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Symmetry and sex differences in knee kinematics and ACL elongation in healthy collegiate athletes during high-impact activities revealed through dynamic biplane radiography

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Abstract

The objectives of this study were to determine symmetry and sex differences in knee kinematics and anterior cruciate ligament (ACL) elongation waveforms in healthy athletes without a history of a knee injury during fast running, drop jump, and 180° internal/external rotation hops. It was hypothesized that knee abduction angle and ACL relative elongation would be greater in women than in men during all activities. Bilateral knee kinematics and ACL relative elongation were determined in 19 collegiate athletes using dynamic biplane radiography. Sex differences in kinematics and ACL relative elongation waveforms were identified using statistical parametric mapping. Average absolute side-to-side differences (SSD_A) in kinematics and ACL relative elongation waveforms were determined for each activity. Women had up to 2.3° (all $p < 0.05$) less knee adduction angle and had greater ACL relative elongation (max. 4.8%–9.2%; all $p < 0.01$) than men during all activities, in support of the hypotheses. SSD_A in kinematics were 1.4 mm and 5.5° or less in all components of translation and rotation, respectively, while SSD_A in ACL relative elongation was 3.6% or less across all activities. Greater ACL relative elongation across a variety of activities may make women more susceptible to ACL injury than men. This study provides valuable reference data for identifying abnormal asymmetry in knee kinematics and ACL elongation in athletes after the ACL injury. These novel results improve our understanding of ACL elongation during demanding athletic activities and may help guide the development of sex-specific risk screening metrics, return to play assessments, and rehabilitation protocols after the ACL injury.

KEYWORDS

ACL, biomechanics, knee, rehabilitation

1 | INTRODUCTION

The number of anterior cruciate ligament (ACL) injuries has increased dramatically in recent years, leading to an estimated 134,000 ACL reconstruction surgeries performed annually in the United States.[1] Women have a fourfold to sixfold increased risk for ACL tear compared to men who play the same sport.[2] The high number of ACL injuries and elevated risk to women has led to the development of screening tests to identify athletes at increased risk for ACL injury[3] and return to playtests to determine when athletes can return to competition after treatment for ACL injury.[4]

Standard clinical measurements of movement quality that are used to screen for injury risk and to evaluate return to play capability are not accurate enough to detect small movement abnormalities that may increase ACL injury risk or indicate insufficient rehabilitation. Superficial measurements of joint kinematics made using motion capture or inertial measurement units do not reflect underlying bone kinematics during dynamic activities due to soft tissue artifact,[5] that is, the relative motion between the skin and underlying bone, which can be as high as 29 mm in translation and 24° in rotation, depending on the marker location and activity.[6,7] This inaccuracy is due to soft tissue artifact, in addition to marker placement error,[8] makes it difficult to assess ACL mechanics during dynamic activities using standard motion capture or inertial measurement units.

Dynamic biplane radiography (DBR) was developed in the late 1990s to accurately measure dynamic 6 degree-of-freedom (6DOF) knee kinematics.[9] The sub-millimeter and subdegree accuracy in bone kinematics provided by DBR can be combined with ligament attachment sites measured from magnetic resonance imaging (MRI) to estimate *in vivo* ACL elongation during dynamic activities.[10,11] DBR studies of the knee during hopping[12] and jogging[13,14] revealed that ACL reconstruction fails to restore native knee kinematics during those moderately challenging tasks, and that ACL elongation is related to 6DOF kinematics, primarily knee extension, during those activities.[10,11,15] However, the moderately challenging activities evaluated in those studies may not replicate ACL mechanics during athletic competition, risk screening, or return to playtests that require large external forces and muscle loading which have been shown to affect ACL strain.[16] Currently, the mechanical environment of the ACL during challenging activities remains unknown. Increased knowledge of ACL elongation and knee kinematics during more challenging tasks may help to identify activities that are most appropriate for screening and return to play evaluations as well as help guide improvements to rehabilitation protocols and surgical procedures, such as the optimal knee joint pose for ligament tightening during ACL reconstruction.

One common criterion used to assess functional recovery after ACL injury is symmetry, with the contralateral uninjured side serving as the reference standard for evaluating the injured or treated side.[17,18] Enduring side-to-side differences (SSD) in kinematics after ACL injury and treatment are believed to reflect inadequate surgery or insufficient rehabilitation. However, there are currently no data unaffected by soft tissue artifacts that quantify the SSD in knee kinematics or ACL elongation in healthy knees of athletes during high-demand activities. That data on healthy symmetry is crucial for providing context to determine if knee kinematics and ACL elongation have been adequately restored during challenging dynamic activities after ACL injury and treatment.

The higher incidence of ACL injury in women than in men[19] has been attributed to increased knee abduction angle,[20] which is consistently reported as the mechanism of noncontact ACL injury[21,22] and is associated with greater ACL elongation *in vitro*.[23,24] It is commonly believed in the research and clinical sport setting that women have greater knee abduction angle at initial contact during running at 3.7 m/s than men,[25] however, empirical support for this theory is inconsistent.[26,27] Contradictory data may exist due to the different tasks used to assess knee abduction angle and due to soft tissue artifact which varies among subjects and activities. There are currently no data unaffected by soft tissue artifacts that quantify sex differences in knee kinematics or ACL elongation in athletes performing injury screening, rehabilitation, or athletic activities. These data are needed to improve our understanding of the role kinematics play in the higher ACL injury rate in women, and subsequently in the development of appropriate prevention and surgery/rehabilitation strategies in female versus male athletes.

The objectives of this study were to determine symmetry and sex differences in 6DOF knee kinematics and ACL elongation without soft tissue artifact in athletes during dynamic activities that replicate athletic movements, injury risk screening tests, and return to sport evaluations. Based upon previous literature, we hypothesized that knee abduction angle and ACL elongation would be greater in women than in men during fast running, during a drop jump, and during a single-leg hop with rotation.[23,24,28] Symmetry in knee kinematics and ACL elongation was also calculated for each activity to provide context for future evaluation of athletes after ACL injury and clinical intervention.

2 | METHODS

2.1 | Subjects

Following Institutional Review Board approval, 19 participants provided informed consent and were enrolled in this study. The sample size was based upon previous studies that used DBR and dynamic MRI to measure kinematics after ACL reconstruction and to compare male versus female kinematics. Those studies were sufficiently powered to detect effects of ACL reconstruction and sex on knee kinematics using 18 subjects[29] and 20 subjects.[30] All participants were healthy collegiate athletes with no history of knee injury who were active in sports that require running, jumping, and/or cutting. All participants were varsity-level athletes, or, if their institution did not have a varsity-level team in their sport, they were on the top-level club team (Table 1).

