Variations in Varus/Valgus and Internal/External Rotational Knee Laxity and Stiffness Across the Menstrual Cycle

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Abstract:

Cyclic variations in genu recurvatum (GR), general joint laxity (GJL), varus–valgus (VV), and internal–external (IER) rotational laxities and stiffnesses were examined in 64 females and 43 males at two time points during the females' menstrual cycle [days of minimum (T1) and maximum (T2) anterior knee laxity (AKL)]. Cyclic increases in AKL (9.5%), GR (37.5%), and GJL (13.6%) were observed in females but not males from T1 to T2 ($p < 0.001$). Cyclic increases in VV and IER laxity were negligible (1.5–3.2%, $p > 0.320$). Females compared to males had lower overall VV stiffness at T2 (F 37% <M) vs. T1 (F 26.9% <M; $p = 0.011$), but no difference across time points for IER stiffness ($p = 0.452$). Across both time points, females had consistently greater VV (30.2%) and IER (20%) laxity and less VV (32.5%) and IER (24.3%) incremental stiffness ($p < 0.001$). Low-to-moderate associations were observed between AKL, GR, and GJL with VV and IER laxities and stiffnesses in females as measured at T1 and the change in values from T1 to T2. Whether these findings reflect ligament-specific responses to hormone changes, or implicate changes in injury risk potential across the menstrual cycle requires further study.

Article:

The potential consequences of varus–valgus (VV) and internal–external (IER) rotational laxity and stiffness of the tibiofemoral joint on knee joint biomechanics have recently been examined. Where rotational laxity is a measure of the rotation of the tibia relative to the femur between fixed torque magnitudes, torsional stiffness represents the torque–rotation response of the joint across a range of applied torques, which may be useful in determining where in the range the knee joint is more or less resistive to the applied torques.[1] Together these measures may help us understand the factors that influence knee joint biomechanics when physiological loads are applied to the knee.

Females have greater VV and IER laxity compared to males,[2–5] which has been associated with decreased torsional stiffness,[1, 4, 6] particularly at lower magnitudes.[1] Greater VV and IER laxities have been associated with at risk landing biomechanics,[7] which also tend to be more predominant in females.[8–12] Specifically, females with higher laxity values were reported to land in greater hip adduction and knee valgus and demonstrated prolonged hip adduction and knee varus internal moments as they moved towards greater hip adduction and internal rotation of the femur during the landing.[7] As increased valgus coupled with internal or external rotation about

the knee has been associated with a common non-contact ACL injury mechanism,[13, 14] greater VV and IE laxity may explain, at least in part, why females are more likely to demonstrate these at risk motions early in the landing phase.[7]

Current consensus indicates that the likelihood of suffering an ACL injury is not uniform across the menstrual cycle, and that sex hormones can mediate changes in knee laxity across the cycle.[15, 16] However, only anterior knee laxity (AKL)[17–19] and more recently genu recurvatum (GR) and general joint laxity (GJL)[20] have been investigated in this regard. While the actual magnitude and timing of these changes are not uniform across individuals,[18, 20] these studies indicate that, on average, lower magnitudes of laxity are observed in the early follicular phase when estrogen and progesterone are at their nadirs, and higher magnitudes are observed near the time of ovulation and early luteal phase once estrogen levels begin to rise and prior to significant rises in progesterone levels. Whether VV and IER knee laxities also change in females across the menstrual cycle in a similar manner, or are constant over time, has not been investigated. Identifying these cyclic variations may be clinically important, as it is plausible that the observed greater magnitudes of "at risk" knee motions associated with greater magnitudes of VV and IER laxity may also vary. Our prior work suggests that AKL and GJL may be strong predictors of VV and IER laxity,[3] but this was examined in a combined sample of males and females that were not matched on baseline laxity values, and the extent to which cyclic variations in AKL, GR, and GJL are associated with cyclic variations in VV and IER laxity in females is unknown. Moreover, examining the associated stiffness characteristics throughout the entire torque–rotation response of the knee joint may provide further insight into where the joint is least resistance to applied torques if cyclic variations occur.

