

ASSESSMENT OF MUSCLE STIFFNESS, ELECTROMECHANICAL DELAY, AND
MUSCLE EXTENSIBILITY OVER THE COURSE OF THE MENSTRUAL CYCLE

by
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Abstract

MEGAN PATE: Assessment of Active and Passive Muscle Stiffness, Electromechanical Delay, and Extensibility Over the Course of the Menstrual Cycle (Under the direction of Darin Padua, Troy Blackburn, Kevin Guskiewicz, and David Bell)

Anterior cruciate ligament injuries are more common in females than in males. Research has failed to isolate a cause for this bias. Hormones have been implicated in increased injury rates, increased ligamentous laxity, decreased stiffness, and increased electro-mechanical delay. We compared active and passive muscle stiffness, electro-mechanical delay, and extensibility of the knee flexor group between menses and ovulation in eumenorrheic women. No significant differences in active and passive muscle stiffness, electromechanical delay, or extensibility of the knee flexor group were measured between menses and ovulation. Menstrual cycle phase may not have an effect on active and passive muscle stiffness, electromechanical delay, or extensibility. Therefore, increased anterior cruciate ligament injury rates in females may not be caused by hormonal changes in muscle stiffness.

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List of Abbreviations

ACL	Anterior Cruciate Ligament
EMD	Electromechanical Delay
MCP	Menstrual Cycle Phase
MTS	Musculotendinous Stiffness
MTT	Musculotendinous Tissue

Chapter One

INTRODUCTION

Anterior Cruciate Ligament (ACL) injuries occur more frequently in women than in men (Arendt and Dick 1995, Powell and Barber-Foss 2000, Rozzi et al. 1999).

Differences in knee anatomy, knee and hip biomechanics, neuromuscular control, and hormones have been implicated as causative factors. However, the reason for the sex bias in ACL rates is likely multi-factorial.

In the attempt to define the role of hormones in the increased risk of ACL injury in females, researchers have identified correlations between menstrual cycle phase (MCP), and the associated hormonal fluctuations and ACL injury risk (Beynnon et al. 2006, Slauterbeck et al. 2002, Wojtys et al. 2002). In addition, a key fluctuating hormone of the menstrual cycle, estrogen, has been shown to influence collagen metabolism in ligament and therefore may have potential weakening effects on the ACL (Liu et al. 1997, Yu et al. 1999). This relationship between estrogen concentration and ACL laxity could be a potential reason for increased knee laxity in females as compared to males (Beynnon et al. 2006). However, there is conflicting evidence for an actual correlation between MCP and ACL laxity (Belanger et al. 2004, Deie et al. 2002, Heitz et al. 1999, Karageanes et al. 2000, Romani 2003, Shultz et al. 2004). Some agree that there is a relationship between menstrual cycle phase and knee laxity and others do not. Therefore, it is possible that the fluctuating reproductive hormones of the menstrual cycle may affect

the active tissues of the knee joint such as, muscle and their connective tissues such as, tendon and fascia in addition to the ACL. This theory is supported by recent research that has demonstrated decreased muscle stiffness in females that cannot simply be attributed to anthropometric differences or less strength (Blackburn et al. 2006, Eiling 2006). Because active tissue is important in providing stability to the knee joint (Duan et al. 1997), potential changes in active tissue stiffness caused by fluctuating hormone levels during the menstrual cycle may leave the ACL more vulnerable to injury (Granata et al. 2002).

Because hormone induced changes such as, collagen metabolism have been shown in ligaments and in smooth muscle (Copas et al. 2001, Liu et al. 1997), it is probable that reproductive hormones are capable of changing the properties of skeletal muscle as well. We are aware of only one study that has investigated the effects of MCP on active tissue (Eiling et al. 2006). Eiling et al. observed musculotendinous stiffness (MTS) of the lower limb at four different points in the menstrual cycle. They observed a significant decrease in MTS during the ovulatory phase, which is associated with a peak in estrogen concentration. This is consistent with reported increased ACL injury rates during the ovulatory phase (Wojtys 2002).

Therefore, the purpose of this study is to determine the effects of MCP on various muscle properties hypothesized to contribute to joint stability, including active and passive stiffness, electromechanical delay (EMD), and extensibility of the knee flexor group.

1. Research Questions

- 1.1 Is there a significant difference in active muscle stiffness of the knee flexor group between post menses onset and post ovulation during a single menstrual cycle?
- 1.2 Is there a significant difference in passive muscle stiffness of the knee flexor group between post menses onset and post ovulation during a single menstrual cycle?
- 1.3 Is there a significant difference in flexibility of the knee flexor group between post menses onset and post ovulation during a single menstrual cycle?
- 1.4 Is there a significant difference in electromechanical delay of knee flexor contraction between post menses onset and post ovulation during a single menstrual cycle phase?

2. Null Hypothesis

- 2.1 There is no significant difference in active muscle stiffness of the hamstring muscle group between post menses onset and post ovulation during a single menstrual cycle?
- 2.1 There is no significant difference in passive muscle stiffness of the hamstring muscle group between post menses onset and post ovulation during a single menstrual cycle?
- 2.2 There is no significant difference in flexibility of the hamstring muscle group between post menses onset and post ovulation during a single menstrual cycle?
- 2.3 There is no significant difference in electromechanical delay of hamstring muscle contraction between post menses onset and post ovulation during a single menstrual cycle phase?

3. Research Hypothesis

- 3.1 There will be a significant decrease in active muscle stiffness of the hamstring muscle group post ovulation compared to post menses onset.

- 3.1 There will be a significant decrease in passive muscle stiffness of the hamstring muscle group post ovulation compared to post menses onset.
- 3.2 There will be a significant increase in flexibility of the hamstring muscle group post ovulation compared to post menses onset.
- 3.3 There will be a significant increase in electromechanical delay of hamstring muscle contraction post ovulation compared to post menses onset.

4. Definition of Terms

- 4.1 Passive muscle stiffness (k_p) is the slope of the linear relationship between passive moment and angular position of the knee ($\Delta F/\Delta^\circ$).
- 4.2 Active muscle stiffness is defined as $k_a=4\Pi^2mr^2f^2$ where k_a is active stiffness, m is the total mass of the lower leg (kg), r is the distance from the lateral femoral condyle to the lateral malleolus, and f is the damped frequency of oscillation (s^{-1}).
- 4.2 Frequency of oscillations (f) is defined as the inverse of the time interval between the first two recorded oscillations of lower leg movement following a perturbation during active stiffness assessment.
- 4.3 Onset of muscle activity (ms) will be defined as the time at which the EMG exceeds two standard deviations of the average baseline muscle activity during EMD assessment.
- 4.4 Baseline activity is defined as the average voltage (V) recorded 500ms before presentation of the visual stimulus during EMD assessments.
- 4.5 Onset of force (ms) will be defined as the time at which force (V) exceeds two standard deviations of the baseline force being registered by the dynamometer during the EMD assessments.

4.6 Extensibility was defined as the angle formed by the longitudinal axis of the femur and a line parallel to the ground.

4.7 Post menses was defined as the time period between the self reported first day of menstrual flow and the three days following.

4.8 Post ovulation was defined as the time period between the occurrence of a positive ovulation test and the three days following.

5. Assumptions

5.1 Participants accurately and honestly reported their medical histories.

5.1 Participants accurately and honestly reported their menstrual cycle histories and information about contraceptive use.

5.2 Participants accurately reported day of current menstrual cycle.

5.3 Participants returned on days that are consistent with the repeated measures guidelines (i.e. three days after the onset of their menstrual flow and three days after ovulating).

