Sex Comparisons of In Vivo Anterior Cruciate Ligament Morphometry

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Context: Females have consistently higher anterior cruciate ligament (ACL) injury rates than males. The reasons for this disparity are not fully understood. Whereas ACL morphometric characteristics are associated with injury risk and females have a smaller absolute ACL size, comprehensive sex comparisons that adequately account for sex differences in body mass index (BMI) have been limited.

Objective: To investigate sex differences among in vivo ACL morphometric measures before and after controlling for femoral notch width and BMI.

Design: Cross-sectional study.

Setting: Laboratory.

Patients or Other Participants: Twenty recreationally active men (age = 23.2 ± 2.9 years, height = 180.4 ± 6.7 cm, mass = 84.0 ± 10.9 kg) and 20 recreationally active women (age = 21.3 ± 2.3 years, height = 166.9 ± 7.7 cm, mass = 61.9 ± 7.2 kg) participated.

Main Outcome Measure(s): Structural magnetic resonance imaging sequences were performed on the left knee. Anterior cruciate ligament volume, width, and cross-sectional area measures were obtained from T2-weighted images and normalized to femoral notch width and BMI. Femoral notch width was measured from T1-weighted images. We used independent-samples t tests to examine sex differences in absolute and normalized measures.

Results: Men had greater absolute ACL volume (1712.2 ± 356.3 versus 1200.1 ± 337.8 mm³; t₉₈ = −4.67, P < .001) and ACL width (8.5 ± 2.3 versus 7.0 ± 1.2 mm; t₉₈ = −2.53, P = .02) than women. The ACL volume remained greater in men than in women after controlling for femoral notch width (89.31 ± 15.63 versus 72.42 ± 16.82 mm²/mm; t₉₈ = −3.29, P = .002) and BMI (67.13 ± 15.40 versus 54.69 ± 16.39 mm²/kg/m²; t₉₈ = −2.47, P = .02).

Conclusions: Whereas men had greater ACL volume and width than women, only ACL volume remained different when we accounted for femoral notch width and BMI. This suggests that ACL volume may be an appropriate measure of ACL anatomy in investigations of ACL morphometry and ACL injury risk that include sex comparisons.

Key Words: anterior cruciate ligament volume, femoral notch width, knee, magnetic resonance imaging

Key Points

- Whereas anterior cruciate ligament (ACL) volume and width were greater in men than in women, sex morphology differences existed only for ACL volume after accounting for femoral notch width and body mass index.
- Anterior cruciate ligament volume may be an appropriate measure when investigating intrinsic ACL injury risk factors between males and females.
- High-risk populations with smaller ACL sizes and weaker ACLs need to be identified.

Higher anterior cruciate ligament (ACL) injury rates in females have been consistently reported.1-5 Even after sport and competition level have been taken into consideration, female athletes are twice as likely to sustain a first-time ACL injury than are male athletes.3 This sex disparity in injury risk has remained consistent despite prevention efforts over the past 2 decades. To date, these prevention efforts have largely focused on biomechanical and neuromuscular risk factors because they are believed to be modifiable through training. However, addressing these factors alone has not yielded a major shift in the incidence of ACL injury among the physically active.6 Whereas anatomic risk factors are not as easy to modify, we still need to understand their effects on knee-joint health and how to best protect against or counteract this risk.

One anatomic risk factor is a smaller ACL volume. In a prospective case-control study, Whitney et al7 reported that a smaller ACL volume was an independent predictor of ACL injury among a combined-sex sample. In another case-control study8 of a combined-sex sample, participants with ACL injuries had less ACL volume on their uninjured side than did control participants. As the size of connective tissue increases, it is generally associated with greater resistance to deformation.9 Specific to the ACL, a larger ACL graft volume has been associated with greater failure
load,\textsuperscript{10} implying that large ACLs (those with greater volume, width, or cross-sectional area [CSA]) would have a greater capacity to resist external forces.