2.2 | Collecting and processing dynamic *in vivo* kinematics data

All data collection and processing were conducted in the University of Pittsburgh Biodynamics Lab. Each athlete performed three activities within a custom biplane radiography system: fast running, drop jump, and single-leg hop with 180° internal/external rotation (Figure 1A). Synchronized biplane radiographs were collected at 150 images per second for each activity (imaging parameters: 90 kV, 160 mA maximum, 1 ms pulse width) (Figure 1B). Ground-reaction forces (GRFs) were obtained using a dual-belt instrumented treadmill (Bertec Corp) sampling at 900 Hz. Following a standardized warm-up, each participant underwent two submaximal practice trials of each activity before test trials were captured. Fast running was the athletic movement selected for evaluation because it is a common activity across a range of sports. The drop jump has been suggested as a valuable tool for screening athletes at risk for ACL injury[31] and was selected as the injury risk screening activity to be evaluated. The single-leg hop with rotation, which requires the athlete to hop off one foot, rotate in the air 180°, and land on the same foot, tests the rotational strength and stability of the knee[32] and was selected as the return-to-sport test to be evaluated. The fast running was performed at 5.0 m/s on the instrumented treadmill. For the drop jump, participants dropped off a 60 cm platform then performed a counter movement jump. For the single-leg rotating hop, participants began balancing on a single leg, then jumped and performed 180° of medial rotation or 180° of lateral rotation relative to the leg they were jumping on, and then landed while maintaining balance on the same leg. Only the take-off portion of the rotating hop was included in this analysis. Three trials were collected, processed, and analyzed for each side during fast running and drop jump activities, and two trials of internal and two trials of external rotating hops were collected for each side. Recovery of at least 30 s occurred between trials.

Bilateral volumetric femur and tibia bone data were obtained via computed tomography (CT) (GE Lightspeed 16; GE Medical Systems) (0.322 mm/pixel, 0.625 mm thickness) (Figure 1C). Commercial software was used to identify bone tissue within each scan (Mimics; Materialize) and three-dimensional (3D) models of each femur and

TABLE 1 Participant demographics

	Total	Male	Female	p Value
N	19	11	8	
Age (years)	20.1 ± 1.3	19.9 ± 1.2	20.4 ± 1.5	0.502
Height (cm)	174.2 ± 10.1	182.0 ± 3.5	163.5 ± 5.3	<0.001
Weight (kg)	72.5 ± 11.0	77.4 ± 8.2	65.8 ± 10.6	0.031
BMI	24.0 ± 2.8	23.5 ± 2.1	24.8 ± 3.4	0.416
Sports		4 track and field	2 track and field	
		3 ultimate frisbee	2 ultimate frisbee	
		2 rugby	2 softball	
		1 cross-country	1 tennis	
		1 soccer	1 lacrosse	

Note: Average value ±1 standard deviation.
Abbreviation: BMI, body mass index.

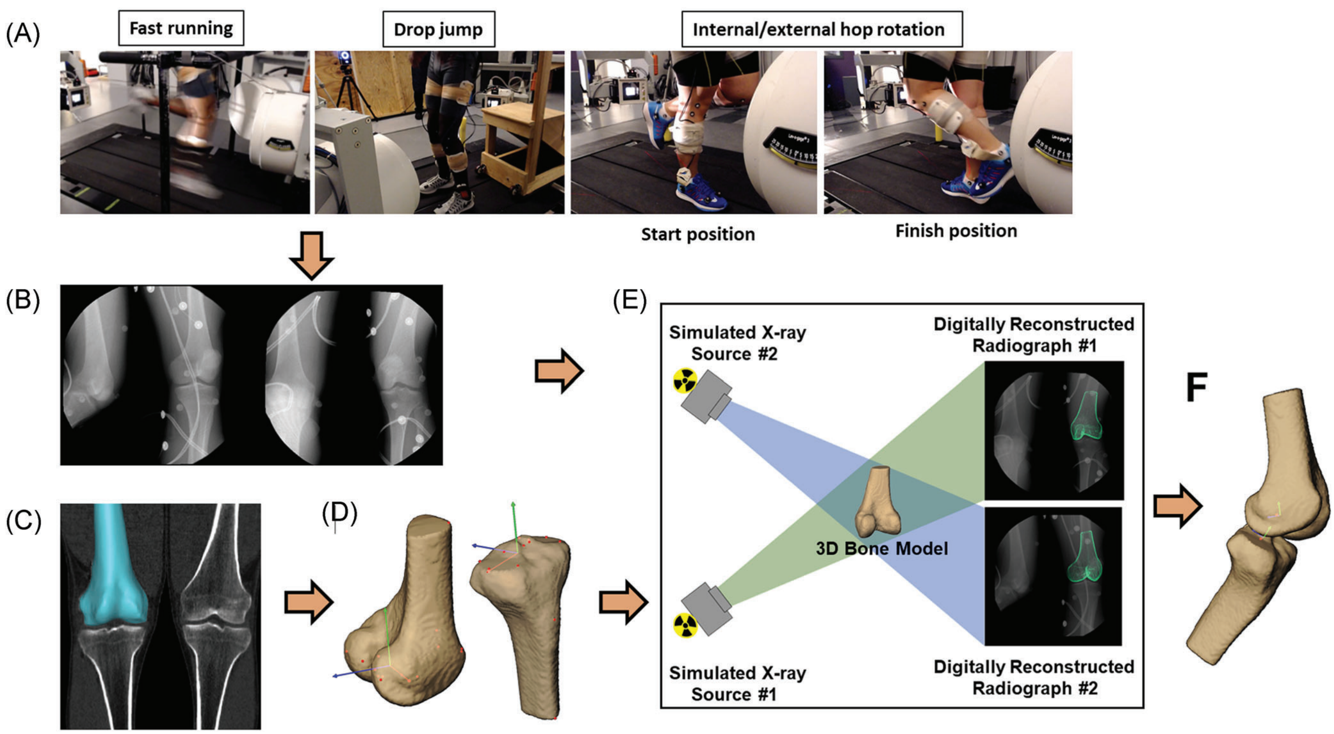

FIGURE 1 Data collection and processing using the biplane radiography system. (A) Participants performed fast running, drop jump, and single-leg rotating hop (internal/external rotation) within a biplane radiographic imaging system. (B) Synchronized biplane radiographs were collected at 150 Hz. (C) Subject-specific three-dimensional (3D) bone models of the bilateral femur and tibia were created from a high-resolution computerized tomography scan. (D) Landmarks were placed on the 3D bone models to establish an anatomical coordinate system. (E) A validated volumetric matching process was used to match the digitally reconstructed radiographs to the distortion corrected biplane radiographs. (F) Joint kinematics were calculated according to the anatomical coordinate systems [Color figure can be viewed at wileyonlinelibrary.com]

tibia were created separately for the left and right knees. Anatomic coordinate systems were constructed in each right femur and tibia based upon femoral condyle and hip joint center locations, and tibial plateau and ankle joint center locations according to previously established methods[33] and mirror imaged onto the left side after coregistering the corresponding right and left bones for each subject (Figure 1D).

