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In-situ mechanical behavior and slackness of the anterior cruciate ligament at multiple knee flexion angles

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ABSTRACT

In this study the *in-situ* tensile behavior and slackness of the anterior cruciate ligament (ACL) was evaluated at various knee flexion angles. In four cadaveric knees the ACL was released at the tibial insertion, after which it was re-connected to a tensiometer. After pre-tensioning (10 N) the ACL in full-extension, the knee was flexed from 0° to 150° at 15° increments, during which the ACL tension was measured. At each angle the ACL was subsequently elongated and shortened under displacement control, while measuring the ACL tension. In this manner, the pre-tension or the slackness, and the mechanical response of the ACL were measured. All ACL's displayed a higher tension at low (0°–60°) and high (120°–150°) flexion angles. The ACL slackness depended on flexion angle, with the highest slackness found at 75°–90°. Additionally, the ACL stiffness also varied with flexion angle, with the ACL behaving stiffer at low and high flexion angels. In general, the ACL was stiffest at 150°, and most compliant at 90°. The results of this study contribute to understanding the mechanical behavior of the ACL *in-situ*, and may help tuning and validating computational knee models studying ACL function.

1. Introduction

The anterior cruciate ligament (ACL) plays an important role in maintaining knee stability, particularly for restraining anterior tibial translation [1–3]. It is also the knee ligament that is ruptured most frequently [4–6]. Surgical reconstruction of the ACL is a common intervention to treat disability or chronic instability of the knee [7]. Unfortunately, in some cases the results are still unsatisfactory, resulting in the development of secondary injury, or progressive degeneration of the injured knee joint [3], and failed restoration of the native physiological characteristics [8].

The function of the ACL can be analyzed in three-dimensional computational models of the knee joint, which in turn can be used to optimize the results of surgical interventions. Computational models allow to vary and isolate factors affecting knee joint functioning, making them suitable for analysis of the different structures around the knee. However, a correct mechanical representation of the intact native ACL is required to reliably simulate the effects of ACL interventions.

Several studies have analyzed the inherent mechanical properties of the ACL by performing tensile tests in experimental set-ups [9–13]. However, these experiments only focused on a tensed ACL, with the knee in full extension or deep flexion. To properly simulate the ACL function, additional information is needed on the slackness of the ligament in the range between full extension and deep flexion. When the slackness of the ACL is underestimated, the ligament becomes active over a larger flexion range, which affects the joint kinematics and cartilage contact pressure simulated in computational models. On the other hand, overestimating the slackness of the ACL results in joint instability. In addition, the ACL is composed of two main fiber bundles, the anteromedial bundle and posterolateral bundle. Due to this specific morphology, different parts of the ligament may become active during knee motion, [9,10,14] making the mechanical response of the ACL dependent on the flexion angle of the knee. Hence, the best way to assess the mechanical behavior of the ACL is *in-situ* in the knee joint.

The goal of this study was therefore to quantify the slackness and mechanical behavior of the ACL *in-situ*, at different flexion angles. The

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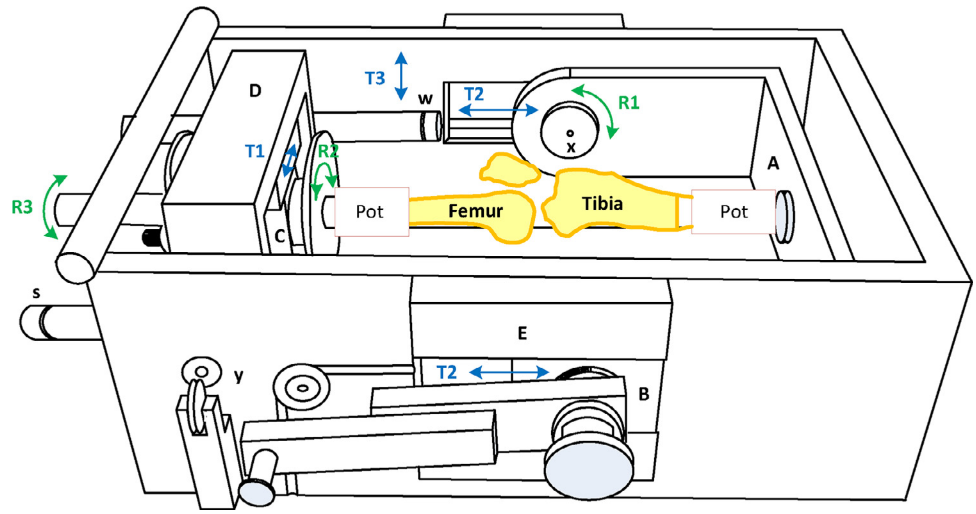