Researchers\textsuperscript{11} have suggested that females have smaller ligaments, which may be a contributing factor to their higher rate of ACL injury. Specifically, in a cadaveric study, Chandrashekar et al\textsuperscript{11} reported that females had less absolute ACL volume and CSA than males. Anderson et al\textsuperscript{12} observed that ACL widths and CSAs were smaller in female than in male high school basketball athletes. These indications of smaller morphometric characteristics among females could be associated with less ligamentous restraint capacity that, in turn, could potentially increase the ACL injury risk. However, measurements of ACL size have lacked consistency. For example, ACL size can be determined from dimensions such as ACL volume, width, and CSA.\textsuperscript{7,8,11–18} These have been measured from images such as coronal-, sagittal-, and axial-plane views\textsuperscript{12–14} and in different physiological environments.\textsuperscript{11,12} Importantly, reports of ACL morphometry\textsuperscript{11–14} offered little consistency in accounting for body size. Sex-specific in vivo comparisons in which researchers have comprehensively assessed ACL morphometry have produced variable results, as the authors\textsuperscript{7,8} did not comprehensively account for factors that may have influenced ACL size in participants with ACL injuries.

When examining sex differences in ACL morphometric characteristics, researchers need to account for factors, such as bony geometry and body size, that may interact with ligament size to influence restraint capabilities. Specifically, a smaller femoral notch may impinge the ACL\textsuperscript{19} and increase the ACL injury risk.\textsuperscript{7,20} A smaller femoral notch area has also been associated with a smaller ACL CSA, with females having smaller relative notch-width areas than males.\textsuperscript{18} This suggests that the smaller femoral notch width in females may both limit the size of the ligament (resulting in less restraint capacity) and increase tension on the ACL (resulting in greater external loading), potentially increasing the ACL injury risk. Body size may also explain sex differences, as ligament size would reasonably be expected to scale to body size. When accounting for body weight, Anderson et al\textsuperscript{12} noted that the ACL CSA was smaller in females than in age-matched males. Adjusting for body weight has been demonstrated\textsuperscript{7} to increase the relationship between ACL volume and the injury risk in females. Using a multifactorial model, Jamison et al\textsuperscript{16} found that body height was the only predictor of ACL volume. Therefore, body size should be considered when examining ACL morphometry.

A better understanding of in vivo sex-specific differences in ACL size and femoral notch width could contribute to our understanding of the underlying factors that may increase a female’s susceptibility to ACL injury. To date, we are not aware of any researchers who have comprehensively compared ACL morphometric characteristics in men and women before and after accounting for potential characteristics that may affect ligamentous size and thus strength. Therefore, the purpose of our study was to investigate sex differences among in vivo ACL morphometric measures before and after controlling for femoral notch width and body mass index (BMI).

\section*{METHODS}

\subsection*{Participants}

Forty recreationally active men (n = 20, age = 23.2 ± 2.9 years [range = 19–30 years], height = 180.4 ± 6.7 cm [range = 170–192 cm], mass = 84.0 ± 10.9 kg [range = 63–106 kg], Marx Activity Rating Scale score = 9.2 ± 4.1 [range = 4–16]) and women (n = 20, age = 21.3 ± 2.3 years [range = 18–27 years], height = 166.9 ± 7.7 cm [range = 151–182 cm], mass = 61.9 ± 7.2 kg [range = 51–76 kg], Marx Activity Rating Scale score = 10.7 ± 3.9 [range = 4–16]) from local universities participated in this study (Table 1). The participants were part of a larger study in which we examined knee structure and function.\textsuperscript{21} For the current study, they attended a magnetic resonance imaging (MRI) testing session consisting of 3-dimensional (3-D) T1- and T2-weighted MRI of the left knee. All participants provided written informed consent, and the study was approved by the University of North Carolina at Greensboro’s Institutional Review Board.

\subsection*{Magnetic Resonance Imaging Examination}

We acquired MRI data and ACL morphometric measures as described in a previous study.\textsuperscript{21} For femoral notch-width measures, we used T1-weighted, multiplanar MRI scans with a repetition time of 1200 milliseconds, excitation time of 33 milliseconds, field of view of 160 × 160 mm, and voxel size of 0.5 × 0.5 × 0.6 mm.

