The Anterolateral Ligament Has Similar Biomechanical and Histologic Properties to the Inferior Glenohumeral Ligament

Peer-reviewed author version

SMEETS, Kristof; Slane, Josh; Scheys, Lennart; Forsyth, Ramses; Claes, Steven & BELLEMANS, Johan (2017) The Anterolateral Ligament Has Similar Biomechanical and Histologic Properties to the Inferior Glenohumeral Ligament. In: ARTHROSCOPY-THE JOURNAL OF ARTHROSCOPIC AND RELATED SURGERY, 33(5), p. 1028-1035.e1 (Art N° 1028).

DOI: 10.1016/j.arthro.2017.01.038
Handle: http://hdl.handle.net/1942/24339

The anterolateral ligament has similar biomechanical and histological properties as the inferior glenohumeral ligament

Abstract

Purpose

To characterize ~~This study characterized~~ the tensile and histological properties of the anterolateral ligament (ALL), inferior glenohumeral ligament (IGHL), and knee capsule.

Methods

Standardized samples of the ALL (N = 19), the anterolateral knee capsule (N = 15), and IGHL (N =13) were isolated from fresh-frozen human cadavers for uniaxial tensile testing to failure. An additional 6 samples of the ALL, capsule and IGHL were procured for histological analysis and elastin content.

Results

All investigated mechanical properties were significantly greater for both the ALL and IGHL when compared to capsular tissue. In contrast, no significant differences were found for any property between the ALL and IGHL. The elastic modulus of ALL and IGHL samples was 174±92 MPa and 139±60 MPa, respectively, compared to 62±30 MPa for capsule ($P = 0.001$). Ultimate stress was significantly lower ($P < 0.001$) for capsule at 13.4±7.7 MPa relative to the ALL and IGHL at 46.4±20.1 MPa and 38.7±16.3 MPa. The ultimate strain at failure for the ALL was 37.8±7.9 % and 39.5±9.4 % for the IGHL, which was significantly greater ($P = 0.041$ and $P = 0.02$, respectively) for both relative to the capsule at 32.6±8.4%.~~,~~ ~~capsule, and IGHL was 37.8±7.9 %, 32.6±8.4%, 39.5±9.4 (P < 0.05), respectively, while~~ T~~t~~he strain energy density of the ALL was 7.8±3.1 MPa, 2.1±1.3 MPa for the capsule, and 7.1±3.1 MPa for the IGHL ($P < 0.001$). The ALL and IGHL consisted of parallelly aligned collagen bundles, containing elastin bundles, which was in contrast to the random collagen architecture noted in capsule samples.

Conclusion

The anterolateral ligament has similar tensile and histological properties as the inferior glenohumeral ligament. The tensile properties of the ALL are significantly greater than those observed in knee capsule.

Clinical Relevance

The anterolateral ligament is not just a thickening of capsular tissue and should be considered as a distinct ligamentous structure comparable to the IGHL in the shoulder. The tensile behavior of the ALL is similar to the IGHL and treatment strategies should take this into account.

Introduction

Subluxation of the anterior cruciate ligament (ACL) deficient knee was described as early as 1845 by Bonnet,[1] but it was not until 1919 when Hey Groves first specified anterolateral instability;[2] a phenomenon later to become known as the pivot shift.[3] Despite the use of state-of-the-art intra-articular ACL reconstruction techniques, a remaining pivot shift has been reported to persist in 11-60% of patients.[4–6] Therefore, several authors have favoured an ACL reconstruction combined with an extra-articular augmentation in an attempt to limit persistent rotational laxity after ACL treatment.[7–10]

Recent studies showed that the anterolateral ligament (ALL) is a distinct ligament in the human knee[11,12], playing an important role as stabilizer for internal rotation[13–16] and whereby ALL reconstruction can therefore help control anterolateral instability.[17] The inferior glenohumeral ligament (IGHL) is an anatomically, histologically, and biomechanically well described ligamentous structure, and unlike the ALL, it is not perceived as just a thickening of the shoulder capsule but is widely accepted as an important static restraint.[18,19] Given their microscopic appearance and presumed function in restraining motion of the knee and shoulder, it can be hypothesized that the ALL and IGHL are comparable structures with similar roles as internal stabilizers.

Ligaments are essential structures for stabilizing joints. Knowledge of ligament mechanical properties is therefore key to elucidating their *in vivo* behavior and function and for selecting appropriate grafting materials used in reconstruction techniques. Recently, several review articles have discussed the lack of knowledge on the biomechanical properties of the knee's anterolateral components[20] while highlighting the need for further research.[21]

One study performed tensile testing of the isolated ALL[22] while other studies have characterized the pull-to-failure strength and stiffness of the bone-ALL-bone complex, however these tests only characterize the structural properties of the bone-ligament-bone complex, not the intrinsic mechanical properties.[23,24] Notwithstanding the lack of information regarding the mechanical properties, the renewed interest in the ALL has led to the development of anatomic reconstruction techniques.[15,25–27] Generally, these techniques utilize the gracilis tendon or a portion of iliotibial band as a graft material with a fixed femoral and tibial screw or anchor fixation.

