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The Anterolateral Ligament Has Similar Biomechanical and Histologic Properties to the Inferior Glenohumeral Ligament Peer-reviewed author version

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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

1 <u>To characterize This study characterized</u> the tensile and histological properties of the

2 anterolateral ligament (ALL), inferior glenohumeral ligament (IGHL), and knee capsule.

## **Methods**

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

## Results

7	All investigated mechanical properties were significantly greater for both the ALL and IGHL
8	when compared to capsular tissue. In contrast, no significant differences were found for any
9	property between the ALL and IGHL. The elastic modulus of ALL and IGHL samples was
10	174±92 MPa and 139±60 MPa, respectively, compared to $62\pm30$ MPa for capsule ( $P =$
11	0.001). Ultimate stress was significantly lower ( $P < 0.001$ ) for capsule at 13.4±7.7 MPa
12	relative to the ALL and IGHL at 46.4±20.1 MPa and 38.7±16.3 MPa. The ultimate strain at
13	failure for the ALL was 37.8±7.9 % and 39.5±9.4 % for the IGHL, which was significantly
14	greater ( $P = 0.041$ and $P = 0.02$ , respectively) for both relative to the capsule at $32.6\pm8.4\%$ .
15	capsule, and IGHL was 37.8±7.9%, 32.6±8.4%, 39.5±9.4 (P < 0.05), respectively, while <u>T</u> the
16	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
17	MPa for the IGHL ( $P < 0.001$ ). The ALL and IGHL consisted of parallely aligned collagen
18	bundles, containing elastin bundles, which was in contrast to the random collagen architecture
19	noted in capsule samples.

## Conclusion

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

## **Clinical Relevance**

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

### Introduction

27 Subluxation of the anterior cruciate ligament (ACL) deficient knee was described as early as 1845 by Bonnet,<sup>1</sup> but it was not until 1919 when Hey Groves first specified anterolateral 28 instability;<sup>2</sup> a phenomenon later to become known as the pivot shift.<sup>3</sup> Despite the use of state-29 30 of-the-art intra-articular ACL reconstruction techniques, a remaining pivot shift has been reported to persist in 11-60% of patients.<sup>4–6</sup> Therefore, several authors have favoured an ACL 31 32 reconstruction combined with an extra-articular augmentation in an attempt to limit persistent rotational laxity after ACL treatment.<sup>7-10</sup> 33 34 Recent studies showed that the anterolateral ligament (ALL) is a distinct ligament in the human knee<sup>11,12</sup>, playing an important role as stabilizer for internal rotation<sup>13–16</sup> and 35 whereby ALL reconstruction can therefore help control anterolateral instability.<sup>17</sup> The inferior 36 glenohumeral ligament (IGHL) is an anatomically, histologically, and biomechanically well 37 38 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.<sup>18,19</sup> Given their 39 40 microscopic appearance and presumed function in restraining motion of the knee and 41 shoulder, it can be hypothesized that the ALL and IGHL are comparable structures with 42 similar roles as internal stabilizers. 43 Ligaments are essential structures for stabilizing joints. Knowledge of ligament

44 mechanical properties is therefore key to elucidating their *in vivo* behavior and function and 45 for selecting appropriate grafting materials used in reconstruction techniques. Recently, 46 several review articles have discussed the lack of knowledge on the biomechanical properties 47 of the knee's anterolateral components<sup>20</sup> while highlighting the need for further research.<sup>21</sup>

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One study performed tensile testing of the isolated ALL<sup>22</sup> while other studies have 48 49 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 50 complex, not the intrinsic mechanical properties.<sup>23,24</sup> Notwithstanding the lack of information 51 regarding the mechanical properties, the renewed interest in the ALL has led to the 52 development of anatomic reconstruction techniques.<sup>15,25–27</sup> Generally, these techniques utilize 53 54 the gracilis tendon or a portion of iliotibial band as a graft material with a fixed femoral and 55 tibial screw or anchor fixation.

Despite the fact that several authors have described the ALL as an anatomical, 56 radiographical, histological and/or functionally distinct ligamentous structure,<sup>12,23,28–30</sup> there is 57 still disagreement within the orthopaedics community, with some suggesting that the ALL is 58 merely a thickening of the knee capsule.<sup>31,32</sup> Therefore, the purpose of this study is to 59 60 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 61 62 ALL, IGHL and knee capsule. It was hypothesized that the mechanical properties of the ALL 63 would be significantly different from the capsule, while being comparable to the IGHL.

