The relationship between female sex hormones and non-contact knee injuries, specifically anterior cruciate ligament and medial cruciate ligament tears

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by

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ABSTRACT

THE RELATIONSHIP BETWEEN FEMALE SEX HORMONES AND NON-CONTACT KNEE INJURIES, SPECIFICALLY ANTERIOR CRUCIATE LIGAMENT AND MEDIAL CRUCIATE LIGAMENT TEARS

By Katherine J. Krummen

Female athletes are two to eight times more likely to experience a serious knee injury than males when participating in the same non-contact activities (Hewett, 2000). The higher incidence of knee injuries in females has been attributed to biomechanical characteristics, muscular strength imbalances, ligament laxity, and movement techniques (Hewett, 2000; Beynnon et al., 2005). In addition, recent studies have reported that fluctuations in female sex hormones may affect ligament laxity, contributing to the risk of knee injuries (Beynnon et al., 2005; Wojtys et al., 1998; Slauterback et al., 2002). A large range of motion relative to the normal range of motion could increase loading on knee structures and amplify injury probability.

The purpose of this study is to compare the effects of cyclical hormones on lower extremity kinematics, specifically, knee joint alignment angle and distance between the knees at landing contact and knee joint alignment angle and distance between the knees during a squat position. Participants included fourteen college-age female subjects who were tested during each of the three phases in the menstrual cycle: once during days 3-6, once during days 12-16, and once during days 23-26. The participants were tested three times per month for a total of four consecutive months. Subjects were asked to perform a drop jump off a 30.5 cm wooden box onto a Bertec force platform while landing symmetrically with both feet. After this they were instructed to immediately perform a maximal vertical jump and land with both feet on the force platform again. Reflective markers attached to joint landmarks on the subjects were used as an aid in digitizing and analyzing video data. Bilateral marker placement included the greater trochanter, the lateral epichondyle of the femur, and the lateral malleolus. No significant differences between the three phases of the menstrual cycle were detected when comparing knee joint alignment angles at landing contact, knee joint alignment angles during the squat position, the distance between the knees at landing contact, and the distance between the knees during the squat position. The results suggest that the increase of knee injury risk in females is due to anatomical differences or other variables as opposed to fluctuations of hormones during the menstrual cycle.

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INTRODUCTION

Following the enactment of Title IX, the Educational Assistance Act of 1972, female participation in sports has increased. Paralleling this, the incidence of sports-related knee injuries in females has also risen. Previous researchers have concluded that this rise in female sports-related knee injuries has increased not simply due to more involvement, but because females are predisposed to injury in comparison to males after puberty. The higher female non-contact knee injury rate has been attributed to anatomical differences such as a wider pelvis, a greater quadriceps angle, muscular strength imbalances, a narrower femoral notch, anterior cruciate ligament size, and also the increased genu valgum position. Researchers have also begun to investigate the relationship between the fluctuation of female sex hormones during the menstrual cycle and joint ligament laxity, as an increase in joint ligament laxity may amplify the risk for knee injuries. The purpose of this study is to explore the effects of cyclical hormone changes on lower extremity kinematics. By examining this relationship and potentially identifying the causes of the increased risk for knee injuries in females, preventative methods could be designed to reduce the overall incidence of serious knee injuries in females, and lessen the physical pain and financial losses associated with these injuries.

STATEMENT OF THE PROBLEM

The purpose of this experiment is to explore the effects of cyclical hormones on lower extremity kinematics, specifically, knee joint alignment angle and distance between the knees at landing contact and knee joint alignment angle and distance between the knees during a squat position. The null hypothesis states that changes in hormone levels throughout the menstrual cycle have no effect on lower extremity kinematics. The alternative hypothesis states that the changes in hormone levels throughout the menstrual cycle have a significant effect on lower extremity kinematics. The dependent variables in this study include sagittal plane knee alignment angles at landing contact, sagittal plane knee alignment angles during the squat position, frontal plane knee joint alignment angles at landing contact, frontal plane knee joint alignment angles during the squat position, and the distance between the knees as measured within the frontal plane at landing contact and in the squat position. The independent variable in the experiment included the three phases of the menstrual cycle: follicular, ovulation, and luteal.

LITERATURE REVIEW

Since the enactment of the Title IX Educational Assistance Act of 1972, women's participation in sports has dramatically increased. Since 1972, female involvement in high school sports has increased about 900% and in collegiate sports, the increase is about 500% (Myer et al., 2004). With this increase in participation, an increase in sports-related knee injuries has also occurred. This increase in injury incidence is not directly caused by the participation in sports, but rather, it has been demonstrated that females have a higher risk for knee injuries in comparison to males after puberty (Myer et al., 2005). For example, anterior cruciate ligament (ACL) tears are 2-8 times more likely in females than in males (Fayad et al., 2003; Wojtys et al., 2002; Ireland et al., 2001; Anderson et al., 2001; Myer et al., 2005; HWHW, 2002). Several hypotheses have been formed to explain this disparity. The higher incidence of knee injuries in females has been attributed to anatomical differences, biomechanical characteristics, ligament laxity, movement technique, muscular strength imbalances, and sex hormone influences (James et al., 2004; Myer et al., 2005). In addition to these differences, several studies have investigated evidence concerning the fluctuation of female sex hormones throughout the menstrual cycle which may increase knee ligament laxity and possibly augment the risk for serious knee injuries (Beynnon et al., 2005; Wojtys et al., 1998; Slauterback et al., 2002; Shultz et al., 2004; Van Lunen et al., 2003; Hewett, 2000; Romani et al., 2003; Aronson et al., 2004). The purpose of this study is to investigate the effects of cyclical hormone changes on lower extremity kinematics.

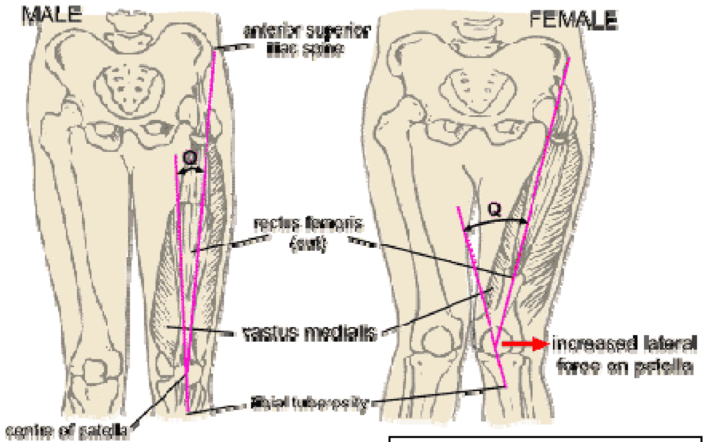


Figure 1 from FAB Website

Anatomical gender differences in females as compared to males include shorter leg length, a wider pelvis, a greater quadriceps angle (Q-angle), the angle formed by the tibial and femoral alignment, an imbalance in the strength of the hamstrings and quadriceps, a narrow femoral notch at the knee, anterior cruciate ligament size, and an increased genu valgum position (Swedan, 2001; Myer et al., 2005; Hutchinson et al., 1995). The Q-angle is measured from the anterior superior iliac spine to the center of the patella and then to the tibial tubercle. A Q-angle of less than 12º is considered normal while an angle greater than 15º is said to be abnormal in women (Hutchinson et al., 1995). There has been focused attention on the theory involving the higher Q-angle which describes the female lower extremity alignment as anteversion at the femoral neck, a varus position at the hip, valgus knee position, external placement of the tibial tubercle, and pronation of the foot (Swedan, 2001; HWHW, 2002; McLean et al., 1999). The hypothesis surrounding how the increased Q-angle is related to the higher incidence of knee injury explains that this inward femoral angle causes a higher load on the inside of the knee, uneven distribution of the body weight on the joint, and increased stress on the ACL. This increased Q-angle is theorized to be the cause of excessive knee cartilage wear and tear because of a valgus force on the patella. The valgus force created increases the risk for lateral patella dislocation and/or subluxation (HWHW, 2002;