\subsection*{Morphometric Data Reduction}

All morphometric data reduction was performed by a single investigator (H.M.W.). The ACL volume data reduction was conducted as described in another study.\textsuperscript{21} The calculation of ACL volume is provided in Figure 1. For the purpose of reliability, the ACL volumes of 10 participants were measured on 2 occasions, at least 1 week apart (intraclass correlation coefficient [ICC] (3,1) of the standard error of measurement [SEM] = 0.97 [36.1 mm$^3$/2.4% of mean ACL volume]) in a pilot study.

We assessed ACL width using MIPAV software (National Institutes of Health, Bethesda, MD) per previously described methods.\textsuperscript{12,22} The sagittal-plane slice that showed the clearest image of the Blumensaat line was selected. A line on the point of the notch outlet that was drawn perpendicular to the Blumensaat line across the ACL was defined as the ACL width (Figure 2). The investigator had previously established intratester measurement consis-

\begin{table}[h]
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\caption{Descriptive Statistics}
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Measure & Men (n = 20) & Women (n = 20) \\
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Anterior cruciate ligament & & \\
Volume, mm$^3$ & 1712.2 ± 356.3 & 1200.1 ± 337.8a \\
 & (1052.0–2261.0) & (805.2–2231.0) \\
Width, mm & 8.5 ± 2.3 & 7.0 ± 1.2a \\
 & (5.3–12.5) & (4.7–9.3) \\
Cross-sectional area, cm$^2$ & 0.9 ± 0.2 & 0.8 ± 0.2 \\
 & (0.6–1.3) & (0.4–1.3) \\
Femoral notch width, mm & 19.1 ± 1.8 & 16.4 ± 1.1a \\
 & (15.9–22.5) & (13.8–18.8) \\
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\begin{flushright}
\textsuperscript{a}Difference between sexes (P < .05).
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\end{table}
tency and precision (ICC [3,1] of the SEM = 0.98 [0.3 mm/3.8% of mean ACL width]).

The ACL CSA was measured with ITK-SNAP software using methods reported by Whitney et al. From an oblique sagittal-plane image perpendicular to the ACL, a point at one-third of the total ACL length from the attachment to the tibia was identified (Figure 3A). After this point was identified, the ACL CSA was segmented and calculated from the oblique axial image (Figure 3B). To establish intratester reliability and precision, the ACL CSA was measured with 10 participants twice, at least a week apart (ICC [3,1] of the SEM = 0.87 [0.7 cm²/87.5% of the mean ACL CSA]) in a pilot study.

Femoral notch width was measured using MIPAV software as reported by Stein et al. First, the clearest images of the Blumensaat line from the sagittal-plane image and the beginning of the Blumensaat line at the anterior outlet from the frontal-plane image were chosen to identify the axial-plane image. Second, from the axial-plane image, the articular surface line tangent to the medial and the lateral femoral condyle was drawn. The notch depth was the line perpendicular to the articular surface line that reached to the most anterior portion of the notch. At two-thirds of the notch depth, the notch width was calculated as the line parallel to the articular surface line (Figure 4). To establish intratester reliability and precision, femoral notch width of 10 participants was measured twice, at least a week apart (ICC [3,1] of the SEM = 0.99 [0.2 mm/1.1% of mean femoral notch width]) in a pilot study.

**Statistical Analysis**

We used independent-samples t tests to examine the differences in ACL morphometry and femoral notch-width measures between sexes. We also examined sex differences in ACL morphometric characteristics after normalization to femoral notch width. Test variables were ACL volume (mm³), ACL width (mm), ACL CSA (cm²), and femoral notch width (mm). Cohen d effect sizes (ESs) were calculated for all comparisons and interpreted as trivial (<0.20), small (0.20–0.49), moderate (0.50–0.79), or large (≥0.80). We set the α level for all analyses a priori at ≤0.05. All calculations were performed using SPSS (version 21.0; IBM Corp, Armonk, NY).

