and have implications for stability and musculoskeletal injury during functional loading tasks (Granata et al. 2002). Vertical leg stiffness is another component of dynamic joint stability that can mimic some activities similar to athletic activity. This can be measured because during hopping, trotting, and running the actions of the body's numerous musculoskeletal springs are combined so that the overall system behaves like a single linear spring (Ferris and Farley 1997). Humans have control over vertical leg stiffness and adjust

leg stiffness in order to accommodate for changes in surface stiffness during hopping (Ferris and Farley 1997). The speed at which a person performs a vertical leg stiffness test also changes the overall stiffness measure. When women and men were allowed to self select a preferred hopping frequency, they selected similar frequencies around 2.2 Hz (Granata et al. 2002). Differences between sexes have been measured in order to address the question of increased female risk for ACL injury. Leg stiffness for females during a hopping task was approximately 77% of the leg stiffness in male subjects (Padua et al. 2005).

Electromechanical Delay

Electromechanical delay (EMD) is defined as the time interval between initiation of neural activity and force production during skeletal muscle contraction. Previous research has shown that the less slack there is in a given muscle, the more efficient and timely the transfer of force to the bony insertion will be due to a greater tension being developed per unit of length change (Muraoka et al. 2004). A less stiff muscle may prolong this delay by requiring a greater change in length to attain a critical level of tensile force. This delay in dynamic stability may lead to increased injury of the ACL. Winter and Brookes(1991) used a sitting plantar flexion task and found a significant difference in soleus EMD between males and female subjects . If similar effects on EMD were found to be true in the hamstrings, this delay in dynamic stability may contribute to the increase in ACL injury found in female athletes possibly due to estrogen. There is currently a gap in the literature concerning the effects the female menstrual cycle on EMD.

Influence of Hormonal Fluctuations on Dynamic Joint Stability

To date there is a lack of evidence that explains the effects of estrogen and progesterone fluctuations on various tissues and structures within the body. Estrogen

receptors have been identified on ligament, muscle, tendon, and the central nervous system. It is currently unclear what role each of these tissues plays in the increased female ACL injury rate. Previously, estrogen has been shown to have an effect on muscle stiffness across the menstrual cycle (Elliott et al. 2005). To the contrary, other researchers hypothesize that estrogen receptors are present on human muscle as a function of mitigating muscle damage, decreasing inflammation, and for tissue repair following injury (Tiidus 2005). Estrogen and progesterone have been shown to have no effect on muscle strength and time to fatigue across the phases of the menstrual cycle (Janse de Jonge 2003). Estrogen and progesterone receptors have been identified on tendon (Hart et al. 1998). The effects of estrogen on tendon are thought to be similar to those found within ligaments. Miller et al.(2007) concluded that there was no effect of menstrual phase on tendon collagen synthesis after exercise or at rest. When looking at post menopausal women using hormone replacement therapy (HRT), Cook et al.(2007) found a significantly smaller achilles tendon in active women taking HRT, and hypothesizes that it may serve a function in preserving tendon collagen. The physiological effects of estrogen on receptors within muscle and tendon are currently unclear. The combined negative effects on muscle and tendon by estrogen concerning stiffness and joint stability may lead to the clues regarding why females have an increased incidence of ACL injuries.

Other plausible explanations for this increased injury rate could lie within the central nervous system. Estrogen receptors have been identified within the central nervous system, yet the effects are not currently fully understood (McEwen 1999). An impairment in neuromuscular control has been found following puberty and across the menstrual cycle (Dedrick et al. 2006; Hewett et al. 2004). Currently there is no conclusive research on

estrogen's effects on the central nervous system. Decreased neural activation may have possible effects on decreased strength, neuromuscular control, and increased EMD leading to a decrease in dynamic joint stability. The hormonal influences on muscle, tendon, and the central nervous system are potentially intertwined. If an increase in estrogen hormone levels near ovulation produces a decrease in muscle stiffness, an increase in EMD would be the product of this interaction. Additionally, if the neural drive to the muscle is delayed or inhibited via the influence of estrogen, this discrepancy would be magnified. The use of monophasic oral contraceptives by active females would control hormone surges across the phases of the menstrual cycle, possibly providing a protective effect through dynamic joint stability to the ACL.

Summary

Female athletic participation continues to increase as new opportunities arise around the world. It is currently unknown why female athletes have a 2-8 times increased risk for an ACL injury. Prior to puberty, adolescent female and male athletes display similar injury statistics. It is after puberty and the onset of the female menstrual cycle that active females' risk increases. Across the female menstrual cycle a female's body has positive and negative fluctuations of sex hormones. Receptors have been identified within human skeletal muscle and ligaments for these particular sex hormones. It is during many of these hormonal fluctuations when an abnormal amount of female ACL injuries seem to occur. Many females today use some form of contraception for a variety of reasons. Monophasic oral contraceptive pills distribute even levels of estrogen and progesterone across a monthly cycle. The purpose of this study is to compare the effects of monophasic oral contraceptive use on muscle properties to female non oral contraceptive users.

Chapter 3 Methodology

Subjects

Thirty subjects from the student population at the University of North Carolina at Chapel Hill volunteered to participate in this investigation. These subjects were selected from a physically active female student population. Subjects that met the study's inclusion criteria were divided into two equal groups consisting of 15 normally menstruating females not using oral contraceptive (non-OC) and 15 monophasic oral contraceptive users (OC). A priori power analysis on previous literature for vertical leg stiffness indicated that a sample size of 30 subjects would allow for a power at 0.80 (Eiling et al. 2007).

Subjects were eligible for participation in this investigation if they met the following inclusion criteria:

- 1). Participation in 20 minutes of activity a minimum of three times per week
- 2). OC group must be using approved monophasic OC for a minimum of six months
- 3). No lower extremity injury or surgery in the past six months.
- 4). Non-OC group must have not taken OCP in past six months.
- 5). Between 18-25 years old
- 7). No history of pregnancy
- 8). Consistent menstrual cycle for last six consecutive months

All subjects were informed of the procedures that were involved prior to participation in this study, and were required to read and sign an approved informed consent form prior to participation.

Protocol

Subjects reported to the Motor Control lab for two separate testing sessions lasting one hour each that took place at both menses and ovulation. The menses testing session occurred three to five days post menstruation for the non-OC group and oral contraceptive pill number three to five for the OC matched group. This was determined by subject recall of menstruation and OCP counting. This session was characterized by low blood hormone concentrations of estrogen and progesterone for the non-OC group and a constant low concentration of estrogen and progesterone for OC group. The ovulation session was determined by a positive ovulation test using urine based ovulation kits daily for the non-OC group. Subjects in the non-OC group reported within 3 days post ovulation and OC pill number 15-17 in the OC matched group. The ovulation testing session was characterized by high blood hormone concentrations of estrogen and progesterone in the non-OC group and a constant low concentration of estrogen and progesterone in the OC matched group. The order of which hormone concentration that subjects first reported for testing was counterbalanced by alternating which menstrual cycle phase or pill number each subject initially reported for testing. All subjects had current height and weight taken prior to dependent variable testing. The order of dependent variable assessments was counterbalanced to prevent an order effect.

Vertical Leg Stiffness

For the vertical leg stiffness protocol, subjects were required to perform a two legged hop on a non-conductive Bertec (Model 4060-nc, Bertec Company, Columbus, Ohio) force

plate for one minute. A metronome was set at 132 beats per minute (2.2 Hz) for the subject to keep pace with, in order to control hopping frequency. This frequency was chosen because past research has identified the preferred hopping frequency of subjects is 2.2 Hz.(Farley et al. 1991) The vertical ground reaction force was sampled at 1,000 Hz via the Motion monitor software system (Innovative Sports Training, Inc., Chicago, IL). All subjects assumed a standardized position with both hands on their hips and were wearing no shoes. Subjects stood stable on the forceplate for 10 seconds to get baseline body mass prior to performing two legged hopping for 60 seconds. Subjects completed one successful trial per test session. The first 10 acceptable hopping trials were selected for analysis. An acceptable hopping trial was defined using the system defined by Padua et al. (2005). Trials were only accepted for analysis if the subjects hopping frequency was within 5% of the designated frequency of 2.2 Hz. This was selected because prior research has shown vertical leg stiffness is directly related to hopping frequency (Farley and Morgenroth 1999; Granata et al. 2002). Secondly, a linear correlation of the vertical center of mass and the vertical ground reaction force had to have a value greater than $r = .80$ to be qualify for analysis. The location of the total body center of mass was estimated from a double integration in the time domain of the acceleration time data (Padua et al. 2005). Vertical acceleration of the center of mass was calculated from the ground reaction force and participants body mass which was measured during a static trial for the first 10 seconds. A linear regression line was fitted to the relationship between the change in ground reaction force and displacement of the total body center of mass (Figure 1). The slope of the resulting regression equation represents vertical leg stiffness (McMahon and Cheng 1990; Padua et al. 2005).

Active Hamstring Stiffness

Subjects laid prone on a custom padded table with the right thigh supported in 30 degrees of flexion (Figure 2). EMG electrodes were placed over the long head of the biceps femoris in order to monitor muscle activity. Electrode placement was verified by manual muscle testing. The system being used was a hardwired Delysis 8-channel surface EMG (Delysis Bagnoli-8, Boston, MA) sampled at a rate of 1000 Hz via the Motion Monitor software system. In order to reduce impedance, the electrode site was shaved and cleaned with an alcohol prep pad. An accelerometer was attached to the subject's foot in order to measure oscillatory motion following an examiner applied perturbation. A standardized cuff weight of 10% of the subject's body mass was secured to the ankle of the right leg. A custom made brace was attached to the right ankle using a compression wrap to keep the ankle in dorsiflexion and for attachment of the accelerometer. When the subject states they are ready, leg support from the examiner was removed from the right leg and the subject was required to hold her lower leg in a standardized position of approximately 30 degrees of knee flexion by contracting the hamstrings. A perturbation was applied by the investigator to the lower leg producing damped oscillatory motion around the knee joint. The subject was asked not to resist the motion and to attempt to keep the knee in the same position. Oscillatory motion about the knee joint was captured from the accelerometer, and the damped frequency of oscillation was used to estimate hamstring stiffness. Using the time periods of the two oscillatory peaks in the tangential acceleration (t_1 and t_2) the damped frequency of oscillation can be calculated by using the formula $(1 / t_2 - t_1)$, Figure 3). Five trials were performed with one minute of rest between each attempt to prevent the likelihood of fatigue. The highest and lowest active hamstring stiffness trials of the 5 trials were thrown out and did not undergo statistical analysis to limit outliers.