5.4 Participants used the ovulation test strips at the right time and as indicated by the manufacturer's instructions.

5.5 Participants did not take any type of hormone altering contraceptive while participating in this study.

5.6 Participants maintained a consistent level of physical activity, abstaining from any new activities that may affect their current level of muscle stiffness or flexibility i.e. new stretching techniques or a different type of exercise.

6. Limitations

6.1 Participants had unavoidable irregularities in their menstrual cycle throughout the testing period.

6.1 Confounding variables such as previous activity, illness, nutrition, or stress levels caused differences in the measured variables.

7. Delimitations

7.1 Participants were asked to keep a calendar that helped them determine day of menstrual cycle more accurately.

7.1 Participants with a history of irregular cycles were eliminated from the study.

7.2 Participants were asked to maintain a consistent level of physical activity throughout the course of the study.

8. Significance of Study

If these muscle properties are negatively affected across the menstrual cycle, this could contribute to our overall understanding of increased ACL injury rates in women. MCP dependent changes may indicate a need to further examine the effects of hormone altering medications such as oral contraceptives. Furthermore, MCP dependent changes in our selected variables will indicate a need to control for MCP when conducting further research on those variables. It may also be necessary to recognize non-MCP hormonal changes in subjects who are pre-pubescent or menopausal when researching active tissue variables.

Chapter Two

REVIEW OF LITERATURE

1. ACL INJURIES

1.1 Epidemiology

Researchers have paid special attention to female ACL injuries over previous decades. Following Title IX, there was a rise in female athlete participation in college athletics. Along with this rise came an increase in recorded ACL injuries. Data collected from the National Collegiate Athletic Association Injury Surveillance System in 1995 revealed that females experience significantly higher ACL injury rates than males competing in the same sport (Arendt and Dick 1995). ACL injuries are one of the most common and costly ligamentous knee injuries. They are potentially season ending and can be devastating to continued athletic and academic performance by jeopardizing scholarship support (Hewett et al. 2006). Researchers are currently trying to pinpoint the causes for increased female ACL injury rates compared to males. Although many causes have been hypothesized, the reason is likely multifactorial.

In the literature, risk factors for ACL injury are consistently grouped into intrinsic and extrinsic factors (Ireland 2002, Huston et al. 2000, Hewett et al. 2006). Ireland (2002) defines intrinsic factors as those that are not changeable, including tibial-femoral

alignment, hyperextension, physiologic rotatory laxity, ACL size, intercondylar notch size and shape, and hormonal influences. Huston et al. (2000) defines intrinsic factors similarly, with joint laxity added to the list. A more recent review (Hewett et al. 2006) is more expansive. Their list of intrinsic factors includes increased available translation of the tibia on the femur, increased foot pronation defined by navicular drop, increased body mass index, changes experienced during puberty, muscle activation timing, proprioception, muscle imbalances, fatigue, and angles at the hip.

Extrinsic factors are defined by Ireland (2002) as those that can be changed and include strength, conditioning, type of shoe, and motivation. Ireland further explains a category of *potentially* changeable intrinsic and extrinsic factors that includes proprioception, neuromuscular patterns, order of muscle firing, and acquired skills.

1.2 *Mechanisms of ACL injury*

Mechanisms of ACL injury can be categorized into contact or non-contact injuries. Contact injuries include those that occur as a result of contact between two individuals, whether the contact occurred on the involved limb or somewhere else on the body (Hewett et al. 2006). However, the most common mechanisms of ACL injury are non-contact in nature. Boden et al. (2000) surveyed 89 injured athletes and found that 72% of them injured their ACL as a result of a noncontact mechanism of injury. Non-contact mechanisms in this study were defined as sudden deceleration prior to a change of direction or landing motion. Video review of the injuries, when available, showed that the involved knees were close to full extension during the deceleration or landing maneuver. Performing decelerations and landing maneuvers at full extension (0 degrees-20 degrees) is a typical movement pattern. In this position the quadriceps have an

increased mechanical advantage over the hamstrings. Therefore, the tibia is subjected to increased anterior tibial translation and the ACL is potentially loaded. Adequate and timely stiffness of the active tissue surrounding the knee joint could play an important role in providing stability that may protect the ACL from excessive loads.

2. Mechanisms of Knee Stability

Joint stability is defined differently among researchers. Different terms such as joint laxity and joint stiffness have been used when describing joint stability. Typically, knee laxity is defined as the amount of motion available at the tibio-femoral joint including translation, transverse plane rotation, and valgus. For example, laxity may refer to degrees of joint opening during the application of a valgus force or millimeters of anterior translation of the tibia during the application of an anteriorly directed force. Therefore, joint laxity and stiffness are related if you consider joint stiffness refers to the ability of the structures comprising the joint to resist changes in position. This is consistent with the definition of stiffness which is the ratio of a change in force to a change in length. When the direction and magnitude of externally applied forces exceed the forces generated by the stabilizing structures of the knee, the tibia will translate in the direction of the externally applied force (Shelburne et al 2004, An 2002). If the tibial translation applies enough tensile or shear strain to the ACL then it will tear or rupture completely. Stability, therefore, is maintained when the resultant joint forces caused by external loads are balanced by the summation of forces generated by the anatomical structures of the knee (An 2002).

Quantitative data concerning the respective contributions of the knee structures to knee stiffness is not well documented. Some consider the ACL to be the primary

stabilizer with knee musculature contributing only when the ACL approaches failure. Other researchers believe that muscles are more important in providing stability. Ideally, the stabilizing structures of the knee work synergistically to increase knee stiffness and minimize tibial motion relative to the femur.

Because females demonstrate less joint stiffness than males it is important to identify the components of joint stiffness and how they may differ between sexes. The structures that contribute to knee stiffness include tibio-femoral contact, ligaments, and muscles (Duan et al. 1997).

2.1 Bony anatomy- TibioFemoral Contact

Because the articular surfaces of the tibia and the femoral condyles are functional in distributing forces across the knee, the alignment of these two bones contribute to knee stability. As valgus/varus and internal/external rotation loads are applied, the tibia maintains an axis of rotation on the femoral condyles. The axis of rotation alternates between the medial and lateral tibial plateaus during movement. As the axis of rotation changes, movement of the tibia causes changes in length and tension in the hamstrings. This stimulates reflexive contractions which provide instantaneous protection against anterior tibial translation and rotation (Besier 2003).

2.2 ACL

The ACL traverses the condylar notch from the posterior lateral femur to the anterior medial tibia, and inserts just posteriorly to the medial meniscus. In this position it is capable of resisting excessive anterior tibial translation and internal and external rotation by undergoing tensile strain. The ACL is able to withstand upwards of 2000N of tensile force before tearing (Woo et al. 1991). Anterior translation or rotation of the tibia

that is experienced with functional movement like landing or cutting increases the forces on the ACL (Dienst et al. 2002). The ability of the ligament to resist applied functional forces is dependent on its strength, loading history, hormone levels, the load applied, geometry of the joint, and the muscles that surround the joint (Besier et al. 2003).

The structural organization of the ACL contributes to its overall strength. It has a hierarchical arrangement of multiple collagen units that are bound together by connective tissue. The first layer consists of cross linked collagen fibers that are bound together into fascicles forming the second layer (Smith et al. 1993). The fascicular nature of the ACL lends to separation of two distinct bundles, the anteromedial (AM) bundle and the posterolateral (PL) bundle which become taut in flexion and extension respectively (Smith 1993). It is this structural organization that contributes to the strength of the ACL. It is suspected that this structure is weakened by fluctuating levels of estrogen in females. It has been demonstrated that estrogen decreases collagen proliferation and may have a weakening effect on ligaments (Liu et al. 1997, Yu et al. 1999).