Knee motion was determined for each trial using a previously validated volumetric model-based tracking process that matches a digitally reconstructed radiograph generated from the CT-based bone model to the biplane radiographs with *in vivo* accuracy of 0.7 mm or better in translation and 0.9° or better in rotation[34] (Figure 1E). Knee rotations were calculated following the Grood and Suntay convention.[35] Knee translations were calculated using the vector from the femur origin, located at the midpoint of the femoral condyles, to the tibia origin, located at the midpoint of the most medial and lateral borders of the tibial plateau[33] (Figure 1F), and expressed in the tibial coordinate system. Left knee kinematics results were mirrored to match the sign convention for the right knee. Knee kinematics were filtered at 8.5 Hz using a fourth-order Butterworth filter, with the optimal filter frequency determined through residual analysis.[36] Fast-running kinematic data were analyzed over the first 70% of the stance phase (0% = initial contact, 100% = toe-off). Drop jump kinematic data were normalized to percent stance, with 0% being initial contact and 100% being toe-off, with the eccentric phase, determined by knee flexion angle, mapped to 0%–50% and the concentric phase mapped to 51%–100% of the movement for each participant. Internal/external rotating hop kinematic data were normalized to the push-off phase (0% = maximum GRFs, 100% = toe-off). Kinematics data from each trial were interpolated to 1% instants of the activity and then averaged over the three (or two for rotational hops) trials, creating an average waveform for each of 38 knees during each activity.

Total radiation exposure associated with biplane radiography for this study was estimated to be 0.27 mSv (estimated using PCXMC, STUK—Radiation and Nuclear Safety Authority) while radiation exposure from the CT scan averaged 1.27 mSv. For comparison, the annual effective dose due to background radiation in the United States is about 3.0 mSv.[37]

2.3 | Identifying ACL attachments

3T MRI scans (T2 weighted DE3D sequence, 0.29 × 0.29 × 0.30 mm/voxel) (Prisma_fit; Siemens) of the knees were obtained. The knee was fully extended during scanning. The ACL attachments were identified by iteratively placing points on the insertion site boundary in each slice and adjusting placements by manually comparing them to neighboring image slices using axial, coronal, and sagittal planes of MRI.[38] This technique was previously validated to have an average bias of 0.6 ± 1.6 mm proximally and 0.3 ± 1.9 mm posteriorly for the femur, and 0.3 ± 1.1 mm laterally and 0.5 ± 1.5 mm anteriorly for the tibia, compared to the "gold standard" insertion measurement by a laser scanner.[38] Subject-specific MRI-based models of the femur and tibia were created and coregistered to the CT-based bone models, providing the 3D transformation needed to correctly place the ACL attachment sites on the 3D bone models.[10,38]

2.4 | Calculating ACL relative elongation

The ACL length was defined as the distance between the center of the femur and the tibia ACL attachments. ACL relative elongation was calculated for each 1% interval of each activity as the ratio of dynamic ACL length divided by static ACL length during the MRI.[10,11] All results were averaged over the three (or two) trials of each activity, creating an average ACL elongation waveform for each of 38 knees in each activity.

2.5 | Data analysis

Male versus female differences in the continuous kinematics waveforms and in the ACL relative elongations from 22 male knees (both knees of 11 male athletes) and 16 female knees (both knees of 8 female athletes) were identified using one-dimensional statistical parametric mapping[39] with significance set at $p < 0.05$ for all tests. As part of a secondary analysis, the continuous translation kinematics were normalized to knee size, with anterior–posterior (AP) translation normalized to the medial tibia AP length, medial–lateral (ML) translation normalized to the tibial plateau ML size, and proximal–distal (PD) translation normalized to femoral condyle height. Average absolute side-to-side differences (SSD_A) in continuous kinematics and ACL relative elongation waveforms were determined for each activity using the 38 paired knees.

3 | RESULTS

3.1 | Male versus female kinematics

No significant differences were observed between men and women in flexion/extension kinematics during fast running (Figure 2A). Women had less knee adduction angle from 0% to 65% of stance during fast running ($p < 0.05$; maximum difference: 2.2°) (Figure 2B). Women had up to 3° more internal tibial rotation than men during fast running, however, those differences did not reach statistical significance (Figure 2C). The tibia was more lateral ($p < 0.001$; maximum difference: 2.4 mm) and distal ($p < 0.001$; maximum difference: 3.5 mm) relative to the femur in men from 0% to 65% of stance (Figure 2D,E). The tibia was more anterior relative to the femur in men from 0% to 3% of stance ($p < 0.05$; maximum difference: 2.1 mm) and from 57% to 65% of stance ($p < 0.05$; maximum difference: 1.6 mm) (Figure 2F). Differences in translation were not observed after normalizing for bone size (Figure 2G–I).