Swedan, 2001; Hutchinson et al., 1995; McLean et al., 1999). However, some studies have tested this hypothesis and have not found a strong correlation been the Q-angle and increased knee injury in females (Myer et al., 2005).

Another theory that attempts to explain the higher incidence of ACL injury in females describes biomechanical differences as part of this cause, such as the shape of the intercondylar notch. The intercondylar notch width (NW) is generally narrower in women than in men. Researchers need to be cautious about how they measure the NW because it has been noted that even a 10º angle change of the knee during measurement produces significantly different results (Ireland et al., 2001). Studies have shown a correlation between a higher risk of ACL tears to narrow notch width because as the ACL has to pass through a narrower notch, it is more susceptible to tearing (Ireland et al., 2001; Hutchinson et al., 1995). Another study suggests that the size and width of the ACL do not proportionately vary with the size of the intercondylar notch. Furthermore, higher risk was found when a normal-sized ACL was matched with a narrow notch which places the ACL at a higher potential of injury during rotational or translational movements (Anderson et al., 2001). Intercondylar notch shape demonstrated less importance than the width of the notch in terms of determining the risk for ACL injury (Hutchinson et al., 1995). One study also measured the notch width index (NWI) which is the ratio of the width of the intercondylar notch to the width

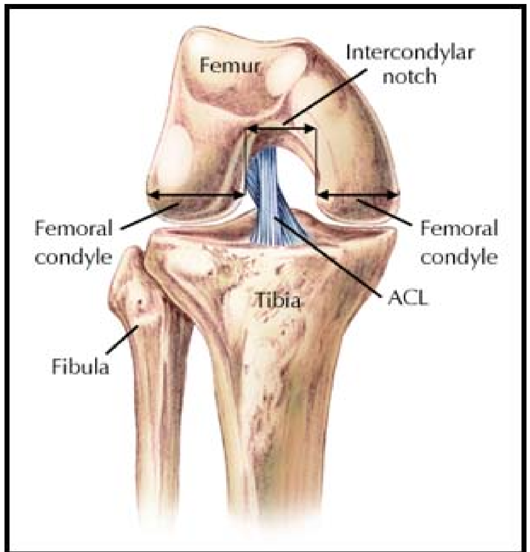


Figure 2 from Hughson Health Alert Website of the distal femur, level with the popliteal groove on a tunnel view radiograph (Ireland et al., 2001). There were no significant differences between male and female NWI, and therefore, it is an ineffective measurement of injury risk (Ireland et al., 2001).

Increased ligamentous laxity and flexibility have been attributed to female knee injuries. Conflicting evidence has been found in regards to this trait as a risk factor for knee injuries in females. While it was found that the hormone relaxin, a hormone only present during pregnancy, relaxes the ligaments and is correlated to increased risk of sprains (Hutchinson et al., 1995), the presence of this hormone is not directly related to athletic female knee injuries during sport-related exercise. Though athletic females have less ligamentous laxity than nonathletic females, no concrete relationship has been proven to exist between looser joints and knee injuries (Hutchinson et al., 1995). Other studies support the idea that ligamentous laxity does not play a significant role in female knee injury rates (Anderson et al., 2001; Myer et al., 2005).

Another possible risk factor for knee injuries in females is movement technique. This is a complex factor in that it comprises anatomical and biomechanical differences as well as control and perceptual influences (James et al., 2004). During landing, decelerating, and cutting movements, female athletes have a higher incidence of knee injury than males (James et al., 2004; Myer et al., 2005). In cutting movements, females tend to execute with more knee extension, more knee valgus, and less hip flexion than male athletes. Lower knee flexion angles (0-30º of maximum knee flexion possible) causes the quadriceps to pull the tibia forward and therefore add stress to the ACL (Myer et al., 2005). Females also show more range of motion (average of 3.5º) in terms of lateral and medial rotation at the knee joint during the cutting motion. This could be indicative of joint control differences between the two sexes, but the exact reason is still unknown. Internal rotation at the knee joint is especially dangerous to the ACL because it must wrap around the PCL during the movement. Therefore, it experiences high amounts of tensile stress (McLean et al., 1999; James et al., 2004). In addition, some females perform the cutting maneuver with more knee abduction than males. Another study reveals that during quick stopping and also vertical landing maneuvers, females tend to demonstrate increased shear force on the anterior tibia than males. This leads to additional loading on the ACL (James et al., 2004). Further studies have connected abduction and adduction movements, increased ground reaction forces (GRF), increased joint forces with a higher incidence of lower extremity injury as a cause and effect continuum (James et al., 2004). Furthermore, during their running stride, females land with a straighter knee than males. Due to the increased load and stress on the ACL caused by these specific motions, women have a higher incidence of ACL injury (Myer et al., 2005).

Muscular imbalances are another theorized risk factor of knee injuries in females. The quadriceps muscles are ACL antagonists and therefore can create enough excess force to tear or stretch the ACL by themselves. The hamstring musculature counteracts the pull of the quadriceps and therefore can help minimize excess force. However, in females, the hamstrings are generally less developed than in males. Consequently, the relatively weaker hamstring musculature is less able to protect the ACL from injury (Anderson et al., 2001). Also, women are slower at creating peak hamstring muscle torque after the protective contraction of the hamstring begins (Anderson et al., 2001). One study found neuromuscular imbalances in females as the presence of knee flexor recruitment was lower in comparison with knee extensor recruitment. In contrast, males display three times more knee flexor recruitment when decelerating after landing (Myer et al., 2005). Another recruitment imbalance is found in the firing of the lateral hamstring. Female athletes have four times more lateral hamstring recruitment than males. Studies also show a high ratio of lateral to medial quadriceps recruitment in females (Myer et al., 2005). This ratio of lateral to medial quadriceps recruitment in combination with the high lateral hamstring musculature firing compresses the lateral portion of the joint, opens the medial portion, and thereby intensifies anterior shear force on the knee joint (Myer et al., 2005). Also, another study has found that the vastus medialis obliquus (VMO) is one of the primary factors compensating for lateral patellar instability (Hutchinson et al., 1995). The VMO counteracts the inward valgus force on the knee joint. In an injured knee, the VMO is the first muscle to atrophy and the last to function normally in rehabilitation. Females are more susceptible to VMO problems after a knee injury and are more likely to have VMO hypoplasia, which is the under-development of muscle size. Some studies have found evidence pointing to genetics as a cause for the VMO weakness in females (Hutchinson et al., 1995).

