The effect of sex differences and hormone fluctuation on ankle stability and function

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The Effect of Sex Differences and Hormone Fluctuation on Ankle Stability and Function

By

Hayley M Hahn, ATC

Submitted as partial fulfillment of the requirements for

the Masters of Science degree in

Exercise Science

Advisor: Dr. Phillip Gribble

Committee Member: Dr. Charles Armstrong

Committee Member: Dr. Mark Timmons

College of Health Science and Human Service

College of Graduate Studies

The University of Toledo

May 2009
An Abstract of

The Effect of Sex Differences and Hormone Fluctuation on Ankle Stability and Function

Hayley M Hahn, ATC

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Objective: To examine the potential hormone contributions to ankle laxity, with an instrumented ankle arthrometer, and dynamic postural control with the Star Excursion Balance Test. These measures were performed on females in their pre-ovulatory and post-ovulatory phases of their menstrual cycle with a cohort group of male matched control subjects tested at similar times of the month. Design and Setting: Three separate ANOVAs were performed for the dependant variables of medial/lateral ankle laxity, anterior/posterior ankle laxity and maximum normalized reaching distance in the Star Excursion Balance Test (SEBT). Significance was set at p<0.05. All data were collected in a research laboratory. Subjects: 14 healthy females (29±7.05 yrs; 172.32±7.91cm; 68.05±9.27 kg) and 27 healthy males (23.22±4.06 yrs; 180.34±6.98 cm; 81.72±11.19 kg) participated in this study. Measurements: Female subjects used ovulation kits for three months to determine the time of ovulation and were tested in the lab with the ankle
arthrometer and SEBT corresponding to their pre-ovulatory and post-ovulatory time periods. Males were tested at similar times and used as a control. To assess ankle stability a portable ankle arthrometer (Blue Bay Medical, Inc, Navarre, FL) was used. Anterior/posterior loading was performed first following by medial/lateral loading. Three trials were done in each direction on each ankle. For dynamic postural control, a custom made mat with eight measuring tapes rigidly fixed at 45º angles to each other was used to assess reaching distance in the SEBT. Only the posterior medial reaching distance was used and four practice trials were completed followed by five actual trials. The reaching test was performed on each leg and leg length was also measured. Results: For ML laxity there was a statistically significant sex main effect (F$_{1,39}$=7.238; p=0.01). There was no statistically significant Time main effect or Side main effect for ML laxity. There were also no statistically significant interactions for Time by Sex, Side by Sex, Time by Side or Time by Side by Sex for ML laxity. For AP laxity there was no statistically significant Sex main effect or Time main effect. There was a statistically significant Side main effect for AP laxity (F$_{1}$=5.280; p=0.027). There were no statistically significant interactions for Time by Sex, Side by Sex, Time by Side or Time by Side by Sex. For maximum normalized reaching distance in the SEBT there was a statistically significant Sex main effect (F$_{1}$=5.093; p=0.030). There was no statistically Time main effect or Side main effect for the average normalized reaching distance. There were also no statistically significant interactions found for Time by Sex, Side by Sex, Time by Side or Time by Side by Sex. Conclusions: The results from this study suggest that hormone fluctuation during the menstrual cycle (pre-ovulatory compared to post-ovulatory) does not have an effect on ankle laxity in the medial/lateral and anterior/posterior directions or dynamic
postural control. This was evidenced through the use of the ankle arthrometer and the results of the Star Excursion Balance Test. There have been few studies that have considered the impact of hormone fluctuation on ankle instability. Further research should aim to strengthen the finding of this study by conducting similar projects looking at more subjects, looking at different points in the menstrual cycle and perhaps looking at different areas of the body to rule out systemic ligament laxity due to hormone influence.
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Chapter One

Introduction

Ankle sprains are common among the physically active and there is a high rate of recurrence after the initial injury; this common pathology is referred to as chronic ankle instability (CAI). CAI results in numerous ankle sprains and repetitive bouts of the injured ankle “giving way.” Mechanical ankle instability (MAI) is one of the contributing factors to CAI. Laxity in the ankle joint ligaments is a primary determinant associated with MAI. The other potential contributing factor to CAI is functional instability and this may be caused by specific deficits in proprioception, postural control, neuromuscular control or strength. After sustaining an ankle sprain, anatomical changes occur in the ankle joint which can lead to insufficiencies that predispose the ankle to further instability, ligamentous laxity is one of those changes. Damage to the ligaments results in laxity and causes the joints to be mechanically unstable. Research has found increased joint laxity in the sagittal plane in functionally unstable ankles.

Impaired postural control has been found in those who have sustained repetitive ankle sprains. These postural control deficits are likely due to the combination of impaired proprioception and neuromuscular control. When one is balancing on one leg, the foot pronates and supinates in an effort to keep the body’s
center of gravity over the base of support. This is called the “ankle strategy” of postural control. It has been found that individuals with CAI show more “hip strategy” of postural control to maintain their stance when compared to healthy subjects. This hip strategy is less efficient than the ankle strategy and this alteration in those with CAI is likely due to the presence of ankle-joint dysfunction and the changes in central neural control that occur with that dysfunction. Deficits in dynamic postural control assessed with the Star Excursion Balance Test (SEBT) have been reported consistently in subjects with CAI.

There is limited evidence to suggest there are differences in ankle injury rates between men and women with some potential similarities to the sex differences seen in the rate of anterior cruciate ligament (ACL) injury in the knee. Among the many factors theorized to be associated with sex differences in ACL injury, the influence of hormonal fluctuations in females has been investigated with conflicting results. This relationship has been investigated in the knee however; it has not been investigated extensively in the function of the ankle.

Not only is there a need to determine if a relationship exists, there is a need to quantify some measure of function, such as dynamic postural control, to determine the level of influence of hormone fluctuations. This will also help determine if there is a need for ongoing research, similar to what has occurred in the ACL research community. By investigating, and perhaps discounting, the impact of hormone fluctuations on two factors that are known to contribute to ankle pathology, ankle laxity and dynamic postural control, important information may be gained for developing successful interventions for ankle instability, especially in females.
Statement of the Problem

Ankle injuries are extremely prevalent in the physically active population. Deficiencies in two factors, mechanical laxity and dynamic postural control, have been established as factors with CAI. Additionally, there is evidence that differences exist in the risk factors of acute ankle injury between males and females. Hormone differences between males and females have been suggested as a risk factor for ACL injury; but this relationship between hormone differences (and fluctuation during the menstrual cycle), and ankle instability has not been assessed adequately.

Statement of the Purpose

The purpose of this project was to examine ankle laxity with an instrumented ankle arthrometer and dynamic postural testing with the SEBT. These measures were performed on females in their pre-ovulatory and post-ovulatory phases of their menstrual cycle with a cohort group of male matched control subjects tested at similar times of the month. Examining potential hormone contributions to ankle laxity has helped to establish data in healthy subjects and determined if this is a potential area of investigation that is needed to address important questions in the understanding of the development of ankle pathology. The intent of this project was to establish information about factors that may affect ankle pathology in females, which contributed to ongoing work in the area of ankle pathology in the laboratory of the faculty advisor.

Significance of the Study

This study evaluated sex differences and the effects of the menstrual cycle on ankle laxity and dynamic postural control contributions to ankle stability in females during the pre-ovulatory and post-ovulatory phases of the menstrual cycle, with
comparisons to males as a control group. Several potential causes of ankle pathology have been previously investigated, but there is limited information available examining potential differences in the pathology between the sexes. One obvious difference between males and females is the menstrual cycle, and with that, the fluctuation of hormones. The influence of the fluctuation of hormones in women on athletic injury has been investigated for the ACL in the knee; but it has not been examined thoroughly in the ankle. The results of this study provided some insight into the potential role that hormone fluctuation plays on ankle stability in women. This line of investigation coincides with the research agenda of the faculty advisor and helps to determine the impact that sex differences may or may not have on established injury factors; and serves as pilot data for continued investigation in this area of inquiry.

