Preliminary Evaluation of Dynamic Knee Valgus and Serum Relaxin Concentrations After ACL Reconstruction

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Background: Athletes who have sustained a tear of the anterior cruciate ligament (ACL) are at a greater risk of re-tear and of developing other adverse outcomes, such as knee osteoarthritis, compared with uninjured athletes. Relaxin, a peptide hormone similar in structure to insulin, has been shown to interfere with the structural integrity of the ACL in female individuals. The purpose of the present study was to evaluate dynamic knee valgus and the serum relaxin concentration (SRC) in athletes who had previously sustained a torn ACL and in those who had not.

Methods: The study included 22 female athletes, divided into 2 groups: those who had previously sustained a torn ACL (4 participants; torn ACL in the dominant leg in all cases) and those who had not (18 participants). Kinematic data were collected at 100 Hz. To assess dynamic knee valgus, participants performed a single-leg squat, a single-leg crossover dropdown, and a drop vertical jump at 2 time points in the menstrual cycle of the patient, pre-ovulatory and mid-luteal. SRC was determined with use of the Human Relaxin-2 Immunoassay using a blood sample obtained during the mid-luteal phase of the menstrual cycle.

Results: Independent samples t tests were utilized to compare the differences in dynamic knee valgus and SRC between groups. For the single-leg squat, participants with a prior torn ACL were found to have significantly higher dynamic knee valgus at the mid-luteal phase but not at the pre-ovulatory phase. For the drop vertical jump and single-leg crossover dropdown, participants with a prior torn ACL were found to have significantly higher dynamic knee valgus at both the pre-ovulatory and mid-luteal phases. SRC was also significantly higher among participants with a prior torn ACL.

Conclusions: Participants who had previously sustained a torn ACL had higher SRC and more dynamic knee valgus compared with those who had not. Further investigation of the effects of hormones as a risk factor for reinjury in participants with a prior ACL tear may be worthwhile. In addition, it may be worth monitoring hormonal and biomechanical properties in athletes during the long-term recovery from ACL reconstruction.

Level of Evidence: Prognostic Level III. See Instructions for Authors for a complete description of levels of evidence.

Female athletes sustain tears of the anterior cruciate ligament (ACL) at disproportionately higher rates than male athletes[1,2]. Despite widespread implementation of neuromuscular training programs, rates of ACL tears remain high among female athletes[3], leading scientists to consider other risk factors, such as hormones. Recently, relaxin, a peptide hormone similar in structure to insulin, has been identified as disturbing the structural integrity of the ACL[4]. Additionally, a high serum relaxin concentration (SRC) has been associated with ACL tears[5]. Relaxin is thought to bind to the ACL in the same manner that it binds to the pubic symphysis: by binding to a G-protein coupled receptor (RXFP1 or RXFP2) and releasing collagenases, matrix metalloproteinase (MMP)-3, and plasminogen activators, which ultimately leads to a breakdown in the tissue fibers and greater joint laxity[6]. The effects of relaxin within the musculoskeletal system extend beyond the ACL. Previous studies have reported that relaxin plays a role in regulating healing and fibrosis in skeletal muscles[7,8], orchestrating bone remodeling[9,10], controlling tendon metabolism and ultimately length[11,12], and decreasing cartilage stiffness[13,14]. Because

TABLE I Demographics*			
	ACL Injury	No ACL Injury	P Value
Age (yr)	25.0 ± 6.8	21.1 ± 2.4	0.330
Weight (kg)	67.2 ± 10.5	63.9 ± 8.4	0.603
Height (m)	1.4 ± 0.6	1.6 ± 0.4	0.283
Body mass index (kg/m²)	23.9 ± 3.1	23.1 ± 2.1	0.660

*Values are given as the mean and standard deviation. P values were determined with use of independent samples t tests; there were no significant differences among demographic variables between groups.

of the expansive effects that relaxin has on the entirety of the musculoskeletal system, it may be important to monitor relaxin in patients following ACL reconstruction.

The purpose of the present study was to compare dynamic knee valgus and SRC between female athletes who had previously sustained a torn ACL and those who had not. It was hypothesized that athletes who had previously sustained a torn ACL would display higher SRC as well as greater dynamic knee valgus during 3 clinical tests.