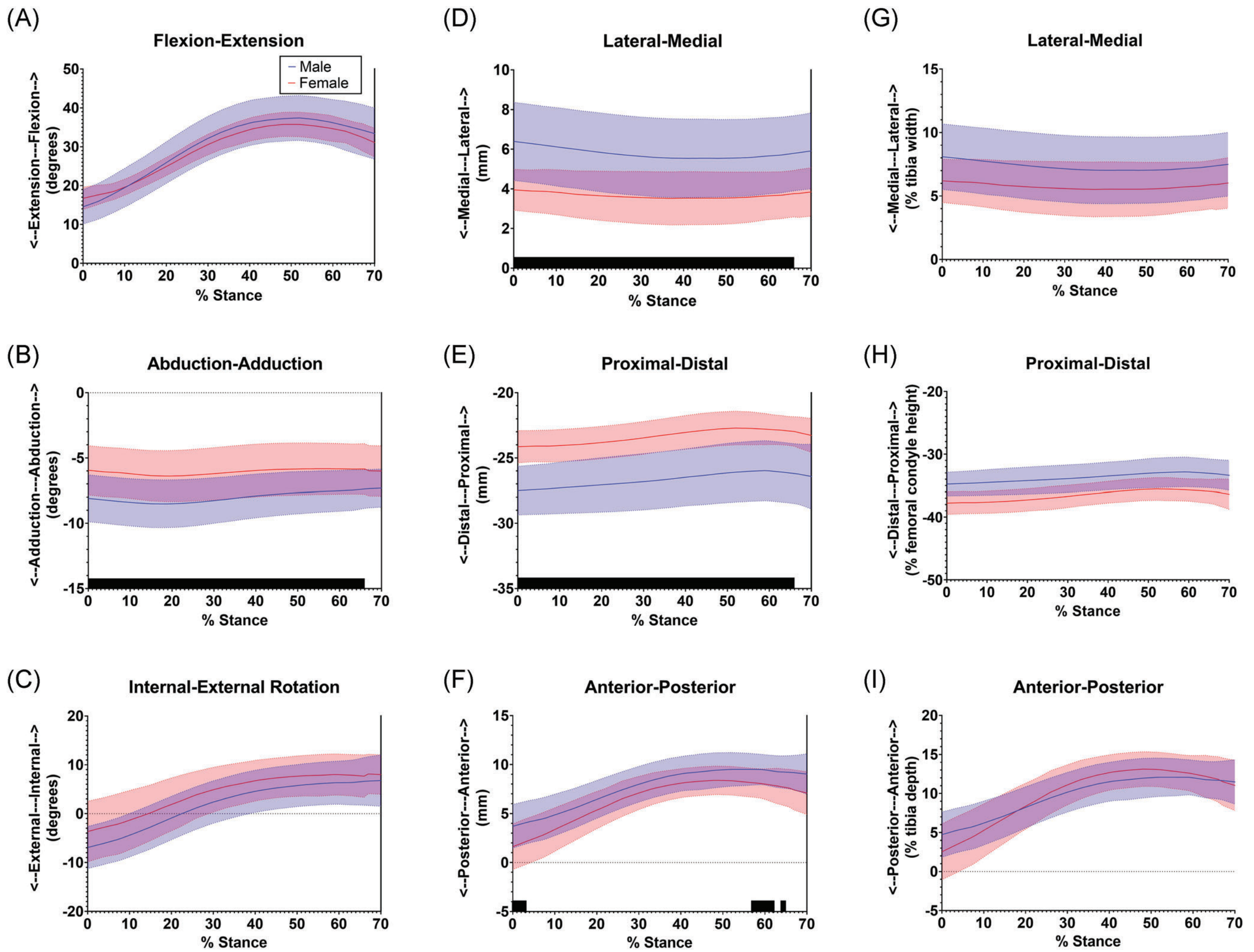

FIGURE 2 Average male and female 6 degree-of-freedom kinematics during fast running for (A) flexion/extension, (B) abduction/adduction, (C) internal/external rotation, (D) lateral/medial translation, (E) proximal/distal translation, (F) anterior/posterior translation, (G) lateral/medial translation normalized to bone size, (H) proximal/distal translation normalized to bone size, and (I) anterior/posterior translation normalized to bone size. For all kinematics values, the first direction listed is positive (flexion, abduction, internal, etc.). Blue curves represent men and red curves represent women. The shaded area represents one standard deviation. The black solid bar on the horizontal axis identifies periods of significant difference between men and women. AA, abduction/adduction; AP, anterior/posterior; FE, flexion/extension; IE, internal/external rotation; LM, lateral/medial; PD, proximal/distal [Color figure can be viewed at wileyonlinelibrary.com]

Men had slightly more knee flexion angle than women during the drop jump, however, those differences did not reach statistical significance (Figure 3A). Women had less knee adduction angle from 10% to 36% ($p < 0.03$; maximum difference: 1.9°) and from 87% to 97% ($p < 0.05$; maximum difference: 1.8°) of the drop jump activity (Figure 3B). No differences in internal/external rotation kinematics were observed during the drop jump (Figure 3C). The tibia was more lateral ($p < 0.001$; maximum difference: 2.6 mm) and distal ($p < 0.001$; maximum difference: 3.6 mm) relative to the femur in men from 10% to 97% of the drop jump activity (Figure 3D,E). The tibia was more anterior relative to the femur in men from 80% to 97% of the drop jump activity ($p < 0.05$; maximum difference: 2.7 mm) (Figure 3F). Differences in lateral–medial and PD (with female tibias being more distal) translation persisted through the middle portion of the drop jump activity after normalizing for bone size ($p < 0.05$ and $p < 0.001$, respectively) (Figure 3G–I).

Men had more knee flexion angle from 0% to 32% of the external rotating hop ($p < 0.05$; maximum difference: 5.7°) (Figure 4A), however, no differences in flexion/extension were observed during the internal rotating hop (Figure 5A). Women had less knee adduction angle from 5% to 95% of the external rotating hop ($p < 0.01$; maximum difference: 2.3°) (Figure 4B) and from 44% to 90% of the internal rotating hop activity ($p < 0.05$; maximum difference: 2.3°) (Figure 5B). No differences were observed in internal/external rotation kinematics during both the external and internal rotating hop (Figures 4C and 5C). The tibia was more lateral ($p < 0.001$; maximum difference: 2.3 mm) and distal ($p < 0.001$; maximum difference: 3.4 mm) relative to the femur in men from 0% to 95% of the external rotating hop ($p < 0.001$; maximum difference: 3.1 mm) and from 0% to 89% of the internal rotating hop activity ($p < 0.001$; maximum difference: 3.5 mm) (Figures 4D,E and 5D,E). The tibia was more

