

Knee ligament behavior following a controlled loading protocol does not differ by menstrual cycle day

By: Christopher R. Carcia^a, Sandra J. Shultz^b, Kevin P. Granata^c, Bruce M. Gansneder^d, and David H. Perrin^e

Carcia, C.R., [Shultz, S.J.](#), Granata, K.P., Gansneder, B.M., [Perrin, D.H.](#) (2004). Knee ligament behavior following a controlled loading protocol does not differ by menstrual cycle day. Clinical Biomechanics 19:1048-1054

Made available courtesy of ELSEVIER:

http://www.elsevier.com/wps/find/journaldescription.cws_home/30397/description#description

*****Note: Figures may be missing from this format of the document**

Abstract:

Background. Females experience a disproportionate number of anterior cruciate ligament injuries compared to males. Increased estradiol concentration has been suggested to alter ligament properties and strength. Determining whether the knee responds differently to an external load at various hormonal levels may be helpful in further explaining the gender disparity.

Methods. Estradiol, progesterone and testosterone were quantified at menses, near ovulation and at the mid-luteal phase. With one knee serving as the control limb and the other as the experimental limb, displacement at 134N and stiffness between 90 and 134N were recorded with a knee ligament arthrometer on both knees before and after a loading protocol. The protocol consisted of three, 3-min, posterior to anterior normalized loads directed to the posterior calf with a ligament testing device.

Findings. The loading protocol produced a measurable increase in displacement but not stiffness. Neither displacement nor stiffness measures however were affected by day of the menstrual cycle. No consistent relationships between hormonal concentrations and displacement or stiffness were evident.

^a Department of Physical Therapy, Rangos School of Health Sciences, Duquesne University, Pittsburgh, PA 15282, USA

^b Graduate Programs in Athletic Training and Sports Medicine, University of North Carolina at Greensboro, School of Health & Human Performance, Department of Exercise and Sport Science, 237B HHP Building, P.O. Box 26170, Greensboro, NC 27402, USA

^c Department of Engineering Science & Mechanics, Virginia Tech, 307 Norris Hall, Blacksburg, VA 24061, USA

^d Curry School of Education, P.O. Box 400265, Ruffner Hall, 268, University of Virginia, Charlottesville, VA 22904, USA

^e School of Health & Human Performance, University of North Carolina at Greensboro, 400-A HHP Building, P. O. Box 26169, Greensboro, NC 27402, USA

Interpretation. Following a controlled, static external load, displacement and stiffness were not affected differently by day of the menstrual cycle.

Keywords: ACL; Estradiol; Displacement; Stiffness; KT-2000

Article:

INTRODUCTION

Despite meticulous research efforts over the last decade, females continue to experience a disproportionate number of anterior cruciate ligament (ACL) injuries compared to males (Arendt et al., 1999; Gwinn et al., 2000). To assist with explaining the disparity, identified risk factors have been classified as anatomical, environmental, neuromuscular and hormonal (Griffin et al., 2000). Some work (Wojtys et al., 2002) has suggested the ACL may be predisposed to greater injury risk during elevated hormone concentrations, specifically estradiol. At the basic science level, estrogen receptors on the ACL have been identified (Liu et al., 1996). Further study revealed a dose dependent decrease in fibroblast proliferation with increasing concentrations of estradiol (Liu et al., 1997). These results suggest the structural integrity of the ACL may be compromised at higher concentrations of estradiol. Supporting this premise, research has identified a decrease in the tensile strength of the ACL in a group of ovariectomized rabbits exposed to estrogen for a one-month period of time compared to a control group (Slauterbeck et al., 1999). More recently, a multi-center prospective epidemiologic study (Wojtys et al., 2002) identified females were more likely to experience a non-contact ACL injury during the ovulatory phase of their menstrual cycle compared to the follicular or luteal phases.

Collectively, these studies suggest that the manner in which the knee is affected by an external load is at least partially dependent upon estradiol concentrations. In fact, some work has identified increases in anterior tibiofemoral displacement (Heitz et al., 1999; Shultz et al., 2004) and decreases in 'ACL stiffness' (Romani et al., 2003) with elevated concentrations of estradiol. Though important, the aforementioned works solely measured displacement and stiffness at different points throughout the menstrual cycle. These studies do not provide insight on how the tibiofemoral joint responds following a loading protocol targeting the structures that restrain anterior tibiofemoral displacement. While unknown, it is plausible that these structures, particularly the ACL, are affected differently following the application of an external load during periods when estradiol concentrations are elevated.

