Monthly Variability in Anterior Knee Laxity and Estradiol Concentration in 18 to 26-Year-Old, Healthy Women with a Regular Menstrual Cycle

Amber Sorensen Van Cleave¹, David A Hawkins^{2*}

¹Biomedical Engineering Graduate Group, University of California, Davis, California, USA

²Department of Neurobiology, Physiology, and Behavior, University of California, Davis, California, USA

***Corresponding author:** David A Hawkins, Department of Neurobiology, Physiology, and Behavior, University of California, Davis, California, USA. Tel: +15307522748; Fax: +15307526681; Email: dahawkins@ucdavis.edu

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Abstract

Background: Increased Anterior Knee Laxity (AKL) is a risk factor for Anterior Cruciate Ligament (ACL) injury, and has been reported to fluctuate throughout a woman's menstrual cycle in proportion to fluctuations in hormone concentrations. Women have a greater rate of ACL injuries compared to men, which might be explained by fluctuations in hormone concentrations and AKL in women. Though studies have reported a linear relationship between hormone fluctuations and AKL within a menstrual cycle, it is not clear if this relationship is consistent between cycles. To gain a better understanding of hormone fluctuation effects on ACL injury risk, we need a better understanding of the relationship between hormone concentrations and AKL, and the repeatability of this relationship.

Hypothesis/Purpose: We hypothesized that AKL would vary linearly with estrogen concentration and this linear relationship would not vary significantly between months in healthy women with regular menstrual cycles.

Study Design: This is a cross-sectional study.

Methods: Thirteen females aged 21±1 years with regular menstrual cycles completed the study. Their AKL and estradiol concentrations were quantified at menses and ovulation for three months. Linear regression analysis was used to determine relationships between AKL and estradiol concentrations for each month and compared for each subject. These relationships were compared using Fisher-Z-transformation and an equality test of correlations.

Results: AKL at menses and ovulation, and the difference in AKL between menses and ovulation, were highly variable between months for 63%, 52%, and 97% of the comparisons respectively. Estradiol concentration at menses and ovulation, and the difference in estradiol concentration between menses and ovulation, were highly variable between months for 69%, 33%, and 81% of the comparisons, respectively. The relationship between estradiol and AKL was significantly different for at least one month for four subjects. Conclusion: There can be high variability between menstrual cycles for AKL at menses, ovulation, and the change between menses and ovulation, and for estradiol concentration at menses and the change between menses and ovulation. The relationship between estradiol and AKL can vary significantly between months for some women. Salivary estradiol concentration changes alone are not sufficient to predict AKL changes.

Keywords: Estrogen; Female; Menses; Ovulation

What is known about the subject: Based on current evidence, the sex hormone estrogen may be involved in the ACL injury disparity that exists between women and men. It has been shown that there is a relationship between AKL and estrogen concentrations within the menstrual cycle [1-4]. Increases in estrogen concentrations are associated with increases in AKL.

Increased AKL has been associated with increased risk for ACL injury [5]. Thus, it is logical to assume that if estrogen concentrations increase significantly during certain phases of the menstrual cycle, resulting in increased AKL, then there is an increased risk for ACL injury during this time. There is evidence that there are associations between phases of the menstrual cycle and ACL injury [6].

What this study adds to existing knowledge: Though studies have reported a linear relationship between hormone fluctuations and AKL within a menstrual cycle, it is not clear if this relationship exists for all women and is consistent between menstrual cycles. This information is important to determine if a woman's AKL changes throughout a month are repeatable and therefore injury risk due to hormone fluctuations are repeatable month-to-month, or if AKL changes vary month-to-month. If ACL injury risk does not change month-to-month, then a woman could be assessed for injury risk in one month. If ACL injury risk does change month-to-month, then a woman would need to be assessed for injury risk more often.