Fig. 1. Knee joint testing rig. The tibia was placed in a bracket A, and the femur was placed in a bracket D through sliding block C, allowing for medial-lateral translation (T1). Bracket A could be rotated around center of rotation *x*, allowing knee flexion and extension (R1). Block B, which was also connected to bracket A, could slide within bracket E, to allow proximal-distal movement of the tibia (T2). Block C could rotate to allow for varus–valgus rotation (R3) and internal–external rotation (R2). Bracket D could rotate around *Y*, to allow for an anterior and posterior movement (T3).

mechanical behavior was assessed by measuring the tensile response of the ACL, when varying its length.

2. Materials and methods

Four fresh-frozen human cadaveric knees (mean age 79 years (range 72–86 years), 2 males, 3 right knees) were used to measure the tension and slackness of the ACL. Before dissection, the knees were examined in a Magnetic Resonance Imaging (MRI) scan to verify that the ACL was intact. The specimens were thawed 24 h prior to dissection, after which the knees were prepared for the measurement in a custom knee loading rig (Fig. 1). The rig allowed for positioning of the knee in each desired position, and was previously used by Barink et al. [15]. In the current study, the position of the tibia was placed in the bracket A, which could be flexed around point *x* (a revolute joint indicated as R1 in Fig. 1) from full extension (0°) to 150° of flexion, with an accuracy of 1°. The rig also allowed for proximal and distal translation by displacing bracket A (T2, Fig. 1). The femur was placed in the bracket D, which had four degrees of freedom: medial-lateral translation (T1), anteroposterior translation (T3), varus–valgus rotation (R3) and internal–external rotation (R2). During the measurements, at each knee flexion angle, all movements of the rig were locked after the knee joint found its self-adjusted position, guided by the soft tissues surrounding the knee.

To fit the knee into the rig, the knees were cut 20 cm proximal and 19 cm distal to the joint line. Soft tissue within 15 cm proximal and 13 cm distal to knee joint cavity was left intact. Subsequently, the bones were potted using polymethylmethacrylate (PMMA).

2.1. Releasing the ACL

To record the ACL tension curve due to the displacement at a specific knee angle from 0° to 150°, the method of Markolf [13] was used to isolate the tibial attachment of the ACL. This technique was later applied by Arnold and colleagues in our laboratory to record ACL tension [16]. The ACL was released from the tibial attachment by an experienced orthopedic surgeon, according to the following procedure.

First, the knee was opened to expose the exact location of the ACL. By using a tibial aiming device *(Arthrex, Naples, FL, USA)*, a guide-wire was drilled into the center of the tibial attachment of the ACL, which was oriented such that it was lining up with the femoral intercondylar notch in the fully extended knee. The guide-wire position was checked under a fluoroscope in the anteroposterior and lateral projection. After confirming the wire position, a 4.5 mm cannulated drill was used over the guide-wire until the subchondral bone under the ACL insertion site was reached. With the guide wire still attached to the bone, a 7 mm cannulated screw was manually driven into the bone over the guide wire, until the screw tip touched the subchondral cortex. Subsequently, a core drill (14 mm outside diameter) was drilled over the guide wire until it reached the subchondral bone plate, effectively loosening the tibial insertion of the ACL. The ACL was then further released by removing the surrounding connective tissue. Additional stitches were made through the distal tibial insertion of the ACL to ensure adequate fixation of the ACL to the tensiometer. Complete release of the ACL was confirmed by pushing and pulling the bone-screw while checking the force response of the ACL.

2.2. Mounting the tensiometer

In the current study, a custom-made tensiometer [16] (Fig. 2) was used, with a force accuracy of $\pm$ 1 N. The tensiometer was calibrated prior to each experiment using dead weights. The electric signal of the sensor was connected to an analog-to-digital converter. The data was then further processed using QuickDAQ (Data Translation, Marlboro, USA).