**Research Hypotheses**

1. Females will have greater ankle laxity in the medial/lateral direction compared to males.

2. Females will have greater ankle laxity in the anterior/posterior direction compared to males.

3. Females will have better dynamic postural control compared to males, as evidenced by increased normalized reaching distances on the Star Excursion Balance Test.

4. Females will not demonstrate a difference in ankle laxity between the pre-ovulatory and post-ovulatory phases.

5. Females will not demonstrate a difference in dynamic postural control between the pre-ovulatory and post-ovulatory phases.
Operational Definitions

CAI = Chronic Ankle Instability
MAI = Mechanical Ankle Instability
FAI = Functional Ankle Instability
ATF ligament = Anterior talofibular ligament
CF ligament = Calcanofibular ligament
SEBT = Star Excursion Balance Test

Limitations

A limitation to this study relates to the ability of the subjects to recall their medical history about previous lower leg injuries and menstrual cycle history. The female subjects used the ovulation kits on their own time and recorded a journal about their menstrual cycle and were expected to administer the kits and record journal entries correctly. There are alternative techniques for monitoring hormone levels that may be more accurate, but they are much more expensive. By incorporating three consecutive months of using ovulation kits, the estimation of the ovulation phases will be improved. Because of the importance of the timing of the testing sessions, it was critical that subjects attend the scheduled testing session during the third month of enrollment. The investigators were diligent in communicating with the subjects regarding correct testing and scheduling procedures.
Chapter Two

Literature Review

Chronic Ankle Instability

Lateral ankle sprains are one of the most common injuries among the physically active, with a rate of recurrence as high as 80%. This common pathology is referred to as chronic ankle instability (CAI). One of the contributing factors to CAI is mechanical ankle instability (MAI). Laxity in the ankle joint ligaments is a primary determinant associated with MAI. Another contributing factor to CAI is functional ankle instability (FAI). Of the many variables identified with FAI, a deficit in dynamic postural control assessed with the Star Excursion Balance Test (SEBT) has been reported consistently in subjects with CAI.

Dynamic Postural Control Contributions to CAI

CAI has been described as an altered mechanical joint stability that is attributed to repeated disruptions in the integrity of the ankle which results in perceived and observed neuromuscular deficits. Neuromuscular control has been quantified through measures of postural control, which can be classified as both static and dynamic. Static postural control can be defined as attempting to maintain a base of support with minimal movement; while dynamic postural control can be defined as attempting to maintain a
base of support while completing a prescribed movement. One commonly used assessment of dynamic postural control is the Star Excursion Balance Test (SEBT), a lower extremity reaching task. It has been found that reaching distances on the SEBT are reduced in subjects with CAI and reduced max distance has been found to correlate with reduced knee and hip joint angles in subjects with CAI. This indicates that there is a possible relationship between SEBT performance and reduced neuromuscular control at the hip and knee because of ankle injury. An injury to the ankle disrupts the integrity of the joint, is theorized to impair afferent-efferent pathways that allow for maintenance of proprioception, kinesthesia and neuromuscular control. A disruption in one joint may create altered neuromuscular activity and compensatory muscle recruitment in other joints that can lead to disruption in movement patterns.

**Ankle Laxity Contributions to CAI**

Ankle laxity results from a tear or lengthening of one or more of the supporting structures in the ankle joint and less than optimal healing of the injured tissues. This pathology results in hypermobility of the joint and increased accessory motion at the joint, which places further strain on injured ligaments. When the ligaments of the ankle are injured, there may also be damage to the mechanoreceptors of the ankle. This may result in altered proprioceptive input from these mechanoreceptors, which may lead to compensations in order to maintain function. The laxity of the supporting structures of the ankle, with damage to the mechanoreceptors, contributes to the giving way and symptoms of chronic ankle instability.
Causes of Ankle Injury in Males and Females

There is recent evidence to suggest that causes of ankle instability differ between men and women. Willems et al\textsuperscript{48, 49} conducted two separate studies looking at intrinsic factors for inversion ankle sprains in both males and females. While these two studies provide information about males and females separately, direct comparisons have not been made. Previous ligamentous laxity is associated with CAI\textsuperscript{19, 25}, but in the two studies described above\textsuperscript{48, 49}, it is not implicated as a risk factor for first time sprains. However, in comparing the two studies, females appear to possess more talocural laxity compared to males, which coincides with a separate study by Beynnon et al\textsuperscript{1}. This is consistent with literature that suggests that post-pubescent females demonstrate greater amounts of knee laxity\textsuperscript{26, 42} and general joint laxity\textsuperscript{8, 27, 33, 40, 44} compared to post-pubescent males. A common factor for both sexes appears to be a deficit in dynamic postural control as measured with the SEBT.\textsuperscript{16, 17, 20, 37} In a recent investigation by the faculty advisor, healthy females were able to perform better on this lower extremity reaching task compared to healthy males.\textsuperscript{18} One potential contribution to inversion ankle sprains that was not suggested in the studies by Willems et al\textsuperscript{48, 49}, and may have an influence on ligamentous structural integrity and perhaps an influence on maintenance of posture, is hormonal fluctuation. However, the relationship that sex differences related to hormonal changes may have on ankle laxity and dynamic postural control has not been studied adequately.

Women’s Menstrual Cycle

During the menstrual cycle, there are three defined phases: follicular or menstrual phase, days 1 to 9; ovulatory phase, days 10 to 14 and luteal phase, days 15 to end.\textsuperscript{2, 51, 55}
The follicular phase begins the first day of menses. This is when follicles are grown under the influence of the hypophyseal follicle-stimulating hormone (FSH). During this phase, there is a lower secretion of both estrogen and progesterone. The ovulatory phase is characterized by peak estrogen secretion. During this time endometrial thickness increases to prepare to receive an embryo.

During the luteal phase, estrogen secretion decreases and progesterone secretion peaks. Progesterone supports the endometrium until the embryo can create a placenta, which will then take over this function. At the end of the luteal phase, secretion of progesterone from the corpus luteum ends and the endometrium is no longer supported which causes it to slough off as menstrual bleeding. The cycle begins again when estrogen levels being to decrease and FSH secretion enhances.

In a recent study published in 2006 by Beynnon et al, female alpine skiers with acute Anterior Cruciate Ligament (ACL) injuries were compared to an age-matched control to investigate the possibility of a relationship between menstrual cycle phase and ACL injury. In their study, they chose not to define the menstrual cycle into three phases but instead chose to look at hormone levels before and after ovulation. Their rationale behind this was that the human menstrual cycle is controlled by two steroid hormones that are produced in the ovaries. These hormones, progesterone and estradiol are secreted at different times during the course of a woman’s monthly cycle. Estradiol secretion has two phases, with peaks both at a follicular (preovulatory) and luteal (postovulatory) time period. The secretion of progesterone is controlled by the corpus luteum and this happens only during the postovulatory period. Menses then begins with the failure of the corpus luteum and the rapid reduction in the estradiol and progesterone hormones. Thus, this
may be a more accurate way of assessing the menstrual cycle and will provide for a more accurate way of determining when a woman is going through each of the phases each month.

The phases of the menstrual cycle are characterized by the fluctuations of estrogen and progesterone levels. During the follicular phase there are low estrogen and low progesterone levels, during the ovulatory phase there are high levels of estrogen and low levels of progesterone and high estrogen and high progesterone characterize the luteal phase.