Our purpose was to first identify for each female the days when her AKL were at their minimum and maximum values, respectively, and compare males and females on VV and IER laxity and stiffness when measured at these two times points. We hypothesized that cyclic increases in AKL would be accompanied by cyclic increases in VV and IER laxity and stiffness in females, resulting in greater values in females compared to males on the day of maximum AKL as compared to the day of minimum AKL in females. A secondary purpose was to examine associations between AKL, GR, GJL and VV, and IER laxity and stiffness between these time intervals in females, in order to explore how the entire envelope of knee laxity and stiffness may change within an individual across her cycle. Our secondary hypothesis was that moderate-to-strong associations would be observed between AKL, GR, and GJL (measures routinely made in the clinical setting) and VV and IER laxity and stiffness between T1 and T2.

METHODS

These data represent secondary analyses from a larger study in which the primary aim was to determine the independent and combined effects of greater absolute baseline and cyclic increases in AKL across the menstrual cycle on knee joint neuromechanics during weight bearing.[21] Appreciating that transverse and frontal plane laxity (thus biomechanics) may be similarly mediated by changes in sex hormone concentrations, we chose to obtain these additional measures of joint laxity and stiffness on the study population to explore these potential effects.

Subjects

Seventy-four females and 50 males who were nonsmokers and recreationally active (2.5–10 h/week); who had a body mass index < 30 (mass/ht^2), and no history of connective tissue disorders or knee injury involving the bone, joint surface, or soft tissue participated in the study. Females were included if they reported normal menstrual cycles (range: 26–32 days, varying no more than ±1 day in length between cycles), no use of exogenous hormones for the past 6 months, and no history of pregnancy or plans to become pregnant. Potential subjects were prescreened to obtain a wide distribution of AKL values in both sexes. Participants signed a University approved consent form prior to enrollment.

Experimental Protocol

Due to the large variation in hormone concentration and temporal profiles among females, there is also substantial variability in the time period during which the knee responds to changing hormone levels, and the magnitude of knee laxity change that a female experiences from the early follicular to luteal phases.[18, 20, 22, 23] This makes it difficult to identify a single day or hormone profile in the early follicular and early luteal phases when knee laxity will change in all women. Because of this, we measured AKL over two cycles on 6 consecutive days after menses onset and 8–10 days post confirmation of ovulation [CVS One Step Ovulation Predictor (sensitivity 20 mIU/ml LH, accuracy 99%); CVS Corporation, Woonsocket, RI] to best capture the days within each individual female that her AKL was at its minimum and maximum value. These days were chosen as the days most likely to yield minimum and maximum values based on previous research.[19, 22] In the subsequent month, males and females were measured on all laxity variables at two time points (T1 and T2). For females, T1 and T2 were the estimated days of minimum and maximum AKL during menses and the early luteal phase, respectively. For males, T1 and T2 were matched to a female with a similar AKL value at T1 (±0.5 mm). AKL, GR, and GJL were measured in the morning (07:00–09:00 a.m., ±30 min at T1 vs. T2), prior to any physical activity. Due to the instrumentation and time required, VV and IER laxity and stiffness were acquired during a biomechanical test session held the same day. However, because of class schedules and other conflicts, this biomechanical test session was often scheduled later in the morning or afternoon (i.e., it did not always immediately follow the morning test session when AKL, GR, and GJL were measured). Because of this, we were careful to schedule the biomechanical test sessions within an individual at a similarly spaced time interval from the morning clinical laxity testing for both T1 and T2 (within ±1 h), and instruct subjects to continue to refrain from all physically activity until the biomechanical test session was complete. To reduce potential differences in resting muscle tension between test sessions and test days, subjects completed a light, standardized stretch routine consisting of three sets of 15 s each of a figure-4 hamstring stretch and a knee flexion quadriceps stretch (hip extended), performed in the same manner prior to each test session. Participants were tested on their dominant limb (stance limb when kicking a ball), and familiarized to all procedures approximately 2 weeks before the first test session.

AKL was measured with the knee flexed 25 ± 5° as the anterior displacement of the tibia relative to the femur when a 133N anterior directed load was applied to the posterior aspect of the tibia using the KT-2000™ Knee Athrometer (MEDmetric Corp, San Diego, CA). GR was evaluated with the subject supine and the distal lower leg supported on a bolster, and measured as the amount of knee hyperextension when the subject maximally extended their knee. GJL was