Active hamstring stiffness was calculated using the following equation:

- 1). $K_a = 4\pi^2 mf^2$ where K_a = active hamstring stiffness, m = summed masses of the shank and foot segment, and f = damped frequency of oscillation.

Electromechanical Delay Protocol

Subjects were positioned prone on the same table used for the active hamstring stiffness protocol with the foot secured to a custom built device containing a load cell (Figure 4). This arrangement prevented knee joint motion, thus making hamstring contraction isometric. Subjects were asked to relax and wait for a light stimulus to turn on. When the stimulus was presented, the subject maximally contracted their hamstring muscles as forcefully and quickly as possible against a load cell until the stimulus was removed. Electromechanical delay was calculated as the time from the onset of muscle activity in the hamstrings measured by EMG to the production of force at the load cell. Muscle onsets were determined by computer algorithm. Force onset was determined as the point when the load cell reaches 5% of peak force. EMG onset was determined as the point when the amplitude exceeds two times the mean amplitude over the first 100ms. EMG activity must stay above this threshold for a minimum of 50ms to qualify as EMG onset. Five trials were recorded for each subject with one minute rest between trials to prevent the likelihood of fatigue. The highest and lowest EMD trials of the 5 trials were thrown out and did not undergo statistical analysis to limit outliers.

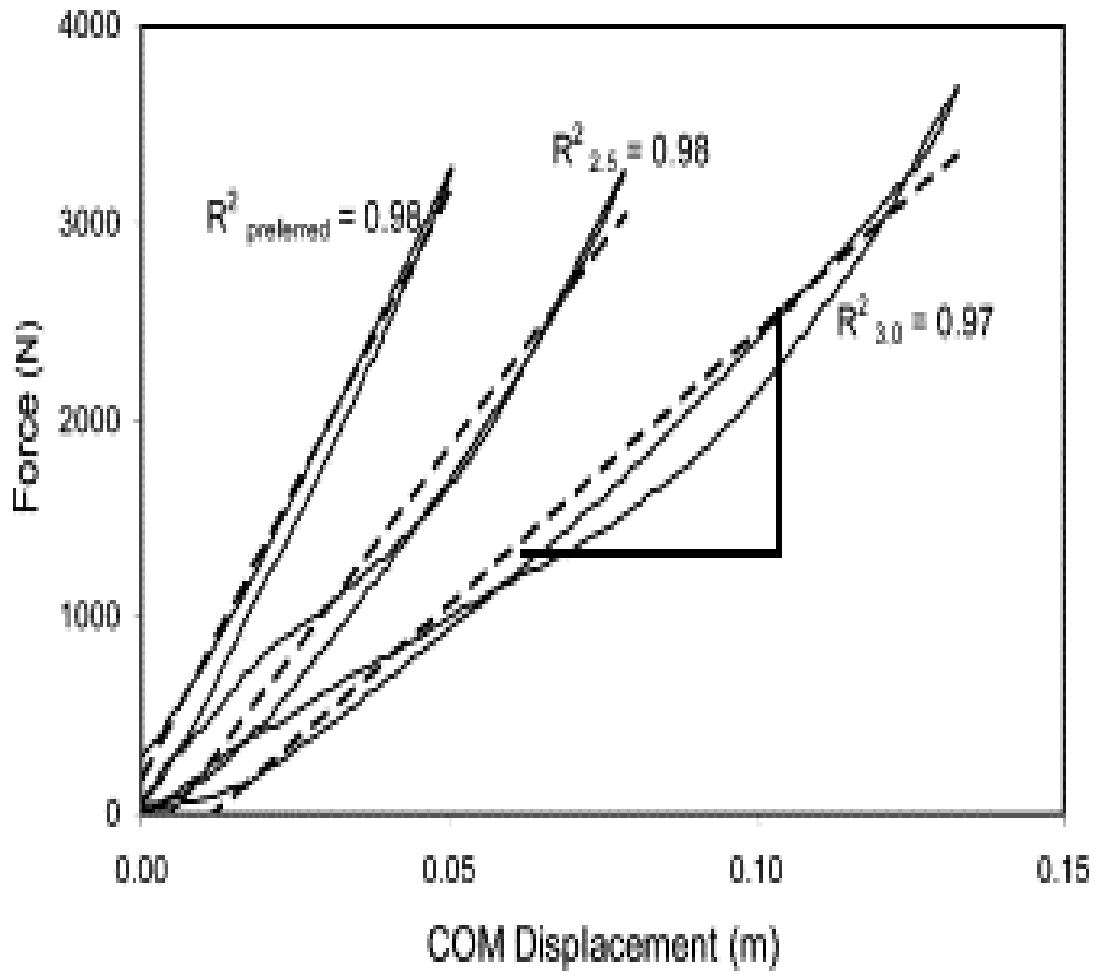
Data Reduction and Statistical Analysis

Active hamstring stiffness and EMD data were processed and reduced using custom software developed in LabVIEW (LabVIEW, National Instruments, San Antonio, TX). Vertical leg stiffness was processed and analyzed using custom software developed in

MATLAB (The Math-Works, Natick, MA). All EMG, load cell, and force plate data were sampled using an A/D converter (National Instruments, Austin, TX) at 1000 Hz to a storage computer. Accelerometer and load cell data were lowpass filtered at 10 Hz (4th order, zero phase-lag Butterworth). EMG data were corrected for DC bias and filtered using a bandpass (20-350 Hz) and notch (59.5-60.5 Hz) filters (4th order, zero phase-lag Butterworth). EMG data were also smoothed using a root mean square sliding window function with a time constant of 20ms.

A 2 (Group: OC, Non-OC) X 2 (Time: menses, ovulation) repeated measures ANOVA was performed for each dependent variable. Significant interaction effects were evaluated post hoc via planned pair-wise comparisons after adjusting for type I error rate with a Bonferroni correction. An alpha level of 0.05 was set a priori. Intraclass correlation coefficients (ICC) and associated standard errors of measurement (SEM) were calculated for active hamstring stiffness and EMD to determine within-session reliability. Separate one way repeated measures ANOVA were performed across trials and ICC's were calculated using ICC equation (2, 1). All statistical analysis were calculated using SPSS 13.0 software (SPSS Inc., Chicago, IL).

Figure 1: Vertical Leg Stiffness Plot



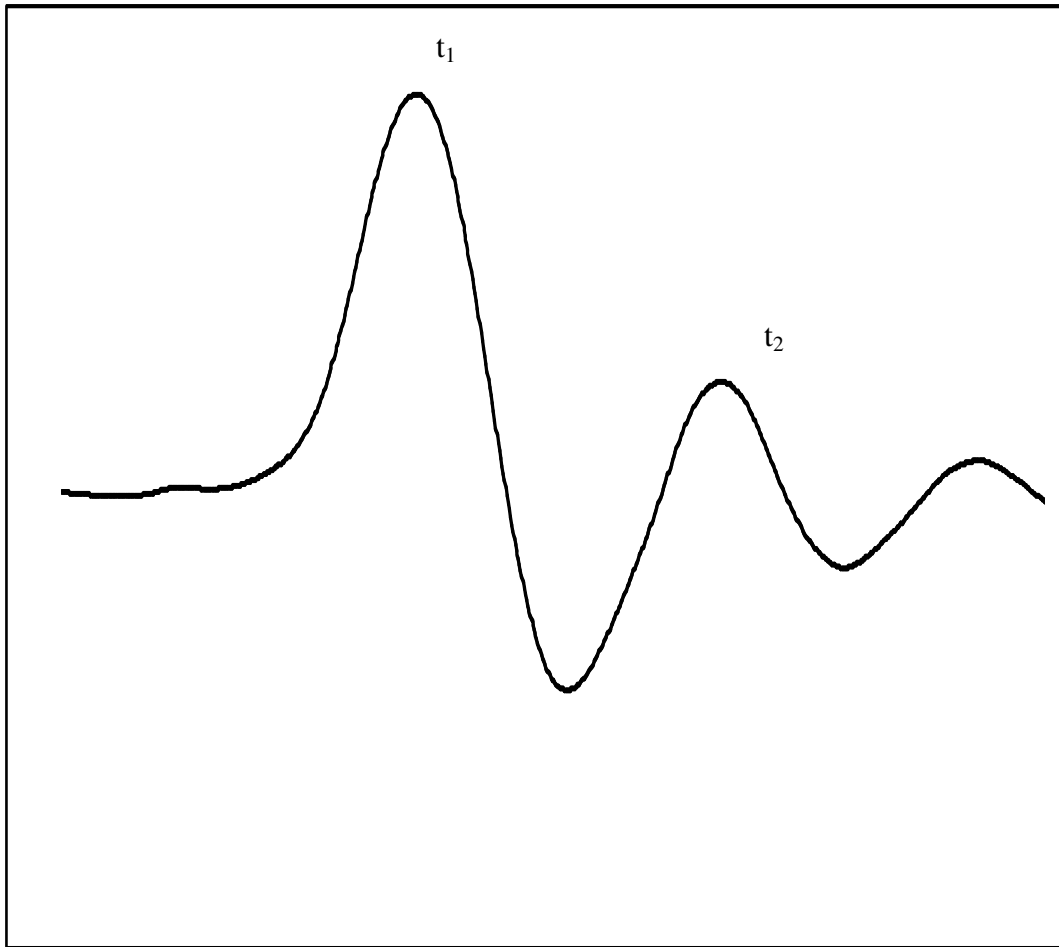
A linear regression line fitted to the relationship between the change in ground reaction force and displacement of the total body center of mass. The slope of this resulting regression equation represents vertical leg stiffness.