**Hormones and Pregnancy**

Relaxation of joints seems to be a normal physiological process during pregnancy. This is essential in the pelvic joints to accommodate a vaginal delivery. Relaxin is a hormone that is secreted during pregnancy and relaxes the pelvic ligaments, allowing for increased extensibility of the soft tissue of the pelvis which increases peripheral joint laxity. During a normal human pregnancy the interosseus ligament of the public symphysis, cervical tissue and uterus stretch and soften. This has also been found to happen after directly administering estrogen and relaxin to animal models.

During pregnancy, relaxin peaks during the first trimester and subsequently peripheral joint laxity occurs. The levels of relaxin then decrease throughout the rest of the pregnancy, while ligament laxity continues increasing until approximately two weeks post-partum. Relaxin may have the ability to initiate ligament laxity and this is propagated with the presence of high estrogen during late pregnancy. The fact that there is ligament laxity present with the fluctuations of hormones during pregnancy,
presents the question of whether that laxity is also present with hormone fluctuations during a normal menstrual cycle.

**Hormones Effect on ACL**

The concept that ligamentous laxity is increased with the fluctuations in these hormones has led to extensive investigation on a similar influence on ligament properties in the Anterior Cruciate Ligament (ACL) in the knee, but have demonstrated conflicting results.\(^1,^{50,54}\) Sex hormones have been found to have a possible effect on the mechanical properties of the ACL, specifically the collagen structure and metabolism. It has also been reported that estrogen, progesterone, and relaxin receptors are present in human ACL tissue.\(^{35}\) This evidence is enough to suggest that the fluctuation of hormones in the female may have an influence on the tensile properties of the anterior cruciate ligament. When the anterior cruciate ligament is exposed to increased levels of estradiol it can effect the ligament in which there can be a reduction in fibroblast proliferation and a reduction in procollagen synthesis in cell cultures.\(^{55}\) The increase in this hormone has also been found to cause a reduction in the load to failure rate in animal models.\(^{55}\)

Estrogen has been found to have a widespread effect on the growth and development of muscle, bone and connective tissue.\(^{6,45,52}\)

The effect of estrogen on ACL tissue was investigated by Faryniarz et al.\(^{10}\) They found estrogen receptor levels to be similar in both males and females. This suggests that estrogen, in itself, may not play a role in gender differences in ACL injuries but it may require other hormones such as relaxin to influence the injury mechanism. Because of relaxin’s role in ligament laxity during pregnancy, Faryniarz et al\(^{10}\) hypothesized that this is the hormone that has the necessary component to activate the estrogen receptors.
An increase in progesterone levels is also associated with changes in the ACL including increased fibroblast proliferation and collagen formation in cell cultures. Estradiol and progesterone have been the focus of many studies in this area. In spite of the large focus of this relationship at the knee, there has been little investigation on the influence of hormone variance on other musculoskeletal joints, such as the ankle.

Beynnon et al reported that uninjured females possess more ankle joint laxity than males, but laxity did not fluctuate across the menstrual cycle. It is challenging to make a conclusion based on a limited amount of previously published work. The role that ankle ligamentous laxity has on measures of neuromuscular control, such as dynamic postural control, is an important relationship to measure in understanding ankle pathology. While evidence exists from the paper mentioned above that ankle laxity is not influenced by hormone fluctuations, additional work is needed to confirm this finding. Simultaneously, there is a need to quantify some measure of function, such as dynamic postural control, to determine the level of influence of hormone fluctuations and if there is a need for ongoing research, similar to what has occurred in the ACL research community. By investigating, and perhaps discounting, the impact of hormone fluctuations on two factors that are known to contribute to ankle pathology, ankle laxity and dynamic postural control, important information may be gained for developing successful interventions for ankle instability, especially in females.

Quantifying Ankle Laxity

There are several manual stress tests that are commonly used clinically to assess ankle stability. This type of manual examination is highly dependent on the clinician’s sensitivity, skill and experience. There is a range of sensitivity with these tests ranging
from 32% to 80% for the anterior drawer and 52% sensitivity on the talar tilt test\textsuperscript{22}. With the wide range of error that could possibly be present with manual tests, for this study a portable ankle arthrometer will be used to assess ankle stability (Blue Bay Medical, Inc, Navarre, FL). This device has been found to be highly reliable and valid in assessing ankle ligamentous laxity.\textsuperscript{23, 28, 30, 32}

Hubbard et al\textsuperscript{23} investigated the mechanical laxity of subjects with self-reported FAI by testing them with instrumented arthrometry and stress radiography. For each subject with self reported unilateral FAI, both ankles were tested for anterior/posterior and inversion/eversion laxity. They concluded that it is important to be able to objectively measure MAI in the functionally unstable ankle in order to understand the nature and cause of the instability; and that ankle arthrometry and stress radiography are objective assessment tools for laxity.\textsuperscript{23} Additionally, Kovaleski et al examined ankle-subtalar joint complex laxity with an ankle arthrometer using cadaver ankle specimens and came to similar conclusions. They found there to be a strong relationship between tibial-calcaneal bone motion and arthrometric measurements of ankle subtalar joint complex laxity; concluding that the ankle arthrometer is a suitable diagnostic tool when evaluating lateral ankle ligament laxity.\textsuperscript{31}

**Star Excursion Balance Test**

The SEBT will be used to assess dynamic postural control in the subjects in this study. The SEBT is a functional test that involves the subject demonstrating a single leg stance and reaching the maximum distance with the opposite leg. The subject performs this test while standing in the center of a grid with eight lines extending in 45 degree increments from the center of the grid. These eight lines are labeled according to their
direction relative to the stance leg\textsuperscript{37}. The subjects in this study will be performing only the posterior medial reach. This is because recent investigations have demonstrated that the reaching directions of the SEBT are redundant and the entire task can be simplified by having the subjects only perform the posterior medial reaching direction.\textsuperscript{20, 41} The SEBT was chosen to be used in this study because it has been used to quantify dynamic postural control differences in healthy male and female subjects\textsuperscript{15, 18}, as well as, in those with chronic ankle instability\textsuperscript{16, 17, 20, 37}; and it has been demonstrated to have strong reliability.\textsuperscript{21, 29, 41}

Olmstead et al.\textsuperscript{37} found the SEBT to have sensitivity in detecting deficits in reach distance both between and within athletes with unilateral CAI. They reported the SEBT to be valid in differentiating the dynamic postural control of those with and without CAI by showing that the non-injured control subjects could reach further than the subjects with CAI while maintaining a stable base of support.\textsuperscript{37} This was further substantiated by two recent papers by the faculty advisor that examined the role of CAI and fatigue on performance of the SEBT\textsuperscript{16, 17}. Additionally, Hertel et al\textsuperscript{20} observed the deficits in performance on the SEBT among those with CAI, but also determined that these differences can be observed more efficiently by reducing the number of reaching distances from 8 down to 3. This will impact how the test is administered in the future.

**Summary**

Lateral ankle sprains are extremely prevalent in the physically active population and the rate of recurrence after initial injury is very high. Deficiencies in two factors, mechanical laxity and dynamic postural control, have been established as factors associated with CAI. Additionally, there is evidence that differences exist in the risk
factors of acute ankle injury between males and females. During pregnancy, with the changes of hormones, pelvic ligaments become more relaxed to accommodate a vaginal birth. Therefore, there is potential for the hormone fluctuations of the monthly menstrual cycle to have an effect on ligament laxity in other joints. The effect of fluctuating hormones on ACL laxity has been investigated, with differing conclusions, but the potential effect of fluctuating hormones on ankle laxity has not been assessed adequately. Ankle laxity will be investigated using an instrumented ankle arthrometer, and dynamic postural control will be investigated with the Star Excursion Balance Test. Both of these methods have been found to be reliable and accurate in assessing laxity and dynamic postural control, respectively.
Chapter Three

Methods

Subjects

Fourteen healthy females (29±7.05 yrs; 172.32±7.91cm; 68.05±9.27 kg) and 27 healthy males (23.22±4.06 yrs; 180.34±6.98 cm; 81.72±11.19 kg) with no history of lower extremity injury were recruited from the university community for this study.