scored from 0 to 9 using the Beighton and Horan Joint Mobility Index.[24] A single tester with strong reliability [$ICC_{(2,3)}$ (SEM) = 0.96 (0.3 mm) for AKL, 0.97 (0.5°) for GR, and 0.99 (0.3) for GJL][20] measured all subjects. Reliability estimates for AKL are consistent with what others have reported.[18] The tester was not blinded to time point, but was blinded to the individual's previous measured values (a separate tester was responsible for recording and reducing the data). VV and IER laxity and stiffness were measured with the Vermont Knee Laxity Device (VKLD).[1, 25] Subjects were positioned supine with the knee flexed to 20°, the thigh securely fixed, the foot and ankle restrained in the foot cradle, and counterweights applied to the thigh and shank to create initial zero shear and compressive loads across the tibiofemoral joint. VV laxity and stiffness were assessed with the knee nonweight bearing by applying 0–10 Nm of valgus and varus torques to the distal tibia with a force transducer (Model SM-50, Interface, Scottsdale, AZ). IER laxity and stiffness were measured with the knee nonweight bearing by applying 0–5 Nm of internal–external torques about the long axis of the tibia using a T-handle connected to a 6 degree-of-freedom force transducer affixed to the foot cradle (MC3A; Advanced Medical Technology, Inc., Watertown, MA). Kinematic and load data were simultaneously acquired (100 Hz) during three continuous cycles for each set of torque–rotations. Electromagnetic sensors (Ascension Technology Corporation, Burlington, VT) and Motion Monitor software (Innovative Sports Training, Chicago, IL) measured joint kinematics. Signals from the position sensors and load transducers were low-pass filtered at 10 and 20 Hz, respectively, using a fourth-order zero lag Butterworth filter. The approach used to locate and orientate the coordinate systems in bone has previously been described.[26]

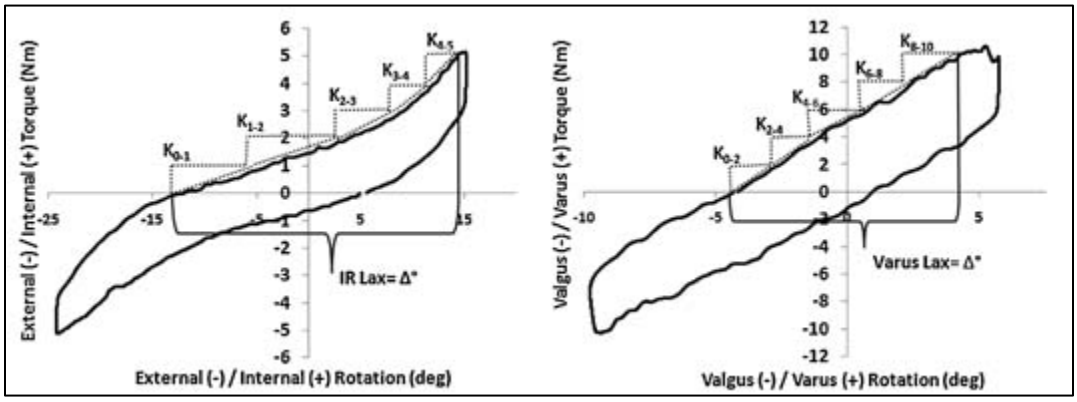
Laxity was recorded for each variable and direction of applied torque as the amount of angular displacement produced by 10 Nm (VV) and 5 Nm (IER) of torque, and averaged over three cycles. To obtain incremental stiffness values, torque–rotation curves were plotted for each trial, then divided into torque increments of 1.0 Nm (e.g., 1–2, 2–3, 3–4 Nm, etc.) for IER, and 2.0 Nm for VV (Fig. 1). For each increment, stiffness was calculated as the change in torque divided by the change in displacement (Nm/deg), averaged across the final two trials (the first trial serving as a conditioning trial). This yielded five stiffness increments for each direction of torque (e.g., varus, valgus) for each variable. Using similar methods, consistent laxity (ICC range 0.70–0.96; measurement error <2° VV and 3–4° IER)[25] and stiffness values (median ICC range across increments = 0.66–0.81 for valgus and varus; −0.07 to 0.75 for internal and external rotation; low ICCs limited to the high-loading range for internal rotation only)1 have been reported.