### Methods

64 Twelve fresh-frozen full body cadavers were obtained (74±7 years, 10 male and 2 female) 65 under ethical approval from our Institutional Review Board. All donors had no history of 66 knee/shoulder injury, instability, or prior surgical intervention. Additionally, 3 knees were 67 excluded because of grade III and IV arthrosis or ACL deficiency. Eight shoulder specimens 68 were reserved for other cadaveric studies and therefore could not be utilized in this work. A 69 total of 21 ALL, 21 capsule, and 16 IGHL samples were dissected from the specimens by a final yearn orthopaedic resident (KS) using previously described techniques.<sup>12,33</sup> Furthermore, 70 71 the capsule specimens were dissected from the area immediately adjacent and anterior to the

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

#### Mechanical Testing

74 One day prior to testing, specimens were removed from the freezer and thawed at room 75 temperature. Using a surgical scalpel, specimens were cut into standardized shaped samples 76 (dog-bone). Specimens were secured to custom tensile grips with cyanoacrylate adhesive and 77 aligned axially (fibers parallel to loading direction) within a materials testing frame (model 78 4467, Instron, Norwood MA, USA) equipped with a 1 kN load cell (Fig. 2). A 1 N pre-load 79 was applied and measurements of the cross-sectional area (assuming rectangular geometry) 80 were taken with a calibrated micrometer five times and the average calculated. The distance 81 between the grip faces was measured and taken as the original gage length. Specimens were 82 pre-conditioned using a series of 10 cycles from 1 to 10 N at a strain rate of 0.1%s<sup>-1</sup>, immediately followed by a test-to-failure using a strain rate of 2%s<sup>-1</sup>. Tests were performed at 83 84 room temperature (~22 °C) and samples were kept moist with saline at all times to prevent

85 dehydration.

## Histological Analysis

86 An additional 6 ALL, capsule, and IGHL specimens were procured from four cadavers to be 87 used for qualitative histological analysis. Dissected specimens were fixed in 10% buffered 88 formol and embedded in paraffin wax in a longitudinal orientation. An automated system 89 (Symphony, Ventana, Tucson AZ, USA) was used to perform section staining with 90 hematoxylin & eosin (H&E). Additionally, extra slides were prepared for histochemical 91 analysis: trichrome (structural collagen and fibrin) and Von Gieson (elastin). All processed 92 slides were digitally scanned (iScan HT, Ventana) and analysed with specialized software 93 (Virtuoso, Ventana).

94 Statistical Analysis

95	Only those specimens that showed mid-substance failure were used for analysis. In
96	total, data from 19 ALL, 15 capsule and 13 IGHL samples could be analyzed. The obtained
97	force and displacement data were converted to stress (force / cross sectional area) and strain
98	(change in length / original length) to allow the calculation of the tissue mechanical
99	properties <sup>34</sup> (Fig. 3): elastic modulus (slope of the linear portion of the stress-strain curve),
100	ultimate stress, ultimate strain, and strain energy density (area under the stress-strain curve,
101	e.g. energy absorbed to failure). The collected mechanical data were found to exhibit non-
102	normal distributions (with the Shapiro-Wilk test) and therefore Kruskal-Wallis tests with
103	pairwise Mann-Whitney U post hoc tests were used. For all statistical analysis, commercial
104	software was used (SPSS 23, IBM, Armonk, NY, USA) and significance level was set to $\alpha$ =
105	0.05. All data are presented as mean $\pm$ standard deviation.

### Results

#### Mechanical Testing

106 The average stress-strain curve obtained for the ALL, capsule, and IGHL specimens is shown 107 in Figure 4. For all curves, specimens were characterized by an initial non-linear toe region 108 followed by a linear stress-strain region and eventual specimen rupture. 109 All calculated mechanical properties were significantly higher for both the ALL and 110 IGHL compared to the capsule (Fig. 5; see appendix for all tabulated values). In contrast, no 111 significant differences were found for any property between the ALL and IGHL. The elastic 112 modulus of ALL and IGHL samples was 174±92 MPa and 139±60 MPa, respectively, 113 compared to  $62\pm30$  MPa for the capsule (P < 0.001). Ultimate stress was significantly lower 114 (P < 0.001) for the capsule at 13.4±7.7 MPa relative to the ALL and IGHL at 46.4±20.1 MPa 115 and 38.7±16.3 MPa. The ultimate strain at failure for the ALL was 37.8±7.9 % and 39.5±9.4 116 % 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%, 117

