Mechanical characterisation of the lateral collateral ligament complex of the ankle at realistic sprain-like strain rates

1 **Abstract**

2 **BACKGROUND**

3 **Synthetic interventions continue to evolve with the progression made in materials**

4 **science, surgical technologies and surgical methods. To facilitate the evolution of**

5 **synthetic devices for lateral ankle repair a better understanding of the mechanical**

6 **properties and failure mechanisms of the lateral collateral ligament (LCL) complex**

7 **is required. This study aimed to improve understanding of the mechanical**

8 **properties and failure modes of the LCL complex at strain rates representative of**

9 **sprain.**

10 **METHOD**

11 **The LCLs were dissected from six human cadavers to produce individual bone-**

12 **ligament-bone specimens. A mechanical testing device uni-axially loaded the**

13 **ligaments in tension. Initially, preconditioning between two Newtons and a load**

14 **value corresponding to 3.5 % strain was conducted for 15 cycles, before extension**

15 **to failure at strain rate of 100 %.s^{-1}. The results were stratified by age, weight and**

16 **body mass index (BMI) to explore potential correlations with ligament ultimate**

17 **failure load or ligament stiffness.**

18 **RESULTS**

ATFL – Anterior Talofibular Ligament
CFL – Calcaneofibular Ligament
LCL – Lateral Collateral Ligament
PTFL – Posterior Talofibular Ligament

19   The mean ultimate failure loads and the 95 % confidence intervals for the ATFL,

20   calcaneofibular (CFL) and posterior talofibular (PTFL) ligaments were 263.6 ± 164.3

21   N, 367.8 ± 79.8 N and 351.4 ± 110.8 N, respectively. A strong positive Pearson

22   correlation was found between BMI and ultimate failure load of the CFL ($r = .919$; P

23    $= .01$). A non-significant relationship was found between the mechanical properties

24   and both age and weight. The ATFL avulsed from the fibula four times, the CFL

25   avulsed from the fibula twice, the PTFL avulsed from the talus twice and all

26   remaining failures were mid-substance.

27   CONCLUSION

28   The results identify the forces required to induce failure of the individual ligaments

29   of the LCL complex and the related failure modes of individual ligaments. A

30   correlation may exist between BMI and the ultimate failure load of the CFL and

31   PTFL, although a greater sample size is required for confirmation.

34   Introduction

35   The lateral collateral ligament (LCL) complex of the ankle (see Figure 1), consists of

36   the anterior talofibular ligament (ATFL), calcaneofibular ligament (CFL) and posterior

37   talofibular ligament (PTFL). The LCLs of the ankle are collectively responsible for the

38   stabilisation of the talocrural joint on the lateral side and the CFL also plays a role in

39   the stabilisation of the subtalar joint.
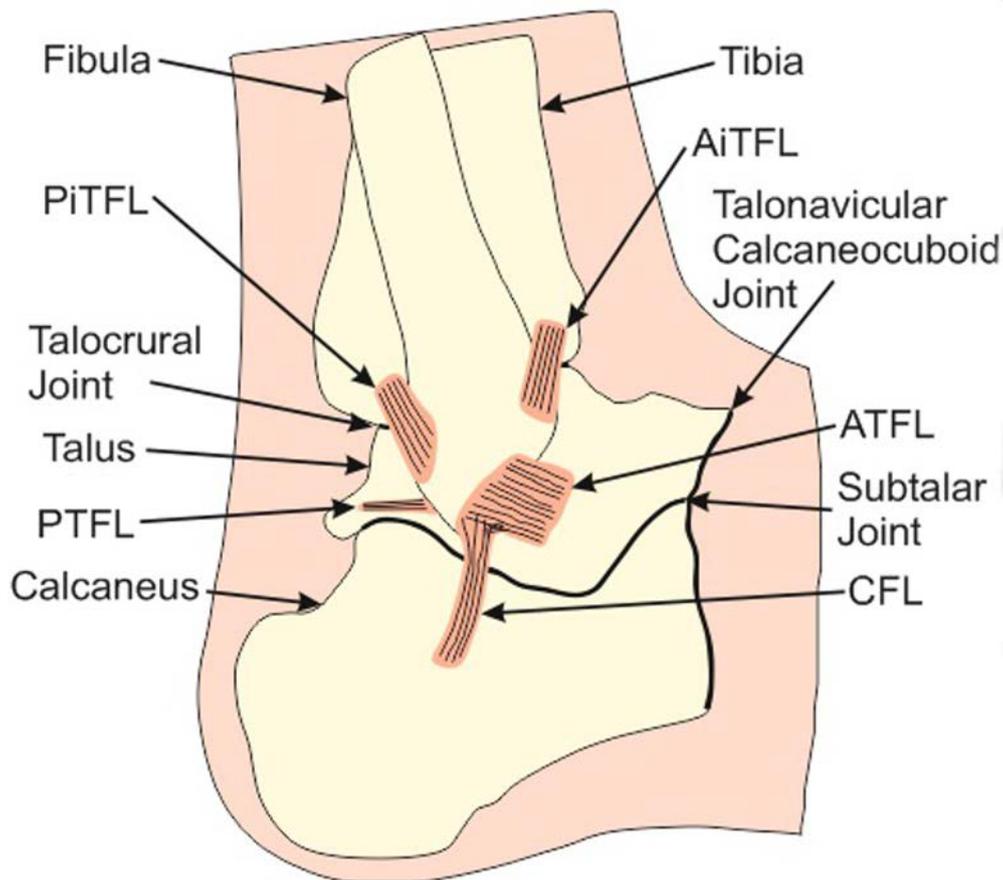
Figure 1. Lateral view of the ankle highlighting the LCL complex (ATFL, CFL & PTFL), the syndesmosis (AiTFL & PiTFL), the talocrural joint, the subtalar joint, the talonavicular calcaneocuboid joint and the bones of the ankle (fibula, tibia, talus and calcaneus).