Statistical Analyses

Separate 2 (sex) by 2 (time) by 2 (direction of motion) repeated measures ANOVA examined cyclic changes in VV and IER laxity. Separate 2 (sex) by 2 (time) by 2 (direction of motion) by 5 (stiffness increment) repeated measures ANOVA examined cyclic changes in VV and IER stiffness. Within each set of laxity and stiffness variables, the alpha level was reduced to $p < 0.025$ (Bonferroni correction = 0.05/2) to account for multiple comparisons and control type I error. To address our secondary purpose, separate multiple linear regressions examined the extent to which AKL, GR, and GJL measured at T1 (herein AKL_{T1}, GR_{T1}, GJL_{T1}) and their change from T1 to T2 (herein AKL_{Δ}, GR_{Δ}, GJL_{Δ}) predicted VV and IER laxity (total motion) and stiffness (aggregate data combined across increments and directions) in females as measured at T1and their change from T1 to T2 (Δ). A two-step modeling procedure was used. On step 1, we examined how much variance in the laxity or stiffness variable was accounted for by AKL

and GR; on step 2, GJL was added to determine if a more global measure of laxity explained additional variance beyond AKL and GR (those measured strictly at the knee)

Figure 1: Exemplar load–displacement curve during the application of (a) 5 Nm external–internal and (b) 10 Nm varus–valgus rotation torques to the knee. Graphic representation of incremental stiffness and laxity calculations are provide for internal rotation and varus loadings. Each incremental stiffness is calculated as the slope (ΔNm/Δ°) of the line between each torque increment ($K_{0–1}$ = 0 to 1 Nm loading, $K_{1–2}$ = 1 to 2 Nm loading, etc., for internal and external rotation; $K_{0–2}$ = 0 to 2 Nm loading, $K_{2–4}$ = 2 to 4 Nm loading, etc., for varus and valgus rotation). Laxity is calculated as the amount of rotation from 0 to 5 Nm (internal, external rotation) or 0–10 Nm (varus, valgus rotation).



RESULTS

From the original sample, complete sets of VV and IER laxity and stiffness data were available on 64 females (21.5 ± 2.7 years, 164.3 ± 6.9 cm, 61.2 ± 8.7 kg) and 43 males (22.3 ± 2.8 years, 177.5 ± 9.5 cm, 80.1 ± 12.0 kg). Consistent with the findings from the original sample,[21] AKL ($p < 0.001$), GR ($p < 0.001$) and GJL ($p = 0.028$) increased from T1 to T2 for females but not males (Table 1). This resulted in greater AKL and GR values for females compared to males at T2 only; however, GJL was greater in females at both T1 and T2 (Table 1). Sex by time interactions were not observed for VV or IER laxities ($p > 0.320$). Rather, each was consistently greater in females compared to males at both T1 and T2 (all $p < 0.001$).

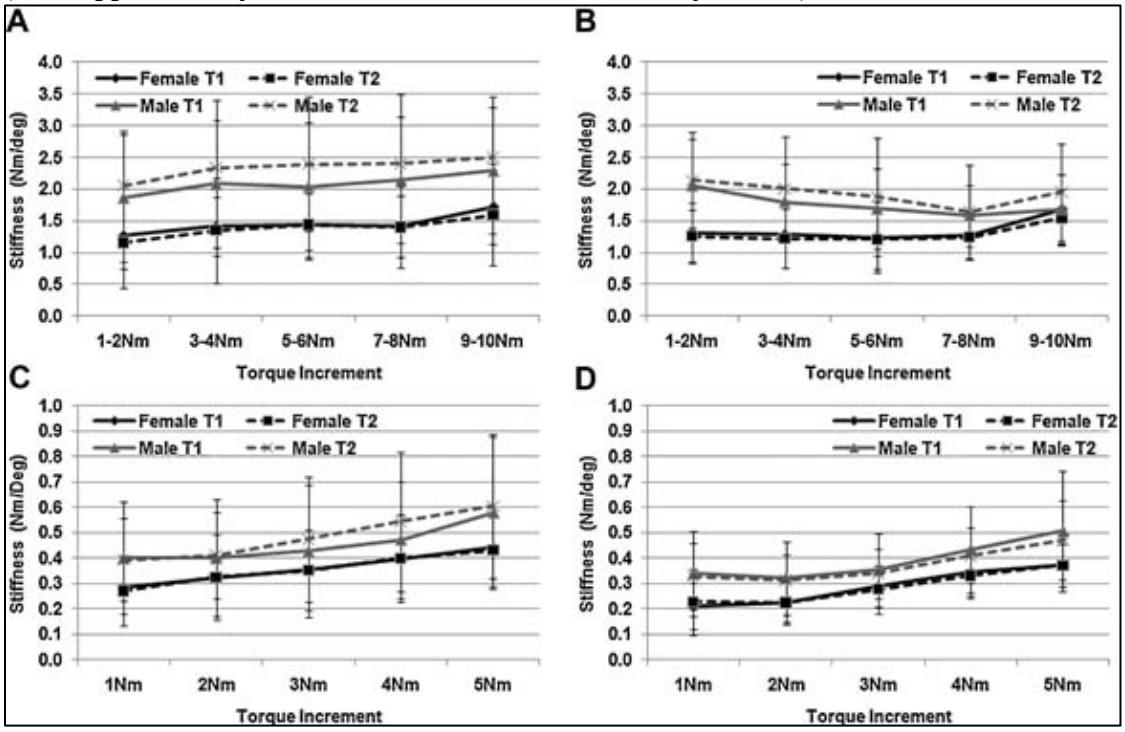
For incremental stiffness, a sex by time interaction was observed for VV ($p = 0.001$). Post hoc analyses revealed a 4.5% decrease in VV stiffness from T1 to T2 in females (1.41 ± 0.37 vs. 1.34 ± 0.31 Nm/deg; $p = 0.05$) and a 9.8% increase in males (1.92 ± 0.48 vs. 2.13 ± 0.71 Nm/deg; $p = 0.01$), resulting in females having 37% lower stiffness values than males at T2 compared to 26.9% lower stiffness at T1 (Fig. 2). Sex differences in VV stiffness from T1 to T2 did not differ by direction of motion ($p = 0.324$), stiffness increment ($p = 0.454$), or interaction between direction and increment ($p = 0.806$; Fig. 2). There was no sex by time interactions for IER incremental stiffness (p-value range for all sex and time interaction terms 0.068–0.758). However, when data were averaged across directions and increments, significant main effects revealed female knees were 32.5% less stiff for VV (1.37 ± 0.32 vs. 2.03 ± 0.54 Nm/deg; $p < 0.001$) and 24.3% less stiff for IER (0.32 ± 0.08 vs. 0.43 ± 0.14 Nm/deg; $p < 0.001$) compared to male knees. (See Supplementary Table 1 for full ANOVA summary results.)