Instrumentation

A custom made mat with eight measuring tapes rigidly fixed at 45º angles to each other was used to assess reaching distances during the SEBT (Appendix A). This test has been used to quantify dynamic postural control differences in healthy male and female subjects\textsuperscript{15,18} and in those with CAI\textsuperscript{16,17,20,37}; and has been demonstrated to have strong reliability.\textsuperscript{21,29,41}

To assess ankle stability a portable ankle arthrometer (Blue Bay Medical, Inc, Navarre, FL) was used. This device has been found to be highly reliable and valid in assessing ankle ligamentous laxity.\textsuperscript{23,28,30,32} The device consists of an adjustable plate that was secured to the plantar surface of the foot. Attached to the plate is a load-measuring handle that distributes a load to the plate and there was a pad that is secured to the tibia. A six-degree of freedom spatial kinematic linkage system that is connected to
the tibial pad denoted the amount of displacement in the designated direction that load is applied and produced information on rotational and translational motion in the ankle complex. The relative motion between the footplate and the reference pad on the tibia was measured and sent through an analog to digital converter to an attached laptop computer where a custom LabView program (LabView 8.1, National Instruments, Austin, TX) processes the information. Based on manufacturer’s recommendations, AP loading is performed first, followed by I-E loading.

**Independent Variables**

1. Sex  
   a. Male  
   b. Female  

2. Time of the month  
   a. Pre-ovulatory  
   b. Post-ovulatory  

3. Side  
   a. Dominant  
   b. Non-dominant  

**Dependent Variables**

1. Ankle laxity  
   a. Anterior linear displacement (AD): cm  
   b. Posterior linear displacement (PD): cm  
   c. Inversion angular translation (INV): °  
   d. Eversion angular displacement (EV): °
2. Dynamic Postural Assessment (SEBT)
   
a. Normalized reach distance in the posterior-medial direction (MAXD):

Procedures

   Once it was determined that a female subject met the inclusion criteria, she reported to the lab for an introduction to the study. She completed the menstrual history questionnaire (Appendix B) and it was determined when she needed to administer the first ovulation kit. She was given three ovulation kits and a journal. (Appendix D) Explicit instructions on how to use the ovulation detection kits, according to the manufacturer guidelines, and procedures for keeping the journal through both months were provided. At the end of the first month of enrollment in the study, she was asked to report the results of the ovulation kit. Once those results were determined, she was told when to administer the second ovulation kit during the second month. At the end of the second month, she again reported the results of the ovulation kit for that month. Once all of the information from both months were collected, the optimal time for her to report to the research laboratory for testing was determined. She then was asked to report to the research laboratory for two separate times in the third month, corresponding with the determination of the pre-ovulatory and post-ovulatory periods from the information gained from the first two months of enrollment in the study. (Appendix F) The females continued to use the ovulation detection kits and record journal entries during the third month to help confirm that the correct testing dates were selected.
Male subjects were recruited in the same procedures of the female subjects. Once the male subject was recruited, they underwent the same laxity and dynamic postural control testing procedures as the female subjects at similar times of the month.

During the testing session, the subjects’ age, height, weight and sex were reported. The subjects completed an injury history questionnaire (Appendix C) and an informed consent for participation. (Appendix D) The following tests were administered in a pre-determined randomized order: laxity measures (anterior/posterior and inversion/eversion) with the ankle arthrometer and dynamic postural testing on the SEBT mat (MAXD in the posteriormedial direction).

For the ankle stability testing, the subjects were sitting on a treatment table with the testing foot extended over the edge of the table and placed in the arthrometer. A strap was placed around the lower leg 1 cm above the malleoli and the sole of the foot was placed onto the footplate of the device. Adjustments were made to the heel and dorsal clamps for comfort. The tibial pad was placed 5 cm above the ankle malleoli and secured with a strap to the lower leg. To begin each assessment, the ankle was positioned in a neutral position by placing a goniometer over the talocrural joint and creating 0 degrees of plantar flexion. This was used as the measurement reference point.\textsuperscript{23, 30}

Motion anterior to the reference point was referred to as anterior displacement (AD) and motion posterior to this reference point was referred to as posterior displacement (PD). When assessing AP displacements, a load of 125 N was applied in the anterior (AD) and then the posterior (PD) directions.\textsuperscript{23, 30} To assess rotational movement, the ankle was rotated about an AP axis and referenced to the measurement reference point. Internal rotation from the measurement reference point was recorded as inversion.
(INV) and external rotation will be recorded as eversion (EV). For INV and EV assessments, a load of 4 N·m (0.41 kp·m⁻¹) was applied.²³, ³⁰

During each of the trials, subjects were instructed to avoid calf contraction and this was monitored by the investigator. If any indication of muscle contraction was observed, the trial was repeated. There were three trials each of anterior/posterior and inversion/eversion done on each ankle and each trial were recorded. (Appendix G)

The SEBT consists of eight lines made using measuring tapes that extending out from the center at 45 degree intervals. The entire SEBT consists of eight separate reaching tasks in each of the eight reaching directions. Recent investigations have demonstrated that the reaching directions of the SEBT are redundant and the entire task can be simplified by having the subjects only perform the posterior medial reaching direction.²⁰, ⁴¹ Therefore, subjects only performed the posterior medial direction in this study.

The subject began the test standing on two feet, with the foot of their support leg in the center of the SEBT. They then reached as far as possible in the posterior medial direction with the non-support leg, lightly touched the line with their toe, and then returned to a double legged standing position. This task was performed without compromising the base of support by keeping the foot of the stance limb firmly on the floor and keeping their hands placed on their hips throughout the entire attempt. If at any point they removed their hands from their hips or their support foot lifts off the floor, that attempt was counted as unsuccessful and the trial was repeated. The investigator recorded the maximum reaching direction of a trial by placing a mark on the tape attached to the floor.
Subjects were given four practice trials on the designated testing limb prior to actual recorded trials to help familiarize the subject with the task. After the practice trials, the subjects were given five minutes of rest before beginning the testing trials. Each subject was required to complete five successful trials. During each trial the maximum reach distance were marked, measured, and recorded as the distance from the touch point to the stance leg at the center of the SEBT. (Appendix H) The recorded maximum reach distances were normalized to the leg length of the stance leg (reach distance/leg length) to produce the variable MAXD, reported as a percentage score. The test was performed on the dominant and non-dominate sides, with the dominant side being defined as the side the subject would chose to kick a ball with.

**Data Processing**

Data for the ankle arthrometer was processed in LabView software. Sagittal and frontal plane variables were extracted as described in the procedures above.

For the SEBT data, the average of the raw distances (cm) from the five test trials were determined. The reported variable, MAXD, represented the mean raw distance divided by the leg length of the stance leg, and was reported as a percentage.