The tensiometer frame was mounted firmly on the tibia, with the tensiometer hook connected to the ACL. Two sharp pins of the frame were driven into the cortical bone alongside the bone tunnel, to anchor the tensiometer. The displacement of the hook was changed through an adjustment screw (part 2 in Fig. 2), while the height of the frame could be adjusted to bring the tensiometer in line with the tibial tunnel. The adjustment screw was used to vary the ACL tension or slackness, at 0.5 mm displacement increments.

2.3. Testing procedure

For each knee, the measurement was started by connecting the ACL to the tensiometer and putting the knee in the “base-position”, after which a pre-conditioning sequence was performed. The pre-conditioning sequence consisted of five consecutive cycles of flexion-extension 0° to 90° in order to obtain a repeatable tissue response for the ACL and other soft tissue structures surrounding the

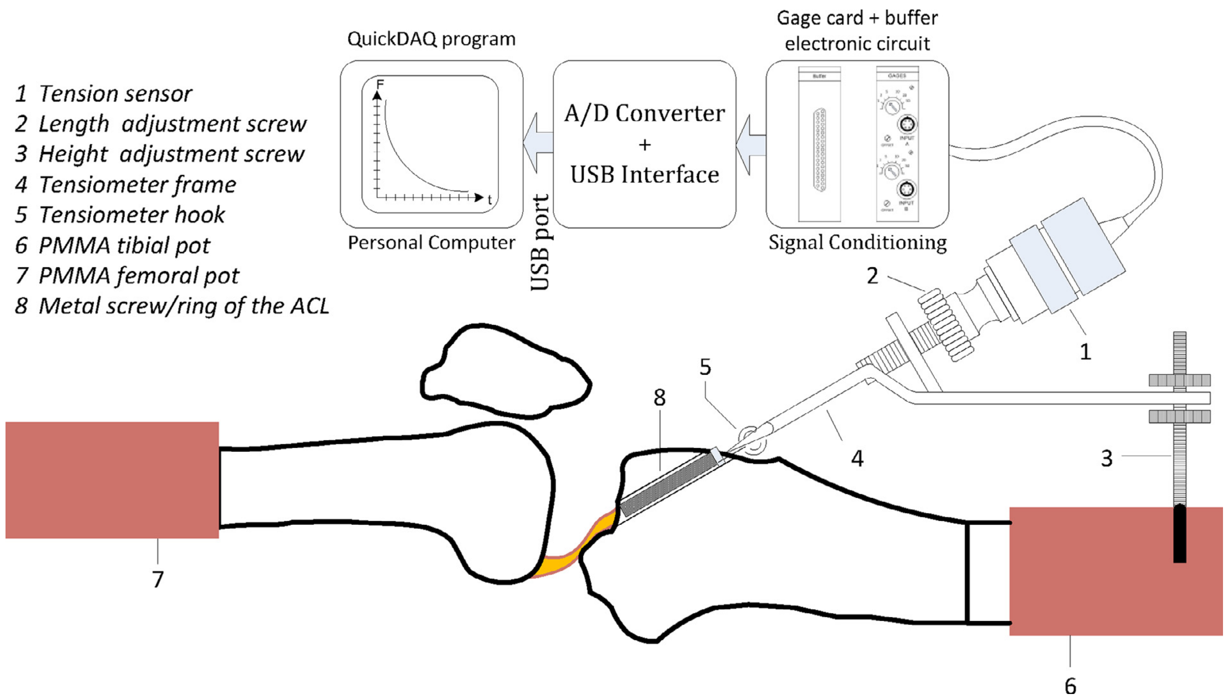

Fig. 2. Set-up of the tensiometer device that was used to measure ACL tension. The displacement of the hook of the tensiometer could be adjusted through an adjustment screw (2), through which the tension or slackness of the ACL could be measured.

knee joint. For the base-position, the knee was placed in full extension, after which the ACL tension was set to 10 N using the adjustment screw. This position was based on the study of Arnold et al. [16].