Table 1: Means ± Standard Deviations (SD) of Laxity Variables by Test Session (T1 and T2) and Sex[a]

Variable		Test Session T1	Test Session T2	Individual Deltas (T2-T1)
Anterior Knee Laxity (AKL)	F	6.7 ± 1.9	7.4 ± 2.1*	0.7 ± 0.7
	M	6.5 ± 1.9	6.5 ± 1.9	0.1 ± 0.4
Genu Recurvatum (GR)	F	3.7 ± 3.3	5.1 ± 3.5*	1.4 ± 0.9
	M	3.6 ± 3.7	3.5 ± 3.5	−0.0 ± 0.7
General Joint Laxity (GJL)**	F	1.9 ± 1.7	2.2 ± 1.7*	0.3 ± 0.7
	M	1.0 ± 1.3	1.0 ± 1.3	0.0 ± 0.0
Varus (VAR)	F	6.0 ± 1.5	6.1 ± 1.4	0.2 ± 1.2
	M	4.1 ± 1.3	4.1 ± 1.3	−0.0 ± 1.1
Valgus (VAL)	F	7.1 ± 2.0	7.1 ± 2.0	0.0 ± 1.6
	M	5.2 ± 1.5	5.0 ± 1.6	−0.2 ± 1.3
Total VAR-VAL**	F	13.0 ± 3.0	13.2 ± 3.0	0.2 ± 2.2
	M	9.3 ± 2.4	9.1 ± 2.6	−0.2 ± 1.6
Internal Rotation (IR)	F	10.6 ± 4.7	10.8 ± 5.8	0.2 ± 5.7
	M	8.9 ± 4.1	8.7 ± 4.4	−0.1 ± 3.5
External Rotation (ER)	F	15.5 ± 4.5	16.2 ± 4.8	0.7 ± 3.7
	M	12.4 ± 3.6	12.4 ± 3.5	0.0 ± 3.1
Total IR-ER**	F	26.0 ± 6.9	26.9 ± 8.1	0.8 ± 7.7
	M	21.2 ± 6.9	21.1 ± 6.3	−0.1 ± 4.8

$N = 64$ F, 43 M.
*Female T2 >female T1 and male T1 and T2 values (sex by Time interaction, $p < 0.001$).
**Female > male @ both T1 and T2 (sex main effect, $p < 0.001$).
[a]See Supplementary Table 1 for full ANOVA summary results.

Figure 2: Incremental varus (A), valgus (B), internal (C), and external (D) torsional stiffness (see Supplementary Table 1 for full ANOVA summary results).