The last hypothesized risk factor of injuries in females is the influence of sex hormones on the structure of the knee. A strong correlation between the phase of the menstrual cycle and the distribution of ACL injuries was found (Wojtys et al., 2002). A larger incidence of ACL injuries took place during the ovulation phase than in the follicular or luteal phases of menstruation in women who did not take oral contraceptives. Additionally, the regular use of oral contraceptives disrupted the correlation between the phase of the menstrual cycle and the distribution of ACL injuries (although there was a slightly higher incidence of injuries during the luteal phase in these cases). Because these findings constitute some of the first completed results, additional information is needed to explain these results. However, the researchers suggest that manipulation of the menstrual cycle with oral contraceptives affects the injury rates of soft tissue. It is possible that oral contraceptives influence the anabolic and catabolic balance in collagen and therefore can cause changes in collagen (types I and III) development and production rates (Wojtys et al., 2002). The studies have also demonstrated a difference in neuromuscular control during menstruation and changes in skeletal muscle function during ovulation (Wojtys et al., 2002). One study specifically tested the effects of oral contraceptives during landing movements. This study observed that female athletes who do not use oral contraceptives have higher peak forces and more knee adduction during landing than female athletes using oral contraceptives (Wojtys et al., 2002). The study reported that ovarian steroids have an effect on cerebral function especially during the menstruation phase of the cycle (Wojtys et al., 2002), although the specific details of how specifically they affect brain function are still unknown.

There are several sex hormones involved in the menstrual cycle. These include estrogen, estradiol, progesterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), sex hormone-binding globulin (SHBG), testosterone, relaxin, estrone, and estriol (Romani et al., 2003; Van Lunen et al., 2003; Hewett, 2000). Previous research has found that estrogen and progesterone have an influence on metabolism and the structure of collagen. Both hormones have receptors on the ACL, and data suggests that when estrogen levels increase, the ligament's fibroblast and procollagen production levels are decreased within three to seven days (Shultz et al., 2004). The ACL's exposure to estrogen also affects the ligament in terms of an increase in collagen synthesis, increased collagen degradation, increased elastin content, decreased total protein and collagen context, and decreased fiber density, although the exact timing of each of these changes during the menstrual cycle is still under investigation (Shultz et al., 2004). It has also been found that the combination of estrogen and progesterone augments some of these effects while progesterone or testosterone in isolation minimizes these effects (Shultz et al., 2004).

Several other sex hormones are involved in the menstrual cycle. Relaxin is produced in the corpus luteum and has been shown to decrease the tension of collagen in soft tissues (Hewett, 2000; Wojtys et al., 1998). When exposed to ACL tissue, estradiol causes a decrease in fibroblast creation and also a decrease of the formation of collagen (Romani et al., 2003). Progesterone, on the other hand, has been shown to increase the growth and production of fibroblasts and also aids in the construction of collagen (Romani et al., 2003). Two weaker, yet important hormones include estrone and estriol. Estrone is produced by androgens and secreted by both ovarian thecal cells and the adrenal cortex (Romani et al., 2003). Estriol is an oxidative by-product of estrone and has short-term effects on tissue properties when compared to effects caused by estrone and estradiol (Romani et al., 2003). Next, the luteinizing hormone (LH) is a glycoprotein hormone that is produced by the anterior pituitary gland. LH plays an important role in controlling ovulation and hormone secretion by the ovaries (and testies) (On-line Medical Dictionary, 2006). Follicle stimulating hormone (FSH) is another example of a glycoprotein that is secreted by the anterior pituitary gland. In females, FSH increases the development of ovarian follicles (eggs) and stimulates the release of oestrogens (sex hormones that control secondary female sex characteristics and manage the cyclical changes in the menstrual cycle). Oestrogens also have an anabolic effect on protein metabolism and water retention in tissues (On-line Medical Dictionary, 2006). Lastly, SHBG is a glycoprotein whose effects change with altering concentrations of progesterone and estradiol during the menstrual cycle. Along with progesterone, it helps maintain the physiological balance when levels of estradiol fluctuate during the cycle. Estradiol, SHBG, and the SHBG binding site on the tissue impacts the pathway of signal transduction which modifies the estrogen's effects on tissue. It has also been suggested that SHBG influences the function of estrogen in the remodeling and reconstruction of ligamentous tissues (Romani et al., 2003).

The cycle of menstruation is based on the endocrine system coordination through hormones in the circulatory system thereby connecting the ovaries, the pituitary gland, and the hypothalamus. During the follicular phase (cycle days 1-9), progesterone and estrogen levels are low. Next, there is a surge of estrogen that immediately precedes the beginning of ovulation (cycle days 10-14). The last phase, the luteal phase (cycle days 15 through the end of the cycle), experiences a rise in progesterone levels due to increased secretion by the corpus luteum while levels of relaxin also increase by 50% (Wojtys et al., 1998; Beynnon, 2005).

There were several methods used to obtain research data about the effects of sex hormone fluctuations on knee joint laxity. One method was to take women between the ages of 18 and 30 who had no history of pregnancy, did not smoke, have not been taking oral contraceptives (or other medications that stimulate hormones) for the last 3-6 months, and have never had any previous knee injuries. This study collected data about the women's hormone levels throughout the cycle by drawing 5-11 ml of blood at the time of testing as well using a KT-2000 knee anthrometer to measure the amount of anterior tibial displacement at 133N of force (Shultz et al., 2004; Van Lunen et al., 2003; Romani et al., 2003). Van Lunen (2003) and his group of researchers also only measured knee joint laxity with the KT-2000 on participants with a dominant right leg to minimize data collection errors that might occur if the test administrator is more comfortable finding measurements for one leg than another. The procedure also minimizes the problems that might occur when comparing dominant leg data with non-dominant leg data (Van Lunen et al., 2003). 133N was a standard force used because previous studies also picked this force value, and maintaining a constant force measurement allows for easier result comparisons (Van Lunen et al., 2003), although some studies did use 89N of force as well (Shultz et al., 2004; Beynnon et al., 2005; Romani et al., 2003). When using the KT-2000 anthrometer (or the KT-1000 anthrometer used by Beynnon and his research group), the researchers measured knee joint laxity by defining it as the total anterior-posterior displacement of the tibia relative to the femur. This was calculated by adding the maximum anterior displacement of the tibia relative to the femur to the maximum posterior displacement of the tibia relative to the femur (Beynnon et al., 2005; Shultz et al., 2004; Romani et al., 2003).

Van Lunen's research (2003) measured anterior knee motion using a radiographic assessment by first drawing a tangent line parallel to the posterior tibial cortex (PTC) to the most posterior aspect of the tibial plateau. Next, a second line parallel to the PTC and tangent to the femoral condyle's posterior aspect. The anterior displacement of the medial compartment (ADm) is the distance between these two tangent lines. The anterior displacement of the lateral compartment (ADl) is the distance between the most posterior aspects of the lateral tibial plateau and lateral femoral condyle. The mean of these two measurements indicates the anterior knee motion (Van Lunen et al., 2003). Van Lunen used the KT-2000 technique to measure total displacement of the knee and a radiographic assessment to measure the changes in bony alignments (Van Lunen et al., 2003).