The ATFL is the most frequently injured LCL in a typical lateral ankle sprain, followed by the CFL and finally the PTFL.[3,16] In cases of severe sprain or in people, such as elite athletes, wherein whom restoration of stability is important, surgical stabilisation may be performed. The current preferred standard is the Broström-Gould procedure in which ruptured ligaments are stabilised with sutures. If this approach is inadequate or has failed or if the patient has an increased BMI, general ligament laxity or is a high-demand athlete, then stabilisation with synthetic ligaments may be attempted.[1]

Natural ligaments exhibit a viscoelastic response to strain, starting with a progressively stiffer nonlinear toe region followed by a linear loading region. The response of ligamentous tissue is believed to be strain rate dependent due to the

55 inherent viscoelastic nature of the tissue.[6] This viscoelasticity causes ligaments to

56 display hysteresis, due to the fluid component of the ligament being redistributed

57 and balanced by the stress carried by the solid component of the ligament. When

58 the lateral collateral ligament of the knee was tested at strain rates greater than 100

59 $\%.s^{-1}$, a strain rate representative of inducing sprain in real-world events, it was

60 found that the strain-rate dependency of the ligament can be neglected as there is

61 insufficient time for appreciable ligament relaxation.[6] The ligaments of the ankle

62 have been reported to be generally insensitive to strain rate.[9] Conversely, the

63 mechanical properties of the LCLs have been reported to be significantly affected by

64 strain rates both above and below 100 $\%.s^{-1}$.[3]

65 Research articles detailing the mechanical characteristics of the LCL complex are

66 scarce.[3,9,16,17] None of the previous papers report mechanical characteristics of the

67 LCL complex tested at realistic sprain-like strain rates. Attarian et al. (1985) and Funk

68 et al. (2000) characterised the LCL complex at strain rates considerably higher than

69 those which occur during a sprain event.[3,9] Although ligaments are considered

70 relatively insensitive to strain rates over 100 $\%.s^{-1}$ the effect on the failure mode of

71 the ligaments is not understood. The absence of literature on this topic is potentially

72 due to the difficulty faced when gripping ankle ligament tissue, as previously

73 reported.[16] A lack of published work in this area has hindered the understanding of

74 the mechanical requirements and failure modes of synthetic interventions for lateral

75 ankle sprain.

76 This study aimed to improve understanding of the mechanical properties and failure

77 modes of the LCL complex at strain rates representative of real-world sprain events.

78 **Materials and Methods**

79 **2.1 Samples**

80 Six fresh frozen human cadaveric feet, sourced from MedCure (USA), were used in

81 the study. Ethical approval was granted by the University of Leeds Research Ethics

82 Committee (MEEC 15-020). Exclusion criteria for the tissues included a reported

83 prior lower limb trauma or surgery, or a history of diabetes. The mean (± 95 %

84 confidence intervals) donor age was 56.2 ± 12.2 years, BMI was 22.3 ± 2.9 kg.m^{-2}

85 (normal) and there were three males and three females. A summary of donor

86 information is shown in Table 1.

87 **Table 1.** Tissue donor demographic details. The mean and 95 % confidence interval (CI) is given for

88 age, weight and body mass index (BMI). (M – male, F – female, A.A – African American, C – Caucasian,

89 R – right & L – left).

Sample	Age (years)	Sex	Race	Weight (kg)	BMI (kg.m^{-2})	L/R Foot
1	72	M	A.A	71	22.4	R
2	60	F	C	53	18.9	L
3	49	F	C	49	20.9	R
4	61	M	C	66	21.4	R
5	38	M	C	85	27.0	L
6	57	F	C	61	23.2	R
Mean	56.2	-	-	64.1	22.3	-
± CI	± 12.2			± 13.8	± 2.9	

90

91 **2.2 Sample Preparation**

92 The feet were stored in a -80 °C freezer, compliant with the Human Tissue Act, until

93 they were tested. Samples were thawed for 48 hours at 4 °C in a refrigerator prior to

94 dissection. After at least 24 hours of thawing, each foot was imaged, at a resolution

95 of 82 μm, using a SCANCO Medical xtreme CT scanner (SCANCO Medical, Brüttisellen,

96 Switzerland). Each scan lasted approximately 90 minutes and was performed to

97 ensure no major undiagnosed damage was present.

98 The LCL complex was dissected intact from each foot while preserving the

99 syndesmosis joint for future study, as shown in Figures 2 & 3. Firstly, all fascia and

100   soft tissue were dissected from around the ankle by a foot and ankle specialist

101   consultant orthopaedic surgeon. Next, the forefoot was removed by transecting

102   along the talonavicular calcaneocuboid joint. Using an oscillating bone saw, a sagittal

103   cut was made through the entirety of the calcaneus and talus, as shown in Figure 2,

104   Panel B. The lateral ankle complex was then removed by a transverse cut through

105   the fibula, separating the LCL complex from the syndesmosis, as shown in Figure 2,

106   Panel C. The cut was made from in-between the attachment points of the ATFL and

107   anterior inferior talofibular ligament (AiTFL) to in-between the attachment points of

108   the PTFL and posterior inferior talofibular ligament (PiTFL). The talus was then split in

109   half with a coronal cut creating an anterior and posterior bone attachment segment

110   for the ATFL and PTFL, respectively. Finally, the calcaneus was reduced in size and

111   shaped to fit within the gripping fixture by performing two parallel coronal cuts

112   either side of the attachment point and one transverse cut distally to the attachment

113   point, as shown in Figure 3. The tissue hydration level of the ankle complex was

114   maintained by wrapping the complex in phosphate-buffered saline (PBS) soaked

115   paper towel.[11]

