Title: An equine tendon model for studying intra-tendinous shear in tendons that have more than one muscle contribution

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Abstract:

Human Achilles tendon is composed of three smaller sub-tendons and exhibits non-uniform internal displacements, which decline with age and after injury, suggesting a potential role in the development of tendinopathies. Studying internal sliding behaviour is therefore important but difficult in human Achilles tendon. Here we propose the equine deep digital flexor tendon (DDFT) and its accessory ligament (AL) as a model to understand the sliding mechanism.

The AL-DDFT has a comparable sub-bundle structure, is subjected to high and frequent asymmetric loads and is a natural site of injury similar to human Achilles tendons. Equine AL-DDFT were collected and underwent whole tendon level (n=7) and fascicle level (n=7) quasi-static mechanical testing. Whole tendon level testing was performed by sequentially loading through the proximal AL and subsequently through the proximal DDFT and recording regional strain in the free structures and joined DDFT and AL. Fascicle level testing was performed with focus on the inter-sub-bundle matrix between the two structures at the junction. Our results demonstrate a significant difference in the regional strain between the joined DDFT and AL and a greater transmission of force from the AL to the DDFT than vice versa. These results can be partially explained by the mechanical properties and geometry of the two structures and by differences in the properties of the interfascicular matrices. In

27 conclusion, this tendon model successfully demonstrates that high displacement discrepancy
28 occurs between the two structures and can be used as an easy-access model for study intra-
29 tendinous shear mechanics at the sub-tendon level.

30 **Keywords (4-5):** Achilles tendon, Equine AL-DDFT, sub-tendon, biomechanics,
31 interfascicular matrix

32 **Abbreviations:**

33 AL: the accessory ligament (of the deep digital flexor tendon)

34 CSA: cross-sectional area

35 DDFT: deep digital flexor tendon

36 IFM: interfascicular matrix

37 SDFT: superficial digital flexor tendon

38

39 **1. Introduction**

40 Achilles tendon injury is prevalent in both athletes and the general population [1], but our
41 limited understanding of the pathogenesis has hampered the development of successful
42 prevention and interventional strategies [2, 3]. Injuries are likely initiated from a combination
43 of several factors including the Achilles anatomy [2, 4, 5], the integrity of the extracellular
44 matrix [6, 7] and activity-related external mechanical demands [1, 3]. The susceptibility
45 however relates to the intrinsic Achilles anatomy and the complex force interaction within the
46 tendon bundles [4, 5, 8]. The Achilles tendon has a unique tri-bundled structure formed by
47 two longer and thinner gastrocnemii tendons and a shorter and thicker soleus tendon.
48 Although the different contributing tendons on visual inspection appear to merge into one
49 homogeneous tendon, in fact the tendon fascicles originating from different muscle bellies do
50 not merge or intertwine within the Achilles tendon until reaching the distal calcaneal

51 tuberosity [9-12]. These fascicle groups or ‘bundles’, termed Achilles sub-tendons [13], are
 52 believed to facilitate movement efficiency by allowing a certain degree of individual control
 53 from contracting muscles to meet the demands from locomotive activities [14-17]. The
 54 gastrocnemii sub-tendons occupy the posterior-lateral-anterior region of the Achilles tendon,
 55 surrounding the soleus sub-tendon which occupies the medial-posterior region [11, 18]. The
 56 gastrocnemii and soleus muscles are activated differently during locomotion [19, 20].
 57 Therefore, the observed mechanical behaviour of the whole Achilles tendon is likely the
 58 result of a combination of factors including the morphology and the mechanical properties of
 59 sub-tendons, various force level from different muscle bellies, and the interface properties
 60 between sub-tendons [8].

61
 62 Individual control of sub-tendons creates non-uniform displacements within the Achilles
 63 tendon, and this non-uniformity has been observed in both passive [21-23] and dynamic [16,
 64 24] movements. Furthermore, this displacement non-uniformity between the sub-tendons
 65 shows an age-related and disease-related decline [17, 25, 26]. At the fascicle level, studies on
 66 the equine superficial digital flexor tendon (SDFT), an extreme example of an energy-storing
 67 tendon, have demonstrated that the high failure strain of this tendon is achieved by having a
 68 compliant interfascicular matrix (IFM), which allows sliding of fascicles [27, 28]. This sliding
 69 capacity decreases with age due to the stiffening of the IFM [29], presumably imposing
 70 premature strains on fascicles and increasing risk of injury. The human Achilles tendon, the
 71 most important energy-storing tendon in human locomotion, exhibits a similar fascicle sliding
 72 mechanism and compliant IFM [30-32] but whether a significant transmission of force occurs
 73 between fascicles remains inconclusive. The matrix between sub-tendons, termed inter-sub-
 74 tendon matrix [33], has even more complex requirements than the IFM within a tendon as it is
 75 subjected to high asymmetric loads due to the aforementioned individual control from
 76 different muscles. The inter-sub-tendon matrix is therefore likely to demonstrate a more
 77 extreme specialisation, which may be structural or compositional, than the presumably more

78 homogeneously loaded IFMs within each sub-tendon [33]. The detailed fascicle level
79 mechanical behaviour between human Achilles sub-tendons is difficult to study both *in vivo*
80 and *in vitro* due to the highly complex, individually variable rotatory morphology of the
81 tendon and sub-tendon [12, 18] in addition to the difficulties of obtaining sufficient healthy
82 specimens to study.

83

84 Here we have used an equine tendon model for studying the mechanism of intra-tendinous
85 shear between sub-structures – the deep digital flexor tendon (DDFT) and its accessory
86 ligament (AL). The horse is a commonly used and widely accepted animal model for studying
87 human tendon, especially the equine SDFT to represent human Achilles tendon as they have
88 comparable energy-storing functions. However, the SDFT does not possess sub-tendon level
89 structures distal to the metacarpo-phalangeal joint [34] and therefore lacks the ability to
90 provide understanding of