**RESULTS**

Sex-specific descriptive statistics for ACL volume, width, and CSA and femoral notch width are shown in Table 1. Independent-samples t tests indicated that men had a larger ACL volume (t38 = −4.67, P < .001, ES = 1.47), ACL width (t38 = −2.53, P = .02, ES = 0.80), and femoral notch width (t38 = −5.52, P < .001, ES = 1.74) than women. We
observed no sex difference in ACL CSA ($t_{38} = -1.89, P = .07, ES = 0.60$). After normalization by femoral notch width, ACL volume was still larger in men than in women ($t_{38} = -3.29, P = .002, ES = 1.04$). However, no sex differences were present in ACL width ($t_{38} = -0.61, P = .54, ES = 0.19$) or ACL CSA ($t_{38} = -0.26, P = .79, ES = 0.08$). After normalization by BMI, ACL volume was still larger in men than in women ($t_{38} = -2.47, P = .02, ES = 0.78$). Yet we noted no sex differences in ACL width ($t_{38} = -0.52, P = .60, ES = 0.16$) or ACL CSA ($t_{38} = -0.16, P = .88, ES = 0.05$). Normalized ACL morphometric values are shown in Table 2.

DISCUSSION

Whereas a higher incidence of ACL injury in females has been consistently reported,1–5 the potential role of ligamentous characteristics in ACL failure and the corresponding factors that may affect them are not well understood.7 We examined sex differences in ACL morphology before and after controlling for femoral notch width and BMI to better understand the role of ligamentous structure in the sex bias of ACL injury. Our primary finding was that men had greater ACL volume before and after accounting for sex differences in bony geometry and BMI. This sex difference became smaller with normalization, suggesting that other anatomic characteristics, such as the tibial slope,26 may need to be considered in conjunction with ligament size.

Our findings for absolute ACL widths between sexes are similar to those from another in vivo study.12 Our results for absolute ACL volume are supported by a cadaveric study11 in which the researchers reported that females had 30% to 35% smaller ACL volume than males. We observed less ACL volume and width in women, but the factors that contribute to these sex differences and the mechanism(s) by which these findings may directly influence ACL injury risk are not well understood.

Theoretically, a smaller connective-tissue size would correlate with a lower capacity to resist external forces.9 Specific to the ACL, a smaller ACL volume has been associated with a lower failure load.10 Furthermore, in a computational study of simulated in situ ACL stress, Westermann et al27 indicated that a smaller ACL-graft diameter was associated with greater ACL stress during loading. Hence, it is possible that the smaller ACL morphometry per unit of BMI in females could be associated with less restraint capacity, increasing their risk of ACL rupture relative to males.1–5

Given that ACL volume is a 3-D measure that uses multiple sagittal-plane images to fully characterize the entire ACL anatomy, this measure is logically the most representative way to encompass the morphometry of the ACL.7,8,16 Investigators28 have suggested that 3-D simulation models using finite element analysis of the ligament could better predict ligamentous biomechanics than 1-dimensional or 2-dimensional models, indicating that ACL volume may be the best predictor of ligamentous function. In future studies, researchers should address which measures of ACL size are most strongly associated with ligamentous function.
We were surprised to observe sex differences in ACL volume and width but not CSA. A potential reason for this finding may be the method by which CSA was obtained in vivo. Whereas ACL width and CSA are both single-plane measures that account for the nonuniform 3-D ACL, the contrast of ligamentous tissue to surrounding tissue was slightly better for ACL width (Figure 2) than measures from the oblique planes used in the ACL CSA calculation (Figure 3). This likely resulted in greater error in the CSA measure, making real differences more difficult to identify. This was demonstrated in our investigation, as the ratios of the SEM to the mean difference were 0.3/1.49 mm (20.1%) in ACL width and 0.7/0.13 cm² (538%) in CSA, which suggests that the precision by which we were able to measure CSA was relatively much greater than the measured difference when comparing the CSA ratio with ACL width. Therefore, for 2-dimensional measures, ACL width may delineate differences in ACL size better than CSA. Collectively, given the better delineation of ACL morphology on standard sagittal-plane images and completely accounting for 3-D structure, ACL volume may be preferred over ACL width and CSA measures to fully represent ACL morphometry.