116
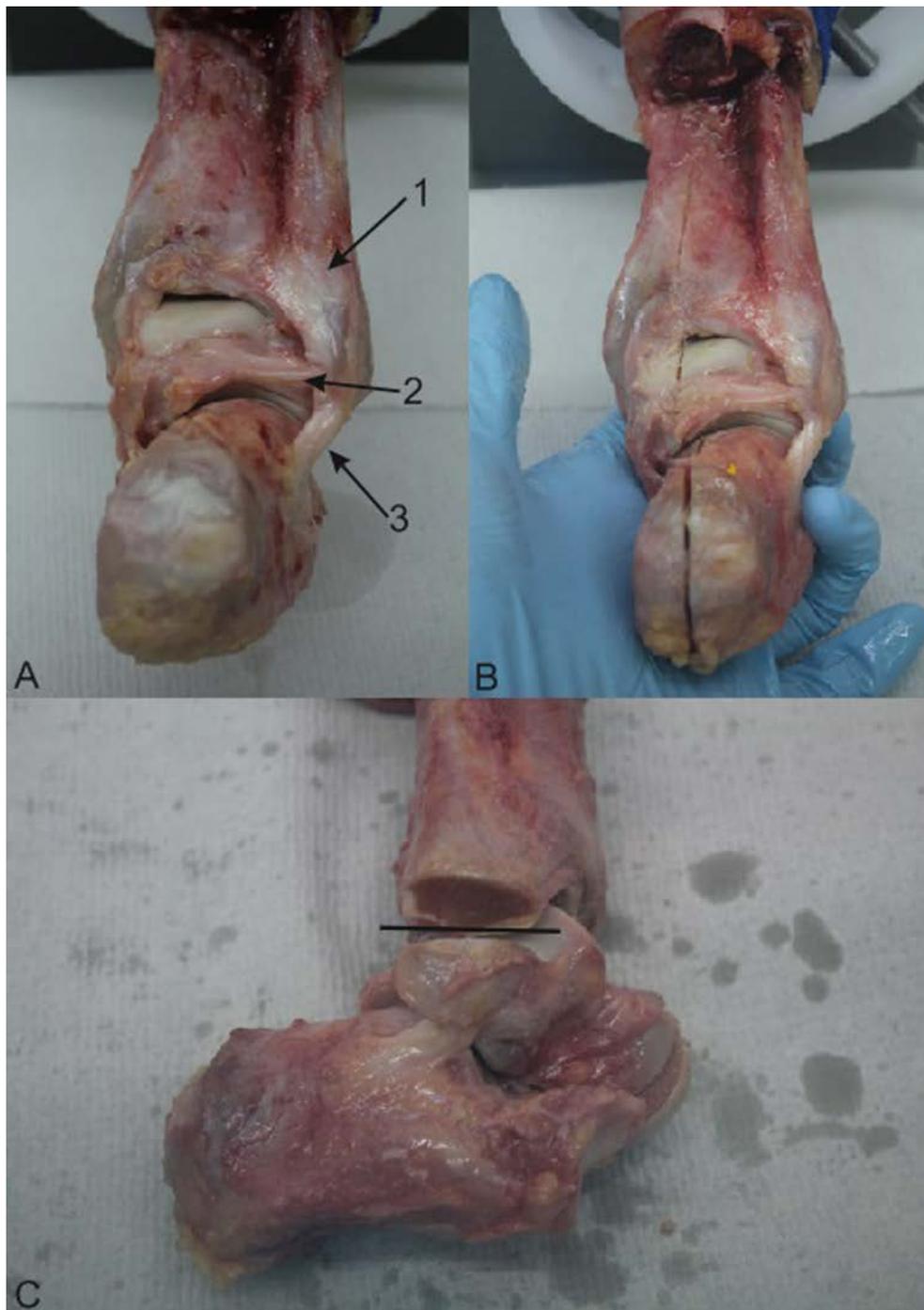
**Figure 2.** The dissection protocol employed to remove the LCL complex from the rearfoot. **A** illustrates

the intact rearfoot and provides a clear view of the PiTFL (1), PTFL (2) and CFL (3). **B** illustrates the

sagittal cut made to separate the medial and lateral aspects of the rearfoot. **C** illustrates the

transverse cut (black line) made through the fibula to separate the LCL complex and syndesmosis.

121
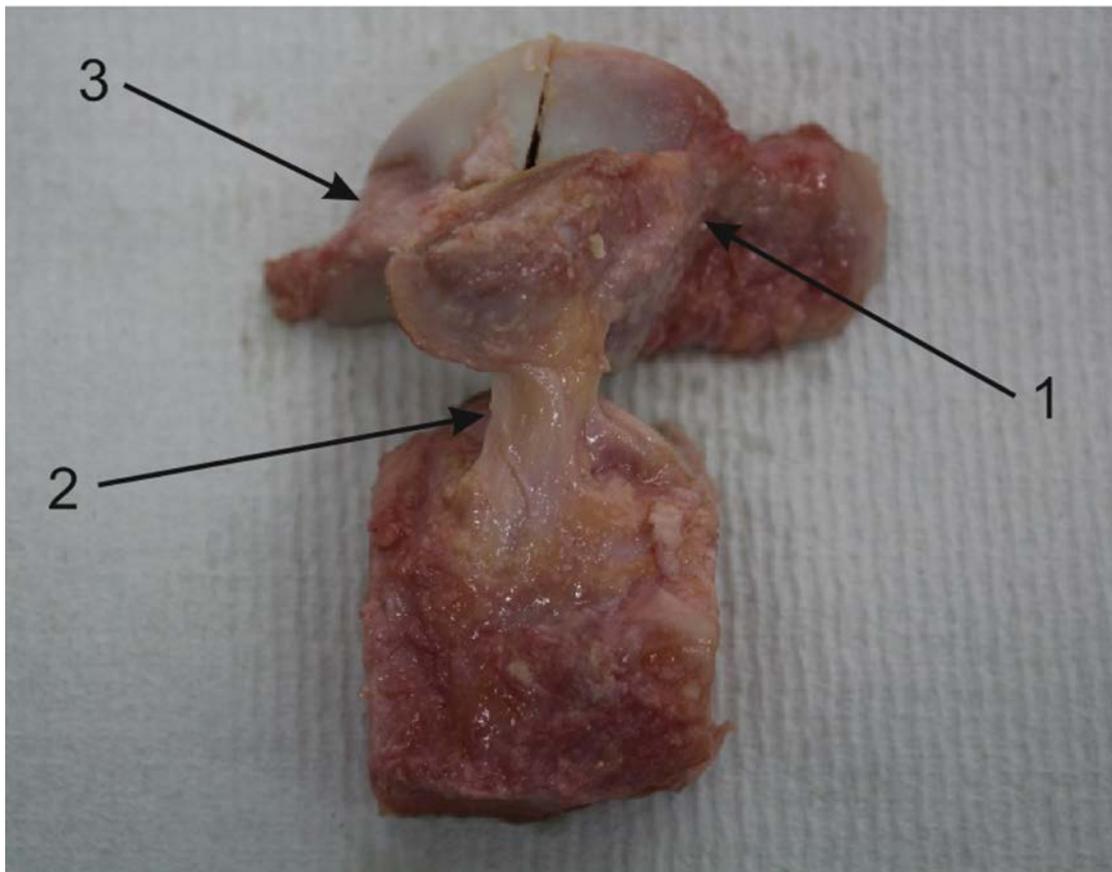
Figure 3. The LCL complex fully dissected prior to testing. The coronal cut into talus has been