Despite the fact that several authors have described the ALL as an anatomical, radiographical, histological and/or functionally distinct ligamentous structure,[12,23,28–30] there is still disagreement within the orthopaedics community, with some suggesting that the ALL is merely a thickening of the knee capsule.[31,32] Therefore, the purpose of this study is to characterize the tensile and histological properties of the ALL, IGHL, and knee capsule. ~~(1) provide a detailed mechanical characterization and (2) histomorphological analysis of the ALL, IGHL and knee capsule.~~ It was hypothesized that the mechanical properties of the ALL would be significantly different from the capsule, while being comparable to the IGHL.

Methods

Twelve fresh-frozen full body cadavers were obtained (74±7 years, 10 male and 2 female) under ethical approval from our Institutional Review Board. All donors had no history of knee/shoulder injury, instability, or prior surgical intervention. Additionally, 3 knees were excluded because of grade III and IV arthrosis or ACL deficiency. Eight shoulder specimens were reserved for other cadaveric studies and therefore could not be utilized in this work. A total of 21 ALL, 21 capsule, and 16 IGHL samples were dissected from the specimens by a final year~~n~~ orthopaedic resident (KS) using previously described techniques.[12,33] Furthermore, the capsule specimens were dissected from the area immediately adjacent and anterior to the

ALL (Fig. 1). Isolated specimens were wrapped in saline-soaked gauze and stored at -80°C until needed.

Mechanical Testing

One day prior to testing, specimens were removed from the freezer and thawed at room temperature. Using a surgical scalpel, specimens were cut into standardized shaped samples (dog-bone). Specimens were secured to custom tensile grips with cyanoacrylate adhesive and aligned axially (fibers parallel to loading direction) within a materials testing frame (model 4467, Instron, Norwood MA, USA) equipped with a 1 kN load cell (Fig. 2). A 1 N pre-load was applied and measurements of the cross-sectional area (assuming rectangular geometry) were taken with a calibrated micrometer five times and the average calculated. The distance between the grip faces was measured and taken as the original gage length. Specimens were pre-conditioned using a series of 10 cycles from 1 to 10 N at a strain rate of $0.1\%s^{-1}$, immediately followed by a test-to-failure using a strain rate of $2\%s^{-1}$. Tests were performed at room temperature (~22 °C) and samples were kept moist with saline at all times to prevent dehydration.

Histological Analysis

An additional 6 ALL, capsule, and IGHL specimens were procured from four cadavers to be used for qualitative histological analysis. Dissected specimens were fixed in 10% buffered formol and embedded in paraffin wax in a longitudinal orientation. An automated system (Symphony, Ventana, Tucson AZ, USA) was used to perform section staining with hematoxylin & eosin (H&E). Additionally, extra slides were prepared for histochemical analysis: trichrome (structural collagen and fibrin) and Von Gieson (elastin). All processed slides were digitally scanned (iScan HT, Ventana) and analysed with specialized software (Virtuoso, Ventana).

Statistical Analysis

Only those specimens that showed mid-substance failure were used for analysis. In total, data from 19 ALL, 15 capsule and 13 IGHL samples could be analyzed. The obtained force and displacement data were converted to stress (force / cross sectional area) and strain (change in length / original length) to allow the calculation of the tissue mechanical properties[34] (Fig. 3): elastic modulus (slope of the linear portion of the stress-strain curve), ultimate stress, ultimate strain, and strain energy density (area under the stress-strain curve, e.g. energy absorbed to failure). The collected mechanical data were found to exhibit non-normal distributions (with the Shapiro-Wilk test) and therefore Kruskal-Wallis tests with pairwise Mann-Whitney U post hoc tests were used. For all statistical analysis, commercial software was used (SPSS 23, IBM, Armonk, NY, USA) and significance level was set to $\alpha = 0.05$. All data are presented as mean ± standard deviation.

Results

Mechanical Testing

The average stress-strain curve obtained for the ALL, capsule, and IGHL specimens is shown in Figure 4. For all curves, specimens were characterized by an initial non-linear toe region followed by a linear stress-strain region and eventual specimen rupture.