We were unable to identify any published studies that examined the effect of an external load on knee ligament behavior at various hormonal concentrations in females. Therefore, the purpose of our study was to evaluate the effects of a controlled anterior tibiofemoral load on passive anterior tibiofemoral displacement and stiffness in a group of healthy females across select points of the menstrual cycle. We hypothesized that following a standardized loading protocol, anterior tibiofemoral displacement would increase and anterior tibiofemoral stiffness would decrease to a greater extent at time points in the menstrual cycle associated with higher concentrations of estradiol compared to time points with lower concentrations of estradiol.

METHODS

Subjects

Twenty healthy, recreationally active (hours engaged in exercise per week, 4.0 (SD, 3.0)) females (age, 20.9 (SD, 1.6 years); height, 1.6 (SD, .07m); mass, 59.6 (SD, 7.4kg)) between the ages of 18 and 26 were recruited from the University community. An a priori power analysis for displacement and stiffness was calculated based on data available from the literature (Heitz et al., 1999; Nawata et al., 1999). With effect sizes of 0.98 and 1.00 for displacement and stiffness respectively, inclusion of 20 subjects was expected to yield a power of 85%. Inclusion criteria included: (1) normal menstrual cycle lasting 28–32 days for the past six months; (2) no history of pregnancy; (3) no use of oral contraceptive or other hormone stimulating medications for the past six months; (4) healthy knees bilaterally and (5) no known co-existing medical conditions affecting the connective tissue. Prior to testing, all participants read and signed an informed consent form approved by the University's Human Investigations Committee.

Experimental setting and design

All data were collected at the General Clinical Research Center (GCRC) at the University Health Sciences Center. Participants reported for testing within 36 h following three occasions: (1) menses; (2) near ovulation and (3) during the mid-luteal phase. Menses (M) was defined by the onset of menstrual bleeding as reported by the participant; near ovulation (O) was identified by a positive test detected with a commercially available urine ovulation kit (CVS Pharmacy Inc.; Woonsocket, RI, USA) and the mid-luteal phase (L) was predicted by having the participant subtract eight days from the expected end of their current cycle (Michaud et al., 1999). The timing of each visit was strategically selected to assess knee ligament behavior at the peaks and valleys of various hormonal levels. Menses was intended to represent the period when both estradiol (E2) and progesterone (P) concentrations were low. Near ovulation was selected in an effort to capture peak estradiol levels while progesterone concentration remained low and the mid-luteal phase was chosen to detect the surge in progesterone while estradiol levels remained elevated.

With the assistance of a co-investigator, participants initiated the study by contacting the principal investigator at one of the three menstrual cycle events. This process ensured the principal investigator was blinded to the participant's day of the menstrual cycle. Both knees were used for the study with one knee serving as the control and the other as the experimental. As participants were enrolled in the study, control and experimental sides were counterbalanced by limb dominance. Limb dominance was defined as the leg a participant would use to kick a ball.

Instrumentation and procedures

The same procedures were followed each test session. All participants refrained from physical activity on the day of testing until after each session had been completed. Upon reporting for testing, 5–7cc of venous blood was drawn to quantify serum hormone levels of estradiol, progesterone as well as testosterone. Testosterone (T) was analyzed not only to gain a broader depiction of each participant's hormonal environment but also to ensure our participants had stable and normal circulating levels of this endogenous hormone.

Next, the KT-2000TM (MEDmetric Corp.®; San Diego, CA, USA) knee joint arthrometer with an attached masonry bubble level (Stanley Works; New Britain, CT, USA) was used per manufacturer's instructions with the knee in 30° of flexion to record three pre-measures of

anterior tibiofemoral displacement at 134N bilaterally. All knee arthrometer measures were performed by the principal investigator with a previously established intra-rater reliability ($ICC_{[2,k]}$ (SEM)) of 0.97 (0.37mm) for displacement. Raw force and displacement data were acquired with the CompuKT® software (MEDmetric Corp.®) and stored on a personal computer.