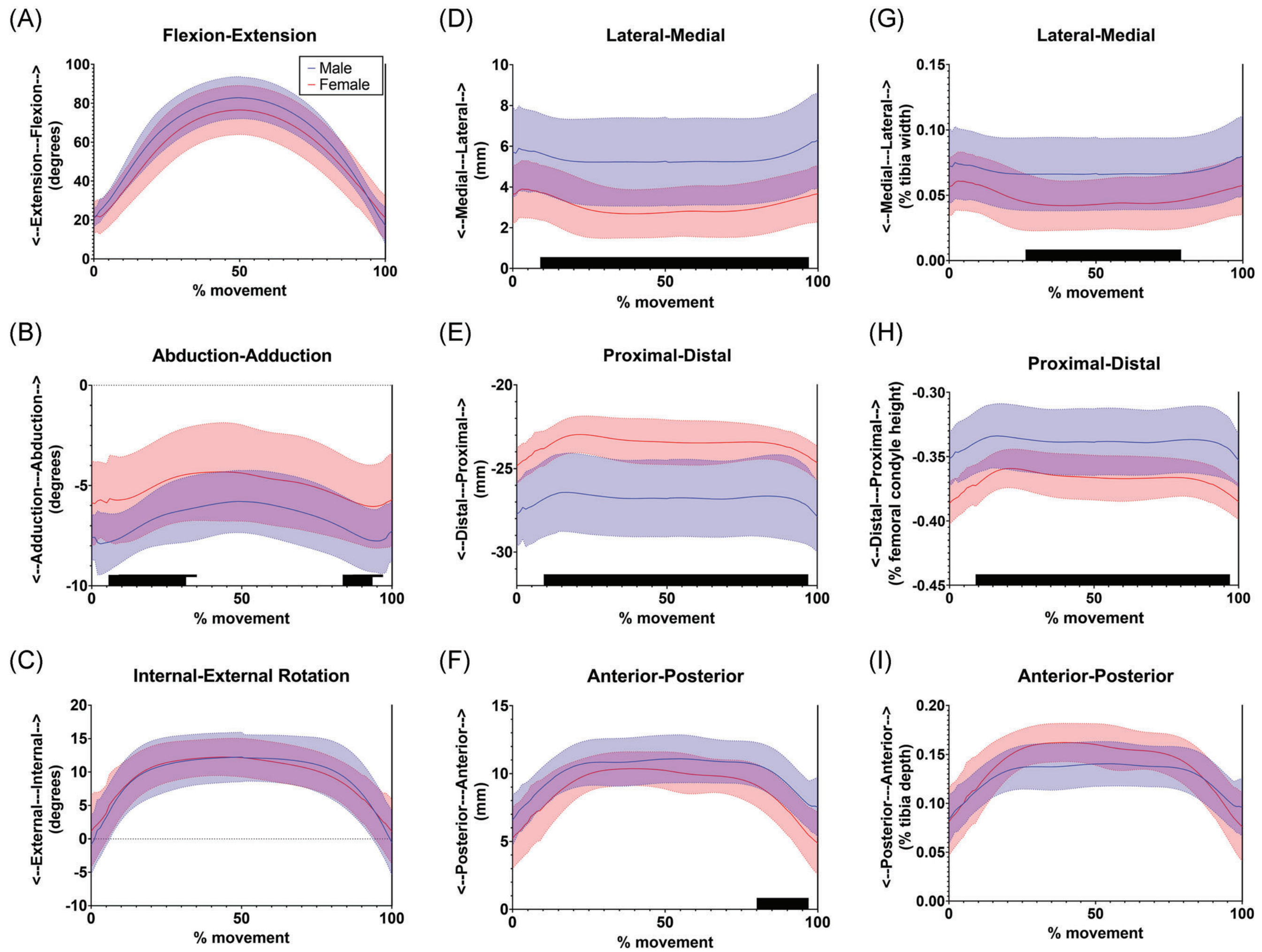

FIGURE 3 Average male and female 6 degree-of-freedom kinematics during the drop jump for (A) flexion/extension, (B) abduction/adduction, (C) internal/external rotation, (D) lateral/medial translation, (E) proximal/distal translation, (F) anterior/posterior translation, (G) lateral/medial translation normalized to bone size, (H) proximal/distal translation normalized to bone size, and (I) anterior/posterior translation normalized to bone size. For all kinematics values, the first direction listed is positive (flexion, abduction, internal, etc.). Blue curves represent men and red curves represent women. The shaded area represents one standard deviation. The black solid bar on the horizontal axis identifies periods of significant difference between men and women. AA, abduction/adduction; AP, anterior/posterior; FE, flexion/extension; IE, internal/external rotation, LM, lateral/medial; PD, proximal/distal [Color figure can be viewed at wileyonlinelibrary.com]

anterior relative to the femur in men from 0% to 95% of the external rotating hop activity ($p < 0.001$; maximum difference: 2.8 mm) (Figure 4F) and from 66% to 68% of the internal rotating hop activity ($p < 0.05$; maximum difference: 2.4 mm) (Figure 5F). The differences in lateral–medial translation remained from 0% to 2% and from 53% to 89% of the internal rotating hop activity after normalizing for bone size (Figure 5G), and the differences in PD translation (with female tibias being more distal) persisted after normalizing for bone size for both internal and external rotating hops (Figures 4H and 5H).

3.2 | Male versus female ACL elongation

ACL relative elongation increased as the knee flexed during fast running, and relative elongation was greater in women than in men from 16% to 58% of the stance phase ($p < 0.001$; maximum difference: 4.8%) (Figure 6A). During the drop jump, ACL relative elongation decreased as the knee flexed and increased as the knee extended, with relative elongation greater in women than in men from 6% to 81% of the activity ($p < 0.001$; maximum difference: 9.2%) (Figure 6B). ACL relative elongation increased as the knee extended during the rotating hop take-off, plateauing between 60% and 70% of the take-off phase for both internal and external rotating hops, and relative elongation was greater in women than in men from 0% to 60% of the take-off during internal rotating hop ($p < 0.01$; maximum difference: 7.3%) (Figure 6C) and from 0% to 27% of the take-off during the external rotating hop ($p < 0.01$; maximum difference: 5.2%) (Figure 6D).

There were no differences in peak relative elongation between men and women for any of the activities (Table 2).

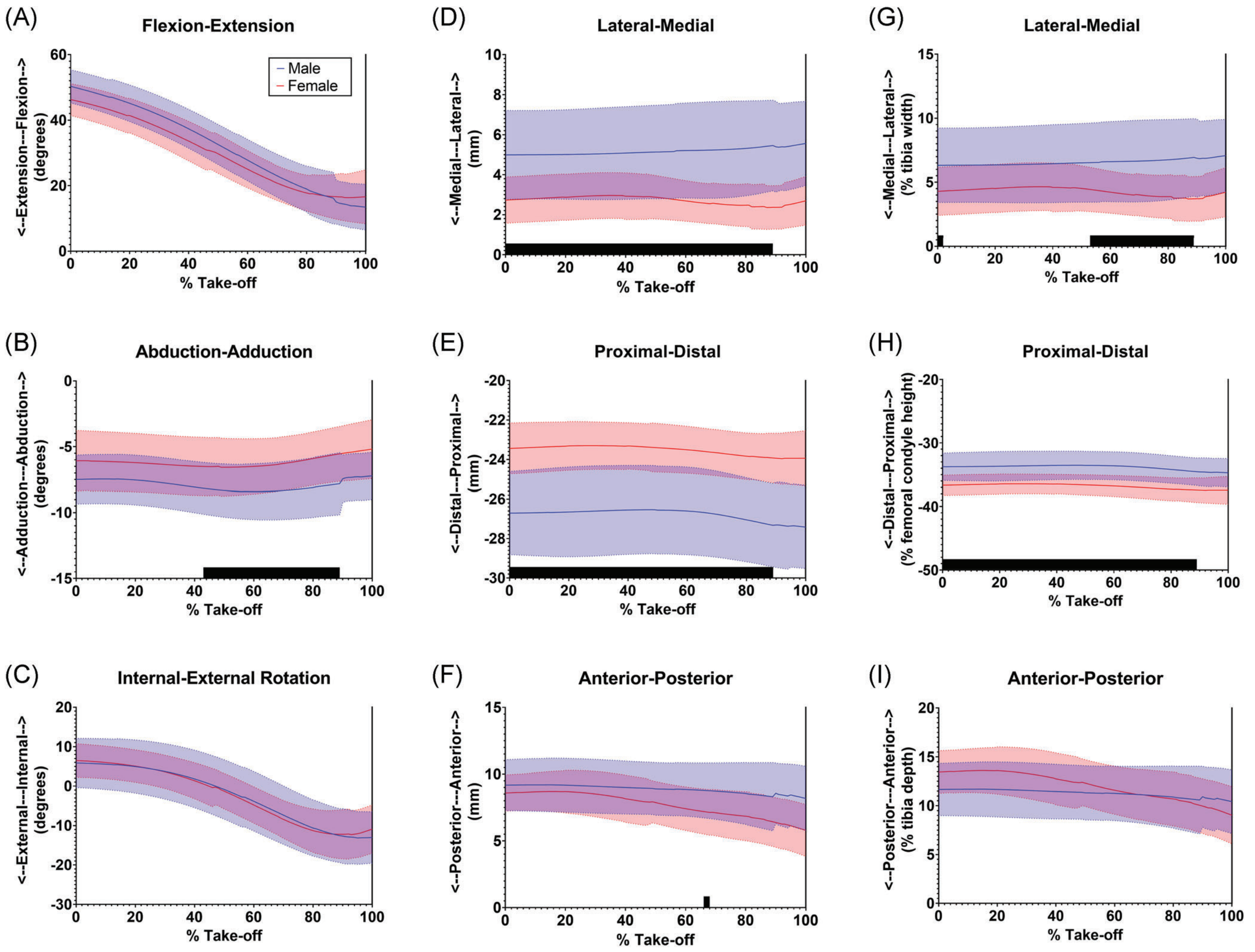

FIGURE 4 Average male and female 6 degree-of-freedom kinematics during the internal rotation hop for (A) flexion/extension, (B) abduction/adduction, (C) internal/external rotation, (D) lateral/medial translation, (E) proximal/distal translation, (F) anterior/posterior translation, (G) lateral/medial translation normalized to bone size, (H) proximal/distal translation normalized to bone size, and (I) anterior/posterior translation normalized to bone size. For all kinematics values, the first direction listed is positive (flexion, abduction, internal, etc.). Blue curves represent men and red curves represent women. The shaded area represents one standard deviation. The black solid bar on the horizontal axis identifies periods of significant difference between men and women. AA, abduction/adduction; AP, anterior/posterior; FE, flexion/extension; IE, internal/external rotation; LM, lateral/medial; PD, proximal/distal [Color figure can be viewed at wileyonlinelibrary.com]

3.3 | Bilateral symmetry

The average SSD_A in peak ACL elongation were 2.7 ± 2.2%, 3.1 ± 2.8%, 2.8 ± 4.4%, and 4.5 ± 6.0% during fast running, drop jump, internal rotation hops, and external rotation hops, respectively (Table 2). There were no differences in SSD_A in peak ACL relative elongation between men and women for any of the activities (Table 2).

