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The Correlations between Estradiol, Estrone, Estriol, Progesterone, and Sex Hormone-Binding Globulin and Anterior Cruciate Ligament Stiffness in Healthy, Active Females

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ABSTRACT

Background: Injury to the anterior cruciate ligament (ACL) often requires surgery and extensive rehabilitation. Women who participate in collegiate sports and military drills are more likely to injure their ACL than are men participating in similar activities. The influence of the normal fluctuation of sex hormones on the physical properties of the ACL is one potential cause for this disparity. The purpose of this study was to report the correlation between estradiol, estrone, estriol, progesterone, and sex hormone binding globulin (SHBG) and ACL stiffness during three phases of the menstrual cycle in normally cycling, healthy females.

Methods: We tested ACL stiffness and collected blood from 20 female subjects who were not using oral contraception during three phases of their menstrual cycle. Ligament stiffness was tested with the KT-2000™ knee arthrometer (MEDmetric, San Diego, CA). Concentrations of estradiol and SHBG were assessed via radioimmunoassay (RIA). Progesterone, estriol, and estrone concentrations were determined via enzyme-linked immunoassay.

Results: Spearman rank correlation analysis indicated a significant correlation between estradiol concentration and ACL stiffness (-0.70, $p < 0.001$) and estrone concentration and ACL stiffness near ovulation (0.46, $p = 0.040$). With the effects of the other variables controlled, there was a significant partial correlation between estradiol (-0.80, $p < 0.001$), estriol (0.70, $p = 0.003$), and progesterone (0.66, $p = 0.005$) and ACL stiffness near ovulation.

Conclusions: Our results indicate that there is a significant correlation between estradiol, estriol, and progesterone and ACL stiffness suggesting that fluctuating levels of sex hormones may influence the stiffness of the ACL near ovulation. Future studies that examine the relationship between sex hormones and the physical properties of the ACL should be focused near the ovulation phase of the menstrual cycle.

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INTRODUCTION

ANTERIOR CRUCIATE LIGAMENT (ACL) INJURY is a serious condition that often requires surgical reconstruction and extensive rehabilitation. Women are 2.75[1]–9.74[2] times more likely to injure their ACL than men participating in similar activities. These findings are consistent in intercollegiate basketball and soccer,[1] scholastic basketball,[3] and military training.[2] Several investigatiors have examined potential causes for this disparity in injury rate. One area that has received significant attention is the influence of fluctuating concentrations of sex hormones on the physical properties of the ACL.

Receptor sites for estradiol and progesterone have been identified on human and rabbit ACL tissue.[4,5] *In vitro* studies have shown that ACL tissue exposed to increased concentrations of estradiol has decreased fibroblast proliferation and a reduction in collagen formation.[6–8] Conversely, increased concentrations of progesterone have been associated with increased fibroblast proliferation and collagen formation.[8]

Previous *in vivo* studies that have quantified the influence of circulating levels of sex hormones have reported conflicting results. Slauterbeck et al.[9] showed that ovariectomized rabbits who were given supraphysiological concentrations of an estradiol analog had ACLs with a lower load to failure than rabbits who were not given the analog. Strickland et al.[10] and Belanger et al.[11] on the other hand, reported no changes in the physical properties of the ACL in rats and ovariectomized and normally cycling sheep exposed to different levels of estradiol and estradiol analogs throughout a normal menstrual cycle.

In humans, the influence of hormone levels on ACL laxity, or change in ligament length, has also been inconclusive. Heitz et al.[12] found changes in ACL laxity during the ovulation and luteal stages of the menstrual cycle of young females, when estradiol levels were at their highest concentrations. In a similar study, Karageanes et al.[13] reported no changes in ACL laxity among three phases of the menstrual cycle in high school-aged girls. These two previous studies examined the relationship between sex hormone level or menstrual cycle phase with ACL laxity. In our laboratory, we have identified a significant relationship between estradiol concentration and ACL stiffness near ovulation, when estradiol concentrations are near their peak levels.[14] These findings suggest that as the ACL is exposed to increased concentrations of estradiol near ovulation, there may be fewer collagen fibers to resist the load placed on the ligament, causing decreased stiffness. It is unclear how long this exposure to estradiol may have to last to cause measurable changes in stiffness.

Although estradiol and progesterone have been the focus of the research on sex hormones and ACL remodeling, there are also other sex hormones and binding globulins that circulate throughout a normal menstrual cycle that may also influence the ACL. Estrone and estriol are two other circulating estrogens.[15] Estrone is formed from androgens secreted by the adrenal cortex and ovarian thecal cells.[15] Even though estrone is 12 times less potent than estradiol, it still has some physiological effects on target tissue.[15] Estriol is an oxidative by-product of estrone and has a shorter-term physiological effect on target tissues compared with the other two estrogens.[15,16] To date, we have found no studies that attempted to investigate the influence of estrone and estriol on the physical properties of ligamentous tissue.