Regression summary results addressing our secondary purpose in females (Table 2) revealed that the combination of AKL_{T1}, GR_{T1}, AKL_{Δ}, and GR_{Δ} were significant predictors of VV and IER

laxity as measured at T1, and the cyclic change in VV laxity and stiffness from T1 to T2, explaining 15–19% of the variance in these variables (all $p < 0.041$). GJL_{T1} and GJL_{Δ} explained additional variance in IER stiffness as measured at T1 (R^2 change 0.148, $p = 0.006$) and the cyclic change from T1 to T2 (R^2 change 0.111, $p = 0.033$), resulting in a combined R^2 value of 0.256 ($p = 0.008$) and 0.127 ($p = 0.239$), respectively.

Table 2: Regression Summary Statistics When Predicting Baseline (T1) and Delta (Δ = T2 − T1) Varus–Valgus and Internal–External Rotational Laxity and Stiffness Values in Females

Variable	Step 1 R^2 (p-Value)	Step 2 R^2 Change (p-Value)	Unstandardized Coefficients*					
			AKL_{T1}	AKL_{Δ}	GR_{T1}	GR_{Δ}	GJL_{T1}	GJL_{Δ}
Varus–valgus								
Laxity @ T1	0.153 (0.041)	0.003 (0.893)	0.113	0.553	0.201‡	−0.916†	—	—
Laxity Δ	0.184 (0.016)	0.010 (0.697)	0.126	−0.082	−0.205†	0.803†	—	—
Stiffness @ T1a	0.062 (0.426)	0.004 (0.892)	—	—	—	—	—	—
Stiffness Δa	0.190 (0.013)	0.046 (0.189)	0.020	−0.077‡	0.004	−0.072†	—	—
Internal–external								
Laxity @ T1	0.188 (0.014)	0.050 (0.164)	1.218†	1.668#	0.099	−1.167	—	—
Laxity Δ	0.029 (0.781)	0.050 (0.220)	—	—	—	—	—	—
Stiffness @ T1a	0.108 (0.142)	0.148 (0.006)	−0.014†	−0.012	−0.001	−0.002	0.007	0.056†
Stiffness Δa	0.016 (0.913)	0.111 (0.033)	0.001	−0.017	−0.001	0.022#	−0.002	−0.056†

Step 1: Combined R^2 for the variables include in the model = AKL_{T1}, AKL_{Δ}, GR_{T1}, GR_{Δ}.
Step 2: R^2 Change when GJL_{T1} and GJL_{Δ} are added to the model.
*Coefficients significant at: †$p < 0.05$; ‡$p < 0.10$; #$p < 0.20$.
[a]Negative coefficients indicate greater laxity change predicting less stiffness.

