

## A comparison of three methods for establishing a zero-strain ACL reference length in vivo

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**INTRODUCTION:** As a ligament under greater tension is closer to its point of failure, measurements of anterior cruciate ligament (ACL) strain have been used to reflect risk of rupture [1]. To measure strain, it is necessary to normalize elongation of the ACL to a 'reference length' which corresponds to the point at which the ligament transitions from being slack to carrying tension. Some have suggested that it may be possible to identify this transition point by applying anterior and posterior loads to the tibia, often in the form of a Lachman's or an anterior-posterior (AP) drawer test [2]. Others have used the length of the ACL with the knee in a supine position during magnetic resonance (MR) imaging to represent this slack/taut transition length [3]. Finally, quiet standing has also served as a reference position for measuring ACL strain [4]. The goal of this study was to compare the length of the ACL in these three different reference positions (Figure 1) for their utility in measuring ACL strain. We hypothesized that the ACL length would be similar between all three of the positions studied here.

**METHODS:** A total of 5 participants (2 males, 3 females; Age: 22-28 years; Height: 1.57-1.78 m; BMI: 19.7-28.7 kg/m<sup>2</sup>) participated in this IRB approved study. All participants underwent MR imaging (sagittal/axial/coronal DESS sequences with an 8-channel knee coil: voxel size 0.3x0.3x1 mm<sup>3</sup>, flip angle 25°, TR 17 ms, TE 6 ms) and high-speed biplanar radiography imaging (matrix size 1152x1152 px<sup>2</sup>, pulse width 1000 μs, frame rate 40 Hz). Using previously established techniques, models of the femur and tibia were generated from the MR scans and the attachment site footprints of the ACL were identified, such that ACL length was determined as the centroid-to-centroid distance between the attachment sites on the femur and tibia [3]. For x-ray imaging, the 3D models of the bones were registered onto both planes of the radiographs to obtain the position of the knee at the time of imaging, allowing for measurement of ACL length throughout [3]. For comparison of ACL length, three reference positions were selected (Figure 1). The supine length was measured during the MR scan, and the x-ray system was used to record quiet standing and an AP drawer test. For the AP drawer test, participants lay supine with their knee flexed to 90°. A fellowship trained orthopaedic surgeon with greater than 15 years of experience stabilized the participant's foot and applied a posterior force on the tibia followed by an anterior force, resulting in the full range of translation in both directions. From this, tibial displacement was plotted versus ACL length for each participant, and a uniform sampling of data points were used to fit the data to a third-order polynomial. The inflection point of this curve was defined as the zero-strain length [2]. Thus, each participant contributed 3 total values to evaluate the ACL reference length (supine, quiet standing, and AP drawer). A repeated measures ANOVA, with the level of significance set at  $p < 0.05$ , was used to test for differences in ACL length between the positions. A 2-way random effects ICC was used to test for consistency in measurement between the positions.

**RESULTS:** No significant differences in ACL length between positions were detected ( $p = 0.24$ ), with at most a 1.2mm difference in mean ACL length between the three positions (Figure 2). Additionally, there was a high degree of reliability between the three ACL length measurements for each subject, with an ICC=0.91.

**DISCUSSION:** This study compared three different positions (supine, quiet standing, and AP drawer) for approximating the reference length of the ACL, which may be used to study the in vivo strain of the ligament. Across all three positions tested, ACL length was highly similar, and there were no significant differences in the mean ACL lengths measured at each position. There was also a high degree of reliability between the three measurements for each participant.

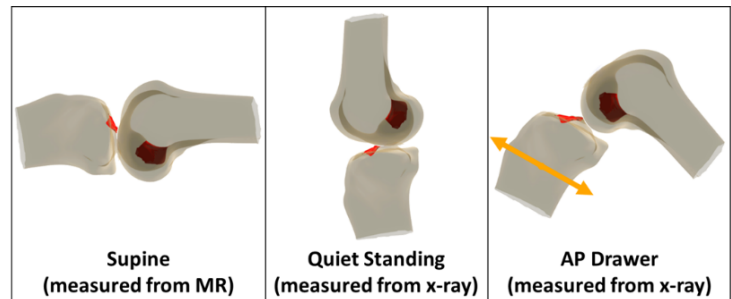
In recent literature, a supine resting position as measured during an MRI scan has been used to establish the reference length of the ACL [3]. Quiet standing is commonly used as the reference position from which kinematics are measured for other imaging modalities, such as motion capture [5]. The AP drawer test is commonly utilized to assess the integrity of the ACL, which undergoes elongation and slackening during anterior/posterior translation [2]. The inflection point of a curve fit to ACL strain data during AP loading has been also been hypothesized to be the position at which the ligament transitions from slack to taut [2]. Therefore, this study compared these three positions to better understand their utility for studying the ACL in vivo.

As a reference length is typically chosen for the purpose of measuring ligament strain, based on these results, a 3 mm change in ACL length from each of the three positions equates to 11.0, 10.9, and 11.4% strain for supine, standing, and AP drawer, respectively. Thus, all three reference positions result in similar strain measurements. Some have speculated that using a reference position where the knee is in extension, such as when supine, overestimates 'true' strain [6]. However, the results of this study indicate that if the ACL length from a quiet standing position or a supine resting position were used as the reference length, it might be under-estimating the amount of strain compared to the length obtained from a test of AP laxity. Regardless, all three references produce strains that are within a reasonable margin of error for in vivo measurement techniques. While it is exceedingly difficult to pinpoint the length of the ACL at its slack-taut transition in vivo, the results of the present study indicate that these three measures of reference length produce comparable strain measurements.

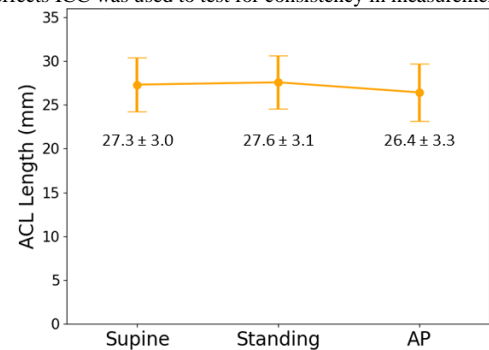
**SIGNIFICANCE:** This study shows that while determining the precise zero-strain length of the ACL in vivo remains a challenge, the commonly used reference positions utilized here all produce comparable measurements of ACL strain. These findings are important because measurements of in vivo ACL strain have the potential to serve as biomarkers for propensity for injury.

**REFERENCES:** [1] Bates 2019, [2] Howe 1990, [3] Englander 2019, [4] Taylor 2013, [5] Althomali 2023, [6] Englander 2020

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**Figure 1 – Anterior cruciate ligament (ACL) length was measured in three different reference positions.** During the MR scan, resting length was measured as the participant lay supine. A biplanar x-ray system was used to measure ACL length during quiet standing and during an AP drawer test. For the AP drawer test, the knee was positioned at 90° as an orthopaedic surgeon applied anterior and posterior loads to the tibia. The reference length from the AP drawer test was defined as the inflection point of the tibial displacement versus ACL strain curve.



**Figure 2 – The points and error bars show the mean and standard deviation of ACL length, respectively, measured from standing, supine, and during the AP drawer test.** A repeated measures ANOVA did not find a significant difference ( $p = 0.24$ ) between the three positions.