Sex hormone-binding globulin (SHBG) is a glycoprotein that has a strong affinity for estradiol and fluctuates with changing concentrations of estradiol and progesterone throughout the menstrual cycle.[17] As estradiol levels increase near ovulation, SHBG levels also increase to help maintain the physiological balance with progesterone.[15,18] The interactions among SHBG, estradiol, and the SHBG binding site expression influence the signal transduction pathway, which has been shown to modulate the effects of estrogen on other target tissues.[19,20] It is possible that SHBG may influence estrogen's role in ligamentous tissue remodeling as well.

The influence of sex hormones and SHBG on the physical properties of the ACL in females is still unclear. Although previous studies have investigated the influence of progesterone and estradiol on the physical properties of the ACL *in vitro*, on animals, and in humans, we are unaware of any studies that have used an *in vivo* model to study the influence of estrogen, progesterone, and SHBG on the stiffness of the ACL in healthy, active women. As a result, the purpose of this study is to report the correlation between estradiol, estrone, estriol, progesterone, and SHBG

and ACL stiffness during three phases of the menstrual cycle in normally cycling, healthy females.

MATERIALS AND METHODS

Subjects

Prior to participation, subjects read and signed a written informed consent form approved by the university Institutional Review Board and completed a health history questionnaire. A physician conducted a brief examination and interview to determine if subjects met the inclusion criteria for the study. The subject's collateral and cruciate ligaments were manually tested via Lachman, posterior drawer, and varus and valgus stress tests. An initial test with a device used to measure ACL laxity and stiffness, the KT-2000™ arthrometer (MEDmetric, San Diego, CA), was conducted to familiarize the subjects with the instrument. Subjects were eligible to participate in the study if they were between 18 and 40 years old, had intact collateral and cruciate ligaments bilaterally, had a regular menstrual cycle lasting between 28 and 32 days for 3 months prior to participation in the study, and exercised for at least 3 days a week for a minimum of 20 minutes per day. Exclusion criteria included an irregular menstrual cycle; a history of pregnancy; any known medical problems that may have influenced the normal fluctuation of hormones during their menstrual cycle (cardiovascular, neurological, or orthopedic disease); previous ligamentous, tendinous, capsular, or meniscal injury to the knee; consumption of more than an average of one alcoholic beverage per day, cigarette smoking; using oral contraception; or being considered elite athletes (marathon runner, elite-Olympic level athlete).

This study was part of a larger project that investigated the influence of sex hormones on ACL stiffness. Thirty-two subjects volunteered to participate in this project. Of this group, 6 did not meet the enrollment criteria, 3 did not comply with the study protocol for reporting to the primary investigator, 1 withdrew during data collection, and 1 became pregnant during the course of the study. The data of 1 subject were eliminated because her assay indicated an estradiol concentration above physiological levels at onset of menses (555.04 pg/ml). All other samples were within normal physiological levels. Twenty female subjects completed this study (mean age = 25.9 years ± 5.1; mean height 166.2 cm ± 8.4; mean weight 71.3 kg ± 25.9).

Experimental procedures

Subjects were randomly assigned in nearly equal proportion to one of three experimental groups. Group one began the protocol at the onset of menses, group two began the protocol near ovulation, and group three began between days 22 and 24 of their cycle.

An exercise log was completed by each subject throughout their participation in the study. They recorded the type of activity they performed (weightlifting, running, aerobics) and how long the activity lasted. Subjects also marked the day they began menses, the day they ovulated, and the day between days 22 and 24 of their cycle when they were tested. Subjects were instructed not to exercise for 1 hour prior to testing with the KT-2000.

Onset of menses was defined as that point when a subject required feminine protection. Ovulation was determined with the OvuQuick™ One-Step Ovulation Predictor (Quidel Corp., San Diego, CA). Subjects began using the ovulation kit approximately 10–12 days after the onset of their menstrual cycle, and each test stick was used one time according to the manufacturer's instructions. Subjects continued to use the test kit daily until the result was positive. Ovulation lasts 24–36 hours in a normally cycling female.[15] Thus, it is possible that KT-2000 arthrometry and blood draws were completed several hours after the peak in estradiol concentration. We referred to this phase in the menstrual cycle as "near ovulation" to reflect this possibility.