After pre-conditioning, the tension and slackness of the ACL measured as a function of the flexion angle. The measurement was performed at flexion angles ranging from 0° to 150°, with 15° increments. For each flexion angle, the measurement was started by first placing the knee in the base-position (full extension, 10 N pretension). Next, the knee was placed in the desired flexion angle. After the knee joint found its self-adjusted position, all degrees of freedom of the rig were locked. Next, the ACL tension was determined, which would show either a specific level of tension (1.) or slackness (2. – no reaction force measured). Based on the initial measurement, the experiment proceeded as follows:

1. In case the ACL was already tensed, the tension was reduced by increasing the ACL slackness at 0.5 mm increments. For each increment the drop in tension was recorded, until full slackness was reached (tension level of 0 N). At this point, the adjustment screw was rotated back to the initial (tensed) position, and the ACL response was measured by continuing stretching the ACL at 0.5 mm increments. The measurement was stopped when the tension exceeded 20 N to prevent damage occurring to the ACL-tensiometer connection, which could affect subsequent measurements.
2. In case the ACL was initially slack, the ACL only was stretched by increasing the displacement using the adjustment screw, at 0.5 mm increments. Again, at each displacement increment, the ACL tension was recorded, until the ACL tension exceeded 20 N. Initial slackness of the ACL at the current flexion angle was defined as the displacement required to get a tensile reading of the tensiometer.

After measuring the tension-displacement characteristics for a specific flexion angle, the ACL was released again, the knee was placed back in full extension, after which the procedure was repeated for the next flexion angle.

2.4. Data processing

As a verification step, and to provide a comparison with previous studies, first, a standard flexion-tension graph was reproduced. For this purpose the ACL pre-tension was set to 10 N in full extension, after which the knee was flexed to 150° in 15° increments while monitoring the ACL tension.

Results from the measurements in which the ACL tension and slackness were recorded as a function of flexion angle were processed as follows. As the experiments were performed at different flexion angles, tension-displacement curves were obtained with a horizontal (displacement) offset that depended on the pre-tension or slackness. To determine the slackness at a specific flexion angle, a cut-off value of 0.5 N was taken. The horizontal offset at this tension level was then taken as either the slackness (positive displacement values), or the level of pre-tension (negative displacement values).

The nominal tension-displacement curves were then determined, by removing the calculated slackness or pre-tension. Theoretically, if the nominal mechanical response of the ACL would be similar in each flexion angle, this would result in nearly identical tension-displacement curves, while the curves would be divergent if the nominal response would depend on the flexion angle. To quantify this difference the linearized ACL stiffness was calculated for each knee, and at each flexion angle. The linearized stiffness was determined at a tension cut-off value of 15 N, and calculated by dividing the tension level (15 N) by the nominal displacement (corrected for slackness/pre-tension).

2.5. Statistical analysis

Friedman's non parametric test was performed using SPSS Statistics 19 (SPSS Inc., IL, Chicago) to determine the mean difference of the linear stiffness of the ACL relative to each group of knee flexion angles. In this study, knee flexion angles (ranging from 0° to 150°, with 15° increments) were divided into three different groups i.e. low knee flexion angle (from 0° to 60°), intermediate knee flexion angle (from 75°to 105°) and high knee flexion angle (from 120° to 135°).

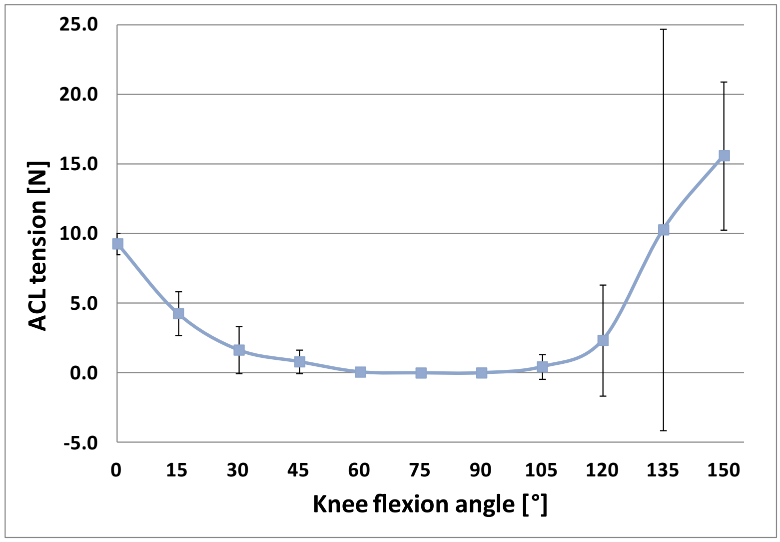

Fig. 3. Average ACL tension as measured in the four knees during unloaded flexion (error bars indicate the standard deviation). For one knee (Knee 3), no measurement was recorded at 150°, due to a too high ACL tension caused by excessive fat tissue surrounding the knee joint. This knee was also responsible for the large standard deviation at 135°.