performed, creating separate bone pieces for the ATFL and PTFL, and the calcaneus has been shaped

to fit within the bespoke testing grip. The ATFL (1), CFL (2) and PTFL (3) are shown.

Post-dissection ligament lengths were measured using Vernier callipers with the

ligaments orientated in line with their collagen fibres and the slack in the ligament

was removed by hand. The ligaments were measured once, from the centre of one

insertion to the centre of the other.

2.3 Testing Protocol

Each ligament of the LCL complex was tested individually whilst the complex was

kept intact. The CFL was characterised first, then the ATFL followed by the PTFL.

Tissue rehydration was performed to ensure the viscoelastic nature of ligaments

could act efficiently during the testing. Immediately before the characterisation of

the CFL, the complex was submerged in PBS for 30 minutes. The complex was then

submerged for 15 minutes prior to testing the ATFL and a further 15 minutes prior to

136   testing the PTFL due to the short length of time taken for each test. Testing the

137   individual ligaments as an intact complex was facilitated by a bespoke gripping

138   fixture. The bone segments at each end of the ligaments were fixed within the

139   gripping fixture using six gripping bolts for each bone attachment segment ensuring

140   collagen fibre alignment, as shown in Figure 4.
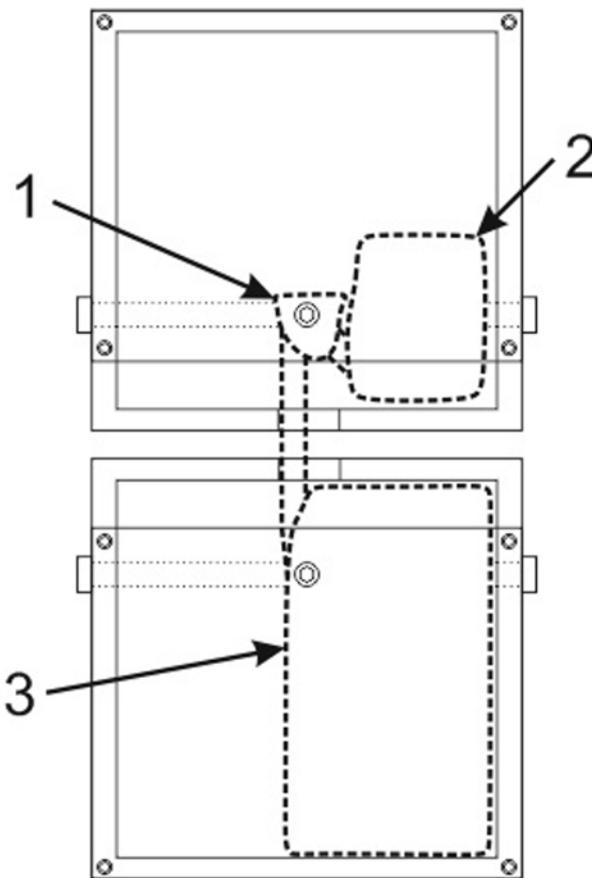
141

142   **Figure 4.** The LCL complex fixed into the bespoke gripping fixture with the CFL prepared for

143   characterisation. The ATFL and PTFL, and their bony attachments from the fibula (1) to the talus (2)

144   are within the top pot and the calcaneus (3) is within the bottom pot.

145   The mechanical characterisation was performed using an Instron ElectroPuls E10000,

146   with a 1 kN load cell (Instron, Buckinghamshire, UK). A floating joint was used to

147   attach the top grip to the Instron to correct for any unintended malalignment within

148   the setup.

149   Preconditioning was completed to ensure specimens were in an appropriate

150   physiological state of readiness prior to failure testing and fluid redistribution had

151   occurred within the specimens.[14] Fifteen cycles of preconditioning following a

152   sinusoidal waveform, ranging between two Newtons and a load value corresponding

153   to 3.5 % strain, were performed at a frequency of 0.83 Hz. The 3.5 % strain value

154   represents the minimum amount of strain accumulated by any of the LCLs during

155   one step of a normal walking cycle (10 degrees dorsiflexion through to 20 degrees

156   plantarflexion).[7] The preconditioning load values representing 3.5 % strain were

157   determined in a preliminary test of each ligament tested under strain control at a

158   rate of 10 %.s[-1]. The frequency of 0.83 Hz is equivalent to the rate of normal walking

159   (approximately one full gait cycle per second).

160   Following preconditioning, the specimens were then ramp loaded to failure at a

161   strain rate of 100 %.s[-1]. A strain rate of 100 %.s[-1] was selected to be representative of

162   sprain, having previously been suggested to be a suitable injury strain rate for

163   anterior cruciate ligament injury.[4] The following equation, incorporating real-world

164   inputs, also suggests that a strain rate of 100 %.s[-1] is appropriate to replicate ankle

165   ligament sprain.