All calculated mechanical properties were significantly higher for both the ALL and IGHL compared to the capsule (Fig. 5; see appendix for all tabulated values). In contrast, no significant differences were found for any property between the ALL and IGHL. The elastic modulus of ALL and IGHL samples was 174±92 MPa and 139±60 MPa, respectively, compared to 62±30 MPa for the capsule ($P < 0.001$). Ultimate stress was significantly lower ($P < 0.001$) for the capsule at 13.4±7.7 MPa relative to the ALL and IGHL at 46.4±20.1 MPa and 38.7±16.3 MPa. The ultimate strain at failure for the ALL was 37.8±7.9 % and 39.5±9.4 % for the IGHL, which was significantly greater ($P = 0.041$ and $P = 0.02$, respectively) for both relative to the capsule at 32.6±8.4%.~~, capsule, and IGHL was 37.8±7.9 %, 32.6±8.4%,~~

~~39.5±9.4 ($P < 0.05$), respectively, while the~~ The strain energy density of the ALL was 7.8±3.1 MPa 2.1±1.3 MPa for the capsule, and 7.1±3.1 MPa for the IGHL ($P < 0.001$).

Histological Analysis

Histological analysis revealed substantial differences between the ALL/IGHL and the capsule (Fig. 6). The ALL was characterized by the presence of dense, parallel oriented collagen bundles with strong collagenization and regularly distributed fibroblasts. This was also illustrated by the trichrome staining, which depicts collagen as dark blue. Generally, the ALL presented as a homogeneous, hypovascular structure containing elastin bundles. On the outside of the dense collagenous structures, some nerve fibre could be noted. Likewise, the IGHL displayed structural characteristics very similar to the ALL, however, the ALL appeared to be even more strictly organized and a higher concentration of loose connective tissue separating the collagen bundles in the IGHL could be observed. In contrast, the capsule showed a disorganized architecture consisting of 'islands' of collagenized tissue, where in between fat, loose connective tissue, and neurovascular bundles are present. Although some fine and thin dense collagenized bundles and elastin could be noted in the capsule, these were not all comparable with the ALL.

Discussion

~~This study characterized the tensile and histological properties of the anterolateral ligament (ALL), inferior glenohumeral ligament (IGHL), and knee capsule.~~ The primary finding of this work demonstrated that the tensile properties of the ALL are significantly higher than those of the knee capsule, while being comparable to the IGHL. This finding, coupled with the results from the histological analysis of the tissues, suggest that the ALL is a ligamentous structure that is distinct from the knee capsule; as is the case with the IGHL, one should refrain from stating that the ALL is just a simple thickening of the knee capsule.

Previously, a number of surgeons vaguely described the ALL as a ‘pearly, fibrous band’, the mid-third lateral capsular ligament, the anterior band of the lateral collateral ligament, the anterior oblique band or the capsulo-osseous layer of the iliotibial tract,[35–39] however, it wasn’t until recently that a more detailed anatomic description was given.[12] Subsequently, additional anatomic studies followed and despite differences in identification of attachment sites, it is generally believed that the ALL is a well-defined ligamentous structure originating around the lateral femoral condyle and running antero-distally to its tibial attachment approximately midway between the center of Gerdy’s tubercle and the anterior margin of the fibular head.[23,28,40] With internal rotation and flexion of the knee, the fibers of the ALL could clearly be distinguished from the slack and thin joint capsule lying just anterior of it.

Information regarding the mechanical properties of knee ligaments and surrounding soft tissues, in particular the ALL and capsule, are sparse. With respect to the ALL, this is surprising given the recent interest in reconstruction techniques using various types of grafts and fixation methods. The majority of studies previously performed have characterized the structural properties of these structures using pull-to-failure tests on either isolated bone-tissue-bone complexes or on entire knee cadavers.[23,24] Properties derived from these tests, such as stiffness, failure load, and toughness, are extrinsic and depend on the geometry of the tissue as well as the properties of the bony insertion sites.[34] In contrast, the mechanical properties measured in this study characterize the intrinsic behavior of the tissue. From a clinical point-of-view, an increase in a property such as the elastic modulus could be indicative of increased collagen content, larger collagen fibrils, and/or the tissue being made of a stiffer material.[34]

In this study, samples were isolated from cadavers and cut into standardized, dog-bone shaped specimens. This technique enabled for the characterization of the intrinsic mechanical, not structural, properties of the tissues. This method was chosen since replicating the loading of ligaments/tissues *ex vivo* is difficult and moreover insuring uniform load distribution to a ligament using an intact knee cadaver is particularly challenging. Cutting the tissue into dog-bone shaped-samples insured uniform loading while simultaneously mitigating potential end-effects that occur form gripping samples within the testing frame fixture.[41]

The tensile testing results obtained in this study provide strong evidence that the ALL is a distinct structure from the anterolateral knee capsule. All measured mechanical properties were significantly higher for the ALL relative to the capsule. The relative percent differences between the ALL and capsule were 95%, 110%, 17%, and 115% for the modulus, ultimate stress, maximum strain, and strain energy density, respectively. Additionally, one of the key strengths of this work relative to previous studies is the high number of specimens tested, done in an effort to mitigate the variability in biological tissue testing.