Introduction

Anterior Cruciate Ligament (ACL) injury is an injury that affects many young people with women having an injury rate between two and seven times greater than for men in the same activity [7]. Approximately 120,000 ACL injuries occur annually within the United States [8,9]. Up to 70% of these injuries are noncontact and occur during cutting maneuvers, landing from a jump, and rapid stops [7]. About 50% of ACL injuries occur among athletes 15 to 25 years old [10]. In response to the high incidence of ACL injuries and the fact that the ACL provides the primary restraint to anterior displacement of the tibia relative to the femur, scientists and clinicians have sought to use knee laxity tests to quantify ACL structural integrity for prognostic, diagnostic, and therapeutic applications [10]. The ACL has been cited as providing, on average, 86% of the force developed to restrain anterior displacement of the tibia relative to the femur (defined as Anterior Knee Laxity (AKL)) when tested at 30 and 90 degrees of knee flexion (0 degrees being full extension) [11]. Thus, changes in resting AKL may be used as an indirect measure of changes in ACL compliance. Increased ACL compliance can result from: [12] ACL injury, [13] an acute viscoelastic response to a bout of cyclic loading, and/or [14] increases in estrogen concentrations in women. Understanding the

effects these ACL responses have on the magnitude and timing of AKL changes is critical to differentiate AKL changes resulting from ACL injury, acute viscoelastic behavior, and changes in estrogen concentrations. An increased resting AKL relative to an individual's baseline AKL has been associated with existing ACL injury [5]. Baseline AKL may vary between people without ACL injury due to anatomical structure, movement mechanics, and metabolic and inflammatory responses.

However, if resting AKL changes within an individual relative to their baseline, then this can be indicative of ACL injury. Daniel et al. performed a study that measured AKL in subjects with and without acute ACL disruptions. They concluded that a laxity difference between knees of 3 mm or greater is indicative of an ACL injury and a laxity difference between knees of 2.0 to 2.5 mm has a high probability of being an ACL injury. Subjects who had a laxity difference between knees of less than 2.0 mm were considered to have normal knee laxity [15].

Due to the viscoelastic properties of knee structures, AKL naturally increases during physical activity and recovers following exercise. In various studies, AKL has been shown to increase by 13-33% in activities including running, basketball, using a bicycle ergometer, and performing a field exercise protocol [16-20]. Increased laxity has been shown to recover post exercise within an hour [18,21,22,23]. Therefore, if AKL varies by more than 13% on separate days when measured an hour or more post physical activity or by more than 33% within an hour of physical activity, then AKL is considered to be highly variable.

Hormone concentration fluctuates through the menstrual cycle and can change the metabolism of the ACL fibroblasts and may affect ACL strength, AKL, and ACL injury risk in females [24]. Estrogen concentrations are highest typically one to two days (up to four days) before ovulation occurs [13,25]. The maximum change in AKL may not be detected until 3-4 days after a peak in estradiol concentration has occurred [26].

Based on current evidence, the sex hormone estrogen may be involved in the ACL injury disparity that exists between women and men. It has been shown that there is a relationship between AKL and estrogen concentrations within the menstrual cycle [1-4]. Increases in estrogen concentrations are associated with increases in AKL. Increased AKL has been associated with increased risk for ACL injury [5]. Thus, it is logical to assume that if estrogen concentrations increase significantly during certain phases of the menstrual cycle, resulting in increased AKL, then there is an increased risk for ACL injury during this time. There is evidence that there are associations between phases of the menstrual cycle and ACL injury [6].

Though studies have reported a linear relationship between hormone fluctuations and AKL within a menstrual cycle, it is not clear if this relationship exists for all women and is consistent be-

tween menstrual cycles. This information is important to determine if a woman's AKL changes throughout a menstrual cycle are repeatable and therefore injury risk due to hormone fluctuations are repeatable or vary across cycles.

We hypothesized that AKL varies linearly with estrogen concentration and this linear relationship does not vary significantly between months in healthy women with regular menstrual cycles. To test this hypothesis, we 1) quantified AKL at menses and ovulation and compared these values and their difference between three menstrual cycles; 2) quantified estradiol concentration at menses and at ovulation and compared these values and their difference between three menstrual cycles; and 3) quantified and compared the linear relationship between AKL and estradiol concentration over 3 menstrual cycles in healthy women with regular menstrual cycles. Although athletic women are at greater risk for an ACL injury, non-athletic women were chosen for this study to establish a baseline of "Normal" responses.