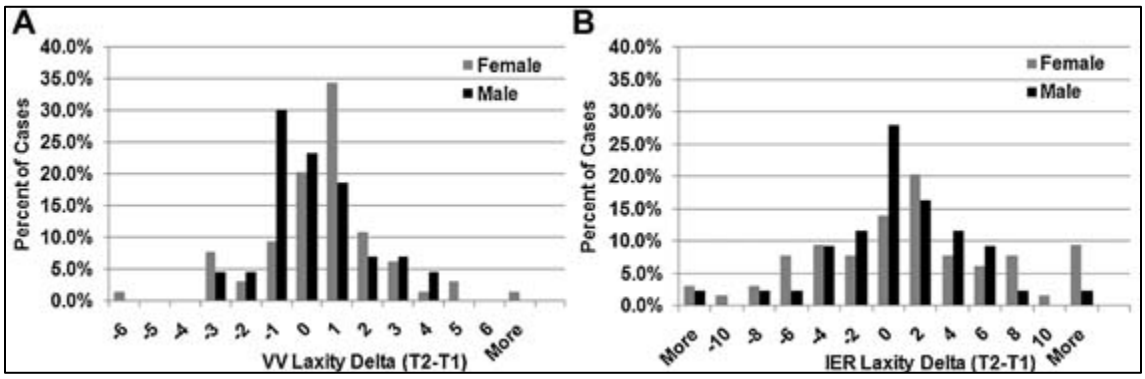
DISCUSSION

This study built on our prior reports of daily cyclic changes in AKL and GR across normal, ovulatory menstrual cycles[20] by adding measurements of VV and IER laxity and stiffness at days of minimum (T1) and maximum (T2) AKL in an effort to determine if the entire envelope of knee laxity changed in a similar cyclic manner. As greater magnitudes of VV and IER knee laxity have been associated with hip and knee motions during landing which resemble ACL injury mechanisms,[13, 14] variations in VV and IER laxity and stiffness across the cycle may represent a potential physiological mechanisms by which ACL risk may vary cyclically. Our primary finding was that cyclic increases in AKL, GR, and GJL in females from T1 to T2 were accompanied by cyclic increases in VV stiffness, but not in VV or IER laxity or IER stiffness. As males increased their VV stiffness values, females either maintained or decreased their stiffness values from T1 to T2, and as expected, this resulted in greater differences between males and females for all increments of VV stiffness at T2. While this finding was consistent with our hypothesis, the mean cyclic variation in VV stiffness from T1 to T2 were relatively small in females (effect size = 0.20).

Although mean cyclic increases in VV and IER laxity were not observed, careful inspection of the deviations around the individual difference scores from T1 to T2 (Table 1, last column and Fig. 3), suggests the deltas were greater in females than males, particularly for IER laxity. A larger proportion of females compared to males had deltas greater than zero for VV (57.8% vs. 37.2%) and IER (53.1% vs. 44.1%) laxity, and more females were represented in the largest deltas observed in IER laxity (18.8% vs. 4.7% > 8°). Thus, while group means were not different, a small portion of the females may have experienced cyclic increases in these variables as expected. This is supported, at least in part, by our secondary analysis, as AKL and GR were moderate predictors of the change in VV laxity from T1 to T2 (R^2 = 18%); although it was primarily lower initial GR values and higher cyclic changes in GR that were the strongest

predictors of increased VV laxity from T1 to T2. Neither baseline nor cyclic changes in AKL or GR were predictors of the change in IER laxity from T1 to T2.

Figure 3: Frequency distribution reporting the percentage of cases for each sex that experienced positive versus negative delta values for (a) VV and (b) IER laxities.



Cyclic variations in VV and IER stiffness were more apparent, primarily because male knees became stiffer while female knees either maintained or slightly decreased their stiffness. Thus, while the interaction across time (greater sex differences at T2 vs. T1) was as expected, it was not based on males maintaining stiffness and females decreasing their stiffness as hypothesized. Because T1 testing always preceded T2 testing, we attempted to control for any time-order effects due to learning or task familiarity by thoroughly familiarizing subjects to all testing procedures before actual data collection began, and by comparing females to males as male values were not expected to change over time (i.e., males essentially serving as a control group). The higher stiffness values in males at T2 suggest some type of time-order effect still occurred from T1 to T2 (as we know of no other plausible explanation for changes across time in males), which may have potentially masked the expected changes in VV and IER laxity and stiffness in females from T1 to T2. As with laxity, our secondary analyses support that cyclic variations in VV and IER stiffness did occur in some females, as greater cyclic increases in AKL and GR was associated with greater reductions in VV stiffness from T1 to T2 and greater cyclic increases in GJL with greater reductions in IER stiffness from T1 to T2.

Although mean cyclic variations in VV and IER laxity and stiffness were relatively small (all effect sizes <0.20) and appear to have occurred in only some of the females, we observed substantially greater magnitudes of VV and IER laxity (20–33%) and lower overall VV and IER stiffness (24–33%) in females compared to males at both time points (effect sizes ranging from 0.8 to 1.2). These sex differences are consistent with values reported for VV and IER laxity,[2–5] as well as those for VV and IER stiffness both in terms of the magnitude[1, 27] and the pattern of change in stiffness across torque magnitudes.[1] These sex differences were clearly apparent even when males and females were similar with regard to their AKL and GR values at T1. In this regard, an unexpected finding was that cyclic variations in AKL and GR were stronger predictors of the absolute magnitude of VV and IER laxity as measured at T1, than the cyclic changes in VV and IER laxity from T1 to T2. Specifically, females had greater VV and IER laxity at T1, if they had greater AKL and GR values at T1, and greater cyclic changes in AKL and lower cyclic changes in GR from T1 to T2. Hence, based on the two time points examined, greater cyclic

variations in AKL and GR were more closely associated with chronically higher VV and IER laxity values than the cyclic change in VV and IER laxity values from T1 to T2.