Following the pre-measures, while the control limb remained relaxed in a non-weight-bearing posture, a standardized, static loading protocol with the knee in 30° of flexion was performed on the experimental side. The loading protocol consisted of three, 3-min, posterior to anterior normalized static loads directed to the posterior calf, 10 cm inferior to the joint line with the LigMaster testing device (Sport Tech, Inc., Charlottesville, VA, USA) (Fig. 1). This device allowed continuous digital readouts of the applied force while the loading protocol was performed. The applied load or force was normalized to body weight and tibia length and calculated using a static moment equation:

$$\text{Force}_{\text{applied}} = \frac{(18\% \text{ BodyWeight [N]})(\text{Tibia Length [cm]})}{\text{Tibia Length [cm]} - 10 [\text{cm}]}$$

The quantity of force utilized with the loading protocol was selected based on the results of other published work (Henning et al., 1985) and pilot testing. Pilot testing indicated a load of 18% body weight was sufficient to

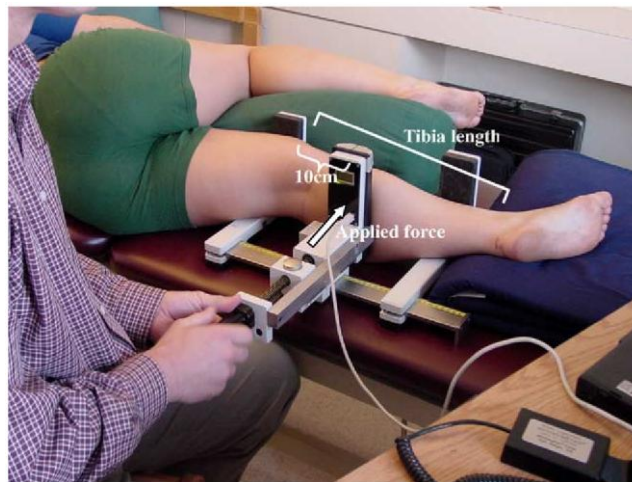


Fig. 1. Participant in standardized position within the Ligmaster Ligament Testing Device.

produce a measurable increase in displacement. Furthermore, this quantity of load was close to the maximum load that a subject could reasonably tolerate during the loading protocol. Once applied, the load was closely monitored and adjusted as necessary to ensure a constant force for the entire trial. Immediately following the loading protocol, post-measures of anterior tibiofemoral displacement using identical procedures as the pre-measures were recorded bilaterally.

Data reduction

Blood assays were analyzed at the GCRC Core Lab. Specifically, progesterone and testosterone were analyzed using a chemiluminescence assay and estradiol by Solid-Phase RIA (radioimmunoassay). Raw data (force and displacement) were exported from the CompuKT® software as a text file (.txt) and imported into Excel™ where displacement and stiffness values were calculated. For displacement and stiffness data, an average of the three trials was used for data analysis. Displacement was defined as the amount of anterior tibial translation at 134N of

force expressed in millimeters. Stiffness (N/mm) was defined as the change in force divided by the change in displacement between 90 and 134N.

Statistical analysis

All data were analyzed with SPSS® Version 10.0. Separate repeated measures analysis of variances (ANOVAs) were utilized to determine if significant differences in estradiol, progesterone and testosterone levels existed between the days of the menstrual cycle. Repeated measures ANOVAs with three within variables (loading [pre, post], side [control, experimental] and menstrual cycle day [menses, ovulatory, mid-luteal]) were used to analyze displacement (134N) and stiffness (90–134N) data. Post-hoc testing as necessary ($P < 0.05$) was performed with Tukey's Honestly Significant Difference test. Pearson correlation coefficients were calculated to examine the relationships between hormone concentrations and the dependent measures.

RESULTS

Tables 1 and 2 provide descriptive statistics for hormonal concentrations and the dependent measures of displacement and stiffness before and after the loading protocol. On average, 145N (SD, 18; range, 114–184N) of force was applied to the subject's posterior calf with the Ligmaster Testing Device during the loading protocol. While concentration levels of estradiol and progesterone were significantly different ($P < 0.001$) between the measured days of the menstrual cycle

Table 1
Means (SD) of hormonal levels by cycle phase

	Estradiol (pg/mL)	Progesterone (ng/mL)	Testosterone (ng/dL)
Menses	73.6 (24)	1.1 (0.5)	33.8 (12)
Normal range	19–83	0.5–1.4	14–76
Near ovulation	123.1 ^a (40)	2.2 (3)	38.8 (15)
Normal range	150–258	3.34–25.56	14–76
Mid-luteal	137.8 ^a (60)	8.3 ^b (6)	41.3 (17)
Normal range	60–211	4.4–28.03	14–76

^a Estradiol at mid-luteal and near ovulation significantly greater than estradiol at menses ($E2_L = E2_O > E2_M$) ($P < 0.001$).