166 $$\dot{\varepsilon} = \frac{\Delta L}{Lt}$$

167   Where $\dot{\varepsilon}$ is the strain rate, ΔL is the change in length of the ATFL from neutral

168   position to maximum plantarflexion (4.5 mm),[2] L is the length of the ATFL in the

169   neutral position (16.3 mm)[2] and t is the time taken for the sprain motion of an ankle

170   (0.3 s).[8]

171   **2.4 Data Analysis**

172   The mode of failure was determined via physical and visual examination of the

173   specimens. Any specimens where the ligament had torn away from bone, torn

174   cartilage away from bone or torn a small fragment of bone away from bone were

175   categorised as an avulsion. Any intra-ligamentous failures were defined as mid-

176 substance failures. After the experimental testing, post-processing was completed to

177 calculate the ultimate failure load and stiffness of each ligament from each donor.

178 The linear stiffness value (k1) was calculated using a custom Matlab algorithm.[11]

179 Mean values and 95 % confidence intervals for the ligament ultimate failure load,

180 stiffness and length, as well as the donor BMI, weight and age were calculated for

181 the ATFL, CFL and PTFL. A repeated measures ANOVA with a Greenhouse-Geisser

182 correction ($p < .01$) was performed to calculate any significant differences in ultimate

183 failure load or stiffness between the ATFL, CFL and PTFL. Analysis of the data

184 stratified by age, weight and BMI was performed to identify any potential

185 correlations with these patient-specific factors and both ultimate failure load and

186 stiffness. Correlations were calculated for the ATFL, CFL and PTFL individually using a

187 two-tailed Pearson correlation test ($p < .01$).

188 **Results**

189 The post-dissection ligament lengths used to calculate the ligament specific

190 preconditioning limits are provided in Table 2. The CFL was the longest of the three

191 ligaments forming the LCL complex, with mean (± 95 % CI) length of 20.0 ± 1.9 mm.

192 The PTFL and ATFL followed in order but were similar in length with mean lengths of

193 13.4 ± 3.2 mm and 12.6 ± 0.9 mm, respectively.

194 **Table 2.** Ligament lengths (mm) for each individual ligament and the mean ligament length and 95 %

195 confidence intervals (CI) for ATFL, CFL and PTFL.

196

197

Sample	ATFL	CFL	PTFL
1	11.62	17.60	10.50

2	11.76	20.66	14.66
3	12.90	19.24	10.54
4	13.50	23.00	18.34
5	12.08	20.06	14.80
6	13.54	19.66	11.66
Mean ± CI	12.6 ± 0.9	20.0 ± 1.9	13.4 ± 3.2

198

199   The mechanical characterisation results for the ATFL, CFL and PTFL are shown in

200   Table 3. The CFL had the highest mean ultimate failure load (± 95 % CI) of 367.8 ±

201   79.8 N followed by the PTFL 351.4 ± 110.8 N, while the ATFL was the weakest 263.6 ±

202   164.3 N. No significant differences were found for the ultimate failure load (p = .24)

203   or stiffness (p = .30) between the ATFL, CFL and PTFL.

204   **Table 3.** The mean and 95 % confidence intervals (CI) for the ultimate failure load and stiffness results

205   of the ATFL, CFL and PTFL. As well as the failure mode (A – avulsion and M – mid-substance) and

206   avulsion location.

	ATFL	CFL	PTFL
Mean Ultimate Failure Load ± CI (N)	263.6 ± 164.3	367.8 ± 79.8	351.4 ± 110.8
Mean Stiffness ± CI (N/mm)	44.7 ± 16.6	45.8 ± 19.0	59.0 ± 10.7
Failure Mode (A/M)	4/2	2/4	2/4
Avulsion Site	Fibula	Fibula	Talus

207

208   The ratio of avulsions to mid-substance failures was similar for the ligament types

209   tested, as detailed in Table 3. The ATFL avulsed from the fibula in four of the six

210   tests, the CFL avulsed from the fibula in two of the six tests and the PTFL avulsed

211   from the talus in two of the six tests. No systematic differences in ultimate failure

212   load or stiffness were identified between the different failure modes. When avulsion

213   did occur, the site of avulsion was consistent amongst ligament types (see Table 3).