**Statistical Analysis**

For the dependant variables, the means and standard deviations were used for statistical analysis. For each dependant variable, a separate one between (Sex: male, female), two within (Time: preovulatory, postovulatory, Side: dominant, non-dominant) repeated measures ANOVA was performed. The means level of significance was set at p< 0.05. All statistical analysis was performed using SPSS 15.0 (SPSS, Inc. Chicago, IL.).
Chapter Four

Results

Medial/Lateral Ankle Laxity

A statistically significant Sex main effect (F₁,₃⁹=7.238; p=0.01) was found between males and females for medial/lateral ankle laxity. The average medial/lateral ankle laxity for males was 56.886±9.96 degrees and the average medial/lateral ankle laxity for females was 64.005±7.84 degrees. (Table 1, Figure 1)

There was no statistically significant Time main effect for medial/lateral ankle laxity (F₁,₃⁹=0.789; p=0.380). The average pre-M/L ankle laxity was 60.039±10.6 degrees and the average post-M/L ankle laxity was 60.851±9.305 degrees. (Table 2)

There was no statistically significant Side main effect found for M/L ankle laxity (F₁,₃⁹=0.119; p=0.732). The average for the dominant side was 60.275±10.395 degrees and the average for the non-dominant side was 60.616±9.515 degrees. (Table 3)

There were no statistically significant interactions for Time by Sex (F₁,₃⁹=0.32; p=0.859), Side by Sex (F₁,₃⁹=3.773; p=0.059), Time by Side (F₁,₃⁹=0.897; p=0.349), (Table 4) or Time by Side by Sex (F₁,₃⁹=3.316; p=0.076). (Table 5)
Anterior/Posterior Ankle Laxity

There was no statistically significant Sex main effect for anterior/posterior ankle laxity ($F_{1,39}=0.801; p=0.376$). The average A/P ankle laxity for males was 14.94±3.37 mm and the average a/p laxity for females was 15.66±2.59 mm. (Table 6)

There was no statistically significant Time main effect for A/P ankle laxity ($F_{1,39}=1.126; p=0.295$). The average pre-A/P ankle laxity was 15.097±3.26 mm and the average post-A/P ankle laxity was 15.510±2.98 mm. (Table 7)

There was a statistically significant Side main effect for A/P ankle laxity between the dominant and non-dominant sides ($F_{1,39}=5.280; p=0.027$). The average for the dominant side was 15.780±3.16 mm and the average for the non-dominant side was 14.826±3.08 mm. (Table 8, Figure 2)

There were no statistically significant interactions for Time by Sex ($F_{1,39}=0.778; p=0.383$), Side by Sex ($F_{1,39}=0.005; p=0.943$), Time by Side ($F_{1,39}=0.033; p=0.857$), (Table 9) or Time by Side by Sex ($F_{1,39}=0.011; p=0.918$). (Table 10)

Maximum Normalized Reach Distance in Star Excursion Balance Test

There was a statistically significant Sex main effect for the normalized maximum reach distance ($F_{1,39}=5.093; p=0.030$). The average normalized reach distance for males was 0.906±0.101 and the average normalized reach distance for females was 0.827±0.101. (Table 11, Figure 3)

There was no statistically significant Time main effect from pre-test to post-test for the normalized reaching distance in the SEBT ($F_{1,39}=3.152; p=0.084$). The average pre-normalized reaching distance was 0.861±0.872 and the average post-normalized reaching distance was 0.873±0.886. (Table 12)
There was no statistically significant Side main effect found for the normalized reaching distance between the dominant and non-dominant sides ($F_{1,39}=3.25; p=0.079$). The average normalized reaching distance for dominant side was $0.861 \pm 0.874$ and the average normalized reaching distance for the non-dominant side was $0.872 \pm 0.883$. (Table 13)

There were no statistically significant interactions for Time by Sex ($F_{1,39}=0.837; p=0.366$), Side by Sex ($F_{1,39}=0.445; p=0.508$), Time by Side ($F_{1,39}=0.036; p=0.850$), (Table 14) or Time by Side by Sex ($F_{1,39}=3.238; p=0.08$). (Table 15)
Table 1: M/L ankle laxity Sex Main Effect. Means ± S.D. are provided. Observed power and effect size (E.S.) are provided.

<table>
<thead>
<tr>
<th>Sex Main effect</th>
<th>M/L laxity (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{1,39} = 7.24$</td>
<td>Male</td>
</tr>
<tr>
<td>$p = 0.01$</td>
<td>56.88$6\pm9.96^\circ$</td>
</tr>
<tr>
<td>power = 0.75</td>
<td>(95%CI: 53.76,60.01)</td>
</tr>
<tr>
<td>E.S. = 0.77</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>64.00$\pm7.85^\circ$</td>
</tr>
<tr>
<td></td>
<td>(95%CI: 59.66,68.35)</td>
</tr>
</tbody>
</table>
Figure 1: M/L ankle laxity Sex Main Effect ($F_{1,39} = 7.24 \ p = 0.01$)
Table 2: M/L ankle laxity Time Main Effect. Means ± S.D. are provided. Observed power and effect size (E.S.) are provided.

<table>
<thead>
<tr>
<th>Time Main effect</th>
<th>M/L laxity (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{1,39} = 0.79$</td>
<td>Pre</td>
</tr>
<tr>
<td>$p = 0.38$</td>
<td></td>
</tr>
<tr>
<td>power =0.14</td>
<td>Post</td>
</tr>
<tr>
<td>E.S. = 0.08</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: M/L ankle laxity Side Main Effect. Means ± S.D. are provided. Observed power and effect size (E.S.) are provided.

<table>
<thead>
<tr>
<th>Side Main effect</th>
<th>M/L laxity (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{1,39} = 0.119$</td>
<td>Dominant 60.275±10.395˚</td>
</tr>
<tr>
<td>$p = 0.732$</td>
<td>(95%CI:57.11,63.43)</td>
</tr>
<tr>
<td>power = 0.063</td>
<td>E.S. = 0.03</td>
</tr>
<tr>
<td>Non-Dominant</td>
<td>60.616±9.515˚</td>
</tr>
<tr>
<td></td>
<td>(95%CI:58.10,63.13)</td>
</tr>
</tbody>
</table>
Table 4: Medial/Lateral ankle laxity (degrees) Two-way Interactions. Means ± S.D. are provided. Observed power are provided.

<table>
<thead>
<tr>
<th>Sex by Time Interaction</th>
<th>Side by Sex Interaction</th>
<th>Time by Side Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{1,39} = 0.03$</td>
<td>$F_{1,39} = 3.77$</td>
<td>$F_{1,39} = 0.90$</td>
</tr>
<tr>
<td>$p = 0.86$</td>
<td>$p = 0.06$</td>
<td>$p = 0.35$</td>
</tr>
<tr>
<td>power = 0.05</td>
<td>power = 0.47</td>
<td>power = 0.15</td>
</tr>
</tbody>
</table>
Table 5: Medial/Lateral ankle laxity (degrees) Sex by Time by Side Interaction. Means ± S.D. are provided. Observed power and effect size (E.S.) are provided.

<table>
<thead>
<tr>
<th>Sex by Time by Side Interaction</th>
<th>Male</th>
<th>Male</th>
<th>Female</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Dom</td>
<td>57.75 ± 10.83° (95%CI: 53.40, 58.16)</td>
<td>57.60 ± 10.39° (95%CI: 54.04, 61.17)</td>
<td>61.125 ± 11.82° (95%CI: 55.08, 67.16)</td>
<td>64.62 ± 6.00° (95%CI: 59.66, 69.58)</td>
</tr>
<tr>
<td>Non-Dom</td>
<td>55.37 ± 9.57° (95%CI: 51.94, 58.81)</td>
<td>56.82 ± 9.08° (95%CI: 53.58, 60.05)</td>
<td>65.91 ± 7.09° (95%CI: 61.38, 70.68)</td>
<td>64.36 ± 6.49° (95%CI: 59.87, 68.85)</td>
</tr>
<tr>
<td>p = 0.07, power = 0.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F_{1,39} = 3.32
Table 6: Anterior/Posterior ankle laxity Sex Main Effect. Means ± S.D. are provided. Observed power and effect size (E.S.) are provided.