118 $39.5\pm9.4 \ (P < 0.05)$ , respectively, while the The strain energy density of the ALL was 7.8±3.1119MPa 2.1±1.3 MPa for the capsule, and 7.1±3.1 MPa for the IGHL (P < 0.001).</td>

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## Histological Analysis

121 Histological analysis revealed substantial differences between the ALL/IGHL and the 122 capsule (Fig. 6). The ALL was characterized by the presence of dense, parallel oriented 123 collagen bundles with strong collagenization and regularly distributed fibroblasts. This was 124 also illustrated by the trichrome staining, which depicts collagen as dark blue. Generally, the 125 ALL presented as a homogeneous, hypovascular structure containing elastin bundles. On the 126 outside of the dense collagenous structures, some nerve fibre could be noted. Likewise, the 127 IGHL displayed structural characteristics very similar to the ALL, however, the ALL 128 appeared to be even more strictly organized and a higher concentration of loose connective 129 tissue separating the collagen bundles in the IGHL could be observed. In contrast, the capsule 130 showed a disorganized architecture consisting of 'islands' of collagenized tissue, where in 131 between fat, loose connective tissue, and neurovascular bundles are present. Although some 132 fine and thin dense collagenized bundles and elastin could be noted in the capsule, these were 133 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.

141 Previously, a number of surgeons vaguely described the ALL as a 'pearly, fibrous 142 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, <sup>35–39</sup> 143 however, it wasn't until recently that a more detailed anatomic description was given.<sup>12</sup> 144 145 Subsequently, additional anatomic studies followed and despite differences in identification of 146 attachment sites, it is generally believed that the ALL is a well-defined ligamentous structure 147 originating around the lateral femoral condyle and running antero-distally to its tibial 148 attachment approximately midway between the center of Gerdy's tubercle and the anterior margin of the fibular head.<sup>23,28,40</sup> With internal rotation and flexion of the knee, the fibers of 149 150 the ALL could clearly be distinguished from the slack and thin joint capsule lying just anterior 151 of it.

152 Information regarding the mechanical properties of knee ligaments and surrounding 153 soft tissues, in particular the ALL and capsule, are sparse. With respect to the ALL, this is 154 surprising given the recent interest in reconstruction techniques using various types of grafts 155 and fixation methods. The majority of studies previously performed have characterized the 156 structural properties of these structures using pull-to-failure tests on either isolated bonetissue-bone complexes or on entire knee cadavers.<sup>23,24</sup> Properties derived from these tests, 157 158 such as stiffness, failure load, and toughness, are extrinsic and depend on the geometry of the 159 tissue as well as the properties of the bony insertion sites.<sup>34</sup> In contrast, the mechanical 160 properties measured in this study characterize the intrinsic behavior of the tissue. From a 161 clinical point-of-view, an increase in a property such as the elastic modulus could be 162 indicative of increased collagen content, larger collagen fibrils, and/or the tissue being made of a stiffer material.<sup>34</sup> 163

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 dogbone shaped-samples insured uniform loading while simultaneously mitigating potential endeffects that occur form gripping samples within the testing frame fixture.<sup>41</sup>

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.

178 An overview comparing previously reported mechanical properties for the ALL, capsule, and IGHL is provided in Table 1. Zens et al.<sup>22</sup> reported maximum strain values 179 180 similar to those that were found in the current work, however, their calculated yield stress and 181 modulus was lower (potentially attributable to differences in the modulus calculation 182 technique). It should be noted that only four samples were tested in their study, cross-183 sectional area was measured after the specimen was tested, and no sample pre-conditioning 184 was performed. Likewise for the knee capsule, similar maximum strain values were found in the current study and that of Rachmat H et al.<sup>42</sup> yet substantial differences were noted in the 185 186 modulus and yield stress values. These differences in reported mechanical properties highlight 187 the variability inherent to biological tissue testing and how variations in methodology (pre-188 conditioning, strain rate, cross-sectional area measurement, etc.) can influence the calculation 189 of mechanical properties.