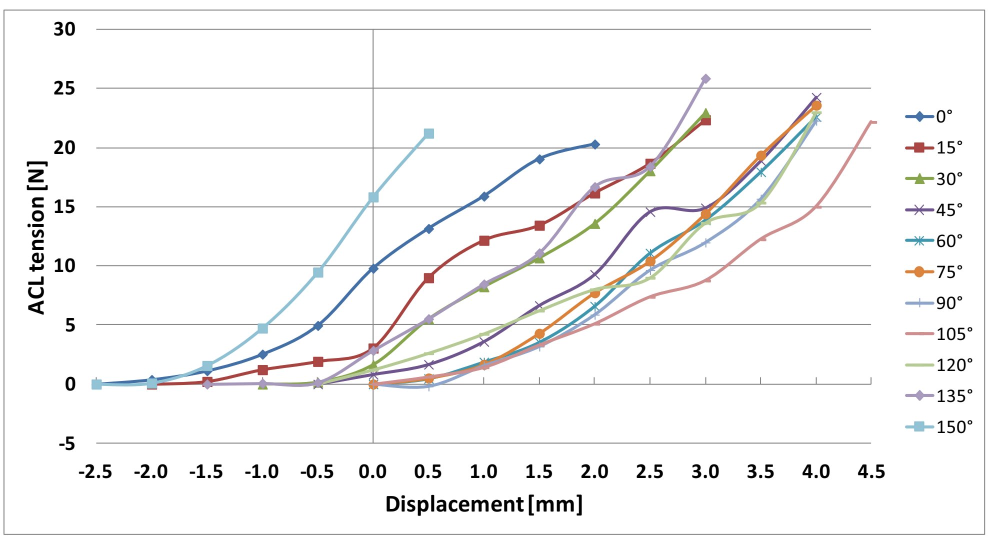

Fig. 4. The ACL tension-displacement curves of one knee (Knee 4: right, 78 years, female). Tension-displacement curves are shown for eleven flexion angles (ranging from 0° to 150° at 15° increments).

Due to the limitation of the experimental data samples and the specific (unique) cadaver's knee properties, subsequently, a General Linear Model (univariate) and a Tukey Post Hoc test were performed to determine the differences between groups. The flexion angle groups (low, intermediate, and high) and the four individual cadaver knees were set as independent variables, whereas the stiffness value was set as a dependent variable**.** A significance level of alpha < 0.05 and a confidence level of 95% were used for all statistical analysis.

3. Results

In three knees the full experimental protocol (0–150°) was completed, while in one knee (knee 3, right, 86 years, female) a flexion angle higher than 135° could not be achieved, due to excessive fat tissue surrounding the knee.

3.1. Standard flexion-tension graph

All knees displayed the characteristic U-curve of ACL tension (Fig. 3), as previously demonstrated by Arnold et al. [16]. The ACL was predominantly tensed in the lower (0–60°) and higher (120–150°) knee flexion angles, and slack in intermediate flexion angles (75–105°). The spread in the data was largest for flexion angles exceeding 105°, caused by the flexion restrictions of knee 3.

3.2. Tension-displacement curves

Fig. 4 shows typical tension-displacement curves produced by a single knee (knee 4; right, 78 years, female). For each flexion angle, a different tension-displacement curve was produced, providing information on the ACL slackness, and the stiffness response at the various angles. Curves passing through the vertical axis (particularly occurring in lower and higher flexion angles) expressed a pre-tension, while curves shifted to the right (mid-flexion range) were slack in the base position.