Figure 2: Active Hamstring Stiffness Positioning



Subjects laid prone on a custom padded table with the right thigh supported in 30 degrees of flexion. EMG electrodes were placed over the long head of the biceps femoris. A custom made brace was attached to the right ankle using a compression wrap to keep the ankle in dorsiflexion and for attachment of the accelerometer. An accelerometer was attached to the subject's foot in order to measure oscillatory motion following an examiner applied perturbation. A standardized cuff weight of 10% of the subject's body mass was secured to the ankle of the right leg.

Figure 3: Damped Frequency of Oscillation (Normal Active Hamstring Stiffness Trial)



Active hamstring stiffness was calculated using the following equation:

1). $K_a = 4\pi^2 mf^2$ where K_a = active hamstring stiffness, m = summed masses of the shank and foot segment, and f = damped frequency of oscillation.

The damped frequency of oscillation can be calculated by using the formula $(1 / t_2 - t_1)$

Figure 4: Electromechanical Delay Positioning



Subjects were positioned prone on the table with the right thigh supported in 30 degrees of flexion and the foot secured to a custom built device containing a load cell. Subjects were asked to relax and wait for a light stimulus to turn on. When the stimulus was presented, the subject maximally contracted their hamstring muscles as forcefully and quickly as possible against a load cell until the stimulus was removed.

Chapter 4 – Results

The sample included in this study consisted of 30 subjects; 15 females who regularly use oral contraception and 15 females who did not use oral contraception. Six females (OCP = 3, Non-OCP = 3) were excluded from the electromechanical delay statistical analysis due to failure of the data reduction algorithm in correctly identifying hamstring EMG onset. Subject demographics are listed in Table 1.

Active Hamstring Stiffness

Means and standard deviations for active hamstring stiffness are listed in Table 2. Statistical analysis revealed that there was no significant main effect for group [$F_{(1, 28)} = 0.452, p = 0.507$] or for time [$F_{(1, 28)} = 0.095, p = 0.760$]. Similarly, there was no significant group x time interaction for active hamstring stiffness [$F_{(1, 28)} = 2.146, p = 0.154$]. Thus there seems to be no effect of hormonal fluctuation or contraceptive use on active hamstring stiffness.

Electromechanical Delay

Means and standard deviations for electromechanical delay of the hamstrings are listed in Table 3. Statistical analysis revealed that there was no significant main effect for group [$F_{(1, 22)} = 3.383, p = 0.079$] or for time [$F_{(1, 22)} = 0.658, p = 0.426$]. Similarly, there was no significant group x time interaction for electromechanical delay of the hamstrings [$F_{(1, 22)} = 0.977, p = 0.334$]. Thus there seems to be no effect of hormonal fluctuation and oral contraceptive use on electromechanical delay of the hamstrings.

Vertical Leg Stiffness

Means and standard deviations for vertical leg stiffness are listed in Table 4. Statistical analysis revealed that there was no significant main effect for group [$F_{(1, 28)} = 1.945$, $p = 0.174$] or for time [$F_{(1, 28)} = 0.333$, $p = 0.568$]. Similarly, there was no significant group x time interaction for electromechanical delay of the hamstrings [$F_{(1, 28)} = 1.506$, $p = 0.230$]. Thus there seems to be no effect of hormonal fluctuation or contraceptive use on vertical leg stiffness.

Intraclass Correlation Coefficients

Reliability analysis revealed moderate ICC values for active hamstring stiffness and EMD. The ICC, SEM values for each dependent variable is (Table 5): active hamstring stiffness (0.50, 1.69 N/cm), EMD (5 trials) (0.20, 214.1 ms) and EMD (highest and lowest trial thrown out) (0.70, 50.69 ms). While an ICC variable of 0.50 for active hamstring stiffness may imply low within session reliability, the narrow window of error measured across the trials (SEM= 1.69) would imply that the active stiffness protocol would be considered moderately reliable within a testing session. Two ICC calculations were performed for EMD due to problems with the computer algorithm correctly picking up muscle EMG onset. The highest and lowest trials were identified for EMD (5 trials) (391,-1873) and EMD with the highest and lowest values discarded for analysis (381,-271) to determine the amount of variability between the two measures. The ICC values reported for EMD (5 trials) are due to variability attributed to data processing and not to the physiological variable itself.

Table 1: Subject Demographics (means \pm sd)

	Age (years)	Height (cm)	Mass (kg)
OCP	19.87 \pm 1.13	165.40 \pm 7.96	61.54 \pm 13.12
Non-OCP	20.40 \pm 1.59	169.93 \pm 5.62	62.75 \pm 10.61

Table 2: Active Hamstring Stiffness (N/cm; means \pm sd)

	Menses	Ovulation	Collapsed Across Time
OCP			
Non-OCP	10.82 \pm 2.00	11.12 \pm 2.06	10.97 \pm 2.00
Collapsed Across	11.64 \pm 1.46	11.17 \pm 2.05	11.41 \pm 1.76
Group	11.23 \pm 1.77	11.15 \pm 2.02	11.19 \pm 1.88

Table 3: Electromechanical Delay (ms; means \pm sd)

	Menses	Ovulation	Collapsed Across Time
OCP			
Non-OCP	130.44 \pm 24.77	144.83 \pm 49.83	137.63 \pm 38.88
Collapsed Across	118.94 \pm 26.88	117.52 \pm 20.80	118.23 \pm 23.52
Group	124.69 \pm 25.95	131.18 \pm 39.57	127.93 \pm 34.14

Table 4: Vertical Leg Stiffness (N/cm; means \pm sd)

	Menses	Ovulation	Collapsed Across Time
OCP			
Non-OCP	196.61 \pm 32.57	191.16 \pm 37.61	193.88 \pm 34.68
Collapsed Across	211.69 \pm 63.50	226.83 \pm 74.37	219.25 \pm 68.38
Group	204.15 \pm 50.18	208.99 \pm 60.68	206.57 \pm 55.25

Table 5: Intraclass Correlation Coefficients

	ICC	SEM
Active Hamstring Stiffness	0.50	1.69 N/cm
Electromechanical Delay (Middle 3 Trials)	0.70	50.69 ms
Electromechanical Delay (5 Trials)	0.20	214.7 ms

Figure 1.

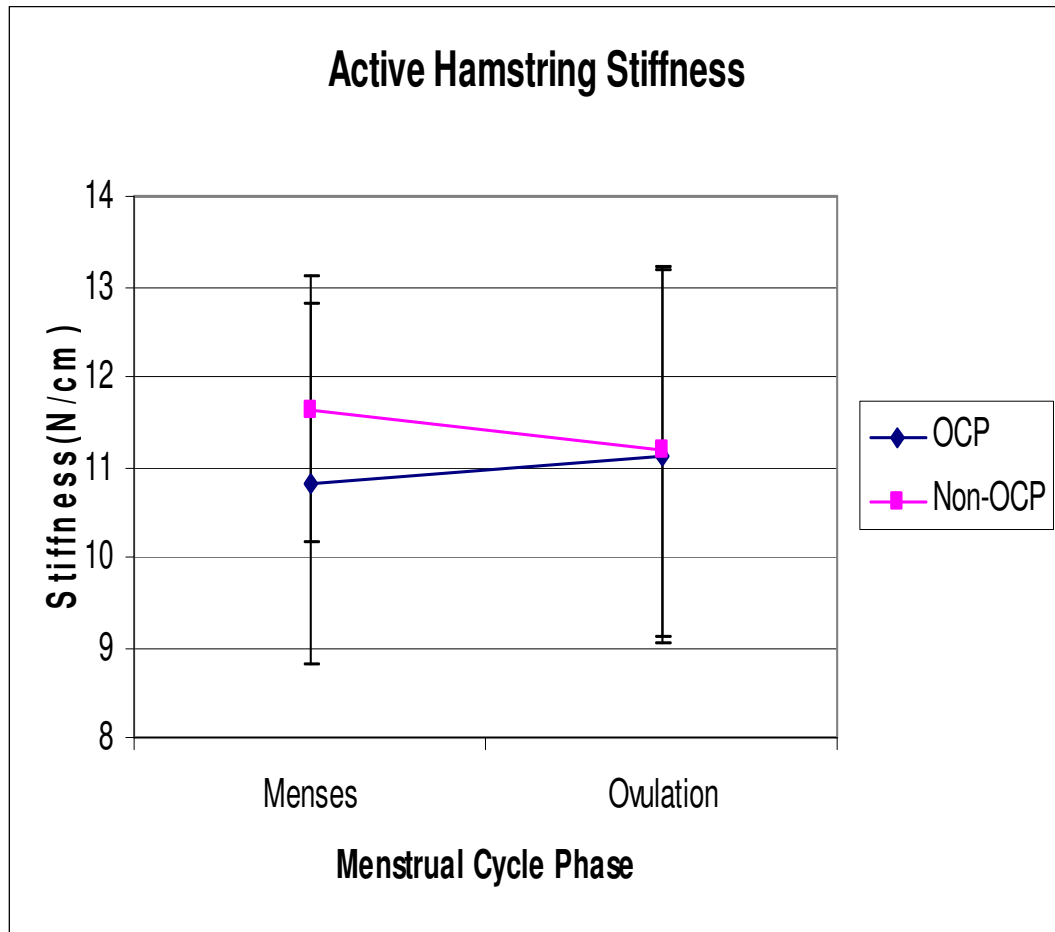


Figure 2.