An overview comparing previously reported mechanical properties for the ALL, capsule, and IGHL is provided in Table 1. Zens et al.[22] reported maximum strain values similar to those that were found in the current work, however, their calculated yield stress and modulus was lower (potentially attributable to differences in the modulus calculation technique). It should be noted that only four samples were tested in their study, cross-sectional area was measured after the specimen was tested, and no sample pre-conditioning was performed. Likewise for the knee capsule, similar maximum strain values were found in the current study and that of Rachmat H et al.[42] yet substantial differences were noted in the modulus and yield stress values. These differences in reported mechanical properties highlight the variability inherent to biological tissue testing and how variations in methodology (pre-conditioning, strain rate, cross-sectional area measurement, etc.) can influence the calculation of mechanical properties.

Several previous studies have performed histological analysis of the ALL. For example, Helito et al.[43] analyzed 20 specimens and demonstrated that the ALL possessed typical histologic characteristics seen in ligamentous structures. Similar findings were shown by Vincent et al.[29] who noted parallel, dense, collagenous fibers suggestive of ligamentous or tendinous tissue within the ALL isolated from 10 cadavers. A recent study from Caterine et al.[11] used magnetic resonance imaging, anatomical dissection, and histological analysis to characterize the anatomical properties of the ALL. They found the morphology of the ALL to be characteristic of ligament tissue and moreover described nerve innervation within of the tissue, which they speculated could be indicative of a proprioceptive role. In the current study, histological analysis agreed with previous reports and showed that the ALL consisted of a dense collagenized and mostly homogeneous ligamentous structure containing thicker elastin bundles. Similar to Caterine et al. the presence of nerve intervention was noted in the current study but was only located outside the densely organized collagenous fibers of the ALL (4/6 samples). As shown in Figure 6, the histological morphology of the ALL was remarkably similar to the IGHL, with one notable difference being thicker elastin fibers within the IGHL. In contrast, the knee capsule contained broad islands of loose collagen with proteoglycans and fatty tissue also present.

Within ligamentous tissue, elastin (elastic fibers) are one of the components responsible for providing elastic recoil to the structure[44] and the distribution of elastic fibers is considered to reflect the physiologic function of the tissue.[45] Previous work from Ticker et al.[46] revealed elastic fibers present within the IGHL and speculated that this is relevant to the ligament's role as a static restraint within the shoulder. The presence of organized elastic fibers within the ALL suggest that it is also capable of providing restraint within the knee joint. While elastin fibers were present within the capsule, they lacked any apparent organization and thus indicate low potential for the capsule to resist tensile loads.

Similar to the ALL, the IGHL is a structure that lies in close proximity to the joint capsule. The IGHL is responsible for providing anterior stability in the glenohumeral joint in 90° of abduction and external rotation.[47,48] Failure patterns are seen in shoulder dislocations, where there can be a capsular stretching, Bankart lesions or even bony Bankart lesions.[46] Similar to the IGHL, the ALL is thought to be an important stabilizer of knee rotation at flexion angles exceeding 35°[13] and the Segond fracture is thought to be a bony avulsion of the ALL.[49] Data from this study supports the hypothesis that the ALL and IGHL are comparable structures with similar biomechanical and histological properties. It can be further postulated that ligaments are a heterogeneous group of connective tissues where subdivisions can be made. For instance, the IGHL and ALL can be classified in the same group of capsuloligamentous structures based on their similar stabilization roles and failure modes. This is in contrast to other ligaments such as the medial and lateral collateral ligaments which have different restraint functions and therefore different mechanical properties.

Limitations

Several limitations of this study should be noted. First, the mean age of the cadavers was 74±7 years which may not represent the typical patient undergoing knee ligament reconstruction. Since ligaments are known to exhibit age-related alterations in mechanical properties[50], data from this study may not be representative of ligament properties from younger patients. Secondly, all testing was conducted at room temperature, however, the mechanical properties of ligaments are known to be temperature dependent.[51,52] Since all specimens were all prepared, stored, and tested under identical conditions, any change in tissue properties resulting from the testing temperature would be carried through all specimens. Third, only the axial tensile properties of the specimens were tested although *in vivo* loading of these tissues is more complex. In this study, axial loading was applied parallel to specimen fibers, thereby approximating a worst-case scenario. Fourth, ligaments and capsular tissue are viscoelastic materials yet only the quasi-static properties were measured in this study. Fifth, the ALL as a distinct ligamentous structure was examined by comparing it to the adjacent knee capsule and an existing ligament (IGHL). Hereby, other interesting anatomical structures, like the shoulder capsule, were not tested in this study.

Conclusion

The anterolateral ligament has similar tensile and histological properties as the inferior glenohumeral ligament. The tensile properties of the ALL are significantly greater than those observed in knee capsule.