Materials and Methods

A convenience sample of 22 female athletes volunteered for participation in the study. Selection criteria included being medically cleared to participate in sports, currently participating in a sport, having no previous injuries or surgeries within the past 6 months, having no history of preg-

nancy, and having a regular menstrual cycle with at least 10 menstrual periods per year[5]. The institutional review board of Auburn University approved all testing protocols. Prior to data collection, all testing procedures were explained to each participant, and informed, written consent was obtained. Data collections were performed at 2 time points during the menstrual cycle of the participant: the pre-ovulatory phase (1 to 2 days after the end of the menstrual period) and the mid-luteal phase (22 to 24 days after the beginning of the menstrual cycle)[15].

Participants completed a health history questionnaire and took a urine pregnancy test (AccuMed Pregnancy Test [human chorionic gonadotropin]). Participants were divided into 2 groups: no history of ACL injury (18 participants) and a history of an ACL tear and reconstruction (4 participants). Participants were excluded from both groups if they had any other surgical procedures in a lower extremity. Participants with a prior torn ACL had undergone surgical treatment of the injury at a mean (and standard deviation) of 6.0 ± 3.3 years prior to the study period, had all returned to sport, and were all actively participating in a sport. In addition, all of these participants had received a patellar tendon graft. Participants were excluded if they had torn multiple ligaments, experienced multiple ACL tears, or undergone reconstruction <1 year prior to the study period.

Kinematic data were collected at 100 Hz with use of an electromagnetic tracking system (trakSTAR; Ascension Technologies) synced with The MotionMonitor (Innovative Sports Training). Eight electromagnetic sensors were affixed to the skin[16,17]. A ninth, moveable sensor was attached to a plastic
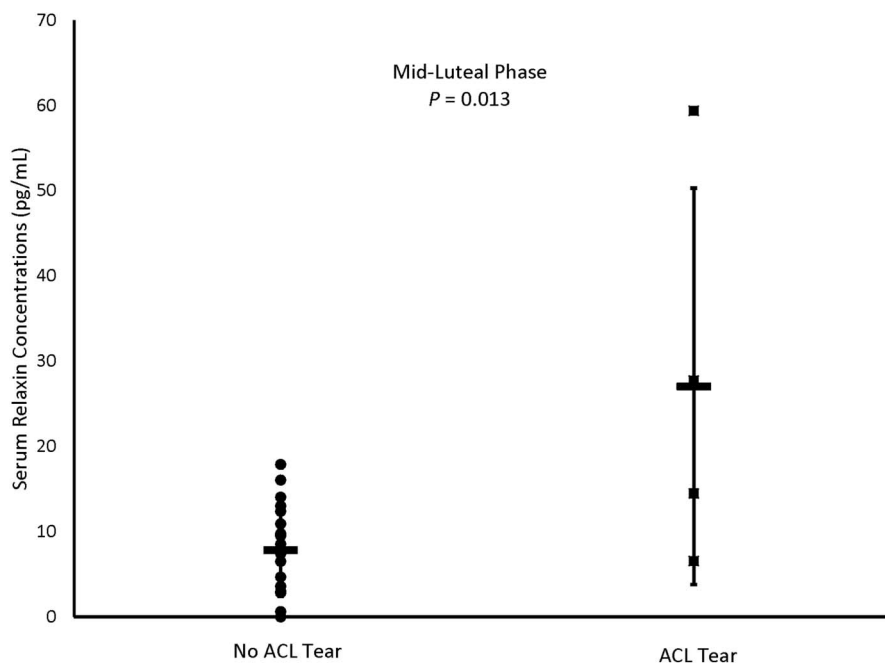