2.3 Muscle Stiffness

Muscles may be more effective at providing joint stiffness than ligaments because muscles operate under reflexive and voluntary control. Antagonist musculature such as the knee flexor group is a crucial component of joint stiffness because it increases and decreases its level of activity in response to changing agonist activity and changing degrees of knee flexion (Baratta et al. 1988, Hagood 1990, An 2002). Furthermore, the hamstrings have medial and lateral attachments on the tibia and fibula and are able to provide rotary stability to the tibia. Voluntary and reflexive control of knee stability provided by musculotendinous tissue (MTT) such as the knee flexors could be crucial in

protecting the ACL during functional maneuvers such as landing, cutting, or sustaining a blow to the knee. MTT includes contractile components, tendons, and connective tissues attached to a muscle.

The importance of reflexive and voluntary use of muscles to increase knee stability is seen when observing ACL deficient patients who lack proprioceptive input and stability that is typically provided by the ACL (McNair et al. 1992). When performing a functional task, ACL deficient individuals have earlier and higher overall muscle activation as compared to controls (Colne and Thoumie 2006). Similarly, elderly patients who experience decreased neuromuscular control have higher and earlier muscle activation during a functional task in anticipation of instability (Hortobagyi and DeVita 2000). This voluntary increase in muscle activity is suspected to increase knee stability by increasing overall joint stiffness.

The knee flexors may be important contributors to joint stiffness during functional tasks because they are in a position to reduce anterior tibial translation (Smith et al. 1993, Baratta et al. 1988, McNair et al. 1992, Li et al. 1999, Liu and Maitland 2000). Moreover, the hamstrings make a unique contribution to knee joint stability by providing rotary stiffness. Because of their medial/lateral attachment to the tibia/fibula they protect the ACL by reducing tibial rotation. Li et al. (1999) demonstrated that voluntary hamstring contractions significantly reduced anterior and lateral translation and internal rotation of the tibia when the quadriceps exerted a 200N extension load (1999).

Voluntary control of joint stiffness is supported by several studies (Wojtys et al 2002, Zhang and Wang 2001). Zhang and Wang (2001) demonstrated that subjects were able increase knee joint stiffness by co-contracting the knee musculature during

application of an abduction/adduction torque to a fully extended knee. Furthermore, the subjects in this study were capable of controlling the amount of joint stiffness by regulating muscle contractions in response to changing amounts of torques. Specific muscle contributions to the increase in joint stiffness were not mentioned. However, it has been reported that activation of the sartorius can increase valgus knee stiffness by an average of 208% and activation of the vastus medialis oblique increases valgus knee stiffness by an average of 164% (Pope et al. 1979).

Wojtys et al. (2002) demonstrated that voluntary co-contraction of the knee musculature is capable of increasing joint stiffness and significantly reducing anterior tibial translation when an external, anteriorly directed force is applied to the proximal tibia. They calculated that anterior shear stiffness of the knee significantly increased as much as 379% and reduced anterior tibial translation by 5mm. The researchers concluded that this significant reduction in tibial translation is capable of protecting the ACL from forces caused by tibial motion, however they could not conclude if the total strain experienced by the ACL during maximal co-contraction of the knee musculature is reduced.

The muscle property that contributes to increased joint stiffness is muscle stiffness. Muscle stiffness is the ratio of the change in force to the change in length of the MTT. Therefore, adequate stiffness of the muscles attached to the tibia can potentially contribute to knee stability by resisting changes in length and reducing total tibial translation (Wojtys et al. 2002, Zhang and Wang 2001, Sherbondy et al. 2003, An 2002). Even when resting, passive muscle stiffness makes an appreciable contribution to knee stability (Wojtys et al. 2002). But more importantly, active stiffness, created by

voluntary or reflexive contractions of the knee musculature, can increase joint stiffness up to more than three times resting stiffness values, and may be more valuable during functional activities (Wojtys et al. 2002, Markolf 1978). The contribution of active muscle stiffness is essential for dynamic knee stability (Granata et al. 2002, Duan et al. 1997). Therefore, low muscle stiffness could increase risk of ACL injury, and could be a contributing factor to increased ACL injury rates in females.

Several studies have reported less musculotendinous stiffness in females. The causes for this are uncertain. Granata et al. (2002a & 2002b) observed in two separate publications that active stiffness of females ranges from 56-81% less than males. In the first investigation they theorized that sex differences in body size, muscle architecture, and material differences secondary to hormonal influences may contribute to decreased stiffness in females. In a different study they concluded that unequal body mass does not fully explain the sex differences and they proposed muscle recruitment strategies and differences in knee and ankle motion as possible contributors. Hormonal differences were not addressed as a cause in the later study.

Other studies have proposed that strength, co-activation levels, and anthropometric qualities contribute to sex differences in stiffness (Markolf et al. 1978, Hagood 1990, Blackburn et al. 2004, Granata et al. 2002a). Blackburn et al. (2004) measured active and passive stiffness and extensibility of the knee flexors in 15 males and 15 females. The females displayed significantly higher extensibility and significantly less active and passive stiffness. However, when the results were normalized, no significant differences were found. The males were significantly taller, heavier, and had significantly longer shanks. Therefore, the authors concluded that differences in stiffness

between males and females are primarily due to anthropometric differences. However, other research suggests that sex differences in stiffness may be more dependent on intrinsic properties of the muscle (An 2002, Wojtys et al. 2002, Blackburn et al. 2006). These intrinsic properties include tendon viscoelastic properties (Blackburn et al. 2006), muscle architecture (Blackburn et al. 2006, Wojtys et al. 2002, An 2002), and the amount and arrangement of passive connective tissue in the muscle (Wojtys et al. 2002). Because fluctuating reproductive hormones have been shown to have an effect on collagen metabolism, it is possible that they may also have an effect on these intrinsic properties of muscle. If this is true, one would expect to see changes in active and passive muscle stiffness during different MCP.

To date, there is only one other study that has measured differences in active muscle stiffness over the course of the menstrual cycle (Eiling et al. 2006). They reported significantly lower MTS on the day of ovulation than during menstruation and day seven of the follicular phase. These findings are consistent with our hypothesis. However, there are some differences in methodology between their study and ours. Eiling et al. defined musculotendinous stiffness (MTS) as $K_{leg} = F_{peak} / \Delta L$ where K_{leg} is stiffness of the leg, F_{peak} was the peak vertical ground reaction force measured during a repetitive hopping on a force plate and ΔL was maximal change of displacement of the leg spring. It is not known if a higher functional demand such as hopping makes differences in stiffness more detectable. The day of testing occurred on the exact day of ovulation in the Eiling study. Because the Eiling study is the only study to date that describes any MCP dependent changes in skeletal muscle, there is little evidence to indicate when testing should occur. The results from a study published by Shultz et al. (2004) suggest that MCP dependent

changes in knee laxity occur within 3-4 days following a peak in estrogen. It is not known if there is a similar delay in the effects of hormone fluctuation on muscle tissue. Shultz et al. (2004) also noted a post hoc regression that showed a relationship between the length of delay in the laxity changes and the length of the follicular phase and the absolute levels of estrogen and progesterone in each subject. Changes in knee laxity occurred later in individuals who had smaller increase in hormone levels. It is possible that potential effects of MCP on MTT would display the same delays.