3.3. Slackness evaluation

For each knee and each flexion angle, the slackness or level of pre-tension were calculated (Fig. 5, Table 1). Slackness was represented

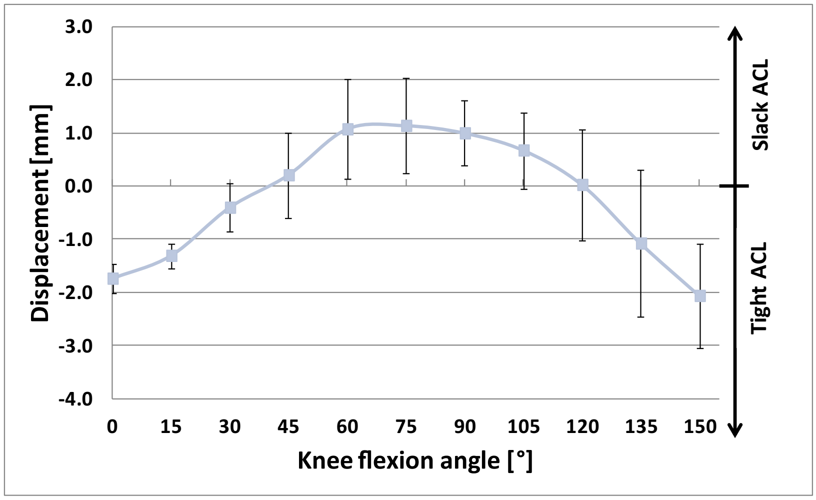

Fig. 5. Average slackness (positive values) or pre-tension (negative values) values for the four knees plotted against the knee flexion angle. In the mid-flexion range ligament slackness values of up to 2.2 mm were found.

Table 1
Slackness measurements for the four knee specimens. Positive values indicate ACL slackness (occurring particularly in the mid-flexion range), negative values indicate ACL pre-tension.

	Slackness [mm]										
	0°	15°	30°	45°	60°	75°	90°	105°	120°	135°	150°
Knee 1	−1.39	−1.06	0.02	1.32	2.22	1.91	1.04	1.32	1.30	0.38	−1.23
Knee 2	−1.94	−1.31	−0.23	0.14	1.42	1.89	1.84	1.15	0.24	−1.44	−3.14
Knee 3	1.65	−1.63	−1.05	−0.59	0.14	0.26	0.59	−0.24	−1.18	−2.83	–
Knee 4	−1.99	−1.26	−0.38	−0.07	0.49	0.46	0.49	0.44	−0.27	−0.44	−1.84
Average	−1.74	−1.31	−0.41	0.20	1.07	1.13	0.99	0.67	0.02	−1.08	−2.07
St. dev.	0.28	0.24	0.46	0.81	0.94	0.89	0.61	0.71	1.03	1.38	0.98

as a positive displacement, while pre-tension was represented as a negative offset. The ACL generally became slack around 45°, and was tensed again around 120°. Maximum slackness was reached around 75°, with a magnitude of 1.13 ± 0.89 mm. The maximum level of pre-tension was reached either at 0° or 150°, with a magnitude of −2.07 ± 0.98 mm.

3.4. Linearized stiffness

After removal of the slackness from the force displacement-curves, comparison of the linearized stiffness indicated the ACL stiffness also varied with flexion angle (Fig. 6). The linearized stiffness ranged from 3.25 N/mm to 10.56 N/mm (Table 2). All knees expressed a similar pattern, with the ACL being stiffer at low and high flexion angles, concurrent with the flexion positions in which the ACL is mostly engaged.

For the statistical analysis, only values for flexion angles ranging from 0° to 135° were used, as there was a missing value for one knee at 150° (knee 3, right, 86 years, female). The linearized ACL stiffness was compared for three ranges of flexion angle (low, intermediate, high) using Friedman's non-parametric tests. The tests showed a significant difference between each knee flexion angle group ($p = 0.039$). The multiple pairwise comparison of the Tukey Post Hoc test revealed a significant difference between low and intermediate knee flexion angles, and between intermediate and high flexion angles ($p = 0.008$ and $p = 0.049$, respectively).

4. Discussion

The goal of this study was to quantify the slackness and mechanical response of the ACL *in-situ*, at different flexion angles. For this purpose, the displacement of the tibial attachment of the ACL was varied while measuring the force response in four fresh-frozen cadaveric knees.

Obviously the current study has a number of limitations. The cadaveric knees used in the current study were of a relatively high age (79.0 ± 5.8 years), not resembling the typical young and active patient. Furthermore, the intended screw fixation in the tibial bone-block as performed in the study of Markolf et al. [13] could not be used to manipulate the tibial insertion site, due to poor bone quality. The suture fixation that was adopted allowed more rotation of the ACL compared to the screw fixation, which potentially could affect the results. However, the bone tunnel provided good visibility of the sutures, and minimal rotational movements of the sutures were observed during the experiments. Furthermore, the results of the measurements were quite reproducible between knees, indicating a limited effect of the manner of fixation. An actual possible benefit of using sutures instead of a bone plug was that possible bone-on-bone friction in the bone tunnel was avoided. The age of the cadaveric tissue most likely also affected the mechanical response of the ACL tissue, as several studies have demonstrated that age is an important factor in the mechanical properties of ligaments [17–21], while ACL injuries occur most frequently in younger patients [13].