^b Progesterone at mid-luteal significantly greater than progesterone near ovulation and at menses ($P_L > P_O = P_M$) ($P < 0.001$).

Table 2

Means (SD) for displacement (mm) at 134 N and stiffness (N/mm) between 90 and 134 N by day of the menstrual cycle before and after the loading protocol for control and experimental sides as well as the overall mean representing a composite measure of both control and experimental sides before and after the load

	Control		Experimental		Overall
	Pre-load	Post-load	Pre-load	Post-load	
<i>Menses</i>					
Displacement	5.77 (1.4)	5.26 (1.4)	5.33 (1.5)	5.93 (1.5)	5.57 (1.3)
Stiffness	45.1 (22)	50.3 (26)	50.5 (25)	48.5 (27)	48.60 (19.0)
<i>Near ovulation</i>					
Displacement	5.48 (1.3)	5.35 (1.5)	5.71 (1.9)	6.17 (1.7)	5.68 (1.4)
Stiffness	51.4 (27)	47.5 (26)	46.1 (20)	45.5 (19)	47.66 (20.6)
<i>Mid-luteal</i>					
Displacement	5.56 (1.3)	5.27 (1.4)	5.67 (1.6)	5.52 (1.7)	5.50 (1.3)
Stiffness	52.7 (29)	50.3 (23)	48.9 (20)	51.8 (24)	50.95 (20.1)

($E_{2L} = E_{2O} > E_{2M}$; $P_L > P_O = P_M$), there were no differences in concentrations of testosterone ($P = 0.11$). We did not observe differences in displacement ($P = 0.40$) or stiffness ($P = 0.36$) when menstrual cycle days were compared. We found a significant interaction between load and displacement ($P = 0.009$) (Fig. 2) with displacement being greater on the experimental side when compared to the control following the loading protocol. This

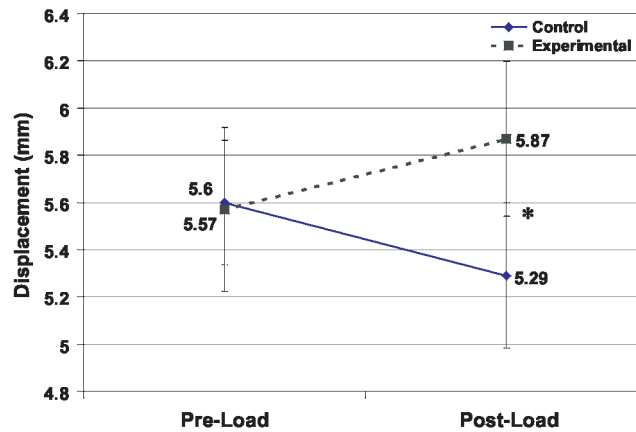


Fig. 2. Averaged across all days, post-loading, displacement (mm) on the experimental limb was greater than displacement on the control limb ($P = 0.009$).

loading effect did not vary by day of the menstrual cycle for either displacement ($P = 0.09$) or stiffness ($P = 0.10$). Table 3 lists correlation coefficients between hormone concentrations and displacement.

DISCUSSION

The primary finding of our study was that following a controlled, static loading protocol, anterior tibiofemoral displacement and stiffness did not significantly vary across the selected days of the menstrual cycle. When menstrual cycle days were collapsed, a difference between experimental and control limbs was evident for displacement following the loading protocol. Additionally, the strength of the correlation coefficients between displacement and stiffness measures and menstrual cycle day were similar between test days.

Effect of cycle day on ligament behavior post-loading

Our findings as they relate to displacement post-loading at select points across the menstrual cycle are consistent with those recently reported by Pollard et al. (2003). In both our study and that by Pollard et al., two important similarities are evident.