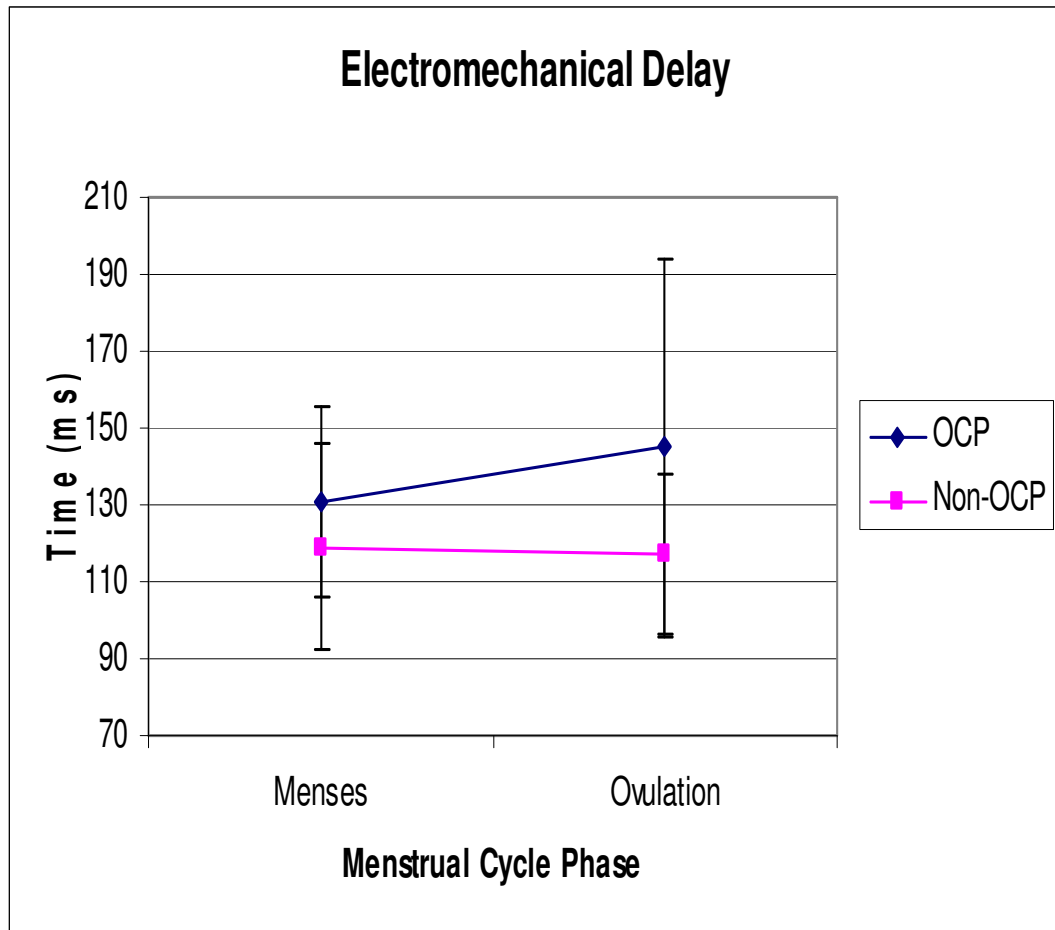
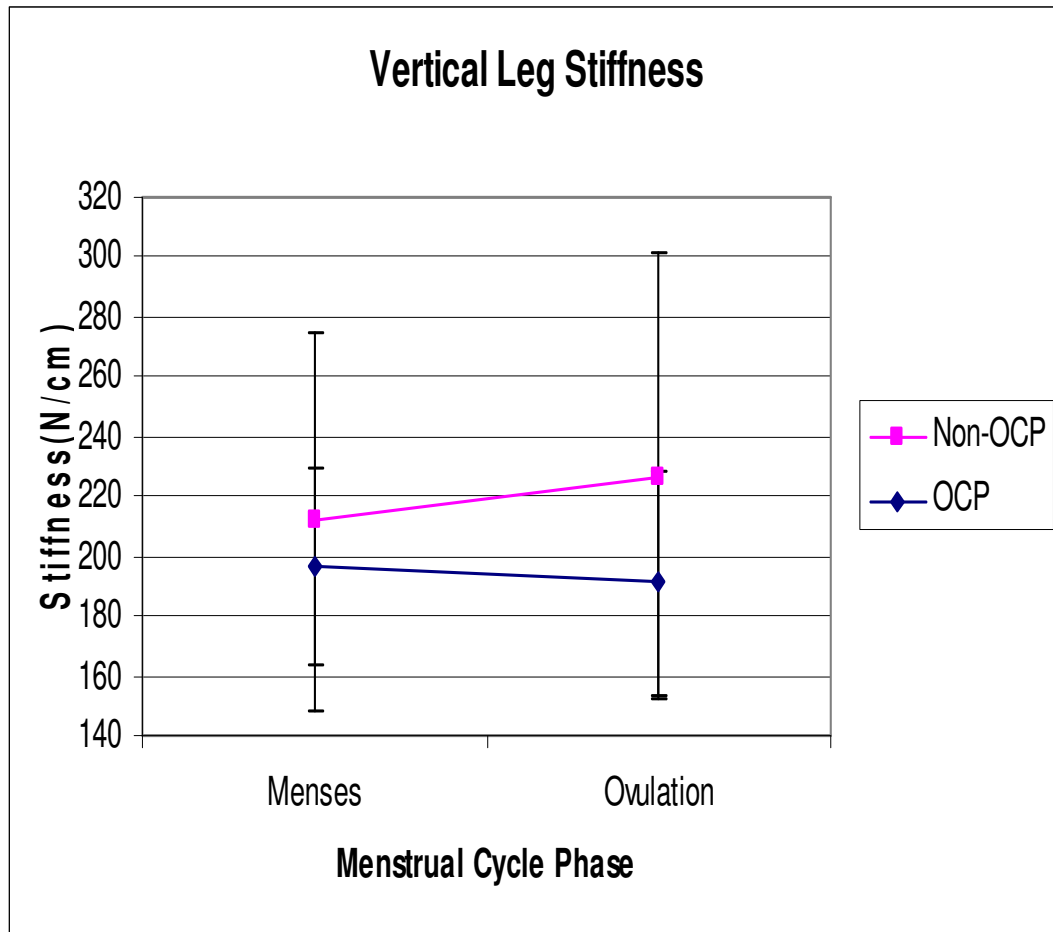


Figure 3.



Chapter 5 - Discussion

The findings of our investigation do not support our hypothesis, and reveal that the oral contraceptive pill does not have a significant effect on active hamstring stiffness, EMD of the hamstrings, or vertical leg stiffness across the menstrual cycle. Our study observed no significant differences in the dependent variables between a sample of females who regularly use a monophasic oral contraceptive pill and a sample of normally menstruating females who do not use oral contraception. Therefore, these findings suggest that the oral contraceptive pill does not likely influence properties of skeletal muscle in manners thought to reduce non-contact ACL injury risk.

Previous investigations have tried to explain the sex related increase in ACL injuries in active females with much debate. One theory at the forefront currently is the effects of female sex hormones on injury rates. Moller-Nielsen and Hammar (1989) found a significantly lower traumatic injury rate in female soccer players currently using oral contraception. The results of our study found no statistical difference in muscle properties between females who are and are not currently taking oral contraception. Heightened hamstring stiffness is thought to provide a greater resistance to lengthening, potentially enhancing joint stability. The hamstrings provide a posterior force to the tibia resisting anterior tibial translation, possibly protecting the ACL. The findings of our investigation support the results of Agel et al. (2006) who found no difference in injury rates between females who are using hormonal therapy and not using hormonal therapy. Our current investigation found no difference in active hamstring stiffness, EMD, and vertical leg

stiffness between the two groups, thus suggesting no differences in dynamic stability of the knee.

Currently, there is limited published research that investigates the effects of oral contraception on muscle properties. Our results contradict those of Eiling et al. (2007) which found a significant change in musculotendinous stiffness during a single leg hopping task between the first day of menses and ovulation. We found no significant change in musculotendinous stiffness between a low hormone concentration and a high hormone concentration. The lack of agreement between these studies may be explained by differences in testing protocol and capture parameters discrepancies. Eiling et al. (2007) had all subjects perform a standardized warm-up on a stationary bike followed by 10 sport specific drills. Subjects were then asked to perform a single leg hopping task on a forceplate. Increased blood flow from a standardized warm up and physical activity may have increased muscle elasticity, and magnified changes in muscle stiffness. The increased loading due to single leg hopping versus double leg hopping, results in increased tissue loading. This increase may have resulted in larger magnitudes of stiffness and provided a significant difference across phase in females not using oral contraceptives in previous literature (Eiling et al. 2007).

The published literature is inconsistent regarding the influence of sex hormones on ACL injury risk. A greater than expected amount of ACL injuries have been identified during the ovulatory phase of the menstrual cycle when estrogen concentration is high (Beynon et al. 2006; Wojtys et al. 1998). Conversely, an increased risk of injury during the first days of the menstrual cycle when estrogen concentration is low has also been noted (Hewett et al. 2007; Myklebust et al. 1998; Slauterbeck et al. 2002). The results of our investigation do not provide support for a possible explanation for an increase in injury rate between menses and

ovulation due to a difference in muscle properties. With no changes in muscle properties across the menstrual cycle in our study, the increased risk for injury noted during the various menstrual phases in these investigations may be due to other factors related to ACL injury not included in this investigation.

Multiple investigations have found no effect of estrogen concentration on the knee laxity (Belanger et al. 2004; Carcia et al. 2004; Hertel et al. 2006; Karageanes et al. 2000; Pollard et al. 2006; Van Lunen et al. 2003; Warden et al. 2006; Wentorf et al. 2006). Estrogen receptors have been identified on the ACL and human skeletal muscle (Kalbe et al. 2007; Komatsuda et al. 2006; Lemoine et al. 2003; Tiidus 2005; Yu et al. 1999). If estrogen receptors on muscle cause a response similar with respect to stiffness to that of estrogen receptors on ligaments, our current findings of no significant change in muscle stiffness properties across the menstrual cycle would be supported. The current investigation found no difference in muscle properties across the menstrual cycle in both OCP users and non-users, thus controlling for circulating estrogen concentrations.