Another group of researchers used saliva to measure the hormone levels throughout the menstrual cycle. They performed a pilot test to investigate whether saliva measurement is as accurate when testing for sex hormone levels as blood samples. Several previous studies demonstrate that saliva collection is an accurate form of hormone level measurement. Because their own pilot study confirmed these findings, the researchers used salivary measurements to determine sex hormone fluctuations throughout their testing as opposed to collecting blood samples (Slauterback et al., 2002).

Additionally, to verify the accuracy of the participants' perceptions of their own menstrual cycles, several studies provided each participant with kits such as the

OvuQuick One-Step Ovulation Predictor to help clarify the beginning and end of menstrual phases (Romani et al., 2003; Van Lunen et al., 2003; Shultz et al., 2004).

Two groups of researchers also conducted research on females who had already experienced ACL injuries. Wojtys (1998) and his group of researchers used a questionnaire to statistically analyze the correlation between the ACL injury and the menstrual phase in which the injury occurred (Wojtys et al., 1998). Slauterback (2002) and his group of researchers used questionnaires to make the same correlation but also added the use of a saliva examination to validate the participants' self-reported phase of menstruation (Slauterback et al., 2002).

Lastly, Hewett (2000) explored the effects of training on female hormone levels. It was found that estradiol levels in plasma were lower in untrained females as opposed to trained females. It was also noticed that 50% of female athletes studied had an overall decrease in estradiol levels throughout the entire cycle and additionally that their ovarian function was altered. Hewett (2000) suggests that female sex hormones, estrogen and relaxin in particular, affect neuromuscular function, but does not explore this topic fully (Hewett, 2000).

Each of these experiments produced varying outcomes that both support and contradict previous research. Beynnon (2005) found that, as predicted, male subjects did not have significant estradiol changes over the course of the study. However, female subjects experienced significant changes in estradiol levels over the course of the menstruation cycle. The highest estradiol levels were found in the late follicular phase directly before the ovulatory phase; the next highest occurred during the mid-luteal phase, and lastly the late-luteal phase and the early follicular phase measured the lowest levels of estradiol (Beynnon et al., 2005). When analyzing levels of progesterone, male subjects again did not demonstrate significant changes over the course of the study. Female subjects, on the other hand, experienced the highest levels of progesterone during the mid-luteal phase and had the second highest levels during the late-luteal phase; and lastly the early and late follicular phases recorded similarly low levels of progesterone (Beynnon et al., 2005). While this study found that females had higher overall values of knee joint laxity, there was no significant variation in knee joint laxity found in females due to sex hormone fluctuation through the menstruation cycle (Beynnon et al., 2005).

In the study conducted by Shultz et al. (2004), the researchers allowed for a time shift between the actual secretion of sex hormones and the response time it took for the tissues to be affected by the hormones. Shultz and her research group found that the average time shifts between secretion and notable tissue effects were about three days for estradiol, four days for progesterone, and about 4.5-8 days for testosterone (Shultz et al., 2004). However, the researchers admitted that the range of shifts was variable among subjects. It was demonstrated that testosterone, estradiol, progesterone, each affect knee joint laxity throughout the menstrual cycle, but this significance is increased when the time delay is taken into account (Shultz et al., 2004). In most of the subject cases, knee joint laxity was increased as estradiol, progesterone, and testosterone levels increased in isolation and also as all three hormones increased together. However, knee joint laxity decreased if only two of the three hormone levels increased at once (Shultz et al., 2004). Additionally, previous studies that demonstrated effects of progesterone on collagen density, collagen content, and elastin content were affected by progesterone were supported. Yet, progesterone in isolation had a smaller effect on collagen tissue than either testosterone or estrogen in isolation (Shultz et al., 2004). Knee joint laxity always increased with increases in testosterone and estradiol, but with the introduction of progesterone, the joint laxity could either increase or decrease depending on the individual subject (Shultz et al., 2004). Another interesting finding included testosterone's contribution to knee joint laxity changes. If testosterone levels increased, then knee laxity was likely to increase. This surprised the researchers because it was a better indicator of knee joint laxity than levels of progesterone (Shultz et al., 2004). However, it is not to be concluded that testosterone itself increases knee joint laxity because that would signify that men would have very high knee joint laxity due to their constantly higher testosterone levels than women. Shultz (2004) suggests that this effect of testosterone in females is dose dependant and also includes its relationship to other sex hormones (Shultz et al., 2004).

When finding the correlations among SHBG, estradiol, estrone, estriol, and progesterone, Romani and his group of researchers (2003) found that SHBG hormone levels did not significantly correlate to ACL laxity throughout the three phases of the menstrual cycle. However, when estradiol levels increased, ACL laxity also increased near ovulation. Estrone also had a positive correlation with ACL stiffness at this time (Romani et al., 2003). This is consistent with previous studies that found that as estradiol levels increased, type 1 collagen (a collagen type that plays a significant role in increasing ligamentous tissues' ability to withstand axial loads) synthesis in ACL tissue decreased (Romani et al., 2003). Other correlations demonstrate that a decrease in ACL laxity was tied to increased progesterone concentrations. Additionally, as estradiol levels increased near ovulation, ACL laxity increased. This antagonistic relationship between progesterone and estradiol has been exhibited in findings from previous studies (Romani et al., 2003). However, this opposing relationship only occurs near the ovulation phase of the menstrual cycle. Romani (2003) suggests that this could be due to a specific dose-dependent relationship between the two hormones and/or the changes of hormone receptor expressions throughout the menstrual cycle (Romani et al., 2003). With estrone levels, there was no significant correlation with knee joint laxity unless this hormone is paired with the other sex hormones and SHBG. For example, when coupled with the other sex hormones and SHBG, as estrone increased, joint laxity decreased. Lastly, near ovulation, as estriol levels increased, ACL laxity decreased (Romani et al., 2003), although it is important to note that estriol is a hormone that creates only a short term effect (4 to 6 hours) on ligamentous tissue.

Van Lunen (2003) and her group of researchers found that there is greater joint laxity measured when using the KT-2000 device as compared to the results of the radiographic assessment, but that there is no significant difference of knee joint laxity throughout the menstrual cycle according to either type of measurement (Van Lunen et al., 2003). This study found no significant correlation among any hormone levels (total estrogens, estradiol, progesterone, LH, and FSH), cycle phase, or measurement technique as compared to ACL laxity (Van Lunen et al., 2003).

Wojtys (1998) and his research group found that there is an increased incidence of ACL injury during the ovulatory phase of the menstruation cycle when the positive surge in the production of estrogen occurs (Wojtys et al., 1998). However, this study is limited in that it depends on the participant's ability to recall the specific menstrual phase they experienced at the time of their ACL injury. Urine or blood samples at the time of injury would have allowed for a more accurate measurement of hormone levels (Wojtys et al., 1998).