Methods

Custom Knee Arthrometer

A Custom Knee Arthrometer (CKA) was designed and built in the UC Davis Human Performance Laboratory to acquire the AKL data needed for this study. It was concluded by Starkel (2013) that in comparison with other knee arthrometers, specifically the commercially available KT-1000, the CKA has equal, if not superior intra-tester precision, test-retest variability, and reliability [21]. The average AKL difference between repeat tests have been reported to be between 0.45 mm and 1.0 mm for the KT-1000 [1,30], and between 0.45 mm [22] and 0.5 mm (current study) for the CKA. The CKA consists of two force transducers (LC101-100 S and LC101-50 S Beam Load Cells, Omega Inc, Stamford, CT) and two string potentiometers (SP1-4 String Pot, Celesco, Chatsworth, CA) to obtain force and displacement data. Signals from the force transducers and potentiometers are acquired through an analog to digital interface at 800 Hz using a custom Virtual Interface (VI) in Lab VIEW (Lab VIEW V2015, National Instruments Corporation, Austin, TX). The VI is used to control the data acquisition process, display the data during testing, and write and save the data to an Excel CSV file. During an AKL test, the subject sits with their torso upright and their thigh securely strapped to a thigh support to ensure 30 degrees of knee flexion. In each test, a small anterior force (-50 to 250 N) is manually and cyclically applied on the upper portion of the tibia. The peak force applied during testing was chosen to be 250 N because this value is: [12] no more than 30% of ACL ultimate load (closer to 9-12% of the ultimate load reported (1730 to 2160 N) for populations similar in age (16-35 years) to our subject population (18-26 years) [27,28] , [13] no more than the force clinicians use to perform a standard knee exam, and [14] less than the peak force present in walking (300 to 1000 N) [29].

The anterior force is applied 20 times sequentially with force application occurring over two seconds for each cycle. Assuming an average ACL resting length of 32 mm and a displacement of 2-4 mm per cycle, the strain rate is approximately 2-4 mm/second or 6.25-12.5 %/s. Electromyography (EMG) is used to monitor activity of the vastus medialis and biceps femoris muscles during a trial, which can affect the AKL results.

Subjects

All recruitment and methodological protocols were approved by the University of California-Davis Institutional Review Board (IRB). Twenty women were recruited for this study. Subjects were excluded if they had a history of irregular menstrual cycles, currently taking oral contraceptives, had any current musculoskeletal injury or known diseases, exercised more than seven hours per week, and/or were outside of the age range of 18-26 years.

Testing Protocol

After enrolling, subjects started the study the next time they started menses. The first month, or cycle, of testing required two visits to lab and was used to determine what day in the menstrual cycle ovulation would occur. Ovulation was detected using an over the counter ovulation test (One Step Ovulation Test Strips, Babi, Beijing, China) that tests urine for a surge in luteinizing hormone. A positive test indicated ovulation would occur within 24-48 hours. The number of days between menses and ovulation were calculated for subjects and used to estimate when ovulation would occur in the next month of testing. In Months 2-4 of testing, subjects were required to come to lab four days before estimated day of ovulation and each day until three days after ovulation actually occurred. On testing days, subjects walked for 10 minutes at 5.15 km/hr on a treadmill, had AKL measured on their right knee, and gave a saliva sample. Subjects were advised to avoid eating 60 minutes prior to sample collection and to rinse their mouth thoroughly with water 10 minutes before the sample was collected. Specimens were stored and frozen (temperature range -20.6 to -15°C) until day of assay when specimens were thawed and the assay procedure was performed to determine estradiol concentrations (17 β -Estradiol Saliva Luminescence Immunoassay, IBL International, Hamburg, Germany) [12].

Data Processing

A custom MATLAB program was used to process the AKL data saved from during a testing session. Signals were converted to appropriate units (Newtons for force and millimeters for displacement) using calibration equations. Net force was calculated by adding the ankle and tibia forces and filtered using a recursive, 6th degree low-pass Butterworth filter with a cutoff frequency of 5 Hz. Relative tibia displacement data were calculated by subtracting the femur position from the tibial tuberosity position and filtered using a 50-point moving average filter.