Subjects contacted the principal investigator (W.R.) at the onset of menses, when they had a positive test with the ovulation kit, and between days 22 and 24 of their cycle. The primary investigator then scheduled a KT-2000 examination with a member of the research staff and scheduled a blood draw at the Veterans Administration or University Hospital phlebotomy laboratories within 24 hours of the onset of menses or a positive ovulation test and between days 22 and 24 of their cycle. All researchers and phlebotomists except for the principal investigator

were blinded as to the phase of the menstrual cycle the subject was in at the time of testing. The two examiners collecting data with the KT-2000 established their intratester reliability above the 0.91 and 0.96 levels prior to the study (ICC 3,1).

The KT-2000 knee arthrometer and X-Y plotter were used to measure the stiffness of the right ACL with the knee at 30 degrees of flexion. Testing was completed according to the manufacturer's instructions, with one modification. We placed a towel roll between the feet of the subject to restrict tibial rotation (Fig. 1). Force-displacement curves were drawn with an X-Y plotter. The x and y axes indicated pounds of force and millimeters of displacement, respectively. During each of the three testing sessions, three force-displacement curves were produced.

The force-displacement curve, illustrated by the X-Y plotter, was used to determine the stiffness measurements between 20 and 30 pounds of force.[21] Pounds of force were converted to newtons (N). The change in force (45 N) between 89 N and 134 N was divided by the difference between the mean tibial displacement measurements (mm) at 89 N and 134 N. The curve with the greatest of the three displacements during the testing at each phase of the cycle was used for statistical analysis.

Subjects had 11 ml of blood drawn into a red-top Vacutainer within 24 hours of the onset of menses and ovulation and between days 22 and 24 of their cycle. Blood was centrifuged at 4°C and 3000 rpm to separate the serum from cells. The serum was transferred into two plastic 5-ml test tubes and stored at −71°C until assay analysis. All samples were run at the same time in each assay. Estradiol and SHBG serum concentrations were determined via radioimmunoassay (RIA) (Diagnostic Systems Laboratory, Inc., Webster, TX). Progesterone, estrone, and estriol serum concentrations were determined via enzyme-linked immunoassay (Diagnostic Systems Laboratory, Inc.). Intraassay and interassay coefficients of variation (CV) (%) for each assay were as follows: progesterone (4.6, 5.1), estrone (3.92, 2.17), estriol (5.3, 3.5), estradiol (5.3, 9.3), and SHBG (2.2, 4.4).

Statistical methods

Descriptive statistics. Along with the mean and standard error, we characterized the sample distributions for estradiol, estriol, estrone, progesterone, SHBG, and ACL stiffness using the geometric mean,[22] the interquartile range,[23] and the minimum and maximum value of the distribution as descriptive measures. The geometric mean and the interquartile range are more robust measures of centrality and dispersion, respectively, than are the arithmetic mean and standard error when the data are nonnormally distributed.

Analysis of variance. All the hormone and SHBG data and the ACL stiffness data were transformed to the logarithmic scale prior to the analysis of

FIG. 1. KT-2000 knee arthrometer (MEDmetric, San Diego, CA) applied to subject's knee. Note the addition of a towel between the subject's feet to help maintain neutral hip and tibia rotation during testing. The compliance index used to determine ligament stiffness is drawn on the X-Y plotter at right.

variance (ANOVA). The measurement rescaling functioned as a variance stabilizing transformation.[24] Comparisons between the hormone and SHBG concentration and ACL stiffness during the onset of menses, near ovulation, and during the luteal phase were conducted via mixed-effect ANOVA.[25] Model parameter estimation was based on the residual-maximum likelihood principle,[26] and the within-subject variance-covariance matrix was modeled in the compound symmetry form[27] to account for the within-subject serial correlation between the pairs of observations from the same person. The paired comparisons between each of the three menstrual stages are presented as the ratio of geometric means. The geometric mean is a location parameter that is similar to the arithmetic mean and median, and its value was computed by simply taking the antilogarithm of the ANOVA estimate for the mean of the logarithmic transformed distribution. The ratio of geometric means is often interpreted as

the fold change in the geometric mean. Under the null hypothesis, we assumed the geometric mean of the distribution was equal at each of the three menstrual stages or, equivalently, that the ratio of geometric means was equal to 1. All of our hypothesis were formulated *a priori*, and a comparison significance level of $p \leq 0.05$ was used as the criterion for rejecting the null hypothesis.