Fig. 1
Graph showing SRC for both groups. Individual values (circles and squares) and means and standard deviations (black bar and whiskers) are reported. The mean SRC was significantly higher among participants with a prior torn ACL.
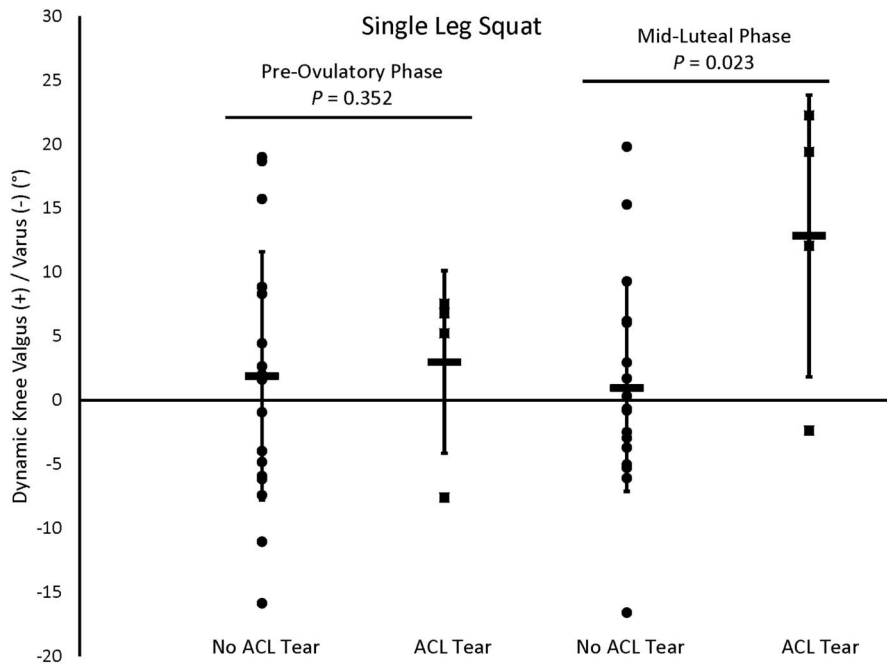
Fig. 2

Graph showing dynamic knee valgus during the single-leg squat at the pre-ovulatory and mid-luteal phases for both groups. Individual values (circles and squares) and means and standard deviations (black bar and whiskers) are reported. Mean dynamic knee valgus was significantly higher among participants with a prior torn ACL during the mid-luteal phase.

stylus for the digitization of osseous landmarks. A link-segment model was developed with use of the digitized joint centers for the ankles, knees, hips, T12-L1, and C7-T1.

Blood samples (3 to 4 mL) were obtained during the mid-luteal phase (22 to 24 days after the start of the menstrual cycle) when SRC is measurable[5]. At the time of
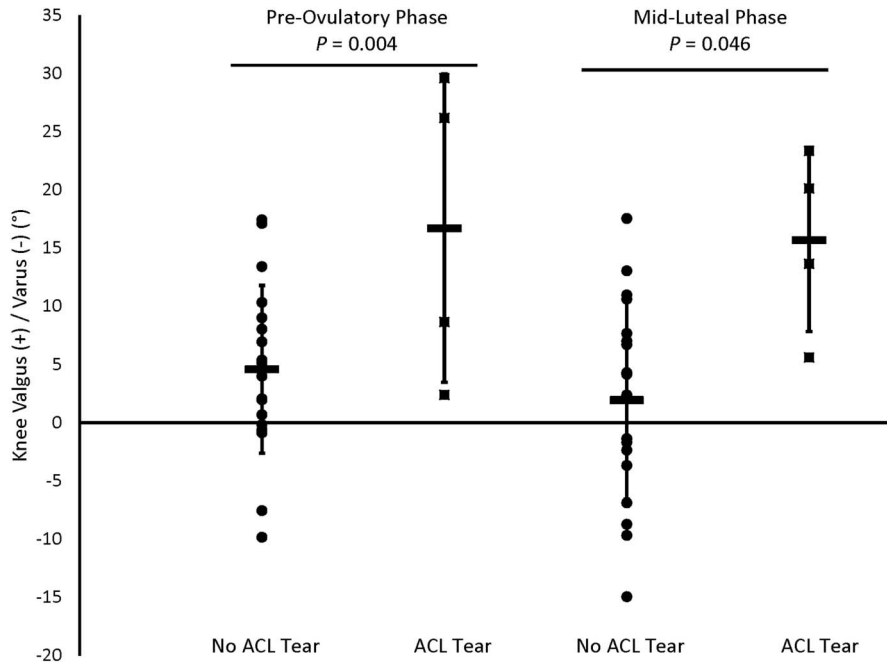