Filtered force-displacement data were plotted for each test and AKL was calculated at a force threshold of 200 N (Figure 1). Trials were not considered if quadriceps or hamstring muscle activity exceeded a maximum threshold. AKL = 4 mm 199

Figure 1: Example of a force displacement curve used to determine AKL at 200 N. For this trial 200 the subject had an average AKL of 4 mm.

Saliva samples were processed and then read by a microplate reader (FLUOstar Omega Microplate Reader, BMG Lab Tech, Ortenberg, Germany). The micro plate reader measured Relative Luminescence Units (RLU) for each well in the plate. The RLU of the standards were used to create a standard curve. Per kit recommendations, a cubic spline fit was used to determine concentration values.

Data Analysis

For Months 2-4, the values of AKL and salivary estradiol concentration were determined for each subject at menses and an average was taken around ovulation (day before ovulation, day of ovulation, and day after ovulation). An average AKL was determined around ovulation for comparison to an average estradiol concentration also taken at that time. An average estradiol concentration was determined to account for any possible variability or unknowns caused by estradiol concentration kit analysis or the subjects not following instructions prior to a test session. The values at menses were subtracted from the values averaged around ovulation.

Appropriate statistical tests to achieve the desired objectives and test the hypothesis were selected following consultation with a statistician from our institution's statistics laboratory. Linear regression analysis was used to test the hypotheses that AKL varies linearly with estrogen concentration and this linear relationship

does not vary significantly between months in healthy women with regular menstrual cycles. A correlation coefficient was calculated for a given subject and month using a parametric (Pearson) correlation. These correlations were compared using Fisher-Z-transformation (Equation 1) and an equality test of correlations (Equation 2). Equation 1 is approximately normally distributed with variance $1/(n-3)$, if the correlation is based on n observations. Equation 2 is approximately normal with mean 0 and variance 1 if the two correlations are equal. The value found using Equation 2 was converted to a p-value using an online calculator (<http://vassarstats.net/tabs.html?#z>). If the p-value is less than 0.05, then it can be concluded that the correlations are significantly different and the null hypothesis that correlations are equal is rejected. If the p-value is greater than 0.05, then it cannot be concluded that the correlations are significantly different but there is not enough evidence to reject the null hypothesis that correlations are equal.

$$\text{Equation 1: } Z = \frac{1}{2} \ln \left(\frac{1+r}{1-r} \right) \quad \text{Equation 2: } (Z_1 - Z_2) / \sqrt{2/(n-3)}$$

Time-delays were incorporated into the linear relationship between AKL and estradiol concentration. For a given estradiol concentration, the correlated AKL value was taken up to 4 days later. Relationships with at least three data points were analyzed. Coefficients of determination were found for each relationship (each month for each subject). The number of instances a time-delay had the highest coefficient of determination for a given subject and month was identified.

Results

Twenty subjects enrolled in the study and thirteen completed the study. Seven chose to discontinue participation in the study due to starting a contraceptive (two), lack of time (three), a leg injury (one), and an unknown reason (one). Thus, results are presented for the 13 subjects who completed the study (age 21 ± 1 years (mean $\pm$ STD dev), height 1.62 ± 0.06 m, mass 63.4 ± 13.0 kg, and BMI 23.9 ± 4.2 kg/m²).

AKL

AKL varied between 0.73 and 8.89 mm across all subjects and months. Monthly average AKL at menses and averaged ovulation ranged from 2.13-7.14 mm (4.07 ± 1.38 mm) and from 1.92-6.60 mm (4.28 ± 1.27 mm), respectively (Table 1). Monthly average change in AKL from menses to averaged ovulation ranged from -0.76-1.35 mm (0.17 ± 0.61 mm). All subjects had an increase in AKL between menses and averaged ovulation for at least one month. Nine subjects had a decrease in AKL between menses and averaged ovulation for at least one month.