190 Several previous studies have performed histological analysis of the ALL. For example, Helito et al.<sup>43</sup> analyzed 20 specimens and demonstrated that the ALL possessed 191 192 typical histologic characteristics seen in ligamentous structures. Similar findings were shown by Vincent et al.<sup>29</sup> who noted parallel, dense, collagenous fibers suggestive of ligamentous or 193 194 tendinous tissue within the ALL isolated from 10 cadavers. A recent study from Caterine et 195 al.<sup>11</sup> used magnetic resonance imaging, anatomical dissection, and histological analysis to 196 characterize the anatomical properties of the ALL. They found the morphology of the ALL to 197 be characteristic of ligament tissue and moreover described nerve innervation within of the 198 tissue, which they speculated could be indicative of a proprioceptive role. In the current study, 199 histological analysis agreed with previous reports and showed that the ALL consisted of a 200 dense collagenized and mostly homogeneous ligamentous structure containing thicker elastin 201 bundles. Similar to Caterine et al. the presence of nerve intervention was noted in the current 202 study but was only located outside the densely organized collagenous fibers of the ALL (4/6 203 samples). As shown in Figure 6, the histological morphology of the ALL was remarkably 204 similar to the IGHL, with one notable difference being thicker elastin fibers within the IGHL. 205 In contrast, the knee capsule contained broad islands of loose collagen with proteoglycans and 206 fatty tissue also present.

207 Within ligamentous tissue, elastin (elastic fibers) are one of the components responsible for providing elastic recoil to the structure<sup>44</sup> and the distribution of elastic fibers is 208 considered to reflect the physiologic function of the tissue.<sup>45</sup> Previous work from Ticker et 209 al.<sup>46</sup> revealed elastic fibers present within the IGHL and speculated that this is relevant to the 210 ligament's role as a static restraint within the shoulder. The presence of organized elastic 211 212 fibers within the ALL suggest that it is also capable of providing restraint within the knee 213 joint. While elastin fibers were present within the capsule, they lacked any apparent 214 organization and thus indicate low potential for the capsule to resist tensile loads.

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215 Similar to the ALL, the IGHL is a structure that lies in close proximity to the joint 216 capsule. The IGHL is responsible for providing anterior stability in the glenohumeral joint in 90° of abduction and external rotation.<sup>47,48</sup> Failure patterns are seen in shoulder dislocations, 217 where there can be a capsular stretching, Bankart lesions or even bony Bankart lesions.<sup>46</sup> 218 219 Similar to the IGHL, the ALL is thought to be an important stabilizer of knee rotation at flexion angles exceeding  $35^{\circ 13}$  and the Segond fracture is thought to be a bony avulsion of the 220 ALL.<sup>49</sup> Data from this study supports the hypothesis that the ALL and IGHL are comparable 221 222 structures with similar biomechanical and histological properties. It can be further postulated 223 that ligaments are a heterogeneous group of connective tissues where subdivisions can be 224 made. For instance, the IGHL and ALL can be classified in the same group of 225 capsuloligamentous structures based on their similar stabilization roles and failure modes. 226 This is in contrast to other ligaments such as the medial and lateral collateral ligaments which 227 have different restraint functions and therefore different mechanical properties.

228 Limitations

229 Several limitations of this study should be noted. First, the mean age of the cadavers 230 was 74±7 years which may not represent the typical patient undergoing knee ligament 231 reconstruction. Since ligaments are known to exhibit age-related alterations in mechanical properties<sup>50</sup>, data from this study may not be representative of ligament properties from 232 233 younger patients. Secondly, all testing was conducted at room temperature, however, the mechanical properties of ligaments are known to be temperature dependent.<sup>51,52</sup> Since all 234 235 specimens were all prepared, stored, and tested under identical conditions, any change in 236 tissue properties resulting from the testing temperature would be carried through all 237 specimens. Third, only the axial tensile properties of the specimens were tested although in 238 vivo loading of these tissues is more complex. In this study, axial loading was applied parallel 239 to specimen fibers, thereby approximating a worst-case scenario. Fourth, ligaments and

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- 240 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
- 242 the adjacent knee capsule and an existing ligament (IGHL). Hereby, other interesting
- 243 <u>anatomical structures, like the shoulder capsule, were not tested in this study.</u>