214   Figures 5A and 5B illustrate clear examples of a mid-substance failure and avulsion,

215   respectively.
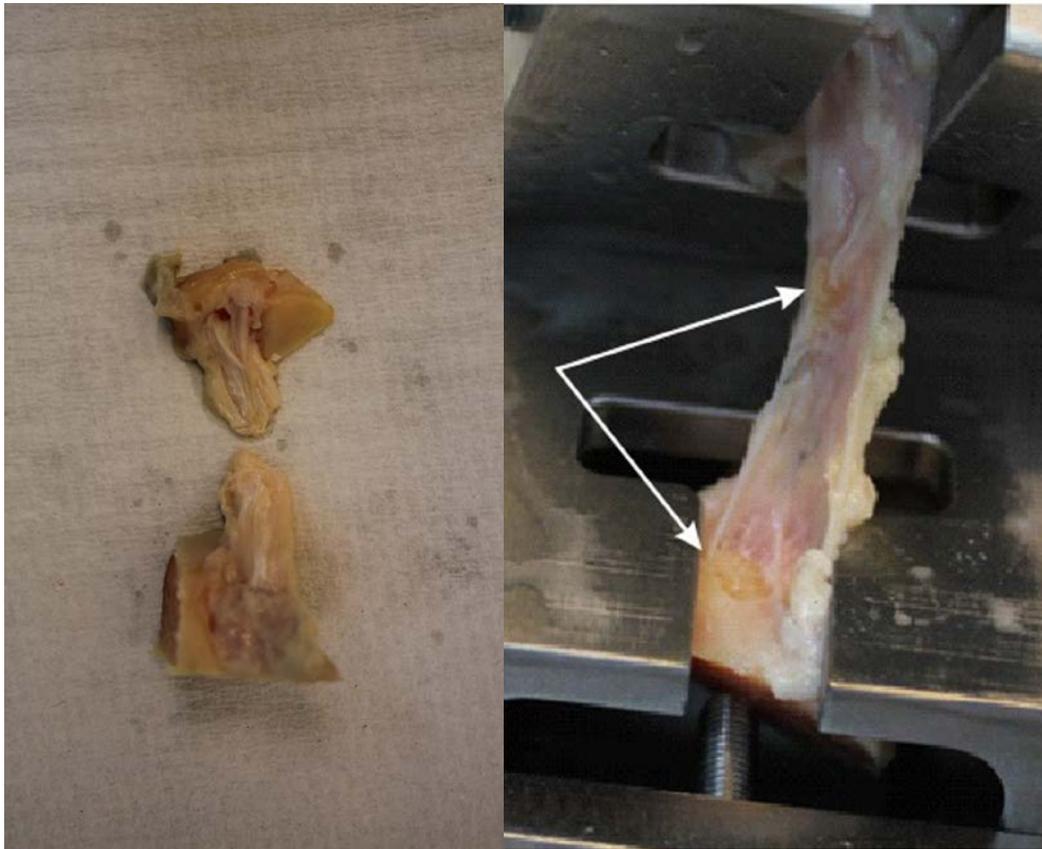
216

217   **Figure 5. A)** A mid-substance failure where intra-ligamentous failure has occurred. **B)** An avulsion

218   failure where a fragment of bone has also been avulsed from the bone surface (white arrows).

219   The correlation results for the ultimate failure load and stiffness to the patient-

220   specific factors: BMI, weight and age are presented in Table 4. The ultimate failure

221   load of the CFL was found to have a significant strong positive Pearson correlation

222   with BMI ($r = .92$; $p = .01$). The ultimate failure load of the ATFL and PTFL had non-

223   significant Pearson correlation scores ($r = .18$; $p = .73$ and $r = .65$; $p = .16$,

224   respectively). A non-significant relationship was found for both age and weight with

225   relation to both the ultimate failure load and stiffness of the ATFL, CFL and PTFL. Any

226   relationship identified between BMI and stiffness of the ATFL ($r = -.05$; $p = .92$), CFL (r

227    $= .22$; $p = .68$) and PTFL ($r = -.01$; $p = .98$) was also negligible.

228   **Table 4.** The correlation (r-value) and respective significance (p-value) of both ligament ultimate

229   failure load and ligament stiffness against the patient-specific factors (PSF): BMI, weight and age.

Ligament Property		PSF	r-value	p-value
Failure Load	**ATFL**	**BMI**	.184	.727
	CFL	**BMI**	.919*	.010
	PTFL	**BMI**	.650	.162
	ATFL	**Weight**	.516	.395
	CFL	**Weight**	.874	.023
	PTFL	**Weight**	.327	.527
	ATFL	**Age**	.560	.248
	CFL	**Age**	-.273	.600
	PTFL	**Age**	-.496	.317
Stiffness (k1)	**ATFL**	**BMI**	-.052	.922
	CFL	**BMI**	.216	.681
	PTFL	**BMI**	-.013	.981
	ATFL	**Weight**	.176	.738
	CFL	**Weight**	.410	.419
	PTFL	**Weight**	.000	.999
	ATFL	**Age**	.750	.086
	CFL	**Age**	-.397	.436
	PTFL	**Age**	-.340	.510

230   *indicates result is significant at the .01 level (two-tailed).

231   The ultimate failure load results of the ATFL, CFL and PTFL are plotted against BMI in

232   Figure 6 with the results for all three ligaments of each donor aligned vertically

233   according to the donor's BMI. There is no evidence of a systematic tendency for the

234   ultimate failure load to vary by ligament type either within or between donors.
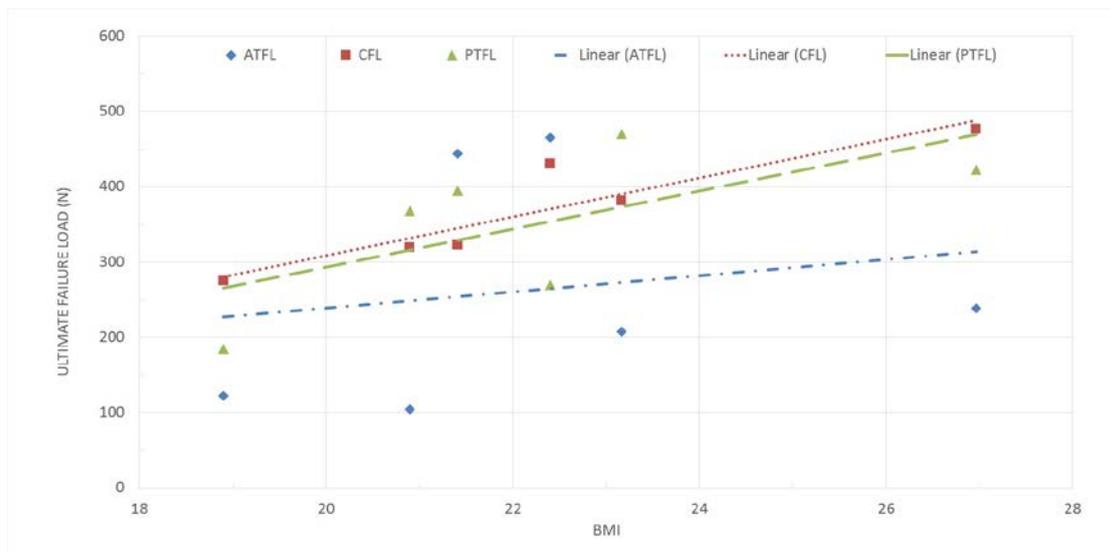
235

236   **Figure 6.** A graphical representation of the relationship between BMI and ultimate failure load. The

237   three ligaments of each donor are vertically aligned according to the BMI of the donor. The ATFL is

238   shown by blue diamond markers, the CFL by red square markers and the PTFL by green triangle

239 markers. Trend lines are shown for the ATFL (blue dash dot), CFL (red dotted) and PTFL (green

240 dashed).