Our finding of smaller femoral notch width in women than in men (16.4 versus 19.1 mm) is supported by a previous report (15.6 versus 17.7 mm).²⁹ Because females are smaller than males and a smaller femoral notch-width area has been correlated with a smaller ACL CSA,⁻¹ it may be relevant to control for skeletal size and BMI when making sex comparisons. Therefore, we also investigated differences in ACL morphology between men and women after normalizing to the femoral notch width and BMI. Men had greater ACL volume than women after normalization to a clinically relevant skeletal geometry measure and BMI. Given the reported sex differences in the bony anatomy of the knee and the association between ACL injury risk and a narrower femoral notch width,²¹ ACL volume may best represent ACL morphometry. The ACL volume is also the only ACL morphometric measure that has been reported as a predictor of ACL injury risk.⁷ Collectively, normalized ACL volume may be an appropriate measure for studying the combined ACL and bony morphology of the knee when considering ACL injuries to both males and females.

Our findings have important clinical implications. Most notably, we demonstrated that, when accounting for sex differences in BMI and bony geometry, women had smaller ACLs than men. Whereas post hoc analysis demonstrated that the magnitude of the sex difference decreased after normalization (decrease in ES of 0.43), both femoral notch width and BMI displayed similarly decreased proportions. This suggests that females are at a distinct disadvantage, as they will likely have smaller ACLs to provide the restraint for a BMI comparable with that of a male. Although the reasons for this smaller anatomy are not yet clear and require further study, it reinforces the need to “shore up” the dynamic support systems through neuromuscular training to stress shield the ACL and maximize knee stability. To our knowledge, researchers have not investigated whether the size or strength of the ACL itself can be increased through training. Empirical evidence is limited, yet in a cross-sectional study of power lifters and non-power lifters, Grzelak et al.³⁰ indicated that chronic training load may affect ACL size, particularly if that training is initiated at a younger age. Further work is needed to determine the extent to which ACL size and strength are modifiable through training designed to specifically load and strengthen the bone-ligament-bone complex. Finally, Wang et al.²² reported a negative relationship between ACL size and anterior knee laxity. If ACL size and structural quality reflect ACL function, measuring laxity may give insights into ligament size as an aspect of screening.

Our study had limitations. All ACL and femoral notch measures required manually segmenting the contour from each MRI image. Depending on the magnetic field strength, chosen sequence, and individual participant variation, a uniform resolution/pixel intensity distinguishing the ACL from surrounding soft tissues was not present, so automated segmentation was not possible. However, our intratester consistency of the ACL measures suggests these measures are reliable. Also, all measures were limited to the left knee because researchers have demonstrated a side-to-side difference in ACL volume of 26.2 mm³, which is equivalent to approximately 1% of the mean measures.¹⁶ Furthermore, even though Marx Activity Rating Scale scores did not differ between men and women, our study was limited by including a generally healthy, active population. However, this concern is somewhat allayed by the Marx Activity Rating Scale scores of our participants (men = 9.2 ± 4.1, women = 10.7 ± 3.9) being similar to those of military cadets with no history of knee injury (males = 12.2 ± 4.3, females = 10.2 ± 4.6).³² We still do not know if the differences we observed would be found in a highly athletic population and would prospectively predict the risk of ACL injury.

CONCLUSIONS

Whereas active men had greater ACL volume and width than active women, only ACL volume differed when we accounted for femoral notch width and BMI. Given the previously established association of ACL volume with ACL injury risk,³ the former may be a relevant measure for investigating sex differences in ACL injury rates. Future studies are needed to determine the factors associated with smaller ACL morphology and weaker ligaments to better target individuals at high risk for injury.

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