The average SSD_A in kinematics waveforms during fast running was 2.8° or less for all rotations and 1.2 mm or less for all translations (Table 3). The average SSD_A in kinematics waveforms during the drop jump was 5.5° or less for all rotations and 1.3 mm or less for all translations (Table 3). The average SSD_A in kinematics waveforms during the rotating hops was 3.5° or less for all rotations and 1.4 mm or less for all translations (Table 3).

The average SSD_A in ACL elongation waveforms was 2.5 ± 1.1%, 3.6 ± 1.7%, 3.1 ± 2.7%, and 2.8 ± 1.0% during fast running, drop jump, internal rotation hops, and external rotation hops, respectively (Table 3).

3.4 | Task-dependency of ACL relative elongation

ACL relative elongation increased as the knee flexed during fast running, however, during the drop jump and rotating hops, ACL relative elongation decreased as the knee flexed and increased as the knee extended (Figure 7). The peak elongation was larger during external rotating hop than during fast running, drop jump, or internal rotating hop ($p < 0.001$, $p < 0.01$, and $p < 0.001$, respectively) (Table 2).

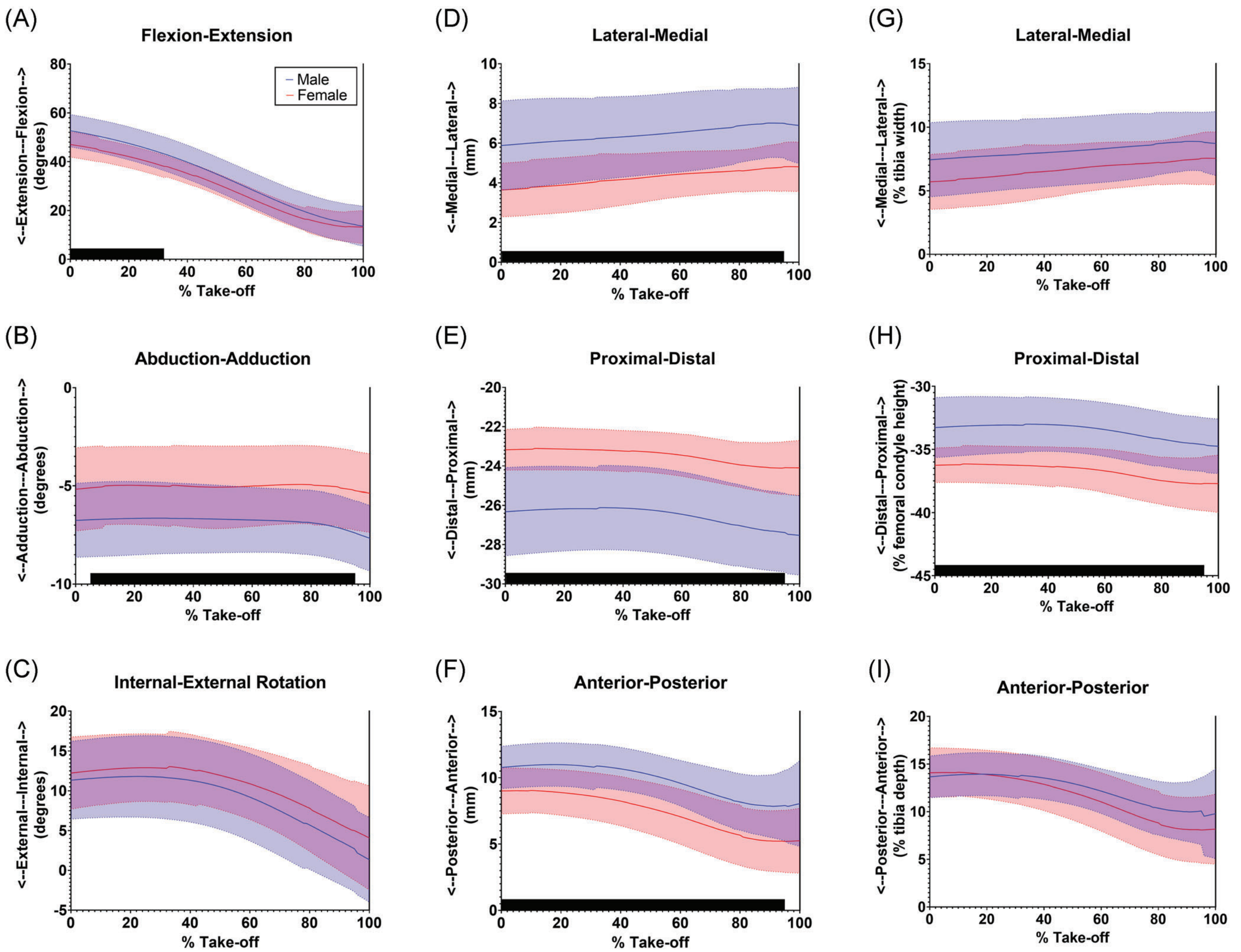

FIGURE 5 Average male and female 6 degree-of-freedom kinematics during the external rotation hop for (A) flexion/extension, (B) abduction/adduction, (C) internal/external rotation, (D) lateral/medial translation, (E) proximal/distal translation, (F) anterior/posterior translation, (G) lateral/medial translation normalized to bone size, (H) proximal/distal translation normalized to bone size, and (I) anterior/posterior translation normalized to bone size. For all kinematics values, the first direction listed is positive (flexion, abduction, internal, etc.). Blue curves represent men and red curves represent women. The shaded area represents one standard deviation. The black solid bar on the horizontal axis identifies periods of significant difference between men and women. AA, abduction/adduction; AP, anterior/posterior; FE, flexion/extension; IE, internal/external rotation; LM, lateral/medial; PD, proximal/distal [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

The aims of this study were to determine bilateral symmetry and sex differences in 6DOF knee kinematics and ACL elongation in healthy athletes during dynamic activities. Our hypothesis that knee abduction angle is greater in females than in male athletes was supported during fast running, drop jump, and internal/external rotating hops. Our hypothesis that ACL relative elongation is greater in women than in men was supported for large portions of each movement, however, the peak ACL elongations were not different between men and women during fast running, drop jump, and internal/external rotating hops. We observed ACL relative elongation increased as the knee extended during drop jumping and rotating hops, but the ACL relative elongation increased as the knee flexed during fast running. Asymmetry was characterized by the SSD_A in kinematics during each activity and was less than 1.4 mm in translation and 5.5° in rotation across all activities. Asymmetry in ACL relative elongation was 3.6% or less across all activities.