With no significant effect of estrogen on muscle properties across the menstrual cycle being noted, a variety of explanations can support our current findings. The effect of estrogen on muscle properties may be too minute for the current testing protocol to quantify. Also, our measures of musculotendinous stiffness are gross in nature, incorporating various other structures within the knee. Testing protocols that are more sensitive to changes in muscle stiffness (i.e. in isolated muscle specimens) may need to be utilized in order to find a significant difference. Conversely, there may be no effect of estrogen directly on muscle properties. The current study tested muscle properties in females at two periods that were characterized by menses and ovulation with no significant differences noted. Schultz et al.

(2004) found a delayed effect of estrogen on knee laxity across the menstrual cycle . If estrogen has similar effects on receptors on skeletal muscle, multiple testing points across the menstrual cycle would be imperative to capture estrogen levels at their peak and identify any delayed effects. Our current study may not have captured the appropriate timeframe during the menstrual cycle when estrogen's effects on skeletal muscle are exhibited. Also, if there is no change in stiffness properties, we would expect to see no change in EMD due to their being no altered force transfer between the musculotendinous unit and bone. If there is no effect of estrogen on muscle properties, we would not expect to see any change in muscle properties across the menstrual cycle with the use of oral contraception, which would be in agreement with our study.

Clinical Application

Previous research has shown that female athletes who have taken the oral contraceptive pill have a reduced risk of ACL injury. Dynamic stiffness of the hamstrings has been theorized to provide a protective effect on the ACL. The hamstrings are thought to prevent anterior translation of the tibia on the femur, which is a common injury mechanism for the ACL. Increased stiffness of the hamstrings may prevent further displacement of the tibia on the femur, increasing the dynamic stability of the knee. The results of our study did not find a significant difference in muscle properties between active females who are regularly taking oral contraceptives, and females with a regular menstrual cycle. The results of this study indicate that the use of the oral contraceptive pill does not affect muscle stiffness properties in a way that would serve as a protective mechanism against non-contact ACL injuries.

Limitations

A limitation of this study is that muscle stiffness measurements were only taken on two separate days across the menstrual cycle. Multiple test sessions across the menstrual cycle may reveal a significant difference in muscle properties that could be due to a delayed effect of estrogen. Shultz et al. (2004) found a delayed effect of estrogen on ACL stiffness during high estrogen levels commonly seen around ovulation. Our study used urine based ovulation kits to identify ovulation which was labeled as the high hormone period. Repeated daily stiffness measurements across the menstrual cycle would be needed in order to identify any possible delayed effect of estrogen on muscle properties.

Verifying estrogen concentrations in the blood is vital to verify that muscle stiffness measurements are truly being taken at a maximal elevated level. Our study did not take blood samples and analyze estrogen concentrations to verify that our high hormone test session was truly performed at an elevated hormone level. Daily blood draws across a complete menstrual cycle would be needed to identify hormone trends and correlate estrogen levels to muscle stiffness properties.

Another limitation to our study is that oral contraception was limited to only monophasic doses. There are a variety of brands of oral contraceptive pills which vary in length of cycle and hormone levels provided, and which are commonly taken by active females that were not allowed in this study. All oral contraceptives have a period in which the female does not take a hormone pill, which allows her body to menstruate. Current contraceptive prescriptions can vary in cycle length by as many as 60 days. Our study was limited to subjects taking oral contraceptives with a 25 – 32 day cycle. Extended periods of elevated estrogen concentrations may have possible effects on muscle properties. Testing

multiple styles of oral contraceptive pills may give insight into a possible protective effect of OCP against ACL injury.

Lastly, only females with a normal menstrual cycle were allowed to participate in this study. Oral contraceptive pills are typically prescribed for females who have an irregular menstrual cycle to stabilize hormone levels and cycle length. Variations in hormone surges that may be seen in females who suffer from irregular menstrual cycles may show variations in muscle properties compared to normally menstruating females. For example, if the ovulatory spike in estrogen concentration is larger in females with an irregular menstrual cycle, increased circulating estrogen in the blood may provide a significant decrease in muscle stiffness. ACL injury rates in this population should be investigated for differences compared to that of normally menstruating females.

Future Research

Currently, there is very limited research that focuses on the effects of female sex hormones and oral contraceptives on muscle properties across the menstrual cycle. Future research should concentrate on tracking contraceptive status and menstrual cycle regularity among female non-contact ACL injuries. Future study samples should include females who have irregular menstrual cycles and females who are using a variety of oral contraceptive delivery methods. These studies should focus on test sessions and blood hormone levels that are to be taken on multiple days in order to better identify changes in muscle properties that may occur across the different phases of the menstrual cycle.

Conclusions

Previous research has shown an increased injury rate for non-contact ACL injuries in female athletes when compared to their male counterparts. The results of this study do not

support our hypothesis and did not find a significant difference in muscle properties across the menstrual cycle between normally menstruating females and females regularly taking the oral contraceptive pill. Finally, this study does not indicate that taking the oral contraceptive pill would alter muscle properties in manners which are suggested to reduce ACL injury risk.

Appendix A: IRB Consent Form

**University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects
Biomedical Form**

IRB Study # 07-0118
Consent Form Version Date: 1/8/2008

Title of Study: The Effects of Hormone Concentration on Muscle Stiffness and Electromechanical Delay.

Principal Investigator: David Bell, MEd, ATC

UNC-Chapel Hill Department: Exercise and Sport Science

UNC-Chapel Hill Phone number: (919) 962-7187

Email Address: bell@email.unc.edu

Co-Investigators: Dr. Troy Blackburn, Dr. Anthony Hackney, Jeff Hudson

Faculty Advisor: Dr. Darin Padua

Funding Source: Injury Prevention Research Center Small Student Grant; National Athletic Trainers Association Osternig Grant

Study Contact telephone number: (919) 962-7187

Study Contact email: bell@email.unc.edu

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The primary purpose of this research study is to determine the influence of hormones on properties of muscle. Specifically, we are interested in determining how hormones affect active hamstring stiffness and electromechanical delay.

Are there any reasons you should not be in this study?

You should not be in this study if you have had a lower extremity injury within the past six months, knee surgery, or have a neurological disorder. You should also not participate in this study if you have a fear of needles or are uncomfortable with donating small samples of blood. If you are a female you should not participate in this study if you are or have been pregnant, or have an irregular menstrual cycle.

How many people will take part in this study?

If you decide to participate, you will be one of approximately sixty five subjects (20 males, 45 females) that will be tested. 30 females will not be using any form of oral contraception and 15 females will be using monophasic oral contraception in pill form.

How long will your part in this study last?

You will be asked to participate in a practice session where you will walk through each of the testing procedures and fill out a questionnaire lasting approximately 30 minutes. If you meet all qualifications you will participate in two separate testing sessions lasting 60 minutes. Each session will be identical to the first.

What will happen if you take part in the study?

If you choose to volunteer for this study, you will first be asked to participate in a practice session where you will fill out a questionnaire and walk through each of the testing procedures. Female participants not using oral contraceptive (OCP) will also be asked a series of questions to determine length and frequency of menstrual cycle and given urine based ovulation kits and instructions to help identify ovulation. Males will be tested twice approximately 7 days apart. Females not on OCP will be tested twice: (1) 3-5 days after the onset of menses and (2) 2-4 days after ovulation. Females using OCP will be tested twice. Testing times will correspond to birth control pills: (1) pills 3-5, (2) pills 15-17. Testing sessions will be exactly the same. Females will be required to return one day after their ovulation or pill 15-17 testing session to have another blood sample taken.

Testing Session

You will have a venous blood specimen analyzed for reproductive hormone levels. Specimens will be obtained by veni-puncture from an antecubial vein using a 3ml syringe (approximately 1 table spoon) with a 23 gauge needle (1 inch length). The veni-puncture procedure will be performed by a nationally certified phlebotomist using standard clinical procedures. All blood samples will take place in the Exercise Physiology Lab in Fetzer Gym on the campus of the University of North Carolina.

You will then be escorted to the Motor Control Lab at the University of North Carolina wearing clothing (t shirt and shorts) and running shoes appropriate for participating in physical activity. When you arrive at the laboratory your height and weight will be measured and you will be given a chance to warm-up on a stationary bicycle for 5-minutes. You will then be asked to hop on two legs, on a force plate at a rate of 2.2Hz for 15 seconds a total of 5 times. Band-aid like electrodes that monitor muscle activity will be attached over the hamstrings muscles on back of the thigh, calf muscle on the lower leg (lateral gastrocnemius), and quadriceps (vastus lateralis), or your dominant leg (the leg used to kick

a ball for maximum distance). Sensors that monitor joint motion will be placed on your lower leg close to your knee and the outer distal thigh and secured with tape. Once the sensors are in place you will lie on a padded table with your leg and thigh supported. Ultrasound gel will be applied over the back of your thigh and an investigator will use the ultrasound to record the size of your hamstring muscle. A sensor that measures movement will be placed on your foot and cuff weights equivalent to 10% of your body mass will be secured at your ankle. When you are ready, the support of the leg will be removed and you will hold your lower leg in a set position (approximately 30 degrees of knee flexion). An investigator will apply a light force to your lower leg and you will hold your leg as still as possible for approximately 5 seconds. Five successful trials will be recorded. This same procedure will be repeated with 15% and 20% of your body weight at your ankle. The investigator will then block the movement of your lower leg at your ankle and you will be asked to relax and look at a light. When the light turns on you will push as quickly as you can into the block as if you were flexing your knee. Five trials will be recorded.

What are the possible benefits from being in this study?

Research is designed to benefit society by gaining new knowledge. You may not benefit personally from participating in this study. However, you will learn techniques for jumping that may help prevent you from sustaining an ACL injury in the future.