References

1. Schindler OS. Surgery for anterior cruciate ligament deficiency: a historical perspective. *Knee Surg Sports Traumatol Arthrosc*. 2012;20(1):5-47. doi:10.1007/s00167-011-1756-x.
2. Groves EWH. The crucial ligaments of the knee-joint: Their function, rupture, and the operative treatment of the same. *Br J Surg*. 1919;7(28):505-515. doi:10.1002/bjs.1800072809.
3. Galway R, Beaupre A, DL M. Pivot shift: a clinical sign of symptomatic anterior cruciate insufficiency. In: *Proceedings of the Canadian Orthopaedic Association*. Jasper, Alberta; 1971.
4. Suomalainen P, Jarvela T, Paakkala A, Kannus P, Jarvinen M. Double-Bundle Versus Single-Bundle Anterior Cruciate Ligament Reconstruction: A Prospective Randomized Study With 5-Year Results. *Am J Sports Med*. 2012;40(7):1511-1518. doi:10.1177/0363546512448177.
5. Karikis I, Desai N, Sernert N, Rostgard-Christensen L, Kartus J. Comparison of Anatomic Double- and Single-Bundle Techniques for Anterior Cruciate Ligament Reconstruction Using Hamstring Tendon Autografts: A Prospective Randomized Study With 5-Year Clinical and Radiographic Follow-up. *Am J Sports Med*. 2016. doi:10.1177/0363546515626543.
6. Jonsson H, Riklund-Ahlström K, Lind J. Positive pivot shift after ACL reconstruction predicts later osteoarthrosis: 63 patients followed 5-9 years after surgery. *Acta Orthop Scand*. 2004;75(5):594-599. doi:10.1080/00016470410001484.
7. Clancy WG, Nelson DA, Reider B, Narechania RG. Anterior cruciate ligament reconstruction using one-third of the patellar ligament, augmented by extra-articular tendon transfers. *J Bone Joint Surg Am*. 1982;64(3):352-359. http://www.ncbi.nlm.nih.gov/pubmed/7061552. Accessed January 6, 2016.
8. Strickler F. A satisfactory method of repairing cruciate ligaments. *Ann Surg*. 1937;105:912-916.
9. Zarins B, Rowe CR. Combined anterior cruciate-ligament reconstruction using semitendinosus tendon and iliotibial tract. *J Bone Joint Surg Am*. 1986;68(2):160-177. http://www.ncbi.nlm.nih.gov/pubmed/3944155. Accessed January 6, 2016.
10. Marcacci M, Zaffagnini S, Giordano G, Iacono F, Presti M Lo. Anterior cruciate ligament reconstruction associated with extra-articular tenodesis: A prospective clinical and radiographic evaluation with 10- to 13-year follow-up. *Am J Sports Med*. 2009;37(4):707-714. doi:10.1177/0363546508328114.