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Between menses and averaged ovulation.	Monthly Average Menses Laxity (mm)	Monthly Stdev Menses Laxity (mm)	Monthly Average Ovulation Averaged Laxity (mm)	Monthly Stdev Ovulation Averaged Laxity (mm)	Change in Laxity (menses to ovulation avg) (mm)	Standard Deviation of Change in Laxity (mm)
Value						
average	4.07	0.79	4.28	0.47	0.17	0.76
Stdev	1.38	0.35	1.27	0.25	0.61	0.51

Table 1: Monthly average AKL values at menses, averaged around ovulation, and difference.

The difference in AKL at menses and ovulation, and the difference in the AKL difference between menses and averaged ovulation between months were 1.02 ± 0.80 mm, 0.65 ± 0.49 mm, and 0.93 ± 0.92 mm, respectively. Of the 39 months compared, more than half had differences that were considered highly variable (Table 2).

Quantity	Months highly variable	Months less than highly variable	Months No data
menses AKL at 200 N	22	13	4
ovulation avg AKL at 200 N	17	16	6
Δ AKL at 200 N	30	1	8

Table 2: Total number of months of all subjects that were highly variable as defined as having a AKL percent difference between months greater than or equal to a magnitude of 13%.

Value	Monthly Average Menses Estradiol Concentration (pg/mL)	Monthly Stdev Menses Estradiol Concentration (pg/mL)	Monthly Average Ovulation Averaged Estradiol Concentration (pg/mL)	Monthly Stdev Ovulation Averaged Estradiol Concentration (pg/mL)	Change in Estradiol Concentration (menses to ovulation avg) (pg/mL)	Standard Deviation of Change in Estradiol Concentration (pg/mL)
average	4.0	1.8	5.2	1.7	1.1	2.5
Stdev	2.6	1.3	2.6	0.9	1.3	1.2

Table 3: Monthly average salivary estradiol concentrations at menses, averaged ovulation, and Difference between menses and averaged ovulation.

The difference in estradiol concentration at menses and ovulation, and the difference in the estradiol concentration difference between menses and averaged ovulation between months were 2.6 ± 2.2 pg/mL, 1.7 ± 1.6 pg/mL, and 2.7 ± 1.7 pg/mL, respectively. Of the 39 months compared, more than half had differences that were considered highly variable (Table 4).

Quantity	Months highly variable	Months less than highly variable	Months No data
menses Estradiol	24	11	4
ovulation avg Estradiol	11	22	6
Δ Estradiol	25	6	8

Table 4: Total number of months of all subjects that were highly variable as defined as having an estradiol concentration percent difference between months greater than or equal to a magnitude of 40%.

Laxity-Estradiol Relationships

The hypothesis was tested by using a linear regression analysis to determine the relationships between AKL and estradiol concentration within a subject within a menstrual cycle for three months. The linear relationships between AKL and estradiol concentration within a menstrual cycle were graphed (example shown in Figure 2). The relationships between AKL and estradiol concentrations for each month for each subject and corresponding coefficients of determination were determined. The average coefficient of determination was 0.13 ± 0.17

Figure 2: Linear relationships between AKL (taken at 200N) and salivary estradiol concentrations for a subject. A: Month 2 relationship. B: Month 3 relationship. C: Month 4 relationship.

Correlations were compared using Fisher-Z-transformation and an equality test of correlations and converted into p-values. The p-values ranged from 0.0006-0.4900 (0.2489 ± 0.1375). Out of 37 conditions, there were 4 p-values less than 0.05 for AKL. Time-delays of up to 4 days were analyzed for the AKL-estradiol relationship. The highest percentage of months with the highest coefficient of determination for a given subject and month was a 3-day time-delay at 30%.

Discussion

The purpose of this study was to test the hypothesis that AKL varies linearly with estrogen concentration and this linear relationship does not vary significantly between months in healthy women with regular menstrual cycles. Estradiol concentrations and AKL values were obtained multiple times per month.

AKL

Minimum AKL values were expected to occur at menses [1-4,30] when estradiol concentrations were low, but this was almost never the case on an individual basis. When the minimum laxity value did occur at menses for a subject, it was not consistent for each month. There were eleven subjects who had a minimum laxity value occur at menses at least once and two subjects had this occur for two months. Since this did not occur every month for each subject, this was inconsistent with what was expected to occur, but not inconsistent with other studies.