After release of the ACL the pre-tension at extension was set to 10 N for all knees, as suggested by Arnold et al. [16]. This is probably a simplification of reality, since pre-tension values are bound to differ between individuals. However, as there currently is no way to assess the pre-tension of the ACL under in-situ circumstances, the choice was made to standardize the pre-tension to 10 N and subsequently assess how the ACL's of four different cadavers respond as a function of flexion angle.

During the experiments the knee was placed at a particular flexion angle, and after the knee found its self-adjusted position all degrees of freedom were locked. This position was guided only by the passive

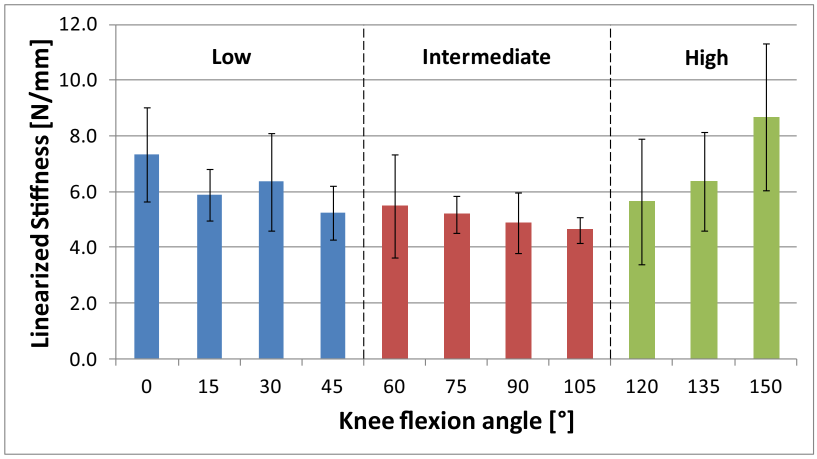

Fig. 6. Average ACL stiffness values for the four knee specimens as measured in the low (blue), intermediate (red) and high (green) flexion ranges. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 2
Linearized stiffness of the ACL for the four knee specimens, showing higher values in extension and deep flexion.

	Linear stiffness at 15 N [N/mm]										
	0°	15°	30°	45°	60°	75°	90°	105°	120°	135°	150°
Knee 1	9.62	6.38	7.94	6.38	7.77	5.30	3.95	4.44	4.56	4.93	9.80
Knee 2	5.75	4.90	3.89	4.09	3.25	4.29	4.03	4.49	4.56	4.72	5.68
Knee 3	7.58	6.91	6.61	5.47	5.32	5.26	5.34	5.30	9.04	7.98	
Knee 4	6.44	5.38	7.01	4.98	5.66	5.91	6.22	4.30	4.46	7.85	10.56
Average	7.34	5.89	6.36	5.23	5.50	5.19	4.89	4.63	5.65	6.37	8.68
St. dev.	1.69	0.92	1.74	0.96	1.85	0.67	1.10	0.45	2.25	1.79	2.63

structures surrounding the knee, while *in vivo* the knee position may be affected by the muscles surrounding the knee. Moreover, while tightening the ACL, the position of the knee was fixed, so that the actual elongation/shortening of the ACL could be measured. *In vivo*, this ACL tension possibly also could affect the knee position, pulling the tibia in the posterior direction relatively to the femur. These limitations may have affected the mechanical response of the ACL, as it was demonstrated that the stiffness depended on the flexion angle, and therefore on the angle of the ACL with respect to the femur and tibia. Allowing for a posterior translation of the tibia, for instance, would have resulted in a steeper orientation of the ACL, with a different mechanical response. Hence, the current measurements give an indication of the stiffness response in a passive flexion position, but may be different in an *in vivo* situation with more degrees of freedom and muscle activation affecting the actual ACL position.