Lastly, in the study conducted by Slauterback (2002) and his research team, findings of previous studies were confirmed by the results of this study as it was found that knee injury is more likely to occur during the early follicular phases and the late luteal phases of the menstrual cycle while both estrogen and progesterone levels are low (Slauterback et al., 2002). The researchers suggest that this result is due to a change in how genes that encode tissue-remodeling proteins and enzymes are expressed. This could lead to either collagen degradation or increased repair rates at specific points in the menstruation cycle (Slauterback et al., 2002). They also provide evidence that the use of saliva is an accurate way to measure the levels of sex hormones, which introduces new conveniences for future studies in this area (Slauterback et al., 2002). Saliva samples are easier to collect and to store, while producing accurate hormone level readings.

Future research needs to be conducted to either support or negate the varied results found in these studies. Technique refinement and consistency will help produce more reliable results among different researchers' studies. Additionally, there are still many aspects of this issue that can be explored. For example, Shultz (2004) discusses the increased presence of relaxin in pregnant women during the first trimester and then that the relaxin level decreases in each subsequent trimester. However, knee joint laxity increased from 105% in the first trimester to 128% in the second trimester and finally, to 170% in the third trimester. What are the effects of the added stress and strain on the knee joint laxity increases due to the relatively rapid weight increases?

Another aspect that requires further exploration, as touched upon by Van Lunen et al (2003), Hewett (2000), and Aronson et al (2004), is the effect of sex hormone levels and ACL injuries when the use of oral contraceptives is considered. Aronson (2004) and his research group briefly discussed some of the findings when they studied quadriceps and hamstring strength, knee joint laxity, and postural control on participants who used oral contraceptives. They found that the female subjects had higher performance rates, in terms of higher average power values and higher peak torques earlier in the cycle than when compared to tests conducted later in the cycle (Aronson et al., 2004). Various unpublished studies by Hewett (2000) found that female athletes using oral contraceptives may have increased hamstring to quadriceps ratios, increased unilateral stability, decreased knee joint laxity, reduced varus and valgus knee torques, and lower impact forces when landing (Hewett, 2000). This suggests that oral contraceptives produce hormone stabilization which in turn helps to maintain the stability of the knee joint (Hewett, 2000). However, like all previous studies mentioned, further investigation and exploration are required before any lasting conclusions can be made from the present research data.

While females do have a higher incidence of ACL injury than males, there are a variety of possible methods of direction for injury prevention. Some basic prevention techniques include maintaining a healthy body weight, stretching after a brief warm up session and before more intense exercise, strengthening both the quadriceps and the hamstrings, and cross training (HWHW, 2002). To resolve the problems involved with female tendencies like the “plant and cut,” landing with straight legs, and the “one-step stop,” it is safer to replace these maneuvers with rounding turns, increasing the angle of flexion in the legs while landing, and stopping within three steps instead of one (Hutchinson et al., 1995). Also, it is advisable to avoid the knee extension machine for injured female athletes because it can cause additional strain on the ligaments. Safe cross training exercises include using stationary bicycles with a high seat and swimming (Hutchinson et al., 1995). Additionally, it is advisable to strengthen the VMO specifically in injured females (Hutchinson et al., 1995). Proper coaching and instruction of safe cutting, landing, and stopping techniques can help reduce the risk of knee injury (Myer et al., 2005). One study demonstrated a 700% decrease in the amount of expected ACL injury with proper proprioceptive training and instruction of landing mechanics (James et al., 2004).

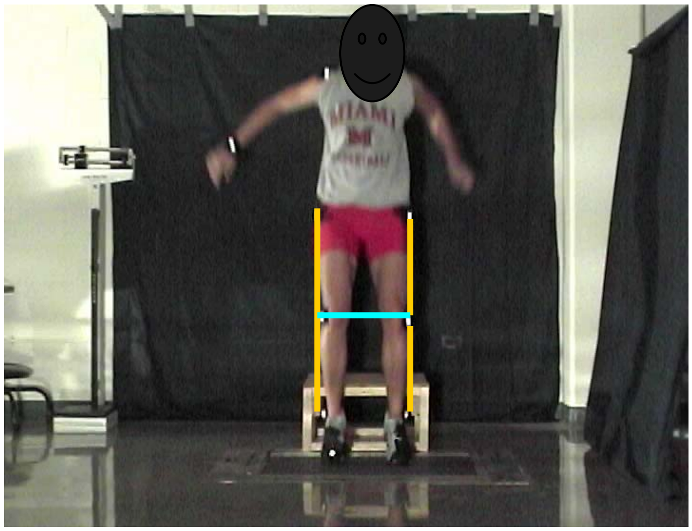
METHODS

Fourteen college-age female subjects were enrolled in this experiment. The mean age, height and body mass of the students were 20.9 ± 0.8 (yrs), 133 ± 11 (cm) and 60 ± 0.5 (kg). The female subjects were tested at three different points during the hormonal cycle: once during days 3-6 of the follicular phase, once during days 12-16 of the ovulation phase, and once during days 23-26 of the luteal phase. Exclusion criteria included previous knee injury, previous knee surgery, or present use of hormone regulators including oral contraceptives. Each participant had three visits per month, one visit per phase, for a total of four consecutive months. Because female menstrual cycles have variable length, questionnaires were distributed and the beginning of each cycle and cycle length was self-reported by each participant monthly.

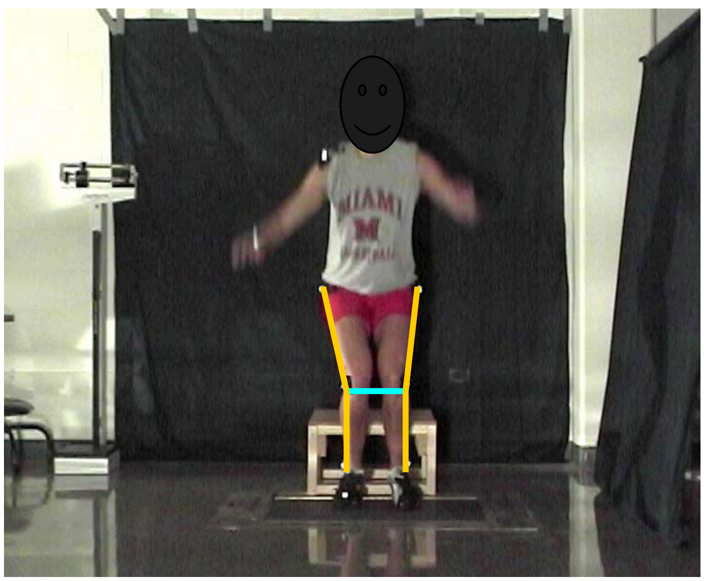
Before testing, the subjects were asked to perform 20 sub-maximal vertical warm-up jumps. For the experiment, the participants completed 6 to 8 measured jumps. The jumps proceeded as follows: subjects were asked to perform a drop jump off a 30.5 cm wooden box onto a Bertec force platform (landing symmetrically with both feet at the same time), after which they were instructed to immediately perform a maximal vertical jump and land with both feet on the force platform again. Reflective markers were affixed to joint landmarks on the subjects before testing began; these markers were used as an aid in digitizing and analyzing video data. Bilateral marker placement included the greater trochanter, the lateral epichondyle of the femur, and the lateral malleolus.