Fig. 3

Graph showing dynamic knee valgus during the drop vertical jump at the pre-ovulatory and mid-luteal phases for both groups. Individual values (circles and squares) and means and standard deviations (black bar and whiskers) are reported. Mean dynamic knee valgus was significantly higher among participants with a prior torn ACL at both time points.

collection, samples were allowed to clot and then spun at 3,500 ×g for 10 minutes at 25°C with use of a benchtop centrifuge. Approximately 1.5 mL of serum were removed and stored at −80°C until all samples were collected. A Quantikine Human Relaxin-2 Immunoassay (R&D Systems) was used to determine SRC, according to the protocol provided by the manufacturer[18]. All samples were run in triplicate, and SRC was calculated with use of a standard curve. The minimum detectable concentration was 1.0 pg/mL.

Dynamic knee valgus was evaluated as participants performed a single-leg squat, a drop vertical jump, and a single-leg crossover dropdown at both the pre-ovulatory and mid-luteal phases of the menstrual cycle[19]. In order to perform the single-leg squat, participants were instructed to cross the arms over the chest, flex the non-dominant knee to 90°, position the non-dominant leg behind the body, and squat as low as possible while maintaining balance, then to ascend to a neutral stance[20-22]. In order to perform the drop vertical jump, participants were instructed to stand on top of a 0.5-meter-tall box, drop off of the box with no leg preference, and, immediately on landing from the box, jump as high as possible, then land again. In order to perform the single-leg crossover dropdown, participants were instructed to stand on top of a 0.5-meter-tall box, cross the dominant leg over the non-dominant leg, and drop from the box onto the dominant leg. The investigator demonstrated these clinical tests for the participants, and the participants were allowed to practice before the data were recorded (average practice time, 5 minutes). If the participant did not comply with 1 of the guidelines (e.g., touched with the non-testing foot before full ascent in the case of the single-leg squat), the trial was rerecorded.

The value of dynamic knee valgus at maximum knee flexion on the dominant leg was used for analyses because the ACL group participants injured their dominant leg. All data were processed using a customized MATLAB (MATLAB R2010a; MathWorks) script. Statistical analyses were performed using Microsoft Excel for all data, with an alpha level set a priori at $\alpha = 0.05$. Prior to analysis, Shapiro-Wilk tests of normality were run. Results revealed approximate normal distributions for all variables. Independent samples t tests were used to compare the differences between dynamic knee valgus during the 3 clinical tests and SRC. For dynamic knee valgus, statistics were analyzed during the pre-ovulatory phase and the mid-luteal phase. A post hoc power analysis revealed the current study has a statistical power of 0.81.

Results

Demographics

Using independent samples t tests to assess demographic variables, no significant differences were found between the 4 participants with a prior torn ACL and the 18 participants without, including in age (25.0 and 21.1 years, respectively), weight (67.2 and 63.9 kg, respectively), height (1.4 and 1.6 m, respectively), and body mass index (23.9 and 23.1 kg/m², respectively) (Table I).
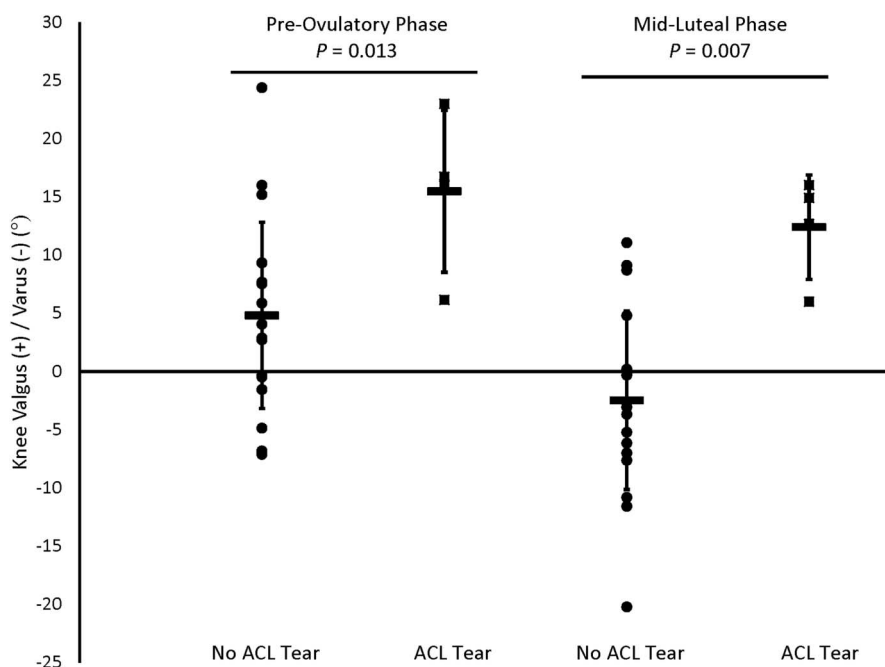