## Conclusion

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

## References

- 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.
- 250 2. Groves EWH. The crucial ligaments of the knee-joint: Their function, rupture, and the
  251 operative treatment of the same. *Br J Surg.* 1919;7(28):505-515.
  252 doi:10.1002/bjs.1800072809.
- 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.
- 265 6. Jonsson H, Riklund-Ahlström K, Lind J. Positive pivot shift after ACL reconstruction
  266 predicts later osteoarthrosis: 63 patients followed 5-9 years after surgery. *Acta Orthop*267 *Scand.* 2004;75(5):594-599. doi:10.1080/00016470410001484.
- 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.
- 271 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.
- 274 9. Zarins B, Rowe CR. Combined anterior cruciate-ligament reconstruction using
  275 semitendinosus tendon and iliotibial tract. *J Bone Joint Surg Am.* 1986;68(2):160-177.
  276 http://www.ncbi.nlm.nih.gov/pubmed/3944155. Accessed January 6, 2016.
- 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.
- 284 12. Claes S, Vereecke E, Maes M, Victor J, Verdonk P, Bellemans J. Anatomy of the
  285 anterolateral ligament of the knee. *J Anat.* 2013;223(4):321-328.
- 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.
- 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.
- 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):585592. doi:10.1177/0363546515618387.
- 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.
- 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.
- 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.
- 311 20. Guenther D, Griffith C, Lesniak B, et al. Anterolateral rotatory instability of the knee.
   312 *Knee Surg Sports Traumatol Arthrosc.* 2015;23(10):2909-2917. doi:10.1007/s00167 313 015-3616-6.
- 314 21. Kosy JD, Mandalia VI. Revisiting the Anterolateral Ligament of the Knee. *J Knee* 315 Surg. 2015. doi:10.1055/s-0035-1569148.
- 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.
- 318 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.
- 321 24. Rahnemai-Azar AA, Miller RM, Guenther D, et al. Structural Properties of the
  322 Anterolateral Capsule and Iliotibial Band of the Knee. *Am J Sports Med.*323 2016:0363546515623500-. doi:10.1177/0363546515623500.
- 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.
- 327 26. Helito CP, Bonadio MB, Gobbi RG, et al. Combined Intra- and Extra-articular
  328 Reconstruction of the Anterior Cruciate Ligament: The Reconstruction of the Knee
  329 Anterolateral Ligament. *Arthrosc Tech.* 2015;4(3):e239-e244.
- doi:10.1016/j.eats.2015.02.006.

- 331 27. Sonnery-Cottet B, Thaunat M, Freychet B, Pupim BHB, Murphy CG, Claes S.
  332 Outcome of a Combined Anterior Cruciate Ligament and Anterolateral Ligament
  333 Reconstruction Technique With a Minimum 2-Year Follow-up. *Am J Sports Med.*334 2015;43(7):1598-1605. doi:10.1177/0363546515571571.
- 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.
- 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):56982.e3. doi:10.1016/j.arthro.2014.12.015.
- 344 31. Dombrowski ME, Costello JM, Ohashi B, et al. Macroscopic anatomical, histological
  345 and magnetic resonance imaging correlation of the lateral capsule of the knee. *Knee*346 *Surgery, Sport Traumatol Arthrosc.* 2015. doi:10.1007/s00167-015-3517-8.
- 347 32. Musahl V, Rahnemai-Azar AA, van Eck CF, Guenther D, Fu FH. Anterolateral
  348 ligament of the knee, fact or fiction? *Knee Surg Sports Traumatol Arthrosc.* 2015.
  349 doi:10.1007/s00167-015-3913-0.
- 350 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.
- 353 34. Woo SL, Debski RE, Withrow JD, Janaushek MA. Biomechanics of knee ligaments.
   354 Am J Sports Med. 1999;27(4):533-543.
- 355 35. Segond P. Recherches Cliniques et Expérimentales Sur Les Épanchements Sanguins
   356 Du Genou Par Entorse. Paris: Progrès Médical; 1879.
- 357 http://gallica.bnf.fr/ark:/12148/bpt6k5712206r. Accessed January 6, 2016.
- 358 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):173179. http://www.ncbi.nlm.nih.gov/pubmed/1254620. Accessed January 3, 2016.
- 361 37. Irvine GB, Dias JJ, Finlay DB. Segond fractures of the lateral tibial condyle: brief
  362 report. *J Bone Joint Surg Br.* 1987;69(4):613-614.
- 363 http://www.ncbi.nlm.nih.gov/pubmed/3611168. Accessed January 5, 2016.
- 364 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.
- 367
   39. Terry GC, LaPrade RF. The posterolateral aspect of the knee. Anatomy and surgical approach. *Am J Sports Med.* 1996;24(6):732-739.
- 369 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.
- Rachmat HH, Janssen D, Verkerke GJ, Diercks RL, Verdonschot N. Material
  properties of the human posterior knee capsule. *Biomed Mater Eng.* 2015;25(2):177187. 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.