<table>
<thead>
<tr>
<th>Sex Main effect</th>
<th>A/P laxity (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>14.94±3.37 mm (95%CI:13.99;15.89)</td>
</tr>
<tr>
<td>Female</td>
<td>15.66±2.59 mm (95%CI:14.34;16.99)</td>
</tr>
</tbody>
</table>

F_{1,39} = 0.801
p = 0.376
power = 0.14
E.S. = 0.24
Table 7: Anterior/Posterior ankle laxity Time Main Effect. Means ± S.D. are provided. Observed power and effect size (E.S.) are provided.

<table>
<thead>
<tr>
<th>Time Main effect</th>
<th>A/P laxity (mm)</th>
</tr>
</thead>
</table>
| $F_{1,39} = 1.126$  
  $p = 0.30$  
  power =0.18  
  E.S. = 0.13 | Pre  
  15.097±3.26 mm  
  (95%CI:14.08,16.11) |
|                  | Post  
  15.510±2.98 mm  
  (95%CI:14.73,16.28) |
Table 8: Anterior/Posterior ankle laxity Side Main Effect. Means ± S.D. are provided. Observed power and effect size (E.S.) are provided.

<table>
<thead>
<tr>
<th>Side Main effect</th>
<th>A/P laxity (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F冰雪 = 5.28</td>
<td>Dominant</td>
</tr>
<tr>
<td>p = 0.027</td>
<td>15.780±3.16 mm</td>
</tr>
<tr>
<td>power = 0.611</td>
<td>(95%CI:14.82;16.74)</td>
</tr>
<tr>
<td>E.S. = 0.31</td>
<td>Non-Dominant</td>
</tr>
<tr>
<td></td>
<td>14.826±3.08 mm</td>
</tr>
<tr>
<td></td>
<td>(95%CI:13.96;15.69)</td>
</tr>
</tbody>
</table>
Figure 2: Anterior/Posterior ankle laxity Side Main Effect ($F_{1,39} = 5.28; p = 0.027$)
Table 9: Anterior/Posterior ankle laxity (degrees) Two-way Interactions. Means ± S.D. are provided. Observed power are provided.

<table>
<thead>
<tr>
<th></th>
<th>Sex by Time Interaction</th>
<th>Side by Sex Interaction</th>
<th>Time by Side Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F_{1,39} = 0.778$</td>
<td>$F_{1,39} = 0.005$</td>
<td>$F_{1,39} = 0.033$</td>
</tr>
<tr>
<td></td>
<td>$p = 0.38$</td>
<td>$p = 0.943$</td>
<td>$p = 0.857$</td>
</tr>
<tr>
<td></td>
<td>power = 0.138</td>
<td>power = 0.051</td>
<td>power = 0.054</td>
</tr>
</tbody>
</table>
Table 10: Anterior/Posterior ankle laxity (cm) Sex by Time by Side Interaction. Means ± S.D. are provided. Observed power and effect size (E.S.) are provided.

<table>
<thead>
<tr>
<th>Sex by Time by Side Interaction</th>
<th>Male</th>
<th></th>
<th></th>
<th>Female</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td></td>
<td>Pre</td>
<td>Post</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dom</td>
<td>Non-Dom</td>
<td>Dom</td>
<td>Non-Dom</td>
<td>Dom</td>
<td>Non-Dom</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F_{1,39} = 0.11</td>
<td>15.07±3.95mm (95%CI: 13.69,16.44)</td>
<td>14.06±3.14mm (95%CI: 12.89,15.23)</td>
<td>15.80±3.18mm (95%CI: 14.69,16.91)</td>
<td>14.84±3.23mm (95%CI: 13.6,16.08)</td>
<td>16.14±2.52mm (95%CI: 14.22,18.05)</td>
<td>15.12±2.71mm (95%CI: 13.5,16.74)</td>
</tr>
<tr>
<td>p = 0.918</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>power = 0.051</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.11±2.01mm (95%CI: 14.57,17.65)</td>
<td>15.28±3.14mm (95%CI: 13.55,17.01)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 11: SEBT Sex Main Effect. Means ± S.D. are provided. Observed power and effect size (E.S.) are provided.

<table>
<thead>
<tr>
<th>Sex Main effect</th>
<th>Normalized Reaching Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{1,39} = 5.093$</td>
<td>Male $0.906 \pm 0.101$ (95%CI: $0.865, 0.948$)</td>
</tr>
<tr>
<td>$p = 0.03$</td>
<td>power $= 0.6$</td>
</tr>
<tr>
<td>$\text{E.S.} = 0.78$</td>
<td>Female $0.827 \pm 0.101$ (95%CI: $0.769, 0.885$)</td>
</tr>
</tbody>
</table>
Figure 3: SEBT Sex Main Effect ($F_{1,39} = 5.093 \ p = 0.03$)
Table 12: SEBT Time Main Effect. Means ± S.D. are provided. Observed power and effect size (E.S.) are provided.

<table>
<thead>
<tr>
<th>Time Main effect</th>
<th>Normalized Reaching Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{1,39} = 3.15$</td>
<td>Pre</td>
</tr>
<tr>
<td>$p = 0.084$</td>
<td></td>
</tr>
<tr>
<td>power =0.41</td>
<td>Post</td>
</tr>
<tr>
<td>E.S. = 0.01</td>
<td></td>
</tr>
</tbody>
</table>
Table 13: SEBT Side Main Effect. Means ± S.D. are provided. Observed power and effect size (E.S.) are provided.

<table>
<thead>
<tr>
<th>Side Main effect</th>
<th>Normalized Reaching Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dominant</td>
</tr>
<tr>
<td>F_{1,39} = 3.25</td>
<td></td>
</tr>
<tr>
<td>p = 0.079</td>
<td></td>
</tr>
<tr>
<td>power = 0.42</td>
<td></td>
</tr>
<tr>
<td>E.S. = 0.01</td>
<td></td>
</tr>
</tbody>
</table>
Table 14: SEBT Two-way Interactions. Means ± S.D. are provided. Observed power are provided.

<table>
<thead>
<tr>
<th>Sex by Time Interaction</th>
<th>Side by Sex Interaction</th>
<th>Time by Side Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{1,39} = 0.837$</td>
<td>$F_{1,39} = 0.445$</td>
<td>$F_{1,39} = 0.036$</td>
</tr>
<tr>
<td>$p = 0.366$</td>
<td>$p = 0.508$</td>
<td>$p = 0.85$</td>
</tr>
<tr>
<td>power = 0.145</td>
<td>power = 0.1</td>
<td>power = 0.054</td>
</tr>
</tbody>
</table>
Table 15: SEBT Sex by Time by Side Interaction. Means ± S.D. are provided. Observed power and effect size (E.S.) are provided.

<table>
<thead>
<tr>
<th>Sex by Time by Side Interaction</th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td></td>
<td>Dom</td>
<td>Non-Dom</td>
<td>Dom</td>
<td>Non-Dom</td>
</tr>
<tr>
<td>F_{1,39} = 3.24, p = 0.08, power = 0.419</td>
<td>0.891±0.099 (95%CI: 0.849, 0.935)</td>
<td>0.903±0.095 (95%CI: 0.861, 0.945)</td>
<td>0.914±0.108 (95%CI: 0.87, 0.958)</td>
<td>0.917±0.105 (95%CI: 0.873, 0.96)</td>
</tr>
</tbody>
</table>
Chapter Five

Discussion

The purpose of this study was to examine potential hormone contributions to ankle laxity to help establish data in healthy subjects and determine if this is a potential area of investigation that is needed to address important questions in the understanding of the development of ankle pathology. This was done by examining ankle laxity with an instrumented ankle arthrometer and dynamic postural testing with the SEBT. These measures were performed on females in their pre-ovulatory and post-ovulatory phases of their menstrual cycle with a cohort group of male matched control subjects tested at similar times of the month. It was hypothesized that 1) females would have greater medial/lateral and anterior/posterior laxity compared to males, 2) females would have better dynamic postural control and 3) that females would not demonstrate a difference in laxity or dynamic postural control between the pre and post ovulatory phases. Most of these hypotheses proved to be correct and will provide important clinical relevance.