Force plate data were collected and analyzed using BioWare software. The relevant data analyzed from this system were the knee joint alignment angles at two points during the jump series.

The first data point occurred as the subjects' feet initially contacted the force platform.



The second data point occurred as the participants' reached the lowest squat position after the drop jump from the 30.5 cm box and before starting the maximal vertical jump.



The video data from the frontal plane and sagittal plane were combined to create a three-dimensional figure which was analyzed using the SIMI motion analysis system to measure changes in joint angles during the jumping process. These data points were correlated with the different points during the hormonal cycle.

Statistical Methods

A two-way ANOVA (group x dependent variable) was performed with repeated measures on the dependent variables to determine significant differences between the three menstruation phases. Means were compared using T-tests. Results were considered significant when $t < 0.05$.

RESULTS

<u>Data for Sagittal Plane Knee Alignment Angles</u>

Angle data were recorded by a camera arranged to view the participant's right lower extremity position at two points during the jumping motion. The first point occurred as the participant's feet first made contact with the forceplate. The knee angle recorded in this case is called the participant's "minimum" knee angle from the sagittal plane. The second data point occurred after the drop jump from the box as the participant crouched down before jumping again to perform a maximal vertical jump. The knee angle during the crouched position constitutes the participant's "maximum" knee angle from the sagittal plane.

Knee Joint Alignment Angles at Landing Contact

During phase 1, the follicular phase, the mean knee joint angle during first contact with the forceplate was 26.2 ± 7.7 degrees (maximum 38.4, minimum 14.0). During phase 2, the ovulation phase, the mean knee joint angle during first contact with the forceplate was 26.6 ± 6.6 degrees (maximum 35.2, minimum 14.5, $p < 0.05$ versus phase 1). During Phase 3, the luteal phase, the mean knee joint angle during first contact with the forceplate was 26.8 ± 7.7 degrees (maximum 38.6, minimum 14.4, $p < 0.05$ versus phase 1 and phase 2).

Knee Joint Alignment Angles during the Squat Position

During phase 1, the mean knee joint angle during the participants' squat position between landing from the box drop jump and jumping vertically a second time from the forceplate was 79.9 degrees ± 12.3 degrees (maximum 96.1, minimum 56.8). During phase 2, the mean knee joint angle during the squat position was 78.5 degrees ± 10.9 degrees (maximum 102.4, minimum 60.7, p = ns versus phase 1). Lastly, during phase 3, the mean knee joint angle recorded during the squat position was 79.9 ± 12.3 degrees (maximum 96.1, minimum 56.8, p = ns versus phase 1 and phase 2).

Data for Frontal Plane Knee Joint Alignment Angles

Concurrent with sagittal plane measurements, frontal plane knee joint alignment angles were recorded using a camera placed directly in front of the participants during two points within the jump series. As for the sagittal measurements, the first data point occurred as the participants' feet first made contact with the forceplate after dropping down from the 30.5 cm box positioned directly behind the forceplate. Similarly, the second data point occurred after the drop jump from the box when the participant squatted down before performing a maximum vertical jump.

Knee Joint Alignment Angles at Landing Contact – Right Leg

During phase 1, the mean knee joint angle measured as the participant made first contact with the forceplate was 1.9 ± 3.3 degrees (maximum 8.1, minimum -2.80). Measurements reflect a valgus knee alignment, 1.9 degrees from a center line perpendicular to the floor (0 degrees). The negative value reflects a varus knee joint alignment, outward from center. During phase 2, the mean knee joint angle was 2.2 ± 3.6 degrees (maximum 9.8, minimum -2.53, p=ns versus phase 1). Finally, during phase 3, the mean knee joint angle was 2.6 ± 4.2 degrees (maximum 9.00, minimum -4.23 degrees, p = ns versus phase 1 and phase 2).

Knee Joint Alignment Angles during the Squat Position – Right Leg

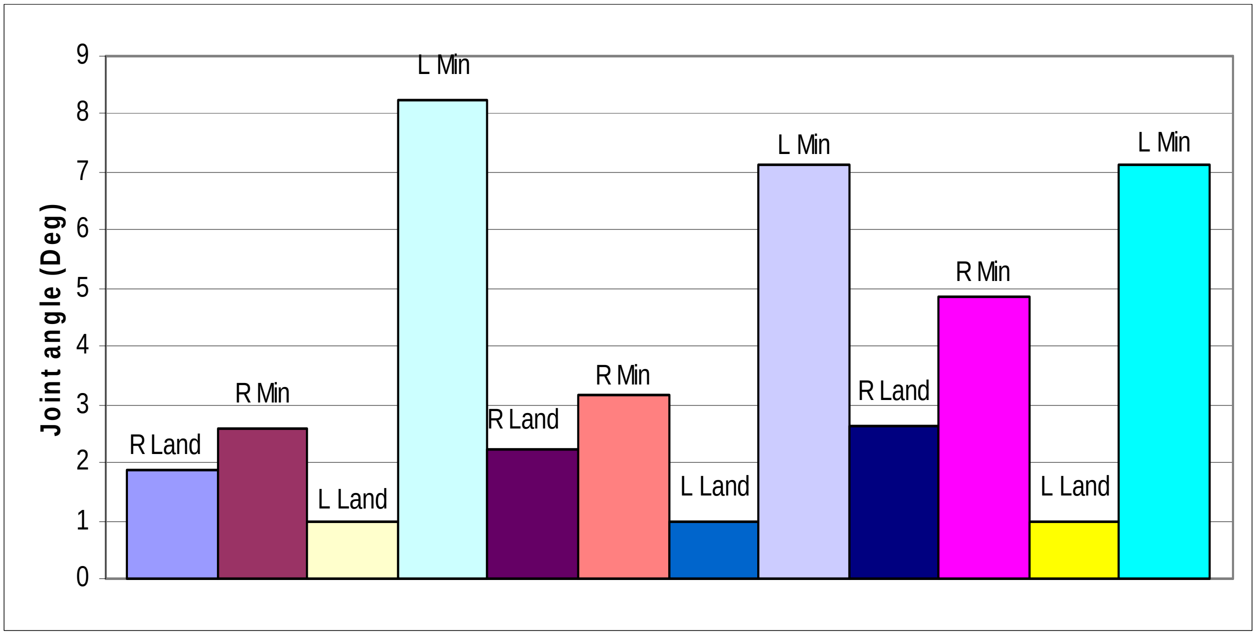
During phase 1, the mean knee joint angle during the participants' squat position between landing from the drop jump and the maximal vertical jump from the forceplate was 2.6 ± 9.0 degrees (maximum 14.0, minimum -21.0). During phase 2, the mean knee joint angle during the squat position was 3.1810 degrees ± 9.09195 degrees (maximum 17.9, minimum -17.4, p = ns versus phase 1). Lastly, during phase 3, the mean knee joint angle recorded during the squat position was 4.9 ± 7.9 degrees (maximum 16.30, minimum -7.80, p = ns versus phase 1 and phase 2).