Discussion

242 The aim of this study was to improve understanding of the mechanical properties

243 and failure modes of the LCL complex when strained at a rate representative of ankle

244 sprain events in real-life. The mechanical characteristics of the entire LCL complex

245 when loaded at a realistic sprain-like strain rate (100 %.s^{-1}) are reported. The mean

246 ultimate failure load results concur with previously published work, that the CFL and

247 PTFL provide similar levels of support under load and that the ATFL is the

248 weakest.[3,16] There is however, a large amount variability between specimens, as

249 shown in Figure 6, and there was no clear pattern for which ligament is the strongest

250 or weakest at an individual donor level. Whilst St Pierre et al. (1983) only reports

251 tensile strength of the ATFL they do so, in most cases, for each foot of each

252 individual donor highlighting the substantial variability in ATFL failure load, ranging

253 from 44 N to 556 N. Notably, the ATFL, which is widely established as the weakest

254 LCL, was the strongest for two donors in this study, contradicting the general

255 consensus.[3,16] The widely established view that the ATFL is the weakest of the LCLs

256 could therefore be incorrect for some people. The cause of this finding is likely

257 multifactorial and a much larger sample size and in-depth patient information is

258 required to substantiate any hypothesis.

259 Stiffness results in this study are similar to those previously reported by Attarian et

260 al. (1985) who strained the LCLs at strain rates considerably higher than 100 %.s^{-1}.

261 This paper therefore supports the theory that the strain-rate dependency of

262 ligaments can be neglected when tested at strain rates greater than 100 %.s^{-1}.[6,9] The

263 current findings indicate a range of indicative ultimate failure loading requirements

264 that can further inform the mechanical property specifications for synthetic ankle

265 ligaments. Through improved matching of the mechanical properties, particularly the

266 stiffness, of synthetic ligaments to their natural counterparts joint mobility and

267 stability have the potential to also improve.

268 Both mid-substance failure and avulsion are abundantly prevalent as failure modes

269 of the LCLs. Categorisation of the failure mode is somewhat subjective due to the

270 fibrous nature of ligamentous failure, the difficulty faced differentiating between

271 failure modes and the lack of a standardised definition of avulsion. The location of

272 ligament avulsion was consistent, at the fibula for the ATFL and CFL and at the talus

273 for the PTFL. Siegler et al. (1988) found the AFTL to avulse 58 % of the time and the

274 CFL and PTFL to avulse in 70 % of tests, with remaining specimen failing mid-

275 substance.[16] Attarian et al. (1985) reported eight mid-substance failures and four

276 talar avulsions for the ATFL, eight mid-substance failures, four calcaneal avulsions

277 and four fibula avulsions for the CFL and four mid-substance failures for the PTFL.[3] St

278 Pierre et al. (1983) reported 18 talar avulsions, 16 mid-substance failures and two

279 unknown failures.[17]

280 The location of ATFL avulsion in this study is inconsistent with those previously

281 reported and an explanation as to why is unclear. Possible explanations include the

282 status of the fibula, the orientation of the ligament or the vastly different strain

283 rates. The fibula was intact for testing in the studies by St Pierre et al. (1983) and

284 Attarian et al. (1985) whereas in this study the fibula was split reducing the amount

285 of bone to be gripped. The orientation of the specimen may differ slightly between

286 this study and the two studies highlighted due to the fibula not being intact,

287 although all studies attempted tensile testing with fibre alignment. The prevalence

288 of avulsion and mid-substance failures are however comparable. The high

289 prevalence of avulsions could be due to the significantly higher local strain proximal

290 to the attachment site of ligaments compared to the central region.[18] The failure

291   mechanism of a ligament is an important consideration prior to a ligament repair

292   being performed as the fixation method may differ depending on whether the

293   ligament needs reattaching to bone or to ligament.

294   A potentially noteworthy finding was the positive correlation between BMI and

295   ultimate failure load values for the ligaments of the LCL complex, specifically the CFL.

296   This finding, from a sample size of six, suggests that the CFL of individuals with a

297   higher BMI have a greater load bearing capacity than those with a lower BMI. This is

298   most likely due to the adaptive remodelling nature of ligamentous structures, as

299   individuals with a greater BMI are likely to apply more stress to the ligament,

300   increasing strength over time.[5] The BMI of an individual could therefore be an

301   important factor when selecting the appropriate material properties of a synthetic

302   intervention, and notably people with a high BMI who are more often candidates for

303   a synthetic ligament replacement.[1] Therefore, the load bearing capacity of the

304   synthetic, and their fixation devices, should match the mean ultimate failure load to

305   ensure the synthetic does not subsequently fail. The stiffness of the synthetic

306   material, along with the tension applied upon insertion, is arguably more important.

307   A stiffness that is too high could reduce the joint mobility and too low could affect

308   the stability of the joint. Therefore it could be recommended that the stiffness of the

309   synthetic material is also matched to that of the natural tissue results reported.

310   The anatomy of ligaments is often depicted incorrectly in illustrations because of

311   stylistic licence. The previously published pictorial essay does however provide

312   detailed images of the ankle ligament anatomy.[10] Figure 3 shows the attachment

313   points of the ATFL and CFL to the fibula. These attachments are often illustrated as

314   separate insertion points however as shown in Figure 3, the two ligaments

315   commonly attach at the same insertion point on the fibula. It is suggested that the

316   inferior aspect of the ATFL and CFL are connected by arciform fibres,[15] thus forming

317 the lateral fibulotalocalcaneal complex.[12] This observation was also made when

318 performing the dissections for this study. The results of this study however suggest

319 that the connecting fibres are not of a sufficient strength to cause both the ATFL and

320 CFL to rupture simultaneously. The CFL was tested first in every instance and the

321 results of the ATFL are still similar to those previously published, where they were

322 tested without the arciform fibres present.[16]

323 The limitations to the study predominantly centre on the use of human cadaveric

324 tissue. The main limitation is the small sample size (n = 6). Research using donor

325 cadaveric tissue should be minimised to only what is essential and performed with

326 maximum efficiency and integrity out of respect for the donors. The characterisation

327 of cadaveric human tissue may not reflect the same response as living tissue.