The female collegiate athletes in this study performed fast running, drop jumps, and internal/external rotating hops with significantly less knee adduction than the male athletes. These kinematics differences are consistent with previous studies that used conventional motion capture or video analysis to compare male and female knee kinematics during running and drop jumps.[40–42] Although the initial findings indicated men had significantly more anterior translation compared to women, those differences did not exist after normalizing translations to bone size. In fact, women had greater PD translation after normalizing for bone size at flexion angles exceeding approximately 20° (Figures 3H, 4H, and 5H). This suggests that bony morphology differences may contribute to greater

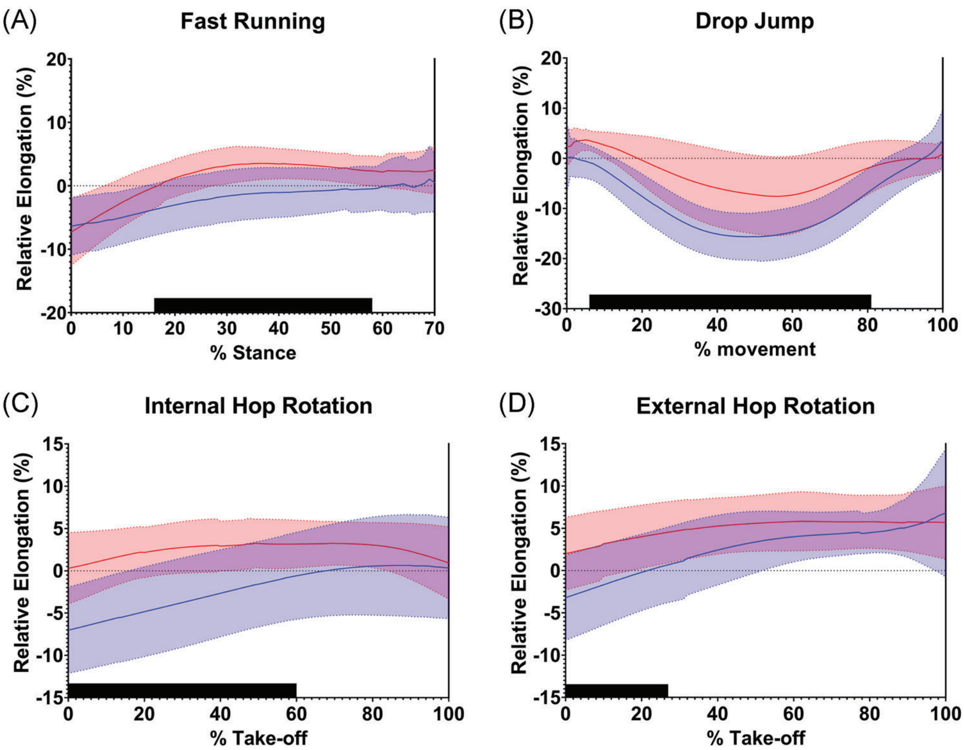

FIGURE 6 Average anterior cruciate ligament relative elongation during (A) fast running, (B) drop jump, (C) internal rotation hop, and (D) external rotation hop. Blue curves represent men and red curves represent women. The shaded area represents one standard deviation. The black solid bar on the horizontal axis identifies periods of significant difference between men and women [Color figure can be viewed at wileyonlinelibrary.com]

ACL relative elongation in female athletes at higher flexion angles (Figure 6).

It is important to measure ACL elongation *in vivo* during dynamic activities, as previous research has shown knee positions that were thought to strain the ACL (valgus collapse) may not do so *in vivo*.[43] The ACL elongation patterns measured in this study during the drop jump and rotating hop concur with previous reports that suggest ACL elongation is positively associated with knee extension.[10,15,44] In contrast, the current study shows that the ACL length increases while the knee flexes during running at 5 m/s (Figures 2A and 6A). Together, these results suggest that ACL elongation patterns are activity-dependent, and likely highly dependent upon external loading and muscle forces. This *in vivo* evidence demonstrates how ACL elongation is affected by different activities and demonstrates the importance of selecting appropriate activities during the course of rehabilitation. The relationship between knee kinematics and ACL elongation during fast running is complex and appears dependent upon tibial rotation and anterior translation in addition to flexion/extension (Video S1). The ACL relative elongation in female athletes was greater than in male athletes over large portions of each activity (Figure 6A–D). Although the measurement techniques make it impossible to determine the exact length at which the ACL transitions from slack to taut, the relative elongation data from this study indicate the female knees are consistently closer to taut (or consistently more elongated) than the ACL in male knees. This suggests that, in female athletes, less slack is available or the ACL is already more elongated when the knee is perturbed due to an unexpected external force or off-balance landing.

Restoring symmetry after treatment for ACL injury is important because athletes who demonstrate limb symmetry before returning to sports may significantly reduce their potential for future ACL injury.[45–47] Knowledge of kinematics symmetry in healthy athletes performing challenging tasks are needed to determine if knee kinematics in injured athletes have been restored before returning to sports. Presently, there are no datasets unaffected by soft tissue artifacts that describe typical symmetry in knee kinematics and ACL elongation during high-impact activities in healthy athletes. In this study, symmetry was calculated as the SSD_A over the entire kinematics (or ACL elongation) waveform for each activity. The average SSD_A is a better standard for comparison when determining if kinematics symmetry has been restored within the typical range after ACL injury and treatment than are group averages of left versus right or dominant versus nondominant differences because those group averages will always underestimate the typical SSD in healthy athletes. The results of this study also provide context for previous studies that used biplane radiography to study patients after ACL injury and clinical intervention. Previous studies have reported statistically significant SSDs in kinematics between injured/repaired/pathologic and contralateral knees ranging from 1.0 to 3.5 mm in translation and 1.3° to 2.3° in rotation.[18,48–50] The results of this study indicate that some of those previously reported differences are within the range of typical SSDs in healthy athletes.

TABLE 2 Peak ACL elongation

	Peak elongation (%)				Ave. SSD_A in peak elongation (%)			
	Total	**Men**	**Women**	***p* Value**	**Total**	**Men**	**Women**	***p* Value**
Fast running	3.6 ± 3.8	2.9 ± 4.2	4.7 ± 2.6	0.15	2.7 ± 2.2	3.4 ± 2.4	1.6 ± 1.3	0.08
Drop jump	5.1 ± 4.3	5.0 ± 5.4	5.2 ± 2.0	0.88	3.1 ± 2.8	3.9 ± 3.0	2.1 ± 2.0	0.19
Internal rotation hops	2.8 ± 4.4	1.6 ± 5.1	4.4 ± 2.6	0.06	2.8 ± 2.6	3.2 ± 2.9	2.3 ± 1.9	0.44
External rotation hops	8.6 ± 5.4	8.8 ± 6.6	8.4 ± 3.1	0.81	4.5 ± 6.0	5.1 ± 7.6	3.6 ± 2.2	0.59

Note: Average value ±1 standard deviation.

Abbreviations: ACL, anterior cruciate ligament, SSD_A, absolute side to side differences.