The linearized ACL stiffness as determined in this study is difficult to be compared directly to other studies at all flexion angles. However, the literature does provide information about the average linear stiffness at knee flexion angles 30° and 45°. In the current study, the average linear stiffness of the ACL from old donors was 6.36 ± 1.74 N/mm at 30° knee flexion angle and 5.23 ± 0.96 N/mm at 45° knee flexion angle. Those values were different from a previous study of Woo et al. [22] and of Noyes and Grood [23] who published an ACL linear stiffness of 180 ± 25 N/mm (60–97 years old donors) at 30° knee flexion angle and 129 ± 39 N/mm (48–86 years old donors) at 45° knee flexion angle, respectively. By analyzing the graphical load-elongation curve at 30° of flexion in the anatomical orientation of Woo et al. [22], the linear stiffness was estimated at 15 N and 20 N loads which were 53 N/mm and 55.4 N/mm, respectively. Hence, the current results show much lower stiffness values which could be partly attributed to the different types of testing and perhaps the age of the knee donors. In the current study, the ACL stiffness was determined at a load level of 20 N with the ACL *in situ*, which is about 28% of the peak load found by Li et al. [24] (71.7 ± 27.9 N), who measured this ACL force at 30° of flexion under a 400 N quadriceps load. In two other studies the ACL was not tested *in* situ, but at a force level of 100 N (Woo et al. [22]) and 600 N (Noyes and Grood [23]). Compared to previous studies, the ACL force was quite low in the current investigation. Markolf et al. [13] showed that the ACL force could reach around 200 N with an external tibial load of 100 N. As the ACL is a highly non-linear material, it is logical that Woo et al. [22] and Noyes and Grood [23] found higher stiffness values than presented here. Hence, the stiffness values found in the current study should be regarded as the 'initial linearized stiffness' of the load-displacement curve. One could argue that a higher load should have been used, but pilot studies showed that such loads would not allow for multiple repetitive measurements as performed here.

The current results indicate that the mechanical response of the ACL varies with the flexion angle. The mean ACL stiffness of the intermediate flexion angle group (4.90 N/mm) was significantly lower than the low (6.07 N/mm) and high flexion angle group (6.01 N/mm), while no significant difference was found between the low and high flexion angle groups. This suggests that ACL slackness was not only influenced by the location of the attachment sites relative to the rotating knee axis, but also by flexion angle. This suggests that during ACL reconstruction, orthopedic surgeons should test the ACL stiffness over the full flexion range. Apparently, the effective stiffness of the ACL is optimized for positions in which the ACL is mostly active (extension and deep flexion), whereas the slackness of the ACL is largest in the mid-flexion angle. Evidently, this is a result of the double-(or multiple) bundle architecture of the ACL, with the anteromedial bundle being stretched in flexion, and the posterolateral bundle being stretched in extension [9,10,14].

The current study may provide further clinical insights, particularly for optimizing the pre-tension and slackness in ACL reconstructions. Although perhaps complex to incorporate in a clinical setting, the results provide guidelines for the amount of slackness in intermediate flexion angles, and for the level of pre-tension in extension and deep flexion. Such a procedure would, however, require a clinically suitable ACL tensiometer which currently is not standard clinical practice. Using a tensiometer to obtain tension data entails a more invasive approach, and more time at the operating room [25]. Moreover, some studies have suggested that the pre-tension applied to ACL grafts is released soon due to remodeling [26] and viscoelasticity [27] post-operatively.

From a knee modeling perspective, the current results facilitate the fine-tuning of the ACL parameters, such as the slackness [28,29], the flexion-angle dependent stiffness, and the level of pre-tension in certain ranges of flexion. These parameters have been shown to have a significant effect on the tibiofemoral joint kinematics [3,30]. A similar experiment could also be performed to determine the mechanical response of the posterior cruciate and collateral ligaments. The final set of parameters could subsequently be validated against *in vivo* laxity tests, such as the drawer test, to verify the functional laxity of the knee [29].

5. Conclusions

The slackness and stiffness of the ACL are dependent on the flexion angle, in such a way that the mechanical properties are optimized for the region in which the ligament is engaged the most, such as in extension (0–30°) and deep flexion (120–150°). These findings may be of clinical relevance for ACL reconstructive surgery, and are furthermore useful for implementing the mechanical response of the ACL in computational models of the human knee.

Conflict of interest

Competing interests: None declared

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Ethical approval: Not required

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