Ankle Laxity

In the measures of medial/lateral ankle laxity, females presented with statistically significant more laxity than males. This finding is consistent with what was hypothesized
and is also consistent with previous research by Willems et al\textsuperscript{48,49} and Beynnon et al\textsuperscript{1} which suggest that females appear to possess more talocrural laxity compared to males.

In contrast, there was no statistically significant Sex main effect for anterior/posterior ankle laxity. This finding was not consistent with what was hypothesized, that females would possess more anterior/posterior ankle laxity than males. While there was not a significant difference found between males and females in anterior/posterior laxity, the average laxity for females (15.66±2.59 cm) was greater than males (14.94±3.37 cm). In general, there is less ankle motion in the sagittal plane, compared to the frontal plane. Therefore, there is less anterior/posterior movement in the joint and is a likely explanation why we observed a non-significant difference between males’ and females’ laxity. Because there is less available movement, the magnitude of the potential differences is smaller making it more difficult to detect potential differences. However, even though there was not a statistically significant difference between sexes, similar to medial/lateral laxity measures, females still had more laxity in the anterior/posterior direction.

There was no statistically significant influence of Time on medial/lateral or anterior/posterior ankle laxity. This is consistent with our hypothesis and is supported by the findings of Beynnon et al\textsuperscript{1} that ankle laxity does not fluctuate across the menstrual cycle. The potential effect of hormones on ligament laxity has been investigated in the anterior cruciate ligament of the knee with conflicting results\textsuperscript{1,50,54}. However, this relationship has not been investigated extensively in the ankle. The finding in this study helps to strengthen the argument that hormone fluctuation does not have a significant influence on ligament laxity in the ankle. This is important because with the knowledge
that hormone fluctuation does not have an impact on ankle laxity, an established factor that contributes to recurrent ankle pathology, clinicians and researchers may be able to focus on other interventions and factors that may be influencing ankle injuries.

Our hypotheses that there would be no differences between the dominant and non-dominant sides in measures of ankle laxity was partially supported. There was a statistically significant Side main effect for anterior/posterior ankle laxity with the dominant side presenting with more laxity than the non-dominant side. However, there was no statistically significant difference in medial/lateral ankle laxity between the dominant and non-dominant sides. A systematic review conducted by Beynnon et al\textsuperscript{4} considered limb dominance as a potential predictive factor for lateral ankle sprains. They noted that limb dominance has been implicated as a risk factor for lower extremity injury because of the greater demand placed on the dominant limb during sport.\textsuperscript{4} However, that review noted that the literature regarding limb dominance and injury risk is divided. In a separate investigation by Beynnon et al\textsuperscript{5}, it was found that limb dominance was unrelated to the risk of ankle injury for male and female field hockey players. Conversely, Ekstrand and Gillquist\textsuperscript{9} found that the dominant leg of male soccer players sustained significantly more injuries than their non-dominant, with 92% of ankle injuries affecting the dominant leg.

As noted by Beynnon et al\textsuperscript{4}, although there are conflicting results in these studies, this may be attributed to the differences in study designs or the data analysis methods used. Therefore, there may need to be more studies to examine limb dominance as a risk factor for ankle sprains, in order to make a definitive conclusion concerning this matter.
Dynamic Postural Control

There was a statistically significant Sex main effect for the normalized maximum reaching distance in the Star Excursion Balance Test that represented the measures of dynamic postural control. It was found that the average normalized reaching distance for the males was further than for the females. This is inconsistent with our hypothesis and also inconsistent with a recent investigation by the faculty advisor in which healthy females were able to perform better on this lower extremity reaching task compared to healthy males. This difference in the results may be explained by noting that the study done by Gribble et al used the anterior, medial and posterior reaches in the SEBT. The reaching direction that was used for this study was the posterior medial reaching direction. To our knowledge, there have not been previous comparisons between males and females on this particular reaching direction of the SEBT. Continued investigation of the differences in males and females on this task is warranted to determine a consistent conclusion of the influence of Sex on this measure of dynamic postural control.

There was no statistically significant influence of Time for the normalized reaching distance in the SEBT. This is consistent with our hypothesis and has important clinical relevance. The SEBT is a dynamic balance reaching test with strong reliability that has been used to quantify dynamic postural control differences in healthy male and female subjects, as well as in those with chronic ankle instability. A finding that hormone fluctuation does not impact performance on the SEBT is important clinically because with the knowledge that hormone fluctuation does not have an impact on ankle dynamic postural control (a known factor that contributes to ankle pathology), researchers and clinicians can focus on other factors that may be contributing to ankle
instability and subsequently limiting dynamic postural control. This may lead to more effective intervention strategies that perhaps do not need to focus on hormone regulation.

Finally, there was no statistically significant influence of Side on the normalized reaching distance between dominant and non-dominant sides in the SEBT. This is consistent with the hypotheses in which there was not expected to be a difference between dominant and non-dominant sides in the normalized reaching distance in the Star Excursion Balance Test and is supported by a previously published stud.15

Limitations

A limitation to this study relates to the ability of the subjects to recall their medical history about previous lower leg injuries and menstrual cycle history. The female subjects used ovulation kits on their own time and recorded a journal about their menstrual cycle and were expected to administer the kits and record journal entries correctly. Alternative techniques for monitoring hormone levels may be more accurate, but they are much more expensive. In this study the estimation of the ovulation phases was improved by incorporating three consecutive months of using ovulation kits. Because of the importance of the timing of the testing sessions, it was critical that subjects attended the scheduled testing session during the third month of enrollment. There was diligent communication between the investigators and the subjects through the duration of the study. This diligent communication helped decrease errors and ensure that the subjects were following as closely to the methods of the study as possible.

The method of testing pre and post ovulatory was based on a previously established method used in a study published in 2006 by Beynon et al.3 This study looked at female alpine skiers with acute Anterior Cruciate Ligament (ACL) injuries and
compared them to an age-matched control to investigate the possibility of a relationship between menstrual cycle phase and ACL injury. This study chose not to define the menstrual cycle into three phases but instead chose to look at hormone levels before and after ovulation. The rationale behind this was that the hormones, progesterone and estradiol are secreted at different times during the course of a woman’s monthly cycle, one peaking during the pre-ovulatory phase and one during the post-ovulatory phase.³ This method provided for a more accurate way of determining when a woman is going through each of the monthly menstrual cycle phases.

Another limitation to this study was the accuracy of the examiner in administering the ankle arthrometer to test ankle laxity. The same examiner administered all the laxity tests, which decreased the possibility of error between and within subjects. The ankle arthrometer has been found to be highly reliable and valid in assessing ankle ligamentous laxity.²³, ²⁸, ³⁰, ³² These studies found the ankle arthrometer to have high reliability, thus the error of the examiner may not be as large of an influence because of this high reliability. Kovaleski et al³¹ also conducted a study using cadaver ankle specimens to examine ankle-subtalar joint complex laxity with an ankle arthrometer. Their results showed there to be a strong relationship between tibial-calcaneal bone motion and arthrometric measurements of ankle subtalar joint complex laxity. Based on this, it was concluded that the ankle arthrometer is a suitable diagnostic tool when evaluating lateral ankle ligament laxity.³¹

**Clinical Relevance**

The results of this study will help clinicians and researchers eliminate hormone fluctuation as a factor that needs to be considered when examining causes and prevention
of ankle pathology in females. Our study demonstrated that hormone changes pre
ovulatory to post ovulatory do not have an effect on ankle laxity in the medial/lateral and
anterior/posterior directions, or on dynamic postural control, through normalized
reaching distances in the Star Excursion Balance Test. The concept that ligamentous
laxity is increased with the fluctuations in these hormones has led to extensive
investigation on a similar influence on ligament properties in the Anterior Cruciate
Ligament (ACL) in the knee, but have demonstrated conflicting results.\textsuperscript{1,50,54} There is a
gap in this literature in the influence of hormone fluctuation on ligaments of the ankle.
This study helped fill that gap and showed that hormones do not have an affect on ankle
ligament laxity and therefore the concern for that risk factor does not need to be as
closely considered as it was in the past. Therefore, clinicians working with female
athletes will not have to worry about changes in hormones through the menstrual cycle
affecting the integrity of the ankle ligaments.