328 However, ligamentous tissue primarily attributes its strength properties to the

329 collagen fibres which form the majority of ligament structure. The collagen would

330 not be greatly affected by the tissue being living or dead, providing it remains well

331 hydrated and is stored appropriately to abate tissue degradation. Although the

332 exclusion criteria required donors to have not reported any lower limb trauma we

333 cannot be certain that a prior sprain had not occurred at some point during the

334 donor's lifespan. It is estimated that ankle injury rates are approximately five and a

335 half times higher than those registered in emergency departments.[13] This could

336 provide some explanation for the inconsistencies in strength between ligament types

337 (Figure 6). Large variations in the results following the mechanical characterisation of

338 ankle ligaments are also reported elsewhere.[16] The use of elderly donor tissue to

339 investigate sprain has previously been suggested to be a limitation of cadaver

340 studies. An effort was therefore made when selecting donor specimens to obtain the

341 youngest specimens possible (mean 56.2 years). A previous study however, reported

342 no correlation between ultimate failure load and age for donors aged 17 to 54 when

343 testing human anterior cruciate ligaments.[4] The link identified between BMI and

344 ultimate failure load of the CFL and PTFL is based on a narrow range of BMI with only

345 one donor having a BMI outside of the normal range and the trend may not be

346 reflected in a population at the extremities of the BMI scale.

Conclusion

348 Limitations aside, the conditions of this study were carefully defined to reflect those

349 experienced by individuals who would suffer an ankle sprain allowing for the entire

350 LCL complex to be characterised at realistic sprain inducing strain rates. In the

351 current study the ultimate failure load and stiffness of the ATFL, CFL and PTFL did not

352 differ systematically but there was a tendency toward greater strength in people

353 with a higher BMI. The maximum likely exposure loads, the BMI of the patient and

354 the failure mode of the LCLs all appear to be factors to be further considered when

355 selecting the material, repair or reconstruction technique to be used for surgical

356 stabilisation of the sprained ankle.

Acknowledgements

References

1. Ajis A, Younger ASE, Maffulli N. Anatomic Repair for Chronic Lateral Ankle Instability. *Foot Ankle Clin*. 2006;11(3):539-545. doi:10.1016/j.fcl.2006.07.005

2. de Asla RJ, Kozanek M, Wan L, Rubash HE, Li G. Function of anterior talofibular and calcaneofibular ligaments during in-vivo motion of the ankle joint complex. *J Orthop Surg Res*. 2009;4(1):7. doi:10.1186/1749-799X-4-7

3. Attarian DE, McCrackin HJ, DeVito DP, McElhaney JH, Garrett WE. Biomechanical characteristics of human ankle ligaments. *Foot Ankle*. 1985;6(2):54-58. doi:10.1177/107110078500600202

4. Blevins FT, Hecker AT, Bigler GT, Boland AL, Hayes WC. The Effects of Donor Age and Strain Rate on the Biomechanical Properties of Bone-Patellar Tendon-Bone Allografts. *Am J Sports Med*. 1994;22(3):328-333. doi:10.1177/036354659402200306

5. Bonnel F, Toullec E, Mabit C, Tourné Y. Chronic ankle instability: Biomechanics and pathomechanics of ligaments injury and associated lesions. *Orthop Traumatol Surg Res*. 2010;96(4):424-432. doi:10.1016/j.otsr.2010.04.003

6. Bonner TJ, Newell N, Karunaratne A, et al. Strain-rate sensitivity of the lateral collateral ligament of the knee. *J Mech Behav Biomed Mater*. 2015;41:261-270. doi:10.1016/j.jmbbm.2014.07.004

7. Colville MR, Marder RA, Boyle JJ, Zarins B. Strain measurement in lateral ankle ligaments. *Am J Sports Med*. 1990;18(2):196-200. doi:10.1177/036354659001800214

8. Fong DT-P, Hong Y, Shima Y, Krosshaug T, Yung PS-H, Chan K-M. Biomechanics of supination ankle sprain: a case report of an accidental injury event in the laboratory. *Am J Sports Med*. 2009;37(4):822-827. doi:10.1177/0363546508328102

9. Funk JR, Hall GW, Crandall JR, Pilkey WD. Linear and Quasi-Linear Viscoelastic Characterization of Ankle Ligaments. *J Biomech Eng*. 2000;122(1):15. doi:10.1115/1.429623

10. Golanó P, Vega J, de Leeuw PAJ, et al. Anatomy of the ankle ligaments: a pictorial essay. *Knee Surgery, Sport Traumatol Arthrosc*. 2016;24(4):944-956. doi:10.1007/s00167-016-4059-4

11. Herbert A, Brown C, Rooney P, Kearney J, Ingham E, Fisher J. Bi-linear mechanical property determination of acellular human patellar tendon grafts for use in anterior cruciate ligament replacement. *J Biomech*. 2016;49(9):1607-1612. doi:10.1016/j.jbiomech.2016.03.041

12. Hertel J. Functional anatomy, pathomechanics, and pathophysiology of lateral ankle instability. *J Athl Train*. 2002;37(4):364-375. doi:10.1017/CBO9781107415324.004

13. Kemler E, van de Port I, Valkenberg H, Hoes AW, Backx FJG. Ankle injuries in the Netherlands: Trends over 10-25 years. *Scand J Med Sci Sports*. 2015;25(3):331-337. doi:10.1111/sms.12248

14. Quinn KP, Winkelstein BA. Preconditioning is correlated with altered collagen fiber alignment in ligament. *J Biomech Eng*. 2011;133(6):575-579. doi:10.1115/1.4004205

15. Sarrafian SK. Anatomy of the Foot and Ankle. *JB Lippincott Co*. 1983.

16. Siegler S, Schneck CD. The Mechanical Characteristics of the Collateral Ligaments of the Human Ankle Joint. *Foot Ankle Int*. 1988;8(5):234-242. doi:10.1177/107110078800800502

17. St Pierre RK, Rosen J, Whitesides TE, Szczukowski M, Fleming LL, Hutton WC. The tensile strength of the anterior talofibular ligament. *Foot Ankle*. 1983;4(2):83-85.

406   doi:10.1177/107110078300400208

407   18.   Stouffer DC, Butler DL, Hosny D. The relationship between crimp pattern and
408   mechanical response of human patellar tendon-bone units. *J Biomech Eng*.
409   1985;107(2):158-165. doi:10.1115/1.3138536

410