Studies on the effect of estradiol on AKL typically report averages and not individual values. The average AKL for the subjects in this study increased between menses and ovulation for Months 2 and 3, but not Month 4. The average response for Months 2 and 3 are consistent with responses reported from several studies [1-4,30]. These studies did not report individual responses. Shultz et al. (2004) reported both group and individual responses [10]. They tested 25 females aged 18-30 years old who had regular menstrual cycles. They found a positive correlation between AKL and the studied hormones (estradiol, progesterone, and testosterone). However, only nine subjects showed this trend on an individual basis, while eight subjects showed a negative correlation, the opposite of the average response [30]. Our results are consistent with these findings. It has been suggested that the maximum AKL may not be detected until 3-4 days after a peak in estradiol concentration has occurred [26]. All but three subjects had a maximum laxity value within a month occur unpredictably. However, a trend was observed where laxity increased in the day or two after ovulation was detected and then decreased again. Subjects were consistent in their individual laxity ranges between months. Overall AKL range for all subjects was between 1.23 and 8.89 mm at 200 N. This range includes values outside reported values for females (average values between 3.77 mm [14] and 9.1 mm [31] at 133 N). The average change in laxity was between 0.97 and 3.07 mm at 200 N,

which is consistent with a change between 1 and 5 mm as reported by other studies at 133 N [4,26].

Between months, AKL varied between 0.9 and 2.73 mm at menses, between 0.02 and 1.54 mm at averaged ovulation, and the difference in AKL between menses and averaged ovulation varied between 0.04 and 3.43 mm. Considering a 13% change in AKL to be highly variable [16-20] out of the 35 comparisons between months at menses, 22 (63%), were highly variable. Out of the 33 comparisons between months at averaged ovulation, 17 (52%), were highly variable. Out of the 31 comparisons between months for the difference between menses and averaged ovulation 30 (97%) were highly variable. It can be concluded that AKL at menses, near ovulation, and the change in AKL between menses and averaged ovulation in a given cycle, can be highly variable between cycles.

Estradiol Concentration

Minimum estradiol concentration was expected to occur at menses. This expected outcome was observed in at least one month for all but one subject, at least two months in six subjects, and three months for two subjects. Since this did not occur every month for each subject, this was inconsistent with what was expected. Estradiol concentrations were expected to increase post menses, peak before ovulation was detected, and then decrease. This trend was observed for at least one month in all subjects, at least two months for twelve subjects, at least three months for seven subjects, and four months for one subject. Though this trend was observed within months, the values between months were not consistent for an individual. Subjects were consistent in their individual estradiol concentration ranges between months. Overall salivary estradiol concentration range for subjects was between 0.001 and 14.8 pg/mL (not including outlier data). This range is consistent with the values stated in the salivary immunoassay kit instructions used for testing the estradiol concentration (values ranged between 0.8 and 14.3 pg/mL for 18 females between the ages of 19 and 43) [1]. Average change in estradiol concentration between menses and ovulation was between 0.1 and 8.2 pg/mL.

According to the salivary immunoassay kit instructions, the lower 10% of the population studied had approximately a 2.7 pg/mL change between menses and ovulation and the top 90% had approximately a 10.5 pg/mL change between menses and ovulation [12]. There was an increase in estradiol concentration between menses and averaged ovulation 70% of the time. Between months, estradiol concentrations varied between 0.1 and 8.3 pg/mL at menses, 0.0 and 6.3 pg/mL at averaged ovulation, and the difference in concentration from menses to averaged ovulation varied between 0.0 and 6.7 pg/mL. Considering a 40% change in estradiol concentration to be highly variable [11], out of the 35 comparisons between months at menses, 24 (69%) were highly variable. Out of the 33 comparisons between months at averaged ovulation, 11

(33%) were highly variable. Out of the 31 comparisons between months for the difference between menses and averaged ovulation, 25 (81%) were highly variable. It can be concluded the averaged ovulation estradiol concentration will not be highly variable between cycles but the estradiol concentration at menses and the change in estradiol concentration between menses and average ovulation can be highly variable.