11. Caterine S, Litchfield R, Johnson M, Chronik B, Getgood A. A cadaveric study of the anterolateral ligament: re-introducing the lateral capsular ligament. *Knee Surg Sports Traumatol Arthrosc*. 2015;23(11):3186-3195. doi:10.1007/s00167-014-3117-z.
12. Claes S, Vereecke E, Maes M, Victor J, Verdonk P, Bellemans J. Anatomy of the anterolateral ligament of the knee. *J Anat*. 2013;223(4):321-328.
13. Parsons EM, Gee AO, Spiekerman C, Cavanagh PR. The Biomechanical Function of the Anterolateral Ligament of the Knee. *Am J Sports Med*. 2015;43(6):669-674. doi:10.1177/0363546514562751.
14. Tavlo M, Eljaja S, Jensen JT, Siersma VD, Krogsgaard MR. The role of the anterolateral ligament in ACL insufficient and reconstructed knees on rotatory stability: A biomechanical study on human cadavers. *Scand J Med Sci Sports*. 2015. doi:10.1111/sms.12524.
15. Spencer L, Burkhart TA, Tran MN, et al. Biomechanical Analysis of Simulated Clinical Testing and Reconstruction of the Anterolateral Ligament of the Knee. *Am J Sports Med*. 2015:0363546515589166-. doi:10.1177/0363546515589166.
16. Rasmussen MT, Nitri M, Williams BT, et al. An In Vitro Robotic Assessment of the Anterolateral Ligament, Part 1: Secondary Role of the Anterolateral Ligament in the Setting of an Anterior Cruciate Ligament Injury. *Am J Sports Med*. 2016;44(3):585-592. doi:10.1177/0363546515618387.
17. Nitri M, Rasmussen MT, Williams BT, et al. An In Vitro Robotic Assessment of the Anterolateral Ligament, Part 2: Anterolateral Ligament Reconstruction Combined With Anterior Cruciate Ligament Reconstruction. *Am J Sports Med*. 2016;44(3):593-601. doi:10.1177/0363546515620183.
18. Bigliani LU, Kelkar R, Flatow EL, Pollock RG, Mow VC. Glenohumeral stability. Biomechanical properties of passive and active stabilizers. *Clin Orthop Relat Res*. 1996;(330):13-30. http://www.ncbi.nlm.nih.gov/pubmed/8804270. Accessed September 29, 2016.
19. Burkart AC, Debski RE. Anatomy and function of the glenohumeral ligaments in anterior shoulder instability. *Clin Orthop Relat Res*. 2002;(400):32-39. http://www.ncbi.nlm.nih.gov/pubmed/12072743. Accessed September 29, 2016.
20. Guenther D, Griffith C, Lesniak B, et al. Anterolateral rotatory instability of the knee. *Knee Surg Sports Traumatol Arthrosc*. 2015;23(10):2909-2917. doi:10.1007/s00167-015-3616-6.
21. Kosy JD, Mandalia VI. Revisiting the Anterolateral Ligament of the Knee. *J Knee Surg*. 2015. doi:10.1055/s-0035-1569148.
22. Zens M, Feucht MJ, Ruhhammer J, et al. Mechanical tensile properties of the anterolateral ligament. *J Exp Orthop*. 2015;2(1):7. doi:10.1186/s40634-015-0023-3.
23. Kennedy MI, Claes S, Fuso FAF, et al. The Anterolateral Ligament: An Anatomic, Radiographic, and Biomechanical Analysis. *Am J Sports Med*. 2015;43(7):1606-1615. doi:10.1177/0363546515578253.
24. Rahnemai-Azar AA, Miller RM, Guenther D, et al. Structural Properties of the Anterolateral Capsule and Iliotibial Band of the Knee. *Am J Sports Med*. 2016:0363546515623500-. doi:10.1177/0363546515623500.
25. Smith JO, Yasen SK, Lord B, Wilson AJ. Combined anterolateral ligament and anatomic anterior cruciate ligament reconstruction of the knee. *Knee Surg Sports Traumatol Arthrosc*. 2015;23(11):3151-3156. doi:10.1007/s00167-015-3783-5.
26. Helito CP, Bonadio MB, Gobbi RG, et al. Combined Intra- and Extra-articular Reconstruction of the Anterior Cruciate Ligament: The Reconstruction of the Knee Anterolateral Ligament. *Arthrosc Tech*. 2015;4(3):e239-e244. doi:10.1016/j.eats.2015.02.006.

27. Sonnery-Cottet B, Thaunat M, Freychet B, Pupim BHB, Murphy CG, Claes S. Outcome of a Combined Anterior Cruciate Ligament and Anterolateral Ligament Reconstruction Technique With a Minimum 2-Year Follow-up. *Am J Sports Med*. 2015;43(7):1598-1605. doi:10.1177/0363546515571571.

28. Helito CP, Demange MK, Bonadio MB, et al. Radiographic Landmarks for Locating the Femoral Origin and Tibial Insertion of the Knee Anterolateral Ligament. *Am J Sports Med*. 2014;42(10):2356-2362. doi:10.1177/0363546514543770.

29. Vincent J-P, Magnussen RA, Gezmez F, et al. The anterolateral ligament of the human knee: an anatomic and histologic study. *Knee Surg Sports Traumatol Arthrosc*. 2012;20(1):147-152. doi:10.1007/s00167-011-1580-3.

30. Van der Watt L, Khan M, Rothrauff BB, et al. The structure and function of the anterolateral ligament of the knee: a systematic review. *Arthroscopy*. 2015;31(3):569-82.e3. doi:10.1016/j.arthro.2014.12.015.

31. Dombrowski ME, Costello JM, Ohashi B, et al. Macroscopic anatomical, histological and magnetic resonance imaging correlation of the lateral capsule of the knee. *Knee Surgery, Sport Traumatol Arthrosc*. 2015. doi:10.1007/s00167-015-3517-8.

32. Musahl V, Rahnemai-Azar AA, van Eck CF, Guenther D, Fu FH. Anterolateral ligament of the knee, fact or fiction? *Knee Surg Sports Traumatol Arthrosc*. 2015. doi:10.1007/s00167-015-3913-0.

33. Bigliani LU, Pollock RG, Soslowsky LJ, Flatow EL, Pawluk RJ, Mow VC. Tensile properties of the inferior glenohumeral ligament. *J Orthop Res*. 1992;10(2):187-197. doi:10.1002/jor.1100100205.

34. Woo SL, Debski RE, Withrow JD, Janaushek MA. Biomechanics of knee ligaments. *Am J Sports Med*. 1999;27(4):533-543.

35. Segond P. *Recherches Cliniques et Expérimentales Sur Les Épanchements Sanguins Du Genou Par Entorse*. Paris: Progrès Médical; 1879. http://gallica.bnf.fr/ark:/12148/bpt6k5712206r. Accessed January 6, 2016.