Spearman correlations. We found in our previous work that even though subjects may have regular menstrual cycles lasting 28–32 days, there are variations in the concentration and the rate of fluctuation of sex hormones at the three phases of the menstrual cycle.[12] Accordingly, we examined the relationship between the hormone parameters (estradiol, estriol, progesterone, and SHBG) and ACL stiffness by a nonparametric analysis that does not require specification of the error distribution. The values of each of the hormone parameters and ACL stiffness were rank or-

TABLE 1. DISTRIBUTION OF EACH HORMONE VARIABLE, SHBG, AND ACL STIFFNESS

Variable	n	Menses	Ovulation	Luteal
Estradiol (pg/ml)	20	41.4 ± 4.7[a] 37.6[b] (29.9, 41.4)[c] [18.9, 100.6][d]	104.2 ± 11.0 92.2 (69.7, 124.1) [28.5, 224.5]	88.7 ± 11.2 73.6 (61.1, 107.1) [15.7, 204.5]
Estriol (ng/ml)	20	0.05 ± 0.01 0.03 (0.01, 0.07) [0.005, 0.12]	0.06 ± 0.01 0.05 (0.03, 0.07) [0.01, 0.12]	0.05 ± 0.01 0.04 (0.03, 0.07) [0.003, 0.19]
Estrone (ng/ml)	20	116.0 ± 11.3 107.9 (78.5, 129.4) [58.1, 285.8]	180.8 ± 12.6 172.6 (146.8, 207.1) [88.9, 298.5]	163 ± 14.1 150.8 (125.1, 195.5) [44.5, 295.0]
Progesterone (ng/ml)	20	6.4 ± 2.1 4.0 (1.0, 45.8) [1.0, 45.8]	10.3 ± 1.7 7.4 (0.5, 27.3) [0.4, 27.3]	11.1 ± 2.6 5.9 (1.0, 37.5) [1.0, 37.5]
SHBG (nmol/ml)	20	41.9 ± 4.0 38.6 (27.5, 52.2) [16.2, 82.2]	40.2 ± 4.3 35.7 (27.3, 56.0) [13.0, 74.2]	44.0 ± 4.7 39.4 (29.7, 61.2) [17.2, 91.8]
ACL stiffness (N/mm)	20	41.7 ± 5.2 36.2 (25.1, 50.3) [14.7, 97.8]	40.3 ± 5.0 34.7 (21.7, 58.2) [13.1, 97.8]	40.4 ± 4.5 35.0 (22.8, 54.9) [11.9, 70.3]

[a]Mean ± SE.
[b]Geometric mean.
[c]Interquartile range.
[d]Minimum, maximum.

dered from 1 to 20 based on their magnitude. Nonparametric Spearman rank-order correlation coefficients (r_s)[28] were used to estimate the correlation between the rank-ordered values of each hormone parameter and the rank-ordered values of ACL stiffness. Nonparametric Spearman partial rank-order correlation coefficients (r_{sp})[29] were estimated to quantify the correlation between the ranks of the hormone variables and ACL stiffness at each of the three stages of the menstrual cycle while controlling for the influence of the other hormone parameters. Note that r_s and r_{sp} are similar to the Pearson's product-moment correlation coefficient (r) in that the value of r_s and the value of r_{sp} are restricted to the closed interval $[-1, 1]$, with an r_s or r_{sp} value of -1 signifying perfect negative correlation between the paired ranks, and an r_s or r_{sp} of 1 signifying perfect positive correlation between the paired ranks. Because of the small sample size, percentile confidence intervals (CI) for r_s and r_{sp} were estimated via the nonparametric bootstrap resampling method.[30] The limits of the CI were estimated based on 1000 bootstrap random samples from the original sample of data. All our hypotheses of association were formulated *a priori*, and a univariate significance level of $p \leq 0.05$ was used as the criterion for rejecting the null hypothesis of no association. All ANOVA calculations were carried out in SAS version 8.2 (SAS Institute Inc., Cary, NC) with the PROC MIXED procedure, and the Spearman correlation analyses were carried out in Splus version 2000 (Insightful Inc., Seattle, WA).

RESULTS

The mean ± SE, the geometric mean, the interquartile range, and the minimum and maxi-mum values of the distribution of the measurements of estradiol, estriol, estrone, progesterone, SHBG, and ACL stiffness in the three phases of the menstrual cycle are listed in Table 1. ANOVA comparisons of estriadiol, estriol, estrone, progesterone, SHBG, and ACL stiffness across menstrual stage are summarized in Table 2. The ratios of the geometric means for estradiol concentrations indicated significant changes between onset of menses and near ovulation (2.45 [1.77, 3.38], $p < 0.001$) and onset of menses and the luteal phase (1.96[1.32, 2.90], $p = 0.002$) and approached significance between near ovulation and the luteal phase (1.25[0.97, 1.61], $p = 0.079$). Estrone concentration ratios also indicated significant changes between onset of menses and near ovulation (1.60[1.34, 1.91], $p < 0.001$) and onset of menses and the luteal phase (1.40[1.17, 1.66], $p < 0.001$). The ratio for SHBG was significant between near ovulation and the luteal phase (0.91[0.84, 0.98], $p = 0.019$) and approached significance between the onset of menses and near ovulation (0.93[0.85, 1.00], $p = 0.061$). The ratios for ACL stiffness and the other hormone variables did not indicate significant changes between menstrual cycle phases.