2.4 Electromechanical Delay

Co-contraction of knee musculature is capable of protecting the ACL by increasing joint stiffness. However, if the muscles are not contracted at the instant of an applied force they must respond quickly enough to offer protection to the ACL. The time it takes for MTT to respond to joint loading is often referred to as total motor time (TMT) (Bell & Jacobs 1986, Moore et al. 2002). TMT was divided by Moore et al.(2002) into premotor time (PMT) and electromechanical delay (EMD). PMT is the time between the application of a load and the onset of electromyographic activity of a muscle (EMG). EMD is the time between the onset of EMG of a muscle and the onset of force or movement created by that muscle (Granata et al. 2000, Moore et al. 2002, Zhou 1996). EMD has been used as a measure of MTT stiffness because EMD is essentially the time it takes for a shortening muscle to apply force to a joint (Granata et al. 2000). Muscles that are pretensioned or intrinsically stiff have shorter EMD. On the other hand, more compliant MTT would be expected to have a longer EMD because cross bridge formation initially functions to take up the “slack” in the series elastic component rather than applying force to move bone segments. Thus, shorter EMD is seen when normal

individuals are compared to subjects who pre-tense their muscles or to patients who suffer from a spastic neuropathy (Granata et al. 2000).

EMD does not affect the magnitude of MTT force, but determines if the force, despite its magnitude, is applied quickly enough to counteract excessive loads at the knee (Granata et al. 2002). Presumably, a prolonged EMD would prevent the knee flexor group from preventing anterior tibial translation or tibial rotation that could result in an ACL tear.

Because of the potential relationship between stiffness and EMD, it is not surprising that females have demonstrated longer EMD than males (Bell and Jacobs 1986, Winter and Brookes 1991, Zhou et al. 1995) as females possess lesser MTS. One study reported no difference in EMD between males and females (Linford et al. 2006) and one study showed prolonged EMD in females only after a fatiguing bout of exercise (Moore et al. 2002). If a discrepancy does exist, it is a possible contributor to the increased risk of ACL injury in females. Therefore, it is important to investigate the reasons for prolonged EMD in females.

The following studies have suggested potential contributors to prolonged EMD. Kaneko et al. (2002) reported an increase in EMD of the quadriceps following ACL reconstructions that used a patellar tendon graft. They speculated that changes in the stiffness of the series elastic component of the patellar tendon secondary to surgical trauma increased EMD. Zhou et al. (1996) also suggested that changes in EMD would be expected when there are changes in the structural and functional properties of MTT. He also cited fatigue, muscle temperature, contraction force, and muscle fiber type as a contributor to changes in EMD. These speculations, however, do not offer an

explanation for the differences in EMD between men and women. One explanation for the sex difference is that EMD is affected by reproductive hormones (Moore et al. 2002, Winter and Brookes 1991). The role of hormones does not contradict the idea that structural changes may contribute to prolonged EMD but offers an explanation for a sex linked change that creates prolonged EMD in women. Hormones have been shown to create changes in collagen metabolism. Therefore, changes in the collagen composition of MTT may make muscle more compliant and prolong its EMD.

3. Menstrual Cycle and Potential Effects of Reproductive Hormones on Active and Passive Tissue

Estrogen is one hormone in the class estradiol. Functions of estrogen in women include stimulating bone and muscle growth, maintaining female secondary sex characteristics, affecting central nervous system activity, maintaining functional accessory reproductive glands and organs, and initiating the repair and growth of the endometrium. The maintenance of the endometrium and production of an egg is the result of a concerted fluctuation of estrogen and other reproductive hormones such as progesterone. It is this fluctuation that has been implicated in increased knee laxity and more frequent knee injuries in females as compared to males. In an attempt to define the relationship between fluctuating hormones and changes in tissue properties at the knee, several observations have been made that warrant further research. The presence of estrogen receptors (ER) has been noted on ligaments, smooth, and skeletal muscle (Copas et al. 2001, Lemione et al. 2003, Liu et al. 1996). Also, it has been observed that estrogen effects the metabolism of collagen in the ACL (Liu et al. 1997), potentially making it weaker. Therefore, it is hypothesized that when there is a high serum

concentration of estrogen, (i.e. during ovulation), ligaments are more lax and more susceptible to injury.

A normal menstrual cycle is typically divided into a menstrual, follicular, ovulatory, and a luteal phase. The follicular phase begins with menstruation (menses) which typically lasts 7 days. During menses estrogen levels are low and follicle stimulating hormone is the predominant hormone and is responsible for stimulating the ovary to produce follicles. The follicles secrete estrogen at an increasing rate as they develop. When the estrogen reaches a threshold concentration, lutenizing hormone is released from the pituitary gland. Lutenizing hormone triggers rupture of the follicle wall and an oocyte is released (ovulation). Ovulation occurs at approximately 14 days after the onset of menses, is accompanied by a sharp rise in estrogen, and followed by a decreased in estrogen and an increase in progesterone approximately twenty days after menses onset. Ovulation marks the start of the luteal phase which is characterized by decreasing estrogen levels and a spike in progesterone levels. If fertilization does not occur, estrogen and progesterone decrease rapidly, and menstruation occurs (i.e. the cycle starts over). (Martini 2006)

There is conflicting evidence that ACL laxity actually increases during the time when estrogen concentration is highest. Shultz et al. (2004), Deie et al. (2002), and Heitz et al. (1999) observed increased ACL laxity during times of the menstrual cycle when estrogen and progesterone are highest. However, Karageanes et al. (2000), Belanger et al. (2004), and Beynnon et al. (2005) were unable to demonstrate any significant changes in knee laxity over the course of the cycle. The distribution of injury frequency across the menstrual cycle is also variable amongst studies. Slauterbeck et al. (2002) observed

increased ACL injury corresponding to the onset of menses. However, Wojtys et al. (2002) observed more ACL injuries than expected during the ovulation phase. These results seem more consistent with the hypothesis that estrogen has a weakening effect on ligaments. The variability in the results of these studies could be due to time delays between hormone concentration changes and tissue property changes (Shultz et al. 2004). Secondly, it is not known if estrogen has an effect on the ability of active tissue to provide stability to the knee. Lastly, it is possible that estrogen is not the only factor influencing changes in tissue property throughout the cycle. Progesterone also demonstrates a huge fluctuation during the cycle.

It has been proposed that estrogen can contribute to increased ACL injury by weakening the ACL itself. Liu et al. (Liu 1997) demonstrated altered collagen metabolism in ACL specimens after exposure to physiologic exposure to estrogen, reporting a 40% reduction in collagen synthesis with increasing estradiol concentrations. Yu et al. (1999) demonstrated decreased Type I procollagen synthesis with increased estradiol exposure in the human ACL. More research is required to determine the influence of menstrual cycle hormones fluctuations on ligaments. Additionally, it is not known if menstrual cycle hormones have an effect on stiffness of skeletal muscle. Since there is collagenous material in the series component of muscles, it is possible that the fluctuation of hormones can change musculotendinous properties and their ability to provide stability to the knee.

Some research has attempted to define the affect of estrogen on muscles. Estrogen has been shown to have a significant influence on muscle membrane stability and possibly on minimizing post exercise muscle damage and soreness (Tiidus 2003, Carter

et al.). Furthermore, some studies have ventured to demonstrate a relationship between menstrual cycle phase and skeletal muscle strength. Three relatively recent studies were unable to identify a correlation between menstrual cycle phase and changes in strength (Friden et al 2003, Janse de Jonge et al 2001, Nicolay et al. 2007). However, Nicolay et al (2007) did report a minimal decrease in endurance of grip strength during the late follicular phase of eumenorrheic women. They also reported that the controls, who were oral contraceptive users, did not demonstrate fluctuations in strength and they were weaker than the eumenorrheic women throughout the entire cycle. Post hoc analysis in the study led the researchers to deny any relationship between oral contraceptive use and decreased strength, but they did suggest a potential interaction between increased estrogen levels and decreased skeletal muscle endurance during late follicular phase in the eumenorrheic women. They proposed that the decreased endurance demonstrated in this study was secondary to a collagenolytic effect of estrogen on ligaments and tendons which supports our theory that MCP may have an effect on stiffness of musculotendinous tissue.