Fig. 4

Graph showing dynamic knee valgus during the single-leg crossover dropdown at the pre-ovulatory and mid-luteal phases for both groups. Individual values (circles and squares) and means and standard deviations (black bar and whiskers) are reported. Mean dynamic knee valgus was significantly higher among participants with a prior torn ACL at both time points.

SRC Measurements

A t test revealed the ACL injury group had significantly higher SRC than the ACL-injury-free group (27.0 and 7.8 pg/mL, respectively) (Fig. 1).

Dynamic Knee Valgus

Independent samples t tests were used to assess differences in dynamic knee valgus at the pre-ovulatory and mid-luteal phases as measured with use of a single-leg squat (Fig. 2), a drop vertical jump (Fig. 3), and a single-leg crossover dropdown (Fig. 4).

For the single-leg squat, participants with a prior torn ACL were found to have significantly higher dynamic knee valgus at the mid-luteal phase but not at the pre-ovulatory phase. For the drop vertical jump and single-leg crossover dropdown, participants with a prior torn ACL were found to have significantly higher dynamic knee valgus at both the pre-ovulatory and mid-luteal phases.

Discussion

Because of the long-term health consequences associated with an ACL tear and the high rates of ACL re-tear among female athletes, it is important to understand and characterize the risk factors for injury following reconstruction. The aim of the present study was to compare dynamic knee valgus and SRC between athletes who had previously sustained a torn ACL (4 participants) and those who had not (18 participants) at 2 time points in the menstrual cycle. We found that athletes with a prior torn ACL had significantly higher SRC and dynamic knee valgus than those who had not sustained a prior torn ACL. Because greater dynamic knee valgus and higher SRC are risk factors for reinjury, it may be beneficial to monitor these and other biomechanical and biochemical properties during the long-term follow-up of patients who have undergone ACL reconstruction. Relaxin has previously been linked to other areas of the musculoskeletal system including regulating healing and fibrosis in skeletal muscles[7,8], orchestrating bone remodeling[9,10], controlling tendon metabolism and ultimately length[11,12], and decreasing cartilage stiffness[13,14]. Understanding how relaxin affects ACL health and ACL reconstruction may provide insight into these other areas as well. Scientists have suggested that injury prevention models should include multiple risk factors to better characterize and understand how various risk factors interact and ultimately contribute to the risk of injury[3]; however, it is also important for evaluation post-reconstruction to include these variables. Although the present study was small and the direct implications of the study are limited, based on the preliminary conclusions drawn from these data, studies of this nature may provide further insight into more efficient methods of long-term monitoring following ACL reconstruction.