Also notable is the smaller amount of variance in VV and IER laxity explained by AKL and GJL in this study compared to previous work,3 which may be explained by two factors. First, the previous work used a smaller sample ($N = 20$) which may have inflated the R^2 values. Second, it examined a combined sample of males and females, where females also had greater magnitudes of AKL and GJL. Recall in this and other studies[4, 5] where males and females had similar AKL values, VV and IER laxity were still higher in females. Hence, analyzing males and females together may have artificially inflated the R^2 value, with greater AKL and GJL in females acting as a surrogate for sex. Thus, while relationships among these variables are still apparent, they may not be as highly correlated as previously reported.[3]

Collectively, these findings suggest that variations in VV and IER laxity and stiffness are of much smaller magnitude than those observed in AKL, GR, and GJL, but may occur in some females who experience larger cyclic variations in AKL, GR, or GJL. Moreover, the absolute magnitudes of VV and IER laxity (rather than their cyclic change from T1 to T2) were more strongly associated with cyclic variations in AKL and GR. The reason for these ligament-specific responses between the two time points is not clear, but may reflect differences in hormone responsiveness associated with intra- versus extra capsular ligament properties. When the knee is flexed to 20–30° and NWB (as the knee was tested in this study), the ACL is the primary restraint to AKL, while the medial collateral ligament (MCL), alone or in combination with the ACL, is the most important structure resisting VV and IER torques.[28, 29] To our knowledge, neither the presence or density of hormone receptors on the ACL versus the MCL, nor the metabolic or mechanical responsiveness of these ligaments to hormone changes in normal menstruating females, have been directly compared. However, studies of pregnant and nonpregnant rabbits suggest that both ligaments possess active hormone receptors,[30] but that their responsiveness to hormone changes may be ligament specific.[31] This is indirectly supported in pregnant females, based on greater increases and decreases in AKL during and postpregnancy, respectively, compared to other joints.[32] Possible reasons for ligament-specific hormone responsiveness to pregnancy may be differences in intrinsic properties, vascularity, mechanical demands, and intra- versus extra articular environment.[31] Currently, there is no clear consensus in the literature as to the underlying mechanism(s) by which sex hormones may mediate changes in collagen metabolism and mechanical properties.[15] As such, it is difficult to determine which of these or other factors may explain the differential changes in anterior–posterior versus transverse and frontal plane joint behavior from T1 to T2. Research comparing cellular responsiveness of the human ACL and collateral ligaments to normal cyclic variations in sex hormone concentrations under both resting and exercise conditions may shed further light on our findings, and the potential for cyclic variations in joint laxity and stiffness to differ by ligament, and thus their control of joint motion in specific anatomic planes.

By nature of the larger study from which these data were derived, the primary limitations are that our measurements of VV and IER laxity and stiffness were obtained at two time points (the days of minimum and maximum AKL in females). Although the timing of cyclic changes in AKL, GR, and GJL are, on average, quite uniform across the cycle, differences in these timings do exist in some individuals.[20] Thus, we may not have always captured true minimum and peak GR,

GJL, or VV and IER laxity and stiffness values. However, this would not explain the much lower cyclic variations observed in VV and IER laxity as compared to GR and GJL, unless the timing of these individual laxity changes was dramatically different. Further, because of the duration of time required to measure VV and IER laxity and stiffness in vivo, we measured these variables as part of a biomechanical test session, which was often scheduled later in the day. While we are not aware of any studies reporting diurnal changes in laxity throughout the day, we cannot completely rule out the potential for subtle VV and IER laxity changes to have occurred from the morning hours (when AKL, GR, and GJL were obtained), which may have affected relationships among these variables to some degree. Future work should examine cyclic changes in all relevant laxity variables during the same test session at multiple time points of the cycle. The development of less time and equipment intensive measurement techniques may enable more time sensitive measures of VV and IER laxity and stiffness in the future.

In summary, females experienced larger cyclic variations in AKL, GR, and GJL as compared to VV and IER laxity and stiffness, yet had substantially greater overall VV and IER laxity, and lower overall VV and IER stiffness values compared to males. These findings indicate that the envelope of laxity does not change equivalently in all planes of motion over the course of a normal, ovulatory menstrual cycle in females. To fully appreciate the clinical implications of these findings, further research is needed to understand (1) potential ligament-specific responses to hormone changes across the menstrual cycle (which may influence the relative balance in secondary movements of the knee in the anterior–posterior, transverse and frontal planes, thought to be important for normal knee joint function[33]), (2) why some females undergo larger increases in joint laxity (potentially making them more susceptible to joint instability and injury risk when magnitudes are increased), and (3) ultimately, the biomechanical implications of cyclic changes in the entire envelope of knee laxity and stiffness on functional knee joint neuromechanics.

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