Table 3
Correlation matrix between hormonal concentrations and displacement values

		Estradiol	Progesterone	Testosterone
Menses–Menses	Control			
	Pre-load	0.289	0.186	–0.152
	Post-load	0.321	0.364	0.024
	Experiment			
	Pre-load	0.484 ^a	0.257	0.156
	Post-load	0.393	0.402	0.120
Near ovulation–Menses	Control			
	Pre-load	–0.256	0.434 ^a	0.056
	Post-load	–0.319	0.552 ^a	0.079
	Experiment			
	Pre-load	–0.032	0.418	0.011
	Post-load	–0.048	0.291	0.124
Mid-luteal–Menses	Control			
	Pre-load	0.036	0.352	0.099
	Post-load	0.156	0.489 ^a	0.045
	Experimental			
	Pre-load	0.137	0.185	0.186
	Post-load	0.136	0.273	0.224

^a Relationship ≤ 0.05 .

First, a significant difference between control and experimental limbs for displacement was noted after the loading protocol, regardless of cycle day. In our study, the significant difference appears to be the result of a concomitant increase in displacement on the experimental side and a slight decrease in displacement on the control side (Fig. 2). The decrease in displacement observed on the control side may be a function of inactivity during the testing period. A decrease in tissue temperature (i.e. cooling from the inactivity) may have played a factor with the observed decrease in displacement though this is only speculative as we did not measure temperature.

Secondly, a significant rise in estradiol from menses to near ovulation was noted. This context is important as it is hypothesized that a rise in estradiol concentration is a prerequisite to initiate the cascade of events that compromise the structural integrity of the ACL (Liu et al., 1997). Though our findings are in agreement, methodologies were substantially different between the two studies. While Pollard et al. used a weight-bearing functional exercise model, we chose a non-weight bearing, controlled loading model. We purposely selected this model to ensure the applied moment was consistent from one test session to the next. Therefore, despite considerable differences in methodology, both studies failed to identify differences in displacement between days of the menstrual cycle following a protocol that stressed the passive structures that restrain anterior tibial translation.

While important, displacement measures alone do not adequately describe ligament behavior. The description of ligament behavior can be enhanced by the addition of stiffness measures. Stiffness represents the ease at which a material can be deformed. A stiff tissue requires a substantial quantity of force to cause changes in displacement whereas a more compliant or less stiff tissue requires significantly less force to cause a change in displacement. Following the loading protocol and contrary to our hypothesis, stiffness measures whether examined

collectively or by menstrual cycle day were similar. Given the inherent relationship between displacement and stiffness, it is reasonable to expect that a change in displacement will be accompanied by a change in stiffness. Our findings of an increase in displacement but no difference in stiffness are not novel (Deie et al., 2002) and are likely due to either statistical or methodological phenomenon. Statistically, the stiffness data variability may have precluded identifying differences. From a methods standpoint, differences in stiffness may have been present at a lower force interval (i.e. 44–89N) of the stress–strain curve thereby resulting in an increase in displacement with no apparent increase in stiffness.

Overall, these findings suggest that ligament behavior, as defined by displacement and stiffness measures, do not vary appreciably across the menstrual cycle following the application of an external load.

Effect of cycle day on displacement

Overall, we did not observe differences in anterior tibiofemoral displacement across the days of the menstrual cycle tested. In fact, the maximum difference between overall means (near ovulation [5.68] — mid-luteal [5.50]) was only 0.18 mm. This is in agreement with some work (Beynon, 2003; Pollard et al., 2003) yet in conflict with others (Shultz et al., 2003; Deie et al., 2002; Karageanes et al., 2000; Heitz et al., 1999). Of those studies that did find a difference in displacement between phases of the menstrual cycle, only Heitz et al. reported absolute hormone levels. Like Beynon et al. (171.4 pg/mL), our mean estradiol level near ovulation (123.1 pg/mL) was dramatically lower than levels reported by Heitz et al. (778 pg/mL). It is possible that a concentration threshold for estradiol exists before increases in displacement can be observed and that our concentrations fell below this theoretical threshold. Furthermore, recent work from our laboratory (Shultz et al., 2004) reported the largest increase in displacement occurred at the beginning of the luteal phase, after the attainment of peak levels of estradiol yet before the rise in progesterone. These results suggest that it is only after several days of exposure to elevated estradiol levels that alterations in displacement are clinically evident. Given the conflicting findings related to displacement across the menstrual cycle, additional work emphasizing frequent if not daily measurement is necessary to clarify this issue.