Knee Joint Alignment Angles at Landing Contact – Left Leg

During phase 1, the mean knee joint angle measured as the participant made first contact with the forceplate was -1.2 degrees ± 3.9 degrees (maximum 6.7, minimum -7.6). For the left leg, the -1.2 degree measurement reflects a valgus knee alignment, while the positive 6.7 degree value reflects a varus knee joint alignment. During phase 2, the mean knee joint angle measured as the participant made first contact with the forceplate was -1.8 ± 3.4 degrees (maximum 5.7, minimum -7.7, p = ns versus phase 1). During phase 3, the mean knee joint angle measured when the participant first made contact with the forceplate was -2.7 ± 3.8 degrees (maximum 4.0, minimum -9.3, p = ns versus phase 1 and phase 2).

Knee Joint Alignment Angles during the Squat Position – Left Leg

During phase 1, the mean knee joint angle during the participants' squat position between landing from the drop jump and the maximal vertical jump from the forceplate was -8.2 ± 10.6 degrees (maximum 12.1, minimum -26.7). Again, for the left leg the negative degree value reflects a valgus knee joint alignment. During phase 2, the mean knee joint angle during the squat position was -7.1 ± 9.6 degrees (maximum 13.4, minimum -24.4, p = ns versus phase 1). Finally, during phase 3, the mean knee joint angle recorded during the squat position was -7.1 ± 12.8 degrees (maximum 10.87, minimum -40.73 degrees, p = ns versus phase 1 and phase 2). Data are displayed in Figure 1 below.



l----------Phase 1 -----------l l-----------Phase 2 -----------l l--------Phase 3------l

Figure 3. The graph depicts the mean joint angle alignments in the frontal plane in degrees through each of the three phases of the menstrual cycle. "R Land" reflects the mean valgus knee joint angle alignment of the right leg as the participants' first contact the forceplate with their feet after dropping down from the 30.5 cm box. "R Min" describes the mean valgus knee joint alignment of the right leg as the participants' reached their lowest squat position on the forceplate before performing a maximum vertical jump. The letter "L" references the same jump positions as "R Land" and "R Min" but signifies left leg measurements.

Data for the Distance between the Knees in the Frontal Plane

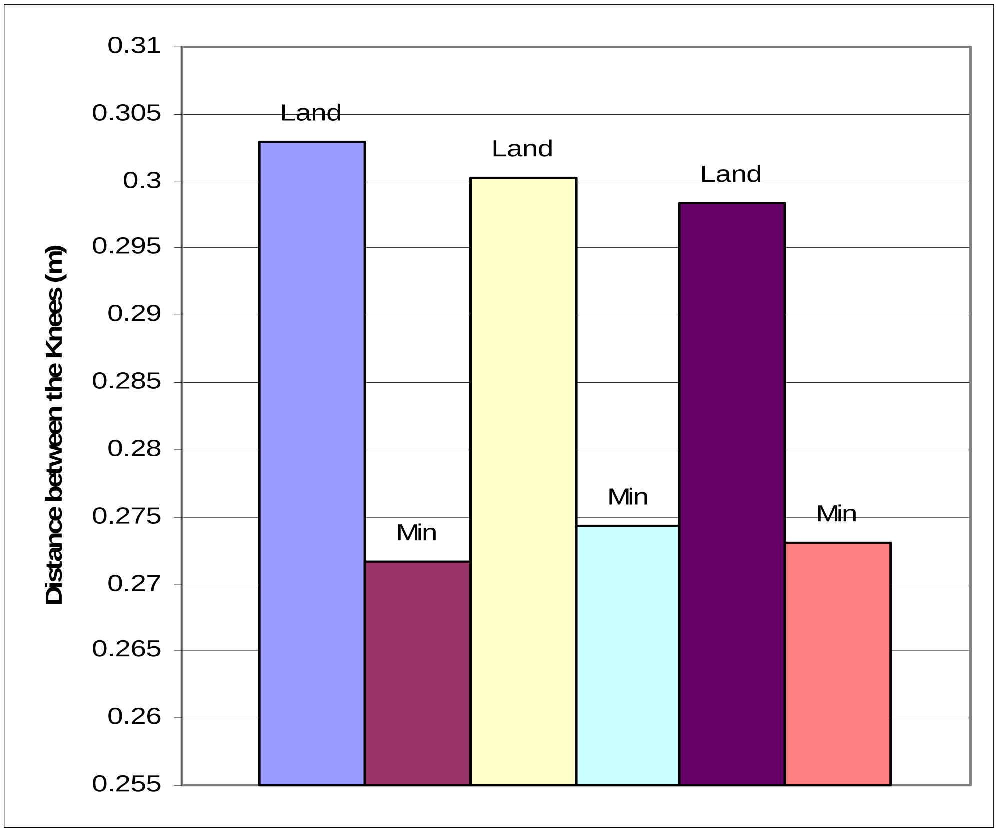
By analyzing the data from the camera which recorded the frontal plane of the jump series, the distance between the participants' knees during the landing contact phase of the jump series and the squatting phase was measured. The distance between the participants' knees at the point the jumping series when the feet first made contact with the forceplate is data point one. The distance between the participants' knees during the squat position after landing from the drop jump and before producing a maximal vertical jump constituted data point two.

Distance between the Knees at Landing Contact

The mean distance between the participants' knees at the point the jumping series when the feet first made contact with the forceplate during phase 1 was 0.303 ± 0.028 meters (maximum 0.350, minimum 0.250). During phase 2, the mean distance between the knees as the participant first made contact with the forceplate was 0.300 ± 0.041 meters (maximum 0.350, minimum 0.220, p = ns versus phase 1). During phase 3, the mean distance between the participants' knees was 0.298 meters ± 0.043 (maximum 0.370, minimum 0.220, p = ns versus phase 1 and phase 2).

Distance between the Knees during the Squat Position

The mean distance between the participants' knees during the squat position after landing from the box drop jump and before making a maximal vertical jump from the forceplate during phase 1 was 0.272 ± 0.043 meters (maximum 0.37, minimum 0.21). The mean distance between the participants' knees during the squat position for phase 2 was 0.274 meters ± 0.046 (maximum 0.350, minimum 0.20, p = ns versus phase 1). Finally, during phase 3, the mean distance between the participants' knees during the squat position was 0.273 ± 0.058 meters (maximum 0.39, minimum 0.19, p = ns versus phase 1 and phase 2). Data are illustrated in Figure 2 below.



l— Phase 1 –--l l—Phase 2---l l--- Phase 3 ---l

Figure 4. This graph illustrates the mean distance between the knees in meters for each of the three phases of the menstrual cycle. "Land" denotes the mean distance between the knees as the participants' feet first contact the forceplate after the drop jump from the 30.5 cm box. "Min" represents the mean distance between the knees as the participants are in the lowest squat position directly before jumping vertically to complete a maximal vertical jump from the forceplate.