The Spearman rank correlations between the hormonal variables, SHBG, and ACL stiffness are shown in Table 3. A significant negative correlation existed between estradiol and ACL stiffness $(r_s = -0.70[-0.85. -0.51], p < 0.001)$ near ovulation, indicating that as estradiol concentration increased, ACL stiffness decreased. In addition, a significant positive relationship existed between estrone concentration and ACL stiffness near ovulation $(r_s 0.46[0.13,0.79], p = 0.040)$. As estrone concentration increased, ACL stiffness also increased. No significant correlation was observed between estriol, progesterone, or SHBG and ACL stiffness at all three phases of the menstrual cycle.

TABLE 2. INTERMENSTRUAL STAGE ANOVA COMPARISONS OF HORMONES, SHBG, AND ACL STIFFNESS GEOMETRIC MEAN

	Ratio of geometric mean					
	Near ovulation/menses		Luteal/menses		Near ovulation/luteal	
Parameter	ratio	p	ratio	p	ratio	p
Estradiol	2.45 [1.77, 3.38][a]	<0.001	1.96 [1.32, 2.90]	0.002	1.25 [0.97, 1.61]	0.079
Estriol	1.50 [0.93, 2.41]	0.092	1.15 [0.72, 1.85]	0.551	1.30 [0.81, 2.10]	0.268
Estrone	1.60 [1.34, 1.91]	<0.001	1.40 [1.17, 1.66]	<0.001	1.14 [0.96, 1.37]	0.128
Progesterone	1.84 [0.90, 3.78]	0.094	1.46 [0.71, 3.00]	0.293	1.26 [0.61, 2.59]	0.517
SHBG	0.93 [0.85, 1.00]	0.061	1.02 [0.94, 1.11]	0.605	0.91 [0.84, 0.98]	0.019
ACL stiffness	0.96 [0.79, 1.17]	0.680	0.97 [0.79, 1.18]	0.740	0.99 [0.81, 1.21]	0.935

[a]Brackets indicate 95% confidence interval.

TABLE 3. CORRELATION (r_{sp}) BETWEEN HORMONE PARAMETER RANKS AND
RANKS OF ACL STIFFNESS AT THREE STAGES OF MENSTRUAL CYCLE

	Menses		Ovulation		Luteal	
Correlates	r_s	p	r_s	p	r_s	p
Estradiol:						
ACL stiffness	−0.26 [−0.60, 0.57][a]	0.269	−0.70 [−0.85, −0.51]	<0.001	−0.24 [−0.57, 0.20]	0.305
Estriol:						
ACL stiffness	0.24 [−0.42, 0.60]	0.307	0.36 [−0.16, 0.80]	0.120	0.30 [−0.16, 0.60]	0.195
Estrone:						
ACL stiffness	0.12 [−0.18, 0.76]	0.609	0.46 [0.13, 0.79]	0.040	−0.18 [−0.55, 0.23]	0.440
Progesterone:						
ACL stiffness	−0.19 [−0.50, 0.55]	0.420	0.20 [−0.26, 0.72]	0.394	−0.11 [−0.47, 0.54]	0.654
SHBG:						
ACL stiffness	−0.11 [−0.55, 0.32]	0.652	−0.39 [−0.74, 0.04]	0.084	−0.23 [−0.60, 0.32]	0.334

[a]Brackets indicate nonparametric 95% confidence interval.

The partial correlations (r_{sp}) between the ranked hormone parameters and ranks of ACL stiffness are summarized in Table 4. Note that r_{sp} represents the correlation between the ranks of the hormone variable and ranked values for ACL stiffness with the influence of the other variables controlled. Our subjects showed a significant negative partial correlation between estradiol and ACL stiffness (r_{sp} −0.80[−0.95,−0.42], $p < 0.001$) and a positive partial correlation for estriol (r_{sp} 0.70[0.18, 0.92], $p = 0.003$) and progesterone (r_{sp} 0.66[0.23,0.90], $p = 0.003$) and ACL stiffness near ovulation. Figure 2 shows the linear relationship between the ranks of estradiol, estriol, and progesterone and the ranked partial residuals for predicting ACL stiffness. The significant correlations between estradiol, estriol, and progesterone and ACL stiffness are showed in Figure 2B, E, and H, respectively.