4. Conclusion

The effects of estrogen on ligaments has been researched in many studies. Less research has been conducted to explain the effects of estrogen on muscle, tendon, and fascia. If, in fact, estrogen does have a significant effect on collagen metabolism in muscle, tendon, and fascia and on the neuromuscular qualities of joint stability, then one might expect significant changes in muscle properties such as active and passive stiffness and electromechanical delay during the three phases of the menstrual cycle.

Chapter Three

METHODS

1. Subjects

Twenty pre-menopausal females were recruited from the University of North Carolina (UNC) to participate in this study. In order to participate the following criteria were met: 1) no history of lower extremity injury within 3 months prior to data collection that has limited physical activity for more than 48 hours, 2) no history of ACL injury, 3) no history of taking oral contraceptive or any hormone altering drug including implants and patches within the 6 months prior to data collection, 4) self reported normal menstrual cycle for the 6 months prior to data collection, 5) no history of a diagnosed neurological disorder or visual impairment, and 6) must be physically active, participating in a minimum of 30 minutes of activity three times a week. During the testing period, subjects were asked to maintain their level of activity and avoid changes in type of activity.

All subjects completed a medical and menstrual cycle history inquiring about the inclusion criteria. As well as an approved informed consent document.

2. Measurements

The following dependent variables were assessed in this study: 1) passive knee flexor stiffness, 2) active knee flexor stiffness, 3) knee flexor electromechanical delay, and 4) knee flexor extensibility. All dependent variables were measured on two separate

occasions for each subject. The first occasion occurred within three days after the onset of menses. This interval falls after the physiological drop in estrogen and the time when tissues are hypothetically not influenced by estrogen. The second occasion of testing occurred within three days after ovulation. Ovulation occurs in response to a peak in estrogen concentrations. It is suspected that within the three days following ovulation tissues experience the greatest influence of estrogen. All testing was performed on the subject's dominant leg. The dominant leg was defined as the leg the subject would self select to kick a ball for maximal distance.

Subjects were asked to complete a medical and menstrual cycle history form. Subjects documented the first and last day of their previous menstrual cycle on a calendar. At the onset of the next menstrual cycle, the subjects were asked to contact the principal investigator. The initial testing session was performed within three days following the onset of menses. Following the initial test session, the subjects were given a commercial ovulation kit. Two days prior to the middle of their menstrual cycle (as calculated from the previous menstrual cycle history) the subjects used the ovulation kit (urine test strip) at the same time everyday until the test was positive, indicating that ovulation had occurred. Within three days after the positive ovulation test the subject underwent the second testing session.

To avoid potential changes in stiffness or flexibility, the participants were asked to abstain from even slight changes in physical activity or stretching for three days prior to each test session. Activity was limited to what they typically performed on a regular basis.

2.1 *Subject Preparation*

Prior to testing, the subject performed a 5 minute warm up on a stationary bike at a pace equivalent to 25% of the individual's perceived maximal exertion level. The subject then performed a standardized stretching routine to prepare the hamstrings, quadriceps, and gastrocnemius complex for testing.

Following the warm up, subjects were prepared for electromyographic (EMG) electrode placement. Preparation of the subject for electrode placement involved shaving, abrading, and cleaning the electrode locations in order to reduce electrical impedance. A telemetered surface EMG system (T42L-8T0 Telemetry, Konigsberg, Inc., Pasadena, CA) was used to assess muscle activation onset time and amplitude throughout testing. The system includes an 8-channel differential preamplifier transmitter and receiver (input impedance = 200k Ω , CMRR>70dB;SNR>40 dB). The EMG signal was amplified by a factor of 10,000 over a bandwidth of 0.01 to 500 Hz and passed via an A/D converter (National Instruments, San Antonio, TX) at 1000Hz to the storage computer. Bi-polar Ag-AgCl surface electrodes (Medicotest, Rolling Meadows IL.) measuring 10mm in diameter with a center to center distance of 2.0 cm were placed in parallel arrangement over the area of greatest muscle bulk within the muscle bellies of the vastus lateralis (VL), biceps femoris (BF), and lateral gastrocnemius (LG). Electrodes were positioned in parallel alignment with muscle fiber directions (Beckman and Buchanan 1995). A reference electrode was placed on the tibial tuberosity. All electrode placements were confirmed and checked for cross talk by performing manual muscle tests (MMT).

2.2 Passive knee flexor stiffness

Passive knee flexor stiffness was assessed with the assumption that a linear relationship exists between passive moment and angular position. Once this relationship was defined, the slope of the moment-angle curve was calculated as passive stiffness. Following the protocol set forth by Blackburn et al. (2004), the moment-angle relationship of the knee flexors was determined with the subject in a seated position on a dynamometer chair. The chair was adjusted so that the subject's trunk was positioned in 30° of extension from vertical to standardize hamstring length. Padded straps were used to secure the subject's thigh, hips, and chest. A strap also was used to attach the subject's ankle to the moment arm of the dynamometer. A rigid splint was used to maintain a neutral ankle position. The axis of rotation of the dynamometer arm was aligned with the axis of the knee joint.

Each trial involved the subject's leg being passively extended by the dynamometer from a position of 90° to 10° of knee flexion. A total of 3 trials was performed and the average passive stiffness across the trials was determined. Passive stiffness was calculated as the slope of the moment angle curve as the knee was passively extended from 90deg to 10deg of knee flexion. During testing the angular velocity of the moment arm was standardized at a low level (5°/s) to reduce the effects of the viscoelastic nature of the tissues and to reduce the risk of stretch reflex contractions. The subject was instructed to remain relaxed and motionless throughout the trial. The EMG activity of the hamstrings, quadriceps, and gastrocnemius was displayed on a computer screen in the subject's line of sight, which allowed them to maintain their

muscle activation at a minimum level. EMG activity from the VL, BF, and LG that exceeded baseline negated the trial and the trial was repeated. Angular displacement and torque data were obtained directly from the dynamometer.

2.3 Active knee flexor stiffness

Active knee flexor stiffness assessment was performed by modeling the lower extremity as a single degree of freedom mass spring system. In this model, active knee flexor stiffness was determined as a function of damped oscillatory motion of the lower limb after a perturbation using the following equation

$$k_a = 4\pi^2 m r^2 f^2$$

where k_a is active stiffness, m was the total mass of the system (shank and foot segments and the added weight), r was the distance from the lateral femoral condyle to the lateral malleolus, and f was the damped frequency of oscillation (s^{-1}) (Blackburn et al. 2004). Mass of the lower leg was approximated using standard anthropometric approximations. Following the protocol set forth by Blackburn et al., the damped frequency of oscillations (f) was determined using the accelerometer. An ankle orthosis designed to maintain the foot in a neutral position was attached to the foot on the ipsilateral side of EMG connections. It was secured with an elastic wrap. A triaxial accelerometer (PCB Piezotronics, Depew, NY, USA) was adhered to the heel of the orthosis using a small amount of wax. The subject was then positioned prone with the knee in 30deg of flexion. The subject was asked to hold the knee in 30 deg of flexion. During assessment, the investigator applied a brief downward force over the posterior aspect of the ankle orthosis. Subjects were instructed to maintain pre-perturbation muscle contraction levels

and to not intervene with the perturbation. In order to monitor hamstring isolation, EMG from the VL and LG were observed. Any trials in which excessive VL or LG activity is observed were discarded and repeated. The perturbation was applied at a random time to reduce the likelihood of subject anticipation. The motion of the foot in response to the perturbation was described as oscillatory motion and was measured with the triaxial accelerometer (PCB Piezotronics, Depew, NY, USA). Frequency of oscillations (f) were defined as the inverse of the time interval between the first two recorded oscillations.