What are the possible risks or discomforts involved with being in this study?

As with any physical activity, participation in this study carries a risk of bodily injury. The motions that you will be asked to perform are ones that repeatedly occur during physical activity. Therefore, you should be familiar and able to perform the tasks with minimal injury risk. To further minimize injury risk, participants will be allowed to warm up and stretch to prepare themselves for testing. In case of injury, medical personnel (certified athletic trainers) will be located in the same building as the testing session. It is also possible that the application of the electrodes may cause minor skin irritation. Physical activity may result in muscle soreness. In phlebotomy there are always risks to the patient; such as, infection, fainting, hematoma and/or bruising. Appropriate first aid procedures and safety pre-cautions will be employed to minimize the likelihood of these events occurring. Furthermore, the procedure will use an experienced, certified phlebotomist, which should also reduce the likelihood and risk of such events occurring. You are free to cease participation at any time.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers.

What if we learn about new findings or information during the study?

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law

to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

All paper documentation will be identified with a subject number. They will be kept in a secured location for the duration of the study and destroyed once they are no longer needed for research purposes.

Any data stored on a computer will be identified by a subject number and protected by a password which only the primary investigator and anyone else directly involved in data collection and reduction for this study will have access to.

What will happen if you are injured by this research?

All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury from being in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. The University of North Carolina at Chapel Hill has not set aside funds to pay you for any such reactions or injuries, or for the related medical care. However, by signing this form, you do not give up any of your legal rights.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will receive \$15 for completing this study.

Will it cost you anything to be in this study?

It will not cost you anything to participate in this study. You are only responsible for your transportation to and from the testing site in Fetzer Gymnasium on the campus of the University of North Carolina at Chapel Hill.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?

Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?

There is no sponsorship for this study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have questions, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

What if you have questions about your rights as a research subject?

All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

Signature of Research Subject

Date

Printed Name of Research Subject

Signature of Person Obtaining Consent

Date

Printed Name of Person Obtaining Consent

Appendix B: Data Collection Form

Data Collection Form

Stiffness Project

Sub ID: _____

Test Session: 1 2 Date: _____

Height: _____ cm

Mass: _____ kg _____ lbs

Amount of weight added to distal shank:

Each bar weighs .2kg/.5lbs

Age _____

Active hamstring stiffness:

Take 5 Trials of the AS protocol
_____ cm

Distance of EMG electrode from jt line:

Females: s#aas# s#bas#

MVIC:

Take 3 MIVC measures of the hamstrings.

s1amax# s1bmax#

EMD:

Take 5 Trials of the protocol

Females: s#aemd# s#bemd#

Hopping Stiffness:

Take a static trial of the subject standing on the forceplate for 10 seconds

Begin collecting data with the person standing still on the forceplate and then have them begin hopping for 1 minute with the metronome (132 bps).

Females: s#ahs s#bhs

s#ahsstatic1 (only one static trial is needed for static and hopping stiffness)

Appendix C: Approved Oral Contraceptive List

Brand names of estrogen-progestin combination oral contraceptive pills

- Alesse®
- Apri®
- Aviane®
- Brevicon®
- Cryselle
- Demulen®
- Desogen®
- Genora® -
- Jenest®
- Junel
- Junel Fe
- Kelnor
- Lessina
- Levlen®
- Levlite®
- Levora®
- Lo/Ovral®
- Loestrin®
- Low-Ogestrel®
- Lutera
- Microgestin®
- Microgestin Fe
- Modicon®
- Necon®
- Norinyl®
- Nordette®
- Norethin
- Nortrel®
- Ogestrel®
- Ortho-Cept®
- Ortho-Cyclen®
- Ortho-Novum®
- Ovcon®
- Ovral®
- Portia
- Reclipsen
- Solia
- Sprintec
- Yasmin®
- Zovia®

Appendix D: Manuscript

The Effect of Oral Contraceptive Use on Muscle Properties Across the Menstrual Cycle

Jeffrey D. Hudson, J. Troy Blackburn, David R. Bell, Darin A. Padua

University of North Carolina, Chapel Hill, NC

Jeffrey D. Hudson, ATC and J. Troy Blackburn PhD, ATC contributed to conception and design; acquisition, analysis, and interpretation of the data; and drafting, critical revision, and final approval of the article. David R. Bell Med, ATC contributed to conception and design; acquisition, analysis, and interpretation of the data; critical revision, and final approval of the article. Darin A. Padua PhD, ATC contributed to conception and design; and critical revision, and final approval of the article.

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THE EFFECT OF ORAL CONTRACEPTIVE USE ON MUSCLE PROPERTIES ACROSS THE MENSTRUAL CYCLE

Hudson JD, Blackburn JT, Bell DR, Padua DA

Objective: To investigate the effects of oral contraceptive use on active hamstring muscle stiffness, vertical leg stiffness, and hamstring electromechanical delay (EMD) in physically active females.

Hypothesis: Non oral contraceptive pill (Non-OCP) users will have altered muscle properties when hormone levels are greatest resulting in decreased muscle stiffness and increased EMD. Oral contraceptive pill (OCP) users will have no difference in muscle properties.

Subjects: 15 physically active females using monophasic oral contraception (OCP; age = 19.87 ± 1.13 years, height = 165.40 ± 7.96 cm, mass = 61.54 ± 13.12 kg) and 15 females not using oral contraception (Non-OCP; age = 20.40 ± 1.59 years, height = 169.93 ± 5.62 cm, mass = 62.75 ± 10.61) with a normal menstrual cycle.

Design and setting: Controlled laboratory setting. Subjects were tested two times across their menstrual cycle (menses and ovulation). Ovulation was detected using urine based ovulation kits. OCP test sessions were matched to non-OCP sessions using a pill counting.

Measurements: Active hamstring stiffness, vertical leg stiffness, electromechanical delay.

Results: There was no significant effect for group or time, nor group x time interaction for active hamstring stiffness, vertical leg stiffness, and EMD of the hamstrings.

Conclusion: This study does not indicate that taking the oral contraceptive pill would alter muscle properties in manners which are suggested to reduce ACL injury risk.

Introduction

Physically active females suffer anterior cruciate ligament (ACL) injuries at an alarming rate. It is estimated that 70% of all ACL injuries are sports related, and each year approximately 38,000 young female athletes injure their ACL. Female athletes' risk of injuring their ACL is 2-8 times higher than males who participate in the same activities (Arendt and Dick 1995). Of these injuries, 80% arise from a non-contact mechanism such as jumping, cutting, and decelerating movements (McNair et al. 1990). The cause of females' elevated injury rate is currently unknown. Possible risk factors include anatomical differences, neuromuscular control, muscular strength, and sex hormone.

As of late, researchers have begun studying the influence of hormones on ACL injury risk. Female sex hormone blood concentrations (e.g. estrogen and progesterone) fluctuate rapidly across the menstrual cycle. Estrogen and progesterone levels are lowest at menses and reach their cyclic peak at or very soon after ovulation (Vollman 1977). Estrogen and progesterone receptors have been identified on the female ACL (Liu et al. 1996). Studies using animals have shown a decrease in tensile stress and linear stiffness of the ACL in a high estrogen hormone group when compared to the lower estrogen hormone group (Komatsuda et al. 2006). Wojtys et al.(2002) found in a study of 69 ACL injured females that a greater than expected number of injuries occurred near the ovulatory phase when estrogen concentration is at its peak. This difference may be due to the effects of sex hormone concentrations on ligament laxity. There is much debate on this issue, but several authors have found an increase in ligament laxity across the various phases of the menstrual cycle (Heitz et al. 1999; Shultz et al. 2004). To date, the effects of female sex hormones on ligament properties and injury risk is still unclear.

Many different structures add to and create the dynamic stability of the knee. The hamstrings have been theorized to prevent anterior displacement of the tibia on the femur and tibial internal rotation. These mechanisms are associated with loading and injury to the ACL and result in lengthening of the hamstring muscles. The hamstrings respond to these motions with a gradual increase in tensile force to resist further lengthening. Active stiffness from various muscles adds to the biomechanical stability to the knee joint. Stiffness refers to the ratio of change of force to change in length ($k = \Delta \text{force} / \Delta \text{length}$). As such, a stiffer hamstring muscle group would provide a greater increase in tensile force per unit of length change compared to a more compliant, less stiff hamstring group. This heightened hamstring stiffness likely provides a greater resistance to lengthening, potentially enhancing joint stability. Interestingly, lesser hamstring and lower extremity stiffness has been demonstrated in females compared to males.

Electromechanical delay is defined as the time interval between initiation of neural activity and force production during skeletal muscle contraction. It is hypothesized that the greater the stiffness of a given muscle is, the more efficient and timely the transfer of force to the bony insertion will be due to a greater tension being developed per unit of length change. A less stiff muscle may prolong this delay, thus delaying the dynamic stability provided by muscle contraction. These notions support the theory that the stiffness of the knee flexors may serve a protective role for the ACL.

In addition to the ACL, estrogen receptors have been identified on human skeletal muscle. Previous literature has found no changes in isometric hamstring strength, fatigability, and electrically stimulated contractile properties across the menstrual cycle (Janse de Jonge et al. 2001). Contradictory to these results, fluctuations in hormonal concentrations across

the menstrual cycle may alter the mechanical properties of muscle similar to the effects noted in isolated ligament specimens. These changes in the mechanical properties of the muscle may limit the dynamic stability provided by muscle contraction. Consequently, Eiling et al.(2007) reported a significant decrease in vertical leg stiffness has been found between ovulation and menses. By limiting peak hormonal fluctuation, oral contraception use may minimize the potentially negative influences on muscle properties which contribute to knee joint stability.

The purpose of this study was to investigate the changes in vertical leg stiffness, active hamstring stiffness, and electromechanical delay of the hamstrings across the menstrual cycle in order to characterize the influence of hormonal fluctuations on properties of skeletal muscle which are thought to contribute to joint stability. The secondary purpose of this study was to investigate the effect of oral contraceptive use on the magnitudes of the changes noted in these muscle properties across the menstrual cycle.