36. Hughston JC, Andrews JR, Cross MJ, Moschi A. Classification of knee ligament instabilities. Part II. The lateral compartment. *J Bone Joint Surg Am*. 1976;58(2):173-179. http://www.ncbi.nlm.nih.gov/pubmed/1254620. Accessed January 3, 2016.

37. Irvine GB, Dias JJ, Finlay DB. Segond fractures of the lateral tibial condyle: brief report. *J Bone Joint Surg Br*. 1987;69(4):613-614. http://www.ncbi.nlm.nih.gov/pubmed/3611168. Accessed January 5, 2016.

38. Campos JC, Chung CB, Lektrakul N, et al. Pathogenesis of the Segond fracture: anatomic and MR imaging evidence of an iliotibial tract or anterior oblique band avulsion. *Radiology*. 2001;219(2):381-386. doi:10.1148/radiology.219.2.r01ma23381.

39. Terry GC, LaPrade RF. The posterolateral aspect of the knee. Anatomy and surgical approach. *Am J Sports Med*. 1996;24(6):732-739. http://www.ncbi.nlm.nih.gov/pubmed/8947393. Accessed January 6, 2016.

40. Dodds AL, Halewood C, Gupte CM, Williams A, Amis AA. The anterolateral ligament: Anatomy, length changes and association with the Segond fracture. *Bone Joint J*. 2014;96-B(3):325-331. doi:10.1302/0301-620X.96B3.33033.

41. Quapp KM, Weiss JA. Material Characterization of Human Medial Collateral Ligament. *J Biomech Eng*. 1998;120(6):757. doi:10.1115/1.2834890.

42. Rachmat HH, Janssen D, Verkerke GJ, Diercks RL, Verdonschot N. Material properties of the human posterior knee capsule. *Biomed Mater Eng*. 2015;25(2):177-187. doi:10.3233/BME-151268.

43. Helito CP, Demange MK, Bonadio MB, et al. Anatomy and Histology of the Knee Anterolateral Ligament. *Orthop J Sport Med*. 2013;1(7):2325967113513546. doi:10.1177/2325967113513546.

44. Smith KD, Vaughan-Thomas A, Spiller DG, Innes JF, Clegg PD, Comerford EJ. The organisation of elastin and fibrillins 1 and 2 in the cruciate ligament complex. *J Anat*. 2011;218(6):600-607. doi:10.1111/j.1469-7580.2011.01374.x.
45. Kielty CM, Baldock C, Lee D, Rock MJ, Ashworth JL, Shuttleworth CA. Fibrillin: from microfibril assembly to biomechanical function. *Philos Trans R Soc Lond B Biol Sci*. 2002;357(1418):207-217. doi:10.1098/rstb.2001.1029.
46. Ticker JB, Flatow EL, Pawluk RJ, et al. The inferior glenohumeral ligament: a correlative investigation. *J Shoulder Elbow Surg*. 2006;15(6):665-674. doi:10.1016/j.jse.2005.11.006.
47. Ticker JB, Bigliani LU, Soslowsky LJ, Pawluk RJ, Flatow EL, Mow VC. Inferior glenohumeral ligament: geometric and strain-rate dependent properties. *J Shoulder Elbow Surg*. 1996;5(4):269-279. http://www.ncbi.nlm.nih.gov/pubmed/8872924. Accessed February 23, 2016.
48. McMahon PJ, Tibone JE, Cawley PW, et al. The anterior band of the inferior glenohumeral ligament: Biomechanical properties from tensile testing in the position of apprehension. *J Shoulder Elb Surg*. 1998;7(5):467-471. doi:10.1016/S1058-2746(98)90196-3.
49. Claes S, Luyckx T, Vereecke E, Bellemans J. The Segond fracture: a bony injury of the anterolateral ligament of the knee. *Arthroscopy*. 2014;30(11):1475-1482. doi:10.1016/j.arthro.2014.05.039.
50. Hammer N, Lingslebe U, Aust G, Milani TL, Hädrich C, Steinke H. Ultimate stress and age-dependent deformation characteristics of the iliotibial tract. *J Mech Behav Biomed Mater*. 2012;16:81-86. doi:10.1016/j.jmbbm.2012.04.025.
51. Bass CR, Planchak CJ, Salzar RS, et al. The Temperature-Dependent Viscoelasticity of Porcine Lumbar Spine Ligaments. *Spine (Phila Pa 1976)*. 2007;32(16):E436-E442. doi:10.1097/BRS.0b013e3180b7fa58.
52. Lam TC, Thomas CG, Shrive NG, Frank CB, Sabiston CP. The Effects of Temperature on the Viscoelastic Properties of the Rabbit Medial Collateral Ligament. *J Biomech Eng*. 1990;112(2):147-152. doi:10.1115/1.2891165.
~~53. Bey MJ, Hunter SA, Kilambi N, Butler DL, Lindenfeld TN. Structural and mechanical properties of the glenohumeral joint posterior capsule. *J Shoulder Elbow Surg*. 2005;14(2):201-206. doi:10.1016/j.jse.2004.06.016.~~

Figure Captions

Figure 1: Knee capsule specimens were dissected from the area immediately adjacent and anterior to the ALL, as shown by the bounding box. LFC – lateral femoral condyle, LCL – lateral collateral ligament, FH – fibular head, ALL – anterolateral ligament.