2.4 Electromechanical delay

Subject positioning was identical to passive knee flexor stiffness with the exception that the knee was placed in 30° of flexion. The subject was instructed to remain still while waiting for a light to be turned on by the examiner. The examiner controlled the light out of the subject's line of sight to reduce the likelihood of anticipation. When the light came on the subject responded as quickly as possible by flexing the knee with a forceful hamstring contraction.

The onset of hamstring muscle activity (ms) was defined as the time at which hamstring's muscle activity exceeded two standard deviations above the average baseline muscle activity. Baseline activity was defined as the average muscle activation amplitude recorded 500ms prior to introduction of the visual stimulus. The magnitude of torque was measured by the dynamometer. Onset of force (ms) was defined as the time at which torque (Nm) exceeded two standard deviations of the baseline torque registered by the dynamometer. Baseline torque was defined as the average torque (Nm) recorded 500ms before the light was turned on. Electromechanical delay was determined by

calculating the difference between the onset of force and the onset of muscle (EMG) activity.

2.5 Knee Flexor Extensibility

Passive hip flexion range of motion while lying in a supine lying position was measured to quantify knee flexor extensibility. During testing, the tester passively moved the participant's hip into flexion while maintaining the knee in full extension. When the participant indicated that the leg could not be pushed any further, an assistant held the leg in place while the degrees of hip flexion were measured using a handheld goniometer. Extensibility was defined as the angle formed by the longitudinal axis of the femur and a line parallel to the ground.

3. Statistical Analysis

Statistical analyses were performed using SPSS 14.0 version statistical software (SPSS, Inc., Chicago, IL, USA). Separate repeated measures analyses of variance (ANOVA) were performed for each dependent variable (knee flexor extensibility, passive knee flexor stiffness, active knee flexor stiffness, and electromechanical delay). The within subject factor was menstrual cycle phase (2 levels: post-menses onset and post-ovulation). Statistical significance was established *a priori* at $\alpha < .05$.

Chapter Four

RESULTS

Descriptive Statistics

A total of twenty subjects were recruited for this study. Sixteen subjects were able to report for testing in a timely manner following onset of menses. Others were unable to report for testing within 3 days of onset of menses for two or more consecutive cycles. Of the sixteen subjects tested at menses, eight subjects returned for a second bout of testing at ovulation and eight subjects were excluded because they never produced a positive ovulation test. Of the eight subjects tested at both time periods, passive stiffness data were unusable for two, and EMD data were unusable for one either due to file corruption or the trials involved EMG activity that was indicated the subject was interfering with passive motion. Data from all eight subjects was used for the active stiffness and extensibility analysis. Demographic data for the eight subjects that were tested at both time periods are presented in Table 1.

Passive Stiffness

Means and standard deviations for passive stiffness are shown in Figure 1. Effect size and power are listed in Table 2. Statistical analysis revealed no significant difference in passive stiffness between the menstruation and ovulation phases ($F_{1,5} = 1.213, p=.321$). This finding indicates that menstrual cycle phase has no significant effect on passive knee flexor stiffness.

Active Stiffness

Means and standard deviations for active stiffness are shown in Figure 2. Effect size and power are listed in Table 2. Statistical analysis revealed no significant difference in active stiffness between the menstruation and ovulation phases ($F_{1,7}=.216$, $p=.655$). This finding indicates that menstrual cycle phase has no significant effect on active knee flexor stiffness.

EMD

Means and standard deviations for EMD are shown in Figure 3. Effect size and power are listed in Table 2. Statistical analysis revealed no significant difference in EMD of the 7 subjects between the menstruation and ovulation phases ($F_{1,6}=.124$, $p=.737$). This finding indicates that menstrual cycle phase has no significant effect on EMD.

Reaction Time

Means and standard deviations for reaction time are shown in Figure 4. Effect size and power are listed in Table 2. Statistical analysis revealed no significant difference in reaction time between the menstruation and ovulation phases ($F_{1,7}=.068$, $p=.802$). This finding indicates that menstrual cycle phase has no significant effect on passive knee flexor stiffness.

Extensibility

Means and standard deviations for extensibility are shown in Figure 5. Effect size and power are listed in Table 2. Statistical analysis revealed no significant difference in knee flexor extensibility between menstruation and ovulation phases ($F_{1,5}=1.26$, $p=.28$).

This finding indicates that menstrual cycle phase has no significant effect on extensibility of the knee flexor group.

Table 1. Means and standard deviations for subject characteristics (height, weight, shank length); mean (\pm SD)

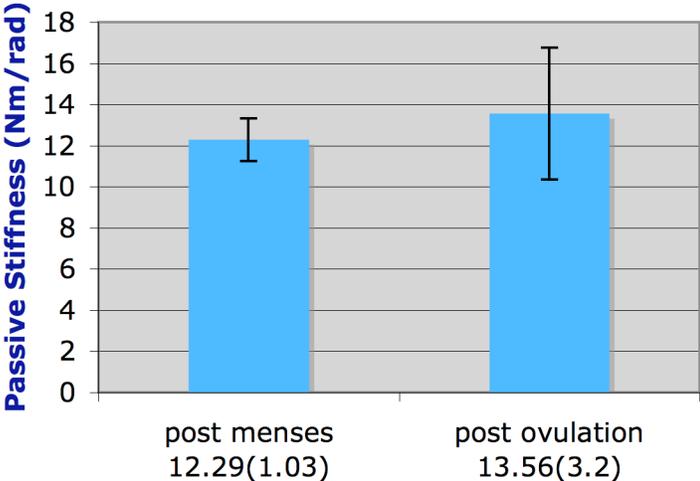
Variables	
N	8
Height (in.)	1.61(.12)
Weight (kg)	65.69(6.31)
Shank Length (m)	0.40(.03)

Table 2. Means and standard deviations, for passive stiffness, active stiffness, EMD, reaction time, and extensibility in post menses and post ovulatory phases with power and effect size; mean (\pm SD).

Variable	Post Menses	Post - Ovulatory	N	p- value	Power	Effect Size
Passive Stiffness (Nm/rad)	12.29(1.03)	13.56(3.20)	6	0.32	0.15	.195
Active Stiffness (Nm/rad)	141.61(49.02)	132.64(45.31)	8	0.66	0.07	.030
EMD (ms)	162.81(81.44)	153.33(82.13)	7	0.74	0.06	.020
Reaction Time (s)	427.94(147.72)	450.92(199.24)	8	0.80	0.06	.010
Extensibility (deg)	90.67(8.47)	95.63(9.16)	8	0.28		

significance (p<0.05)