AKL-Estradiol Relationships

The relationship between AKL and estradiol concentration was expected to be linear and therefore a linear relationship was tested [26,30]. Of the 80 relationships, 47 had a coefficient of determination, or R^2 value, below 0.1. Only one subject had a coefficient of determination above 0.6 with 0.84 for the relationship for Month 3. Estradiol concentration changes alone are not sufficient to predict AKL changes, supporting previous findings [26]. The relationship between estradiol and AKL was significantly different for at least one month for four subjects. Because the p-value was greater than 0.05 for the other subjects and month comparisons, we cannot conclude that the correlations were significantly different, but there is not sufficient evidence to conclude that they are not significantly different. The evidence indicates that for some women the relationship between estradiol and AKL varies between months. From the time delay analysis, a 3-day delay between AKL and estradiol concentration resulted in a higher coefficient of determination more often than any of the other time-delays. This is consistent with previous studies that reported maximum AKL may not be detected until 3-4 days after a peak in estradiol concentration [26].

Limitations

Limitations of this study included the equipment used and how often data were collected. Although the custom knee arthrometer was used by one user when collecting AKL data and repeatability was confirmed before the main study, some of the AKL measurements may have varied. The straps used at the calf and ankle were tightened as much as the user could tighten them. The thickness of the skin, subcutaneous fat, and muscle could affect how well these straps tightened and stayed tight. Subjects were frequently asked if any of the straps felt loose and were readjusted when necessary. It was assumed that there was minimal soft tissue compression during testing since a relatively constant force was applied on the skin around the patella and that the deformation of the material between the surface of the skin and femur did not change during testing [21].

Another limitation was the use of EMG. Electrodes would sometimes slip due to sweat. There were instances when the EMG signals failed (10.5% of test sessions) and it was assumed that the muscles were not active during that test. If the muscles were active, then the AKL values would have been lower than if the muscles

had been relaxed. To account for variability or unknowns caused by estradiol concentration kit analysis or the subjects not following instructions prior to coming into lab for a test session, data around ovulation was averaged using values the day before, day of, and day after ovulation detection. The averaged value was lower than the single day peak value. Averaging was not done around menses since there was only one day of data collection at this time. Although salivary measurements of estradiol can detect changes in the hormone, it may not be representative of the concentrations of estradiol circulating in the body, and therefore, ligament exposure. Salivary measurements were used to save time and money as well as make testing comfortable for subjects who needed estradiol measured multiple days in a row. Saliva can be inconsistent in its properties between different days of sampling. Using serum measurements could result in concentrations better indicative of concentrations at the ligament, but with the consequence of fewer subjects volunteering.

Future Work

Future work on the relationship between hormone concentrations and AKL should test every day for multiple cycles and consider more than just estradiol. This study accounted for estrogen concentrations being highest typically one to two days (up to four days) before ovulation occurs and that the peak AKL may not occur until 3-4 days after a peak in estradiol concentration by collecting data in the days preceding and days following ovulation [13,26]. Only a single day of testing was performed at menses. Including more days of testing would allow averages to be taken around menses as well as to explore more time shifts in the relationship between estradiol concentration and AKL.

Conclusion

The purpose of this study was to investigate if AKL would vary linearly with estrogen concentration and if this linear relationship would vary between months in healthy women with normal menstrual cycles. The averaged ovulation estradiol concentration will not be highly variable between cycles, but the estradiol concentration at menses and the change in estradiol concentration between menses and average ovulation can be highly variable. AKL at menses, averaged ovulation, and the change in AKL between menses and averaged ovulation in a given cycle, can be highly variable between cycles. Estradiol concentrations do not correlate well with menses and ovulation, in 18-26-year-old healthy women with a regular menstrual cycle. AKL varies on average between 1 and 3 mm throughout a menstrual cycle in 18-26-year-old healthy women with a regular menstrual cycle. Estrogen or estradiol concentration changes alone are not sufficient to predict AKL changes.

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