Subjects

Thirty subjects from the student population at the University of North Carolina at Chapel Hill volunteered to participate in this investigation. These subjects were selected from a physically active female student population. Subjects that met the study's inclusion criteria were divided into two equal groups consisting of 15 normally menstruating females not using oral contraceptive (non-OC) and 15 monophasic oral contraceptive users (OC). A priori power analysis on previous literature for vertical leg stiffness indicated that a sample size of 30 subjects would allow for a power at 0.80 (Eiling et al. 2007). Subjects were eligible for participation in this investigation if they met the following inclusion criteria: participation in 20 minutes of activity a minimum of three times per week, OC group must use an approved

monophasic OC for a minimum of six months, no lower extremity injury or surgery in the past six months, non-OC group must have not taken OCP in past six months, between the ages of 18-25 years old, no history of pregnancy, and a consistent menstrual cycle for last six consecutive months. All subjects were informed of the procedures that were involved prior to participation in this study, and were required to read and sign an approved informed consent form prior to participation.

Protocol

Subjects reported to the Motor Control lab for two separate testing session lasting one hour each that took place at both menses and ovulation. The menses testing session occurred three to five days post menstruation for the non-OC group and oral contraceptive pill number three to five for the OC matched group. This was determined by subject recall of menstruation and OCP counting. This session was characterized by low blood hormone concentrations of estrogen and progesterone for the non-OC group and a constant low concentration of estrogen and progesterone for OC group. The ovulation testing session was determined by a positive ovulation test using urine based ovulation kits daily for the non-OC group. Subjects in the non-OC group reported within 3 days post ovulation and OC pill number 15-17 in the OC matched group. The ovulation testing session was characterized by high blood hormone concentrations of estrogen and progesterone in the non-OC group and a constant low concentration of estrogen and progesterone in the OC matched group. The order of which hormone concentration that subjects first reported for testing was counterbalanced by alternating which menstrual cycle phase or pill number each subject initially reported for testing. All subjects had current height and weight taken prior to dependent variable testing. The order of dependent variable assessments was counterbalanced to prevent an order effect.

Vertical Leg Stiffness

For the vertical leg stiffness protocol, subjects were required to perform a two legged hop on a non-conductive Bertec (Model 4060-nc, Bertec Company, Columbus, Ohio) force plate for one minute. A metronome was set at 132 beats per minute (2.2 Hz) for the subject to keep pace with, in order to control hopping frequency. This frequency was chosen because past research has identified the preferred hopping frequency of subjects is 2.2 Hz (Farley et al. 1991). The vertical ground reaction force was sampled at 1,000 Hz via the Motion monitor software system (Innovative Sports Training, Inc., Chicago, IL). All subjects assumed a standardized position with both hands on their hips and were wearing no shoes. Subjects stood stable on the forceplate for 10 seconds to get baseline body mass prior to performing two legged hopping for 60 seconds. Subjects completed one successful trial per test session. The first 10 acceptable hopping trials were selected for analysis. An acceptable hopping trial was defined using the system defined by Padua et al. (2005). Trials were only accepted for analysis if the subjects hopping frequency was within 5% of the designated frequency of 2.2 Hz. This was selected because prior research has shown vertical leg stiffness is directly related to hopping frequency (Farley and Morgenroth 1999; Granata et al. 2002). Secondly, a linear correlation of the vertical center of mass and the vertical ground reaction force had to have a value greater than $r = .80$ to be qualify for analysis. The location of the total body center of mass was estimated from a double integration in the time domain of the acceleration time data (Padua et al. 2005). Vertical acceleration of the center of mass was calculated from the ground reaction force and participants body mass which was measured during a static trial for the first 10 seconds. A linear regression line was fitted to the relationship between the change in ground reaction force and displacement of the total body

center of mass. The slope of the resulting regression equation represents vertical leg stiffness (McMahon and Cheng 1990; Padua et al. 2005).

Active Hamstring Stiffness

Subjects laid prone on a custom padded table with the right thigh supported in 30 degrees of flexion. EMG electrodes were placed over the long head of the biceps femoris in order to monitor muscle activity. Electrode placement was verified by manual muscle testing. The system being used was a hardwired Delysis 8-channel surface EMG (Delysis Bagnoli-8, Boston, MA) sampled at a rate of 1000 Hz via the Motion Monitor software system. In order to reduce impedance, the electrode site was shaved and cleaned with an alcohol prep pad. An accelerometer was attached to the subject's foot in order to measure oscillatory motion following an examiner applied perturbation. A standardized cuff weight of 10% of the subject's body mass was secured to the ankle of the right leg. A custom made brace was attached to the right ankle using a compression wrap to keep the ankle in dorsiflexion and for attachment of the accelerometer. When the subject states they are ready, leg support from the examiner was removed from the right leg and the subject was required to hold her lower leg in a standardized position of approximately 30 degrees of knee flexion by contracting the hamstrings. A perturbation was applied by the investigator to the lower leg producing damped oscillatory motion around the knee joint. The subject was asked not to resist the motion and to attempt to keep the knee in the same position. Oscillatory motion about the knee joint was captured from the accelerometer, and the damped frequency of oscillation was used to estimate hamstring stiffness. Using the time periods of the two oscillatory peaks in the tangential acceleration (t_1 and t_2) the damped frequency of oscillation can be calculated by using the formula $(1 / t_2 - t_1)$. Five trials were performed with one minute of rest between

each attempt to prevent the likelihood of fatigue. The highest and lowest active hamstring stiffness trials of the 5 trials were thrown out and did not undergo statistical analysis to limit outliers.

Active hamstring stiffness was calculated using the following equation:

- 1). $K_a = 4\pi^2 mf^2$ where K_a = active hamstring stiffness, m = summed masses of the shank and foot segment, and f = damped frequency of oscillation.

Electromechanical Delay Protocol

Subjects were positioned prone on the same table used for the active hamstring stiffness protocol with the foot secured to a custom built device containing a load cell. This arrangement prevented knee joint motion, thus making hamstring contraction isometric. Subjects were asked to relax and wait for a light stimulus to turn on. When the stimulus was presented, the subject maximally contracted their hamstring muscles as forcefully and quickly as possible against a load cell until the stimulus was removed. Electromechanical delay was calculated as the time from the onset of muscle activity in the hamstrings measured by EMG to the production of force at the load cell. Muscle onsets were determined by computer algorithm. Force onset was determined as the point when the load cell reaches 5% of peak force. EMG onset was determined as the point when the amplitude exceeds two times the mean amplitude over the first 100 ms. EMG activity must stay above this threshold for a minimum of 50 ms to qualify as EMG onset. Five trials were recorded for each subject with one minute rest between trials to prevent the likelihood of fatigue. The highest and lowest EMD trials of the 5 trials were thrown out and did not undergo statistical analysis to limit outliers.

Data Reduction and Statistical Analysis

Active hamstring stiffness and EMD data were processed and reduced using custom software developed in LabVIEW (LabVIEW, National Instruments, San Antonio, TX). Vertical leg stiffness was processed and analyzed using custom software developed in MATLAB (The Math-Works, Natick, MA). All EMG, load cell, and force plate data were sampled using an A/D converter (National Instruments, Austin, TX) at 1000 Hz to a storage computer. Accelerometer and load cell data were lowpass filtered at 10 Hz (4th order, zero phase-lag Butterworth). EMG data were corrected for DC bias and filtered using a bandpass (20-350 HZ) and notch (59.5-60.5 Hz) filters (4th order, zero phase-lag Butterworth). EMG data were also smoothed using a root mean square sliding window function with a time constant of 20ms.

A 2 (Group: OC, Non-OC) X 2 (Time: menses, ovulation) repeated measures ANOVA was performed for each dependent variable. Significant interaction effects were evaluated post hoc via planned pair-wise comparisons after adjusting for type I error rate with a Bonferroni correction. An alpha level of 0.05 was set a priori. Intraclass correlation coefficients (ICC) and associated standard errors of measurement (SEM) were calculated for active hamstring stiffness and EMD to determine within-session reliability. Separate one way repeated measures ANOVA were performed across trials and ICC's were calculated using ICC equation (2, 1). All statistical analysis were calculated using SPSS 13.0 software (SPSS Inc., Chicago, IL).

Results

The sample included in this study consisted of 30 subjects; 15 females who regularly use oral contraception and 15 females who did not use oral contraception. Six females (OCP = 3, Non-OCP = 3) were excluded from the electromechanical delay statistical

analysis due to failure of the data reduction algorithm in correctly identifying hamstring EMG onset.

Active Hamstring Stiffness

Statistical analysis revealed that there was no significant main effect for group [$F_{(1, 28)} = 0.452, p = 0.507$] or for time [$F_{(1, 28)} = 0.095, p = 0.760$]. Similarly, there was no significant group x time interaction for active hamstring stiffness [$F_{(1, 28)} = 2.146, p = 0.154$]. Thus there seems to be no effect of hormonal fluctuation or contraceptive use on active hamstring stiffness.

Electromechanical Delay

Statistical analysis revealed that there was no significant main effect for group [$F_{(1, 22)} = 3.383, p = 0.079$] or for time [$F_{(1, 22)} = 0.658, p = 0.426$]. Similarly, there was no significant group x time interaction for electromechanical delay of the hamstrings [$F_{(1, 22)} = 0.977, p = 0.334$]. Thus there seems to be no effect of hormonal fluctuation and oral contraceptive use on electromechanical delay of the hamstrings.

Vertical Leg Stiffness

Statistical analysis revealed that there was no significant main effect for group [$F_{(1, 28)} = 1.945, p = 0.174$] or for time [$F_{(1, 28)} = 0.333, p = 0.568$]. Similarly, there was no significant group x time interaction for electromechanical delay of the hamstrings [$F_{(1, 28)} = 1.506, p = 0.230$]. Thus there seems to be no effect of hormonal fluctuation or contraceptive use on vertical leg stiffness.