DISCUSSION

The purpose of this study was to report the correlations between estradiol, estrone, estriol, progesterone, and SHBG and ACL stiffness in healthy, active women during three phases of the menstrual cycle. We found that estradiol had a significant negative correlation with ACL stiffness near ovulation and that estrone was positively correlated near ovulation. This indicated that when concentrations of estradiol increased near ovulation the stiffness of the ACL decreased. When concentrations of estrone increased, ACL stiffness also increased. We recognize that sex hormones may interact with one another to effect a change on a target tissue. Given this, we used a Spearman partial correlation to determine the correlation between individual sex hormones and ACL stiffness while controlling for the influence

TABLE 4. PARTIAL CORRELATION (r_{sp}) BETWEEN HORMONE PARAMETER RANKS AND RANKS OF
ACL STIFFNESS. ORDERED RANKS OF EACH AT THREE STAGES OF MENSTRUAL CYCLE

	Menses		Ovulation		Luteal	
Correlates	r_{sp}	p	r_{sp}	p	r_{sp}	p
Estradiol:						
ACL stiffness	−0.34 [−0.76, 0.24][a]	0.203	−0.80 [−0.95, −0.42]	<0.001	−0.24 [−0.72, 0.42]	0.377
Estriol:						
ACL stiffness	0.35 [−0.34, 0.72]	0.182	0.70 [0.18, 0.92]	0.003	0.38 [−0.12, 0.78]	0.144
Estrone:						
ACL stiffness	0.06 [−0.50, 0.57]	0.813	0.26 [−0.46, 0.71]	0.324	−0.16 [−0.69, 0.38]	0.542
Progesterone:						
ACL stiffness	−0.04 [−0.58, 0.52]	0.876	0.66 [0.23, 0.90]	0.005	0.00 [−0.64, 0.64]	0.996
SHBG:						
ACL stiffness	−0.26 [−0.66, 0.33]	0.325	−0.18 [−0.73, 0.44]	0.497	−0.28 [−0.69, 0.40]	0.301

[a]Brackets indicate nonparametric 95% confidence interval.

A

B

C

D

E

F

G

H

I
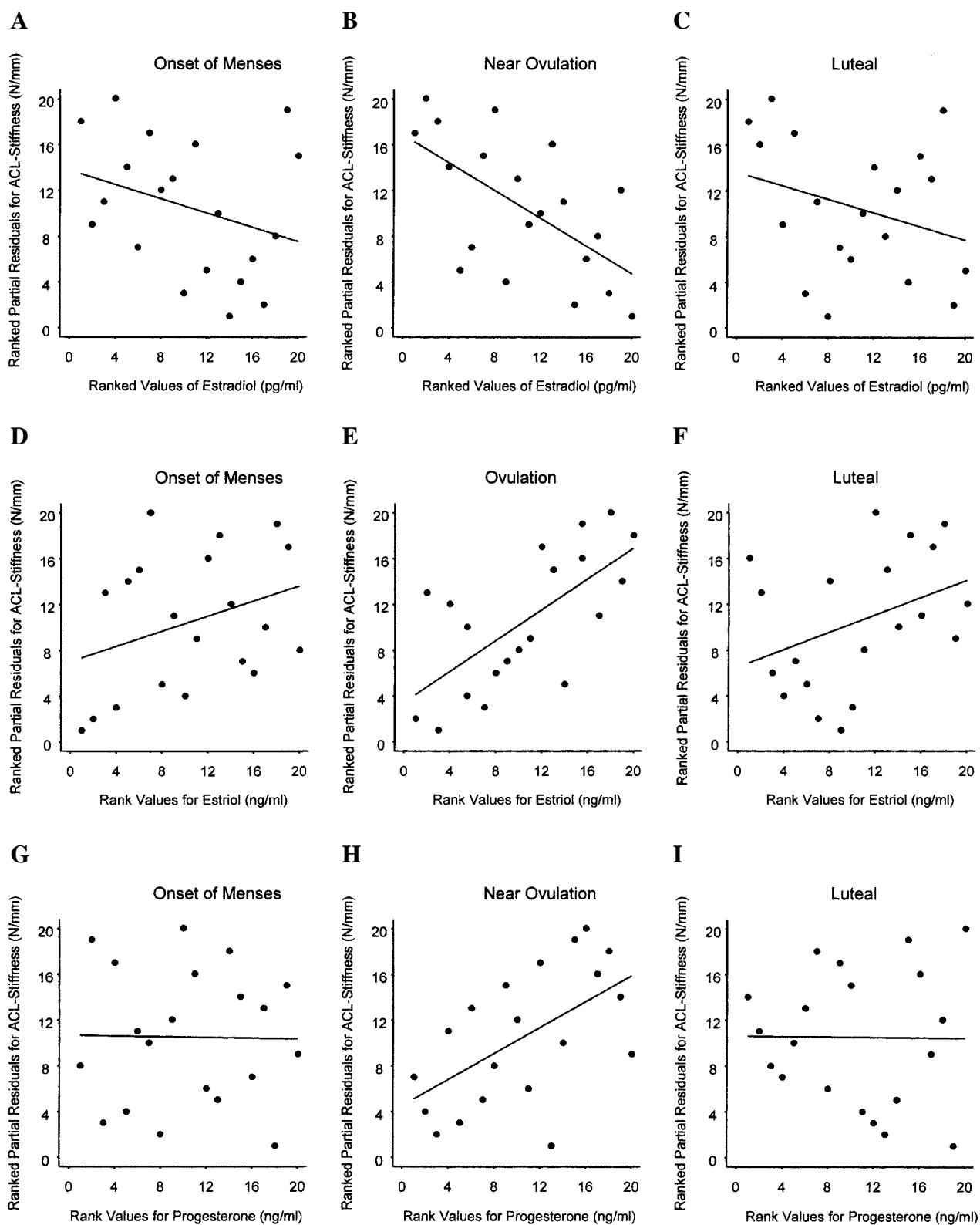
FIG. 2.   Scatter plot diagrams showing the linear relationships between the ranked partial residuals of estradiol (**A, B, C**), estriol (**D, E, F**), and progesterone (**G, H, I**]) and the ranked partial residuals of ACL stiffness at the onset of menses, near ovulation, and at the luteal phase of the menstrual cycle.