Figure 1. Comparison of passive stiffness post menses and post ovulation



Mean and standard deviations at two points in the menstrual cycle

Figure 2. Comparison of active stiffness post menses and post ovulation

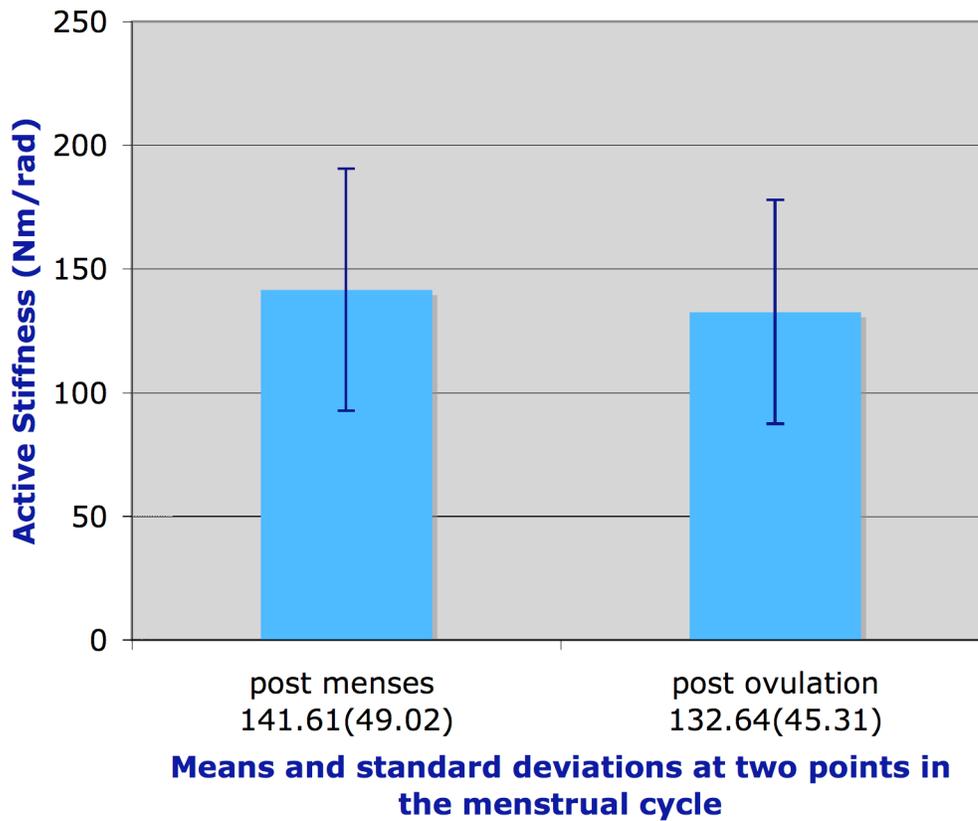


Figure 3. Comparison of EMD post menses and post ovulation

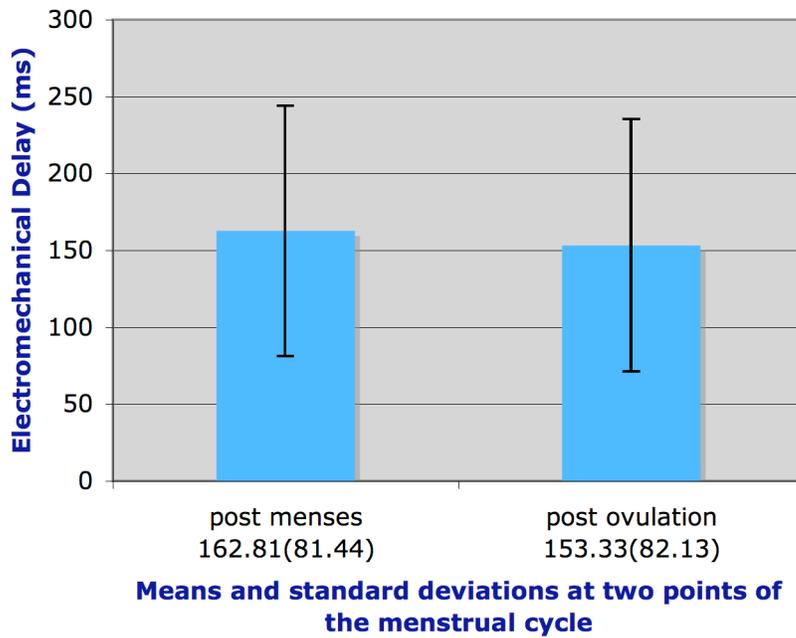


Figure 4. Comparison of reaction time post menses and post ovulation

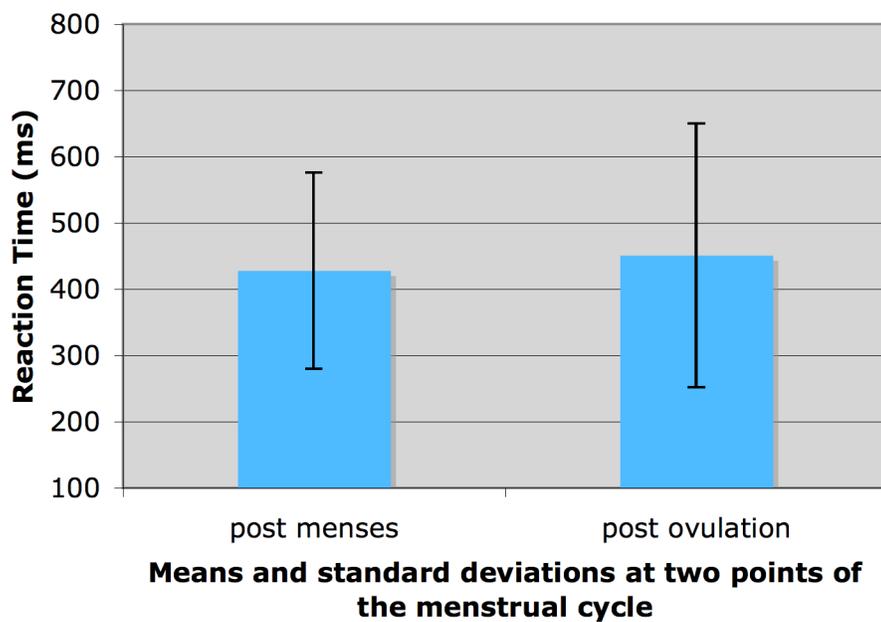
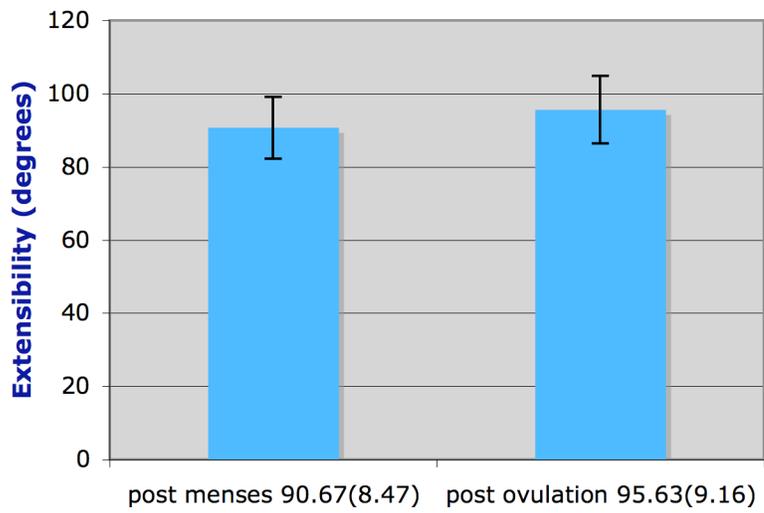


Figure 5. Comparison of extensibility post menses and post ovulation



Mean and standard deviation of extensibility at two points in the menstrual cycle

Chapter Five

DISCUSSION

Current research that has investigated the reasons for increased ACL injury rates in females has produced conflicting results. Many studies have suggested that fluctuating menstrual cycle hormones may contribute to ACL laxity and increased injury risk in females. A relationship has been identified between menstrual cycle phase (MCP) and injury rates (Beynon 2006, Slauterbeck et al. 2002, Wojtys et al. 2002), however there is conflicting support for a correlation between injury rates and a particular phase of the cycle and there is conflicting support between ACL laxity and MCP (Belanger et al. 2004, Deie et al. 2002, Heitz et al. 1999, Karageanes et al. 2000, Romani 2003, Shultz et al. 2004). It is possible that increased injury rates during certain phases of the menstrual cycle are caused by the affects of hormones on active tissue such as muscle, tendon, and fascia rather than ligamentous tissue alone.