In this study, participants with a prior torn ACL had significantly higher levels of SRC (27.0 pg/mL) compared with those without a prior ACL injury (7.8 pg/mL). The observation that individuals with a prior torn ACL had significantly higher SRC than those with no prior ACL injury agrees with previous studies[5]. It should be noted that the SRC values reported for participants with a prior torn ACL (27.0 pg/mL) and those without (7.8 pg/mL) are both higher than the value reported by Dragoo et al. to be the threshold for ACL injury risk (6.0 pg/mL)[5]. Other previous studies have shown that the relaxin receptors RXFP1 and RXFP2 are expressed in substantially higher concentrations in the ACLs of females than those of males; however, the cause of this difference in expression is unclear[23,24]. Conversely, an additional study showed that young, athletic men expressed relaxin in similar concentrations to those of young, athletic women, and showed that there was no correlation between relaxin and joint laxity[25]. The current discrepancies in the literature highlight the need for further study of the relationship between ACL reconstruction and hormonal profiles. Because of the limited data related to relaxin and ACL injury, it is unclear whether higher SRC indicates a higher risk of ACL tear or if the effects of relaxin eventually plateau.

Within the sports medicine community, it has been suggested that athletes who have an ACL reconstruction are more likely to develop knee osteoarthritis and other health problems as a result of the ACL tear and the reconstruction[26]. Anatomic ACL reconstruction has consistently shown the ability to restore knee stability and kinematics, and results in functional scores that are comparable with injury-free knees[27-29]. If knee instability and the resultant forces were the sole factors contributing to the development of knee osteoarthritis, then osteoarthritis theoretically should develop at the same rates in knees that have undergone ACL reconstruction as in uninjured knees[27]. However, regardless of reconstruction, ACL tears have been reported to be associated with a higher risk of knee osteoarthritis. Indeed, knees with a prior ACL reconstruction have a relative risk of 3.62 of developing knee osteoarthritis compared with uninjured knees; similarly, nonoperative treatment of ACL-deficient knees showed a relative risk of 4.98 compared with uninjured knees[27]. Additionally, previous studies have illustrated that the synovial fluid in knees with an ACL injury contains significantly higher concentrations of interleukin (IL)-6 (a cytokine involved in acute inflammatory responses), MMP-3 (an enzyme involved in cartilage destruction), and tissue inhibitor of metalloproteinase (TIMP)-1 (a glycoprotein that counterbalances the effects of MMP-3) compared with uninjured controls[30-32]. Even though there have been few studies examining the changes to the biochemical properties of the knee following ACL reconstruction, such changes may be a key piece to understanding and providing the best treatment to athletes.

Given the known effects of relaxin on the risk of ACL injury, as well as the long-term effects on bone, joint, muscle, and tendon health[33], it is reasonable to question if relaxin contributes to the development of knee osteoarthritis and other joint problems, independently of ACL injury. This speculation further emphasizes the importance of long-term evaluation of hormones following ACL reconstruction and is of interest for further study.

In the present study, participants with a prior torn ACL were found to have significantly greater dynamic knee valgus as measured on the drop vertical jump and single-leg crossover

dropdown during both the pre-ovulatory and mid-luteal phases of the menstrual cycle. Because increased dynamic knee valgus is 1 of the strongest predictors of ACL injury[34] and 1 of the most common mechanisms of ACL injury[35], these findings indicate a greater risk of reinjury, which is not surprising considering that previous evidence suggests that athletes with a prior torn ACL are more likely to sustain a subsequent torn ACL[36].

The present study had limitations. Firstly, there was a low number of participants because of the lack of direct benefits offered to participants. Additionally, blood samples were only taken once to minimize the number of invasive procedures. Future studies should consider taking multiple blood samples over the course of multiple menstrual cycles. Finally, data from only 1 trial of each clinical tests were collected. This study was part of a larger study that included manual muscle testing techniques with electromyography, and the authors did not want to introduce fatigue as a variable.

In conclusion, participants who had a prior torn ACL had higher SRC compared with those without a prior torn ACL. This finding suggests that hormonal risk factors during the long-term recovery from ACL reconstruction may be worth further investigation because of the known correlation between high SRC and risk of ACL tears and because of the known effects of relaxin on other areas of the musculoskeletal system[5]. Additionally, participants with a prior torn ACL displayed greater dynamic knee valgus than those without a prior torn ACL across all 3 clinical tests. Because dynamic knee valgus is a mechanism of ACL injury, these findings suggest that the participants with a prior torn ACL still have different movement patterns compared with the participants without a prior torn ACL, and monitoring biomechanical and biochemical risk factors long-term may provide further insight into other adverse health outcomes associated with ACL reconstruction. ∎

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