of the other hormones and binding proteins. In this way, we could estimate the influence of individual hormones on ACL stiffness without including the influence of the other variables. With this analysis, estradiol had a significant negative partial correlation with ACL stiffness, and estriol and progesterone had positive, significant partial correlations.

The significant negative relationship between estradiol and ACL stiffness is consistent with previous *in vitro* studies and findings in our laboratory. Estradiol receptors have been identified on human and rabbit ACL tissue.[4,5] Increasing concentrations of estradiol also have caused decreased type 1 collagen synthesis in human ACL tissue *in vitro*.[8] It is type 1 collagen that plays a major role in the ability of ligamentous tissue to withstand axial loads.[31] We have shown previously that estradiol is negatively correlated to ACL stiffness at ovulation in an *in vivo* model similar to the one used in this study.[14] A similar decrease in collagen synthesis due to increased exposure to estradiol may have led to the negative correlation between estradiol and ACL stiffness that our subjects showed near ovulation.

Analysis of the present study showed that the correlation between estradiol concentration and ACL stiffness was stronger when the effects of progesterone, estriol, estrone, and SHBG were controlled for through a partial correlation. This finding is consistent with findings from other studies that indicate that the effects of estradiol on target tissues can be modulated by other hormones[8] and binding proteins.[19] With those other variables controlled, the negative correlation between estradiol and ACL stiffness was greater.

Rank-order analysis showed that progesterone did not correlate with ACL stiffness near ovulation in the presence of other hormones and SHBG in the present study. However, when estradiol and the other variables were controlled in the Spearman partial analysis, a significant positive correlation existed. These findings suggest an antagonistic relationship between the increased ACL stiffness that was correlated with higher concentrations of progesterone and the decreased ACL stiffness that was correlated with increased concentrations of estradiol near ovulation. This antagonistic relationship between estradiol and progesterone has been demonstrated previously. Yu et al.[8] showed a dose-dependent increase in fibroblast proliferation and procollagen type 1 synthesis when increasing concentrations of progesterone were added to human ACL tissue *in vitro*. When applied to ACL tissue independently, estradiol and progesterone appeared to act antagonistically. These effects were mitigated when higher concentrations of estradiol were included, however, suggesting that although progesterone may enhance type 1 collagen synthesis, its effects may depend on the concentration of estradiol. It is possible that the higher concentrations of progesterone near ovulation in our subjects may have contributed to increased ligament stiffness, but that increase may have been reduced in the presence of high concentrations of estradiol.

It is not clear why there were no significant relationships between estradiol and progesterone and ACL stiffness in the luteal phase of the menstrual cycle when concentrations of both these hormones were relatively high. It is possible that estradiol only influences genetic activation of fibroblast and procollagen formation once it reaches a certain peak or threshold concentration that is attained near ovulation. Another explanation may be that the influence of estradiol and progesterone depends on the changing concentrations of estrogen and progesterone receptor expression throughout the menstrual cycle. In other connective tissues, the expression of estrogen receptor alpha, estrogen receptor beta, and progesterone receptors changes in response to changes in estradiol and progesterone concentrations.[32,33] If the number of available estrogen and progesterone binding sites change in ligamentous tissue, the influence or antagonism between estradiol and progesterone may be different near ovulation, when estradiol levels are highest, than during other phases of the menstrual cycle.