Conclusion

This study attempted to determine if hormone fluctuation has an impact on ankle
laxity and dynamic postural control, two known factors that contribute to ankle
pathology. The evidence suggests that hormone fluctuation during the menstrual cycle,
(pre-ovulatory compared to post-ovulatory) does not have an effect on ankle laxity, as
evidence through the use of the ankle arthrometer, or dynamic postural control, as
evidenced through the use of the Star Excursion Balance Test. There have been few
studies that have considered the impact of hormone fluctuation on ankle instability.
Further research should aim to strengthen the finding of this study by conducting similar
projects looking at more subjects, looking at different points in the menstrual cycle and
perhaps looking at different areas of the body to rule out systemic ligament laxity due to hormone influence. By concluding that hormone fluctuations do not have an impact on ankle laxity and function, researchers and clinicians will be able to focus on other factors that may more heavily influence ankle instability in order to form more effective ankle pathology prevention programs.
References


Appendix A:

Star Excursion Balance Test Reaching Directions
Anterior
Anterolateral
Lateral
Posterolateral
Anteromedial
Medial
Posteromedial
Posterior

Left-leg stance

Anterior
Anteromedial
Medial
Posteromedial
Posterolateral

Right-leg stance
Appendix B:

Female Menstrual History Questionnaire
1. Age: __________
2. Height: __________
3. Weight: __________
4. Age at start of menstruation __________
5. Number of menstrual periods during the previous 12 months __________
6. First day of last menstrual period ________________
7. Mean number of days between menstrual periods __________
8. Anticipated onset of next menstrual period ________________
Appendix C:

Health History Questionnaire
1. Age: __________
2. Height: __________
3. Weight: __________
4. Which foot would you chose to kick a ball with?: Right______ Left______
5. Have you ever had an injury to your lower extremity? Yes______ No______
6. Have you ever had a concussion?: Yes______ No______
7. Do you suffer from vertigo, or any other neurological disorders?: Yes____
   No____
10. If yes, explain:
   __________________________________________________________
       __________________________________________________________
Appendix D:

Informed Consent for Human Research Study
University of Toledo

Title of Project: “The Effect of Sex Differences and Hormone Fluctuation on Ankle Stability and Function”

Person in Charge: Hayley Hahn
University of Toledo
Athletic Training
2801 W Bancroft St.
Toledo, OH 43606
Office Phone: (419) 530-4303
Email: hayley_hahn@yahoo.com

1. This section provides an explanation of the study in which you will be participating:

   A. The study in which I am participating is part of research intended to establish information about the factors that may affect ankle pathology in females compared to males.

   B. If I agree to take part in this research, I certify that I have not had any history of lower extremity injury, and that I am not suffering from any diseases or illnesses that would prevent me from performing the study.

   C. I will be asked to report to the Athletic Training Research lab in the Health and Human Services building on the campus of the University of Toledo on one occasion. The session will comprise of filling out a health history questionnaire, measurement of the laxity in my ankle, and measurement of my dynamic postural control using the Star Excursion Balance Test.

2. This section describes your rights as a research participant:

   A. I understand that I may ask the investigator any questions about the research procedures, and these questions will be answered.

   B. My participation in this research is confidential. Only the person in charge will have access to my identity and information that can be associated with my identity. In the event of publication of this research, no personally identifying information will be disclosed. To make sure my participation is confidential, only a code number will appear on the data
collection sheet. Only the researchers can match my name with my code number.

C. My participation is voluntary. I am free to stop participating in the research at any time, or to decline to answer any specific questions without penalty.

D. I may contact the Office for Research, 2300 University Hall, University of Toledo, Toledo, OH 43606, (419) 530-2844, for additional information concerning my right as a research participant.

3. This section indicates that you are giving your **informed consent to participate** in the research:

**Participant:**

I agree to participate in the scientific investigation described above, as an authorized part of the education and research program of the University of Toledo.

I understand the information given to me, and I have received answers to any questions I may have had about the research procedure. I understand and agree to the conditions of this study as described.

To the best of my knowledge and belief, I have no physical or mental illness or difficulties that would increase the risk to me of participation in this study.

I understand that my participation in this study does not entitle me to any compensation, financial or otherwise.

I understand that my participation in this research is voluntary, and that I may withdraw from this study at any time by notifying the person in charge.

I am 18 years of age or older.

I understand that medical care is available in the event of injury resulting from research but that neither financial compensation nor free medical treatment is provided. I also understand that I am not waiving any rights that I may have against the University for injury resulting from negligence of the University or investigators.
I understand that I will receive a signed copy of this consent form.

_____________________________________________ ____________  
Signature                                           Date

Researcher:

I certify that the informed consent procedure has been followed, and that I 
have answered any questions from the participant above as fully as possible.

_____________________________________________ ____________

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Appendix E:

Ovulation Kit Testing Journal
Name______________________________________
Phone______________________________________
Email______________________________________

First day of last period______________________
First day of second to last period______________
First day of next period______________________

Length of normal cycle_____________________(from day one of one period to day one of next period.)

Start testing_______days after the first day of last / next period.
Date of start of testing______________
*Please test at the same time every morning*

Results of Testing:
Start testing_______days after the start of last / next period.
Please indicate the results of testing each day by putting a plus sign for positive and negative sign for negative. Please also indicate the dates of when you tested.

1st Test Day___________Date__________
2nd Test Day___________Date__________
3rd Test Day___________Date__________
4th Test Day___________Date__________
5th Test Day___________Date__________
6th Test Day___________Date__________
7th Test Day___________Date__________

Once you get a positive test, you no longer need to administer the rest of the tests. Please keep the unused tests, along with the box and directions and return them to me.
LH surge is indicated when two lines are visible on the test and the color and intensity of the test line is similar to or darker than the reference line.
Appendix F:

Subject Information and Ovulation Predictor
Subject__________

**Date of start of menses:**
First month__________
Second month__________
Third month__________
Fourth month (projected)__________

**Length of cycle:**
First month__________
Second month__________
Average__________

**Date of positive ovulation test:**
First month__________
Second month__________
Third month (projected)__________

Projected date to start ovulation kit testing in third month__________

**Projected ankle testing dates:**
Pre-test__________
Post-test__________
Appendix G:

Ankle Arthrometer Measurements
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<td>cm</td>
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<td>cm</td>
<td>cm</td>
<td>cm</td>
</tr>
<tr>
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<td>°</td>
<td>°</td>
<td>°</td>
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<tr>
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Appendix H:

Reach Distance Data Collection Form
Subject # _____________

Height: ___________cm  Weight: ___________kg

Kicking Limb: _________

Leg Length : L__________cm  R___________cm

Posterior Medial Reach Distances

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<td>5</td>
</tr>
<tr>
<td>Avg</td>
</tr>
<tr>
<td>Normalized (MAXD)</td>
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