Figure 2: The clamping system used to perform tensile testing of the tissue specimens. Samples were kept hydrated at all times during testing with saline.

Figure 3: Representative stress-strain curve obtained from tensile testing of the anterolateral ligament showing the derivation of the calculated mechanical properties.

Figure 4: Average stress-strain curves for the ALL, capsule, and IGHL specimens. The final points represents the average ultimate stress and ultimate strain and the error bars indicate the standard deviation.

Figure 5: Results (mean ± SD) for a) elastic modulus, b) ultimate stress, c) ultimate strain, and d) strain energy density obtained from tensile testing. All measured properties were significantly ($P < 0.001$ a-b-d~~—c~~ and $P < 0.05$ for c~~d~~) higher for the ALL and IGHL relative to the capsule. No significant differences were found between the ALL and IGHL.

Figure 6: Representative histological cross sections of the ALL, capsule, and IGHL using three different staining techniques. The arrows represents the elastin fibers.

Table 1: Comparison of the mechanical properties from the current study with previously reported data. All data are presented as mean ± standard deviation. A dashed line indicates the property was not reported. Note, only mechanical properties, not structural properties (stiffness, load at failure), are reported here.

Reference	**Structure**	***N***	**Modulus (MPa)**	**Ultimate Stress (MPa)**	**Maximum Strain (%)**	**Strain Energy Density (MPa)**
Zens M.[22]	ALL	4	1.2±0.4*	32.8±4.0	36.0±4.5	---
Current study	ALL	19	174±92	46.4±20.1	37.8±7.9	7.8±3.1
Rachmat H.[42]	Capsule	15	9±11	1.8±1.9	35±10	---
Current study	Capsule	15	62±30	13.4±7.7	31.9±8.4	2.1±1.3
Bey M.[53]	IGHL	7	38±19	8.7±3.8	36±15	1.6±1.1
Ticker J.[46]	IGHL	8	115±44	13.9±7.1	17±5	---
McMahon P.[48]	IGHL	11	104±10	8.0±1.0	10±1	---
Current study	IGHL	13	139±60	38.7±16.3	39.5±9.4	4.3±2.3

*this property was 'calculated at 20% strain' and is thus a different calculation methodology than that used in the current study

Appendix~~Table A~~: Raw values obtained from tensile testing of the ALL, capsule, and IGHL

	Elastic Modulus (MPa)			**Ultimate Stress (MPa)**			**Ultimate Strain (mm/mm)**			**Strain Energy Density (MPa)**		
	ALL	**Capsule**	**IGHL**	**ALL**	**Capsule**	**IGHL**	**ALL**	**Capsule**	**IGHL**	**ALL**	**Capsule**	**IGHL**
	389	56	77	90	12	35	0.27	0.38	0.61	11.4	2.3	9.6
	121	56	109	30	12	38	0.32	0.28	0.48	4.2	1.7	8.7
	129	68	180	27	14	47	0.31	0.24	0.36	4.5	1.6	8.1
	102	109	112	24	19	24	0.39	0.24	0.29	4.5	2.2	3.1
	130	115	168	40	31	44	0.47	0.37	0.34	7.6	5.3	7.5
	114	54	83	32	10	18	0.41	0.35	0.27	6.3	2.0	2.1
	60	21	71	20	6	19	0.50	0.35	0.42	4.5	1.3	3.5
	198	54	131	73	18	29	0.44	0.40	0.31	14.1	3.3	5.1
	211	89	147	52	27	45	0.29	0.36	0.48	6.8	4.3	8.8
	227	55	293	62	13	79	0.34	0.28	0.32	10.9	1.7	11.3
	421	8	150	90	2	39	0.28	0.54	0.39	11.1	0.7	7.1
	173	43	189	40	7	55	0.31	0.27	0.44	5.5	1.0	12.2
	150	55	101	49	11	30	0.47	0.24	0.42	11.1	1.4	5.6
	140	98		37	9		0.34	0.22		6.1	0.7	
	159	50		55	10		0.44	0.27		12.1	1.4	
	204			46			0.28			5.7		
	122			35			0.39			5.3		
	147			43			0.43			7.3		
	103			36			0.50			9.0		
Mean	**174**	**62**	**139**	**46**	**13**	**39**	**0.38**	**0.32**	**0.39**	**7.8**	**2.1**	**7.1**
SD	**92**	**30**	**60**	**20**	**8**	**16**	**0.08**	**0.09**	**0.09**	**3.1**	**1.3**	**3.1**