The purpose of this study was to investigate the effects of menstrual cycle phase (MCP) on active and passive muscle stiffness, electromechanical delay (EMD), and muscle extensibility of the knee flexor group. The most important finding of this study is that females do not demonstrate changes in active and passive muscle stiffness, EMD, or extensibility between two time periods of the menstrual cycle when measured within three days after menses and within three days following ovulation.

We hypothesized that a significant decrease in active and passive muscle stiffness, and a significant increase in EMD and muscle extensibility would occur within three days post ovulation in females with a normal menstrual cycle. These hypotheses were made based on three previous findings. 1) Females have less muscle stiffness (Wojtys et al. 2002, Granata et al. 2002) and longer EMD (Winter and Brookes 1991) than males. 2) These differences are not predominately attributed to neuromuscular or anthropometric differences in men and women (Granata et al. 2002, Wojtys et al. 2002). 3) Estrogen has a weakening effect on collagen (Liu et al. 1997, Yu et al. 1999) .

It is commonly speculated that differences in sex hormones, neuromuscular characteristics, and anthropometric characteristics contribute to lesser muscle stiffness, longer EMD, and greater extensibility in females. However, one study demonstrated less stiffness in females even when anthropometric differences were standardized (Blackburn et al. 2006). The authors of the study suggested that lesser stiffness observed in females is likely due to differences in tendon viscoelastic properties and muscle architecture. Viscoelastic properties and muscle architecture could be affected by fluctuating estrogen (Wojtys et al. 2002). Additionally, recent research has failed to support changes in neuromuscular characteristics such as kinematics, externally applied moments, fine motor coordination, and strength in females at different points in the menstrual cycle (Abt et al. 2007, Chaudhari et al. 2007, Hertel et al. 2006). Future research may benefit from knowledge of MCP dependent changes in variables such as muscle stiffness, EMD, and muscle extensibility.

The results of a recently published study that researched the effects of MCP on stiffness were consistent with our hypothesis, but not with our findings (Eiling et al.

2006). They reported significantly lower MTS on the day of ovulation than during menstruation and day seven of the follicular phase. There are several reasons this group may have found significant differences while we did not. First, Eiling et al. assessed total leg stiffness and we isolated stiffness of the knee flexors. Second, gender differences are amplified at higher joint loads (Granata et al. 2002a). Because the demand for muscle activity was much higher in the Eiling study MCP dependent changes in stiffness may have been more apparent. The third and perhaps the most critical difference between the two studies was day of testing. Eiling et al. tested subjects on the day of ovulation, whereas the corresponding day for our study was within 3 days post ovulation. It is possible that this timing is necessary for isolating phase dependent changes. Because the Eiling study is the only published study to date that describes any MCP dependent changes in skeletal muscle, there is little evidence to indicate when testing should occur. We study proposed that circulating hormones do not show maximal effects on skeletal muscle during the peak hormone level but within the days following the peak. This was based on results published by Shultz et al. (2004), who found a time delay between changes in serum hormone concentrations and increased knee laxity. They found increased knee laxity associated with increased progesterone, estradiol, and testosterone, however the knee laxity increase occurred 3-4 days following the change in hormone concentration. It is not known if there is a similar delay in the effects of hormone fluctuation on muscle tissue. Shultz et al. also noted a post hoc regression that showed a relationship between the length of delay and the length of the follicular phase and the absolute levels of estrogen and progesterone in each subject. Changes in knee laxity occurred later in individuals who had smaller increases in hormone levels. Again, it is

not known if effects of MCP on muscle stiffness display the same time delays. This study did not take into account potential time delays unique to each subject.

Although the effect of MCP on muscle stiffness is not clear, previous research does suggest that there is an interaction between estrogen and muscle tissue. Estrogen receptors (ER) have been detected in smooth and skeletal muscle tissue (Lemione et al. 2003, Copas et al. 2001). Lemione et al. detected the presence of ER alpha mRNA in female deltoid and pectoral muscle tissue. Other studies were unable to identify ER in skeletal muscle (Gustafsson et al. 1984, Saartok et al. 1984). However, there were differences in binding techniques in these three studies so there could have been differences in test sensitivity. Furthermore, it is possible that these studies did not control for cyclical changes in ER expression which have been reported in human tissue (Iwai et al. 1990).

Some studies have ventured to demonstrate a relationship between menstrual cycle phase and skeletal muscle strength. Three relatively recent studies were unable to show any correlation between menstrual cycle phase and changes in strength (Friden et al 2003, Janse de Jonge et al 2001, Nicolay et al. 2007). However Nicolay et al (2007) did report a minimal decrease in endurance of grip strength during the late follicular phase of eumenorrheic women. They also reported that the controls, who were oral contraceptive users, did not demonstrate fluctuations in strength and they were weaker than the eumenorrheic women throughout the entire cycle. Post hoc analysis in the study led the researchers to deny any relationship between oral contraceptive use and decreased strength but they did suggest a potential interaction between increased estrogen levels and decreased skeletal muscle endurance during late follicular phase in the eumenorrheic

women. They proposed that the decreased endurance demonstrated in this study is secondary to a collagenolytic effect of estrogen on ligaments and tendons, which supports our theory that MCP may have an effect on stiffness of musculotendinous tissue.

Limitations

A primary limitation of this study was low subject number. There were several contributing factors to low subject number. It was difficult to find subjects that met the inclusion criteria in a college age population. It is common for young women to have irregular menstrual cycles or to take oral contraceptives. Of the limited number of participants that we recruited, many were excluded when they were unable to indicate a day of ovulation using the test strips that we provided. Causes may include poor sensitivity of the test strips, poor compliance with instructions, a true lack of ovulation, or the strips were not used at an appropriate time. Future studies could be better controlled using serum or saliva tests for hormone levels. The implication of low subject number is low statistical power. However, effect size calculations indicate that 100-130 subjects are required to demonstrate statistically significant differences.

Another limitation of this study is a lack of knowledge about the effects of hormones on muscle tissue. As previously mentioned it is not known how long it takes for circulating hormones to have an effect on musculotendinous tissue. Further research may make time delay predictions by measuring serum hormone levels as laid out by Shultz et al. (2004) and testing muscle stiffness in a shorter time window. Further research may also seek to monitor changes in muscle stiffness in consecutive days following ovulation.

There was no direct measure of hormone levels in this study. This may have had an effect on subject number because we were not able to indicate actual day of ovulation. It may have also affected the validity of the study. We do not know how well our testing days correlate with actual serum hormone levels.

The final limitation is low rate and low total loading on the knee flexor group. Because sex differences in stiffness are amplified at higher joint loads (Granata et al. 2002), it is possible that we did not provide loads sufficient for demonstrating different stiffness characteristics. The applied loads were also limited to the sagittal plane. Therefore, rotational stiffness was not measured. The significant difference in stiffness measured in the Eiling (2006) study may have contained a rotational component in response to higher functional loads.

Conclusion

This study was one of the first to investigate the effects of menstrual cycle phase on knee flexor stiffness. There was no significant difference in active or passive stiffness, EMD, or extensibility of the knee flexor group across MCP, variables that are possibly associated with decreased knee stability and increased injury rates in females. This study, however, was only a start in trying to answer the research questions presented. Because the study had many limitations, further research is warranted.

Low subject number was a primary limitation and may have resulted from a difficulty in isolating ovulation in the subjects. Future research may more easily define ovulation phase in subjects by using serum or urinary analysis. In addition, because we were not able to isolate phase dependent changes it may be necessary to test subjects on

the exact day of ovulation (the exact day of peak in estrogen) as outlined by Eiling et al. (2006).

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