Intraclass Correlation Coefficients

Reliability analysis revealed moderate ICC values for active hamstring stiffness and EMD. The ICC, SEM values for each dependent variable is: active hamstring stiffness

(0.50, 1.69 N/cm), EMD (5 trials) (0.20, 214.1 ms) and EMD (highest and lowest trial thrown out) (0.70, 50.69 ms). While an ICC variable of 0.50 for active hamstring stiffness may imply low within session reliability, the narrow window of error measured across the trials (SEM= 1.69) would imply that the active stiffness protocol would be considered moderately reliable within a testing session. Two ICC calculations were performed for EMD due to problems with the computer algorithm correctly picking up muscle EMG onset. The highest and lowest trials were identified for EMD (5 trials) (391,-1873) and EMD with the highest and lowest values discarded for analysis (381,-271) to determine the amount of variability between the two measures. The ICC values reported for EMD (5 trials) are due to variability attributed to data processing and not to the physiological variable itself.

Discussion

The findings of our investigation do not support our hypothesis, and reveal that the oral contraceptive pill does not have a significant effect on active hamstring stiffness, EMD of the hamstrings, or vertical leg stiffness across the menstrual cycle. Our study observed no significant differences in the dependent variables between a sample of females who regularly use a monophasic oral contraceptive pill and a sample of normally menstruating females who do not use oral contraception. Therefore, these findings suggest that the oral contraceptive pill does not likely influence properties of skeletal muscle in manners thought to reduce non-contact ACL injury risk.

Previous investigations have tried to explain the sex related increase in ACL injuries in active females with much debate. One theory at the forefront currently is the effects of female sex hormones on injury rates. Moller-Nielsen and Hammar (1989) found a significantly lower traumatic injury rate in female soccer players currently using oral

contraception. The results of our study found no statistical difference in muscle properties between females who are and are not currently taking oral contraception. Heightened hamstring stiffness is thought to provide a greater resistance to lengthening, potentially enhancing joint stability. The hamstrings provide a posterior force to the tibia resisting anterior tibial translation, possibly protecting the ACL. The findings of our investigation support the results of Agel et al. (2006) who found no difference in injury rates between females who are using hormonal therapy and not using hormonal therapy. Our current investigation found no difference in active hamstring stiffness, EMD, and vertical leg stiffness between the two groups, thus suggesting no differences in dynamic stability of the knee.

Currently, there is limited published research that investigates the effects of oral contraception on muscle properties. Our results contradict those of Eiling et al. (2007) which found a significant change in musculotendinous stiffness during a single leg hopping task between the first day of menses and ovulation. We found no significant change in musculotendinous stiffness between menses and ovulation. The lack of agreement between these studies may be explained by differences in testing protocol and capture parameters discrepancies. Eiling et al. (2007) had all subjects perform a standardized warm-up on a stationary bike followed by 10 sport specific drills. Subjects were then asked to perform a single leg hopping task on a forceplate. Increased blood flow from a standardized warm up and physical activity may have increased muscle elasticity, and magnified changes in muscle stiffness. The increased loading due to single leg hopping versus double leg hopping, results in increased tissue loading. This increase may have resulted in larger magnitudes of stiffness

and provided a significant difference across phase in females not using oral contraceptives in previous literature (Eiling et al. 2007).

The published literature is inconsistent regarding the influence of sex hormones on ACL injury risk. A greater than expected amount of ACL injuries have been identified during the ovulatory phase of the menstrual cycle when estrogen concentration is high (Beynon et al. 2006; Wojtys et al. 1998). Conversely, an increased risk of injury during the first days of the menstrual cycle when estrogen concentration is low has also been noted (Hewett et al. 2007; Myklebust et al. 1998; Slauterbeck et al. 2002). The results of our investigation do not provide support for a possible explanation for an increase in injury rate between menses and ovulation due to a difference in muscle properties. With no changes in muscle properties across the menstrual cycle in our study, the increased risk for injury noted during the various menstrual phases in these investigations may be due to other factors related to ACL injury not included in this investigation.

Multiple investigations have found no effect of estrogen concentration on knee laxity. (Belanger et al. 2004; Carcia et al. 2004; Hertel et al. 2006; Karageanes et al. 2000; Pollard et al. 2006; Van Lunen et al. 2003; Warden et al. 2006; Wentorf et al. 2006). Estrogen receptors have been identified on the ACL and human skeletal muscle (Kalbe et al. 2007; Komatsuda et al. 2006; Lemoine et al. 2003; Tiidus 2005; Yu et al. 1999). If estrogen receptors on muscle cause a response similar with respect to stiffness to that of estrogen receptors on ligaments, our current findings of no significant change in muscle stiffness properties across the menstrual cycle would be supported. The current investigation found no difference in muscle properties across the menstrual cycle in both OCP users and non-users, thus controlling for circulating estrogen concentrations.

With no significant effect of estrogen on muscle properties across the menstrual cycle being noted, a variety of explanations can support our current findings. The effect of estrogen on muscle properties may be too minute for the current testing protocol to quantify. Also, our measures of musculotendinous stiffness are gross in nature, incorporating various other structures within the knee. Testing protocols that are more sensitive to changes in muscle stiffness (i.e. in isolated muscle specimens) may need to be utilized in order to find a significant difference. Conversely, there may be no effect of estrogen directly on muscle properties. The current study tested muscle properties in females at two periods that were characterized by high estrogen and low estrogen concentrations with no significant differences noted. Schultz et al. (2004) found a delayed effect of estrogen on knee laxity across the menstrual cycle. If estrogen has similar effects on receptors on skeletal muscle, multiple testing points across the menstrual cycle would be imperative to capture estrogen levels at their peak and identify any delayed effects. Our current study may not have captured the appropriate timeframe during the menstrual cycle when estrogen's effects on skeletal muscle are exhibited. Also, if there is no change in stiffness properties, we would expect to see no change in EMD due to their being no altered force transfer between the musculotendinous unit and bone. If there is no effect of estrogen on muscle properties, we would not expect to see any change in muscle properties across the menstrual cycle with the use of oral contraception, which would be in agreement with our study.

Clinical Application

Previous research has shown that female athletes who have taken the oral contraceptive pill have a reduced risk of ACL injury. Dynamic stiffness of the hamstrings has been theorized to provide a protective effect on the ACL. The hamstrings are thought to prevent

anterior translation of the tibia on the femur, which is a common injury mechanism for the ACL. Increased stiffness of the hamstrings may prevent further displacement of the tibia on the femur, increasing the dynamic stability of the knee. The results of our study did not find a significant difference in muscle properties between active females who are regularly taking oral contraceptives, and females with a regular menstrual cycle. The results of this study indicate that the use of the oral contraceptive pill does not affect muscle stiffness properties in a way that would serve as a protective mechanism against non-contact ACL injuries.

Limitations

A limitation of this study is that muscle stiffness measurements were only taken on two separate days across the menstrual cycle. Multiple test sessions across the menstrual cycle may reveal a significant difference in muscle properties that could be due to a delayed effect of estrogen. Shultz et al. (2004) found a delayed effect of estrogen on ACL stiffness during high estrogen levels commonly seen around ovulation. Our study used urine based ovulation kits to identify ovulation which was labeled as the high hormone period. Repeated daily stiffness measurements across the menstrual cycle would be needed in order to identify any possible delayed effect of estrogen on muscle properties.

Verifying estrogen concentrations in the blood is vital to verify that muscle stiffness measurements are truly being taken at a maximal elevated level. Our study did not take blood samples and analyze estrogen concentrations to verify that our ovulation testing session was truly performed at an elevated hormone level. Daily blood draws across a complete menstrual cycle would be needed to identify hormone trends and correlate estrogen levels to muscle stiffness properties.

Another limitation to our study is that oral contraception was limited to only monophasic doses. There are a variety of brands of oral contraceptive pills which vary in length of cycle and hormone levels provided, and which are commonly taken by active females that were not allowed in this study. All oral contraceptives have a period in which the female does not take a hormone pill, which allows her body to menstruate. Current contraceptive prescriptions can vary in cycle length by as many as 60 days. Our study was limited to subjects taking oral contraceptives with a 25 – 32 day cycle. Extended periods of elevated estrogen concentrations may have possible effects on muscle properties. Testing multiple styles of oral contraceptive pills may give insight into a possible protective effect of OCP against ACL injury.

Lastly, only females with a normal menstrual cycle were allowed to participate in this study. Oral contraceptive pills are typically prescribed for females who have an irregular menstrual cycle to stabilize hormone levels and cycle length. Variations in hormone surges that may be seen in females who suffer from irregular menstrual cycles may show variations in muscle properties compared to normally menstruating females. For example, if the ovulatory spike in estrogen concentration is larger in females with an irregular menstrual cycle, increased circulating estrogen in the blood may provide a significant decrease in muscle stiffness. ACL injury rates in this population should be investigated for differences compared to that of normally menstruating females.

Future Research

Currently, there is very limited research that focuses on the effects of female sex hormones and oral contraceptives on muscle properties across the menstrual cycle.

Future research should concentrate on tracking contraceptive status and menstrual cycle regularity among female non-contact ACL injuries. Future study samples should include females who have irregular menstrual cycles and females who are using a variety of oral contraceptive delivery methods. These studies should focus on test sessions and blood hormone levels that are to be taken on multiple days in order to better identify changes in muscle properties that may occur across the different phases of the menstrual cycle.

Conclusions

Previous research has shown an increased injury rate for non-contact ACL injuries in female athletes when compared to their male counterparts. The results of this study do not support our hypothesis and did not find a significant difference in muscle properties across the menstrual cycle between normally menstruating females and females regularly taking the oral contraceptive pill. Finally, this study does not indicate that taking the oral contraceptive pill would alter muscle properties in manners which are suggested to reduce ACL injury risk.

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