Effect of cycle day on stiffness

Similar to displacement, we did not detect overall differences in stiffness between 90 and 134N across the three days tested. As can be discerned from Table 2, the differences between our mean stiffness values by day of the menstrual cycle were remarkably small. Adding to this, there was considerable inter-subject variation in stiffness quantified in this range, as noted by the large standard deviations. However, our mean values, small differences between days of the menstrual cycle and large inter-subject variability are consistent with previously reported studies (Romani et al., 2003; Shultz et al., 2003). At comparable estradiol levels, Romani et al. (2003) did not detect differences in passive anterior tibiofemoral stiffness as a function of menstrual cycle phase. Likewise, using a total of 15 days (five days to represent each of the menstrual cycle phases) across one cycle, preliminary results from Shultz et al. (2003) did not identify differences in passive anterior tibiofemoral stiffness measures. Each of these works used the KT-2000 at 30° of knee flexion and measured stiffness between 89 and 134N in healthy normally menstruating female participants. Collectively, these reports imply stiffness does not differ

appreciably across days of the menstrual cycle, although considerable variability exists between subjects.

Hormonal–ligament behavior relationship

Our findings are in contrast to those reported by Romani et al. (2003), who reported a significant inverse relationship between estradiol and 'ACL stiffness' near ovulation. These conflicting findings are likely explained by methodologic and statistical differences. Romani et al. investigated the influence of multiple endogenous hormones on 'ACL stiffness'. The authors were particularly interested in the relationship between estradiol and 'ACL stiffness'. To account for the interactive effect among the multiple hormones on 'ACL stiffness', the authors used partial correlations. Partial correlations likely assisted with identifying this significant relationship by accounting for the influence of other hormones. Despite the identified inverse relationship between 'ACL stiffness' and estradiol near ovulation, it is interesting to note that the authors did not detect a mean difference in stiffness values between menstrual cycle phases. Additional research is necessary to further elucidate these relationships.

Study limitations

Like all studies, our work does present with some limitations. As we placed several constraints on our study to minimize the influence of extraneous variables, our results can only be generalized to the specific loading protocol utilized, measured hormones, and the examined dependent measures. Our findings do not provide insight as to whether the response would have been similar under different loading conditions. As in vivo forces during sport activity occur more rapidly and in a repetitive fashion, a controlled model that produces faster applications of force in a cyclical manner is worthy of pursuit. While the findings of Pollard et al. do not support this contention, their work despite a standardized exercise protocol did not strictly control the applied moment at the tibiofemoral joint from one test session to the next.

Also, our study only examined the effects of loading on displacement and stiffness as it related to the concentrations of three endogenous hormones. Certainly, we appreciate there are numerous hormones simultaneously circulating and interacting within the female hormonal environment. However, we chose to examine only those hormones that have been suspected to at least partially explain the high prevalence of non-contact female ACL injuries (Slauterbeck et al., 1999; Wojtys et al., 2002). Additionally, it is conceivable that we did not see changes in ligament behavior by days of the menstrual cycle as we may have failed to capture peak estradiol levels. Our mean estradiol value of 123 pg/mL near ovulation was below the expected range 150–258 pg/mL of estradiol for the ovulatory phase (Table 1). The likely failure to capture peak estradiol levels was influenced by the fact that we only measured hormone concentrations three times across the menstrual cycle. While these visits were strategically selected and an ovulation predictor was utilized, given the rapid rise and fall of estradiol, we may have missed this small window. Furthermore, more recent work (Shultz et al., 2004) has noted substantial variation in hormone fluctuations across the menstrual cycle, highlighting the importance of taking multiple measurements across the cycle to more accurately depict the rapidly changing hormonal environment.

Future studies should quantify serum hormone concentrations while examining the effects of a cyclic, weight-bearing loading protocol with an emphasis on multiple measurements per cycle

phase to clarify if displacement and stiffness measures are affected. Alternative populations including females taking oral contraceptives and those that are amenorrheic should also be examined.

CONCLUSION

Following a controlled, static non-weight-bearing loading protocol stressing the passive restraints that limit anterior tibiofemoral translation, displacement at 134N and stiffness between 90 and 134N did not vary significantly between three select points across the menstrual cycle in a group of young healthy females. Overall, we failed to detect differences in displacement and stiffness measures by day of the menstrual cycle and likewise did not identify significant relationships between estradiol, progesterone or testosterone with displacement or stiffness measures.

Acknowledgment

This work was supported by grants from the Women's Sport Foundation and the General Clinical Research Center at the University of Virginia (Grant #MO1 RR00847).