- 381 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.
- 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.
- 387 46. Ticker JB, Flatow EL, Pawluk RJ, et al. The inferior glenohumeral ligament: a
  388 correlative investigation. *J Shoulder Elbow Surg.* 2006;15(6):665-674.
  389 doi:10.1016/j.jse.2005.11.006.
- 390 47. Ticker JB, Bigliani LU, Soslowsky LJ, Pawluk RJ, Flatow EL, Mow VC. Inferior
  391 glenohumeral ligament: geometric and strain-rate dependent properties. *J Shoulder*392 *Elbow Surg.* 1996;5(4):269-279. http://www.ncbi.nlm.nih.gov/pubmed/8872924.
  393 Accessed February 23, 2016.
- 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/S10582746(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.
  400 doi:10.1016/j.arthro.2014.05.039.
- 401 50. Hammer N, Lingslebe U, Aust G, Milani TL, Hädrich C, Steinke H. Ultimate stress
  402 and age-dependent deformation characteristics of the iliotibial tract. *J Mech Behav*403 *Biomed Mater*. 2012;16:81-86. doi:10.1016/j.jmbbm.2012.04.025.
- 404 51. Bass CR, Planchak CJ, Salzar RS, et al. The Temperature-Dependent Viscoelasticity of
  405 Porcine Lumbar Spine Ligaments. *Spine (Phila Pa 1976)*. 2007;32(16):E436-E442.
  406 doi:10.1097/BRS.0b013e3180b7fa58.
- 407 52. Lam TC, Thomas CG, Shrive NG, Frank CB, Sabiston CP. The Effects of Temperature
  408 on the Viscoelastic Properties of the Rabbit Medial Collateral Ligament. *J Biomech*409 *Eng.* 1990;112(2):147-152. doi:10.1115/1.2891165.
- 410 53. Bey MJ, Hunter SA, Kilambi N, Butler DL, Lindenfeld TN. Structural and mechanical
   411 properties of the glenohumeral joint posterior capsule. J Shoulder Elbow Surg.
   412 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  $\pm$  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<u>-b-d</u>—e and P < 0.05 for <u>cd</u>) 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. <u>The arrows represents the elastin fibers.</u>

**Table 1:** Comparison of the mechanical properties from the current study with previously reported data. All data are presented as mean  $\pm$  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. <sup>22</sup>	ALL	4	1.2±0.4*	32.8±4.0	36.0±4.5	
Current study	ALL	19	174±92	46.4±20.1	$37.8 \pm 7.9$	$7.8 \pm 3.1$
Rachmat H.42	Capsule	15	9±11	$1.8 \pm 1.9$	35±10	
Current study	Capsule	15	62±30	13.4±7.7	31.9±8.4	2.1±1.3
Bey M. <sup>53</sup>	IGHL	7	38±19	$8.7 \pm 3.8$	36±15	$1.6 \pm 1.1$
Ticker J.46	IGHL	8	115±44	$13.9 \pm 7.1$	17±5	
McMahon P.48	IGHL	11	$104 \pm 10$	$8.0{\pm}1.0$	$10 \pm 1$	
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

-	Elastic Modulus (MPa)			Ultimate Stress (MPa)			Ultimate Strain (mm/mm)			Strain Energy Density (MPa)		
	ALL	Capsul e	IGH L	ALL	Capsul e	IGH L	ALL	Capsul e	IGH L	ALL	Capsul e	IGH L
	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		
Aean	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

Appendix Table A: Raw values obtained from tensile testing of the ALL cansule and IGHI

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