It is important to consider the study's strengths and limitations when interpreting the results. Strengths of the study include a relatively large cohort (compared to previous biplane radiography studies) of 38 healthy knees. Notably, study participants were collegiate athletes and the activities tested were physically challenging and clinically relevant. The tracking system used to measure kinematics was validated *in vivo*[34] during running, providing confidence that the reported tracking accuracy was representative of the accuracy in this study. DBR may not yet be widely available for clinical use, however, results from DBR studies can still be used to inform clinical practice by, for example, identifying activities that are more likely to increase ACL strain and can, therefore, be used to guide progression during rehabilitation. It is important to recognize the small number of trials performed is a study limitation, and the kinematics measured from only two or three trials may differ slightly from the typical kinematics for each participant. However, to minimize radiation exposure and reduce the time needed to process DBR data, only three trials or fewer are typically analyzed in DBR studies. Additional limitations include the participant group (Table 1), which did not include athletes from some sports where ACL injury risk is relatively high, such as basketball and volleyball. In addition, ACL length was normalized by the length at static knee full extension during MRI which may not reflect the true ACL slack length. However, this is the common method currently used to estimate *in vivo* ligament elongation during dynamic activities.[10,15,51] In addition, only the take-off portion of the single-leg rotating hop was included in the analysis. Our athletes were unable to reliably land with the knee within the view volume of the biplane radiography system. Therefore, data were not available for the landings of all subjects as they performed the rotating hop in both directions, so the landing was excluded from the analysis. There is very little existing data describing knee kinematics when the knee is subjected to high rotational torques *in vivo*, so the take-off kinematics during a rotating hop is novel and interesting to compare to more commonly measured planar activities. Finally, setting the running speed at 5.0 m/s for all subjects led to differences in cadence and stance duration that may be related to leg length. Leg length differences were not included in the analysis comparing male versus female knee kinematics and ACL relative elongation.

5 | CONCLUSION

This study of healthy athletes provides valuable reference data for identifying subtle changes in knee kinematics symmetry in athletes after the ACL injury. These reference values can be used in the future to identify excessive asymmetry after surgical or conservative treatments. The kinematics results highlight the importance of sex-specific analysis when assessing the effects of ACL injury, surgery, and rehabilitation on knee kinematics and identify sex-specific kinematics factors that may be associated with

TABLE 3 Average SSD_A in knee kinematics waveforms and ACL elongation waveforms

	Fast running	Drop jump	Internal rotation hops	External rotation hops
Flexion/extension (°)	2.8 ± 2.0	5.5 ± 6.4	3.4 ± 3.1	2.9 ± 2.2
Adduction/adduction (°)	0.7 ± 0.5	0.8 ± 0.6	0.8 ± 0.6	0.8 ± 0.7
Internal/external rotation (°)	2.2 ± 2.0	2.2 ± 2.3	3.5 ± 2.8	1.9 ± 1.7
Medial/lateral (mm)	0.8 ± 0.6	0.8 ± 0.6	0.8 ± 0.5	0.8 ± 0.6
Proximal/distal (mm)	1.1 ± 0.7	1.1 ± 0.7	1.1 ± 0.7	1.1 ± 0.7
Anterior/posterior (mm)	1.2 ± 0.9	1.3 ± 0.9	1.2 ± 0.9	1.4 ± 1.0
ACL elongation (%)	2.5 ± 1.1	3.6 ± 1.7	3.1 ± 2.7	2.8 ± 1.0

Note: Average value ±1 standard deviation.

Abbreviations: ACL, anterior cruciate ligament; SSDA, absolute side to side differences.

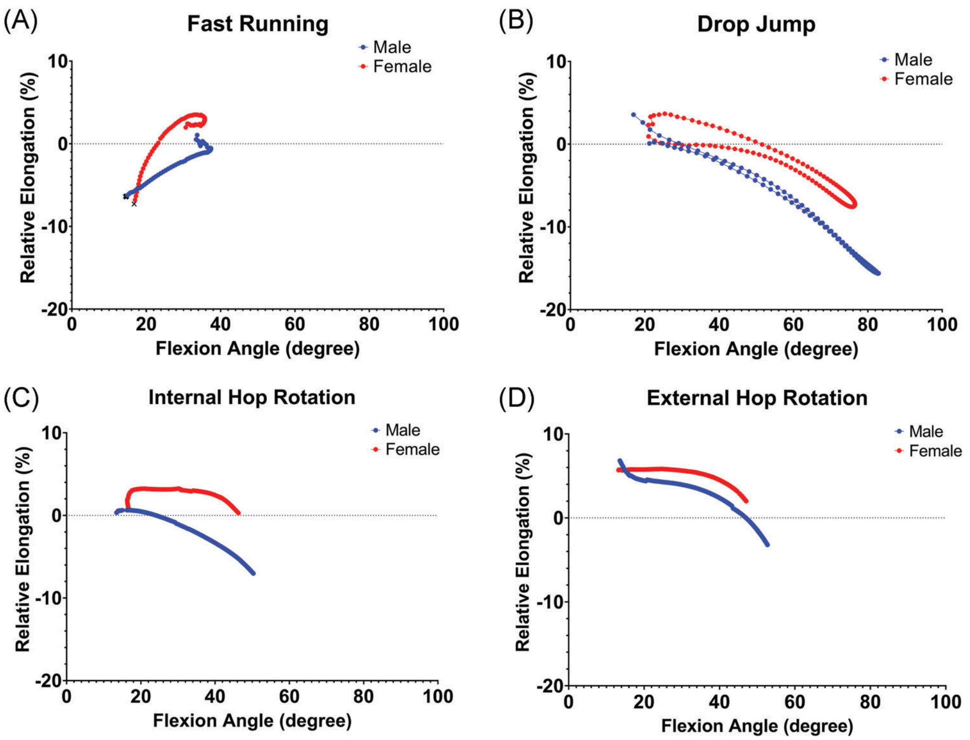

FIGURE 7 The relationship between anterior cruciate ligament relative elongation and knee flexion angle during (A) fast running, (B) drop jump, (C) internal rotation hop, and (D) external rotation hop. The fast-running data begin when the knee is in less flexion and progresses to higher flexion during support. The drop jump data begin when the knee is in less flexion, progress to high flexion, and then return to less flexion at take-off. The rotating hop data begin at higher flexion and progress to less flexion at take-off. Blue plots represent men and red plots represent women [Color figure can be viewed at wileyonlinelibrary.com]

increased incidence of ACL injury in women. The variety of activities studied demonstrates the importance of measuring ACL mechanics *in vivo* under dynamic, physiologic loading. The novel information from this study improves our understanding of ACL elongation during demanding athletic activities and has implications for sex-specific risk screening metrics, return to play assessment, and rehabilitation protocols after the ACL injury. Even in collegiate athletes, sex-dependent differences in kinematics and ACL relative elongation exist when performing injury risk screening tests, return to sport evaluations, and athletic activities.

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AUTHOR CONTRIBUTIONS

Kyohei Nishida chiefly drafted the manuscript and carried out the acquisition of data, processing of data, and the analysis and interpretation of data. Tom Gale carried out the acquisition of data and the analysis of data. Caiqi Xu carried out the acquisition of data and processing of data. William Anderst chiefly drafted the manuscript and carried out the design of the study and revised the manuscript. Freddie Fu contributed to the study design and conducted the final approval of the manuscript to be submitted. All authors have read and approved the final submitted manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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