REFERENCES

- Arendt, E.A., Agel, J., Dick, R., 1999. Anterior cruciate ligament injury patterns among collegiate men and women. *J. Athl. Train.* 34,86–92.
- Beynon, B.D., 2003. Risk factors for knee ligament trauma. *J. Orthop. Sports Phys. Ther.* 33, A10–A13.
- Deie, M., Sakamaki, Y., Sumen, Y., Urabe, Y., Ikuta, Y., 2002. Anterior knee laxity in young women varies with their menstrual cycle. *Int. Orthop.* 26, 154–156.
- Griffin, L., Agel, J., Albohm, M., Arendt, E.A., Dick, R.W., Garrett, W.E., et al., 2000. Noncontact anterior cruciate ligament injuries: risk factors and prevention strategies. *J. Am. Acad. Orthop. Surg.* 8,141–150.
- Gwinn, D.E., Wilckens, J.H., McDevitt, E.R., 2000. The relative incidence of anterior cruciate ligament injury in men and women at the United States naval academy. *Am. J. Sports Med.* 28, 98–102.
- Heitz, N.A., Eisenman, P.A., Beck, C.L., Walker, J.A., 1999. Hormonal changes throughout the menstrual cycle and increased anterior cruciate ligament laxity in females. *J. Athl. Train.* 34, 144–149.
- Henning, C.E., Lynch, M.A., Glick, K.R., 1985. In vivo strain gauge study of elongation of the anterior cruciate ligament. *Am. J. Sports Med.* 13, 22–26.
- Karageanes, S.J., Blackburn, K., Vangelos, Z.A., 2000. The association of the menstrual cycle with the laxity of the anterior cruciate ligament in adolescent female athletes. *Clin. J. Sport Med.* 10, 162–168.
- Liu, S.H., Al-Shaikh, R.A., Panossian, V., Yan, R.-S., Nelson, S.D., Soleiman, N., et al., 1996. Primary immunolocalization of estrogen and progesterone target cells in the human anterior cruciate ligament. *J. Orthop. Res.* 14, 526–533.
- Liu, S.H., Al-Shaikh, R.A., Panossian, V., Finerman, G.A.M., Lane, J.M., 1997. Estrogen affects the cellular metabolism of the anterior cruciate ligament. *Am. J. Sports Med.* 25, 704–708.
- Michaud, D.S., Manson, J.E., Spiegelman, D., Barbieri, R.L., Sepkovic, D.W., Bradlow, H.L., et al., 1999. Reproducibility of plasma and urinary sex hormone levels in premenopausal women over a one-year period. *Cancer Epidemiol. Biomarkers Prev.* 8, 1059–1064.

- Nawata, K., Teshima, R., Morio, Y., Hagino, H., Enokida, M., Yamamoto, K., 1999. Anterior-posterior knee laxity increased by exercise: quantitative evaluation of physiologic changes. *Acta Orthop. Scand.* 70, 261–264.
- Pollard, C., Braun, B., Hamill, J., 2003. Influences of gender and exercise on ACL laxity. *J. Orthop. Sports Phys. Ther.* 33, A27.
- Romani, W., Patrie, J., Curl, L.A., Flaws, J.A., 2003. The correlations between estradiol, estrone, estriol, progesterone, and sex hormone-binding globulin and anterior cruciate ligament stiffness in healthy, active females. *J. Womens Health* 12, 287–298.
- Shultz, S.J., Sander, T.C., Kirk, S.E., Perrin, D.H., 2003. Knee joint laxity but not stiffness changes across the menstrual cycle. *Med. Sci. Sports Exerc.* 35, S-81.
- Shultz, S.J., Kirk, S.E., Johnson, M.L., Sander, T.C., Perrin, D.H., 2004. Relationship between sex hormones and anterior knee laxity across the menstrual cycle. *Med. Sci. Sports Exerc.* 36, 1165–1174.
- Slauterbeck, J., Clevenger, C., Lundberg, W., Burchfield, D.M., 1999. Estrogen level alters the failure load of the rabbit anterior cruciate ligament. *J. Orthop. Res.* 17, 405–408.
- Wojtys, E.M., Huston, L.J., Boynton, M.D., Spindler, K.P., Lindenfeld, T.N., 2002. The effect of the menstrual cycle on anterior cruciate ligament injuries in women as determined by hormone levels. *Am. J. Sports Med.* 30, 182–188.