Estrone was positively correlated with ACL stiffness with rank-order analysis but not with partial correlation analysis in our subjects. In the presence of the other sex hormones and SHBG, as the concentration of estrone increased, the stiffness of the ACL also increased. This positive correlation with ACL stiffness was not present when the other sex hormones and SHBG were controlled for. This indicates that the influence of estrone on ACL stiffness may be dependent on other hormones. Estrone also demonstrated estrogenic effects that were antagonistic to those demonstrated by estradiol. Such an antagonistic relationship between estrone and other estrogens is not novel. Estrone is thought to have limited inhibition of *in vitro* aortic cell proliferation, col-

lagen synthesis, platelet-derived growth factor (PDGF)-induced smooth muscle cell migration, and mitogen-activated protein kinase activity compared with four other estrogens.[34] The effect of estrone on ligamentous tissue is unclear. We believe that this is the first study to examine the effects of estrone on the physical properties of ligament tissue.

We found no significant relationship between estriol and ACL stiffness with rank analysis. However, when the effects of the other hormones and SHBG were controlled for, there was a significant positive partial correlation with ACL stiffness near ovulation. This correlation was antagonistic to that found for estradiol. Estriol is a short-acting estrogen that has the ability to stimulate a short response in target tissues that diminishes after 4–6 hours.[16] In the long term, this response may be antagonistic to the effects of estradiol on the same tissue.[35] Although this antagonism has not been investigated in ligament tissue, it has been examined in *in vitro* aortic and uterine tissue models that showed differences in DNA synthesis, cell proliferation, and collagen synthesis.[35,36] As our subjects potentially had blood draws and stiffness examinations up to 24–36 hours after the sharp rise in estrogen concentration at ovulation, it is possible that the effects demonstrated by our subjects were due to the antagonistic long-term effects of estradiol and short-term effects of estriol on ACL stiffness.

SHBG was not significantly correlated with ACL stiffness during the three phases of the menstrual cycle on rank-order analysis. There was a trend near ovulation, however, that as SHBG concentration increased, ACL stiffness decreased. Previous work has shown that SHBG concentrations increase near ovulation to facilitate the balance between estrogen and progesterone.[18] It is possible that the correlation between SHBG and ACL stiffness that we observed in our subjects was due to the normal response of SHBG to the increases in estradiol concentration near ovulation. If this were the case, we would have expected that the correlation between SHBG and ACL stiffness would decrease when the effects of estradiol were controlled for with Spearman partial analysis. This is what we found. As a result, we believe that the trend between SHBG concentration and ACL stiffness near ovulation was a result of the normal physiological response of SHBG to rising estradiol concentrations, not a physiological influence on the ligament.

Even though we found significant correlations between sex hormones and ACL stiffness near ovulation, there are some limitations to this study. First, our subjects were examined only during one menstrual cycle. Because there are variability in hormone concentration and fluctuations within even the most consistent menstrual cycles, this sampling may or may not be an accurate indication of their hormone concentrations in previous or subsequent cycles. In addition, it is possible that hormone-modulated changes to the ACL do not occur acutely but over a longer period of time spanning several menstrual cycles. If this were the case, our protocol would not have identified these changes. It is unclear how long the ACL would have to be exposed to a sex hormone to demonstrate changes in stiffness that could be measured by the KT-2000. Although the KT-2000 has been shown to be a reliable and valid tool to measure the compliance and stiffness of injured and healthy knees, it is possible that the changes due to ACL remodeling within one menstrual cycle are too small to be detected by this device. We examined the relationship between hormone concentration and one material property of the ACL (stiffness). This study did not look at the relationship between ACL stiffness and injury rate. Thus, our conclusions can only address the relationships between individual hormones and ACL stiffness, with the understanding that changes in ACL stiffness may limit the ACL's ability to resist tensile loads that lead to injury.

CONCLUSIONS

In this study, we identified the potential relationships between individual sex hormones and SHBG with ACL stiffness. Our findings do not identify the mechanism of action of these hormones. Potential mechanistic questions that should be addressed in future studies include the role of hormone receptor expression, interactions between hormones and proteins, and the presence of a potential threshold effect necessary for ligament remodeling. We found a significant negative correlation between estradiol and ACL stiffness and a significant positive correlation between estrone and ACL stiffness near ovulation. In addition, when the influences of the other hormones and SHBG were controlled for, there was a stronger negative correlation between estradiol and ACL stiffness and a significant positive cor-

relation between estriol and progesterone and ACL stiffness near ovulation. It is not clear if sex hormone-mediated changes in ACL stiffness are large enough to predispose women to ACL injuries. As a result, future studies are needed to examine the relationship between fluctuating levels of sex hormones and the physical properties of the ACL near ovulation to better understand the mechanism of collagen remodeling in the ACL and how that remodeling influences the ACL's ability to resist the tensile loads that lead to injury.

ACKNOWLEDGMENTS

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