DISCUSSION

The goal of this experiment was to examine the relationship between cyclical hormone changes and alterations of lower extremity kinematics. By comparing the knee joint alignment angles and distance between the knees throughout the three major phases of menstruation, the objective was to locate differences between knee joint angles and distance between the knees between the three phases. Determining hormonal effects on muscle tension and ligament laxity is important to more clearly understand the mechanism behind increased susceptibility to knee injury in particular times within the menstrual cycle.

While no significant differences were found in the measured variables among the three different phases of the menstrual cycle, one notable trend exists. During the squat position when the participant is in the lowest body position after the drop jump from the 30.5 cm box and before producing a maximal vertical jump from the forceplate, the mean knee joint angle alignment for the left knee is in a more valgus position than the mean knee joint angle alignment for the right knee throughout all three phases of menstruation (Figure 3). It is possible that this occurs because the participants' favored their right leg, resulting in greater stability than the left leg. However, it must also be considered possible that the frontal plane camera captured this trend because the participants may have slightly rotated towards the experiment conductor, located to the subject's right side during each measured jump.

There are several possible reasons to explain why no significant differences in the data were found. First, it is possible that changes in ligament laxity occurred, but were not large enough to elicit changes in the kinetic variables measured in this experiment. Second, enrolling more participants in the experiment could elicit more significant differences between the lower extremity kinematics during the three phases of menstruation. The non-significant results of this study coincide with the results of some studies (Beynnon et al., 2005; Van Lunen et al, 2003) although it negates the results of other studies (Wojtys et al., 2002; Wojtys et al., 1998; Romani et al., 2003).

Other researchers have searched for differences in ligament laxity throughout the menstrual cycle using ligament laxity measurement devices such as the KT-2000 device, radiographic assessment, and electromyographic (EMG) measurements and found contradicting results (Romani et al., 2003; Van Lunen et al., 2003; Hewett, 2000; Wojtys et al., 2002). Future research should aim to incorporate several of these devices during experimentation to aid data collection and comparison with the results of other studies.

Future evaluation of hormonal effects on joint kinematics will require an increased number of subjects and more accurate lower body kinematic measurement techniques. Additionally, electromyography equipment or strength testing could be used to measure changes in muscle tension during the three phases of menstruation. Lastly, comparisons with participants who use hormonal regulation medications such as oral contraceptives, transdermal contraceptives, and/or intravenous contraceptives could elicit differences in knee joint alignment angles and muscle tension from fluctuating cyclic hormone levels.

REFERENCES

Anderson A.F., Dome D.C., Gautam S., Awh M.H., Rennirt G.W. (2001). Correlation of anthropometric measurements, strength, anterior cruciate ligament size, and intercondylar notch characteristics to sex differences in anterior cruciate ligament tear rates. *The American Journal of Sports Medicine* 29(1): 58-65.

Aronson S., Olmsted L.C., Hertel J. (2004). Effects of tri-phasic oral contraceptives on risk factors on anterior cruciate ligament injuries in recreationally active females. *Journal of Athletic Training*, 39(supplement 2): 111-112.

Beynnon B.D., Berstein I.M., Beslisle A., Brattbakk B., Devanny P., Risinger R., Durant D. (2005). The effect of estradiol and progesterone on knee and ankle joint laxity. *The American Journal of Sports Medicine*, 33(9): 1298-1304.

Fayad L.M., Parellada J.A., Parker L., Schweitzer M.E. (2003). MR imaging of anterior cruciate ligament tears: is there a gender gap?. *Skeletal Radiol* 32: 639-646.

Hewett T.E. (2000). Neuromuscular and hormonal factors associated with knee injuries in female athletes: strategies for intervention. *Sports Medicine*, 29(5): 313-327.

Hutchinson M.R., Ireland M.L. (1995). Knee injuries in female athletes. *Sports Medicine* 19(4): 288-302.

Ireland M.L., Ballantyne B.T., Little K., McClay I.S. (2001). A radiographic analysis of the relationship between the size and shape of the intercondylar notch and anterior cruciate ligament injury. *Knee Surg, Sports Traumatol, Arthrosc* 9: 200-205.

Ireland, M.L. Hughston Health Alert. Retrieved April 5, 2007, from the Why Are Women More Prone Than Men Web site: www.hughston.com/hha/a.acl.htm

James C.R., Sizer P.S., Starch D.W., Lockhart T.E., Slauterbeck J. (2004). Gender differences among sagittal plane knee kinematic and ground reaction force characteristics during a rapid sprint and cut maneuver. *American Alliance for Health, Physical Education, Recreation and Dance* 75(1): 31-38.

McLean S.G., Neal R.J., Myers P.T., Walters M.R. (1999). Knee joint kinematics during the sidestep cutting maneuver: potential for injury in women. *Medicine and Science in Sports and Exercise* 31(7): 959-968.

Myer G.D., Ford K.R., Hewett T.E. (2005). The effects of gender on quadriceps muscle activation strategies during a maneuver that mimics a high ACL injury risk position. *Journal of Electromyography and Kinesiology* 15: 181-189.

On-line Medical Dictionary. (2006). Online. Accessed 10 April 2006. Available: <http://cancerweb.ncl.ac.uk/omd/>.

Retrieved April 1, 2007, from Functional Anatomy and Biomechanics Web site: www.gla.ac.uk/.../tutorial/anatomy/kneet.html.

Romani W., Patrie J., Curl L.A., Flaws J.A. (2003). The correlations between estradiol, estrone, estriol, progesterone, and sex hormone-binding globulin and anterior cruciate ligament stiffness in healthy, active females. *Journal of Women's Health*, 12(3): 287-298.

Shultz S.J., Kirk S.E., Johnson M.L., Sander T.C., Perrin D.H. (2004). Relationship between sex hormones and anterior knee laxity across the menstrual cycle. *Medicine & Science in Sports & Exercise*, 36(7): 1165-1174.

Soft-tissue knee injuries in women. (2002). *Harvard Women's Health Watch* 19(11): 4-6.

Slauterbeck J.R., Fuzie S.F., Smith M.P., Clark R.J., Xu K.T., Starch D.W., Hardy D.M. (2002). The menstrual cycle, sex hormones, and anterior cruciate ligament injury. *Journal of Athletic Training*, 37(3): 275-280.

Swedan N. ed. (2001). The knee. Women's Sports Medicine and Rehabilitation. Gaithersburg, Maryland: Aspen Publishers, Inc. 55-85.

Van Lunen B.L., Roberts J., Branch J.D., Dowling E.A. (2003). Association of menstrual-cycle hormone changes with anterior cruciate ligament laxity measurements. *Journal of Athletic Training*, 38(4): 298-303.

Wojtys E.M., Huston L.J., Boynton M.D., Spindler K.P., Lindenfeld T.N. (2002). The effect of the menstrual cycle on anterior cruciate ligament injuries in women as determined by hormone levels. *The American Journal of Sports Medicine* 30(2): 182-188.

Wojtys E.M., Huston L.J., Lindenfeld T.N., Hewett T.E., Greenfield M.L. (1998). Association between the menstrual cycle and anterior cruciate ligament injuries in female athletes. *The American Journal of Sports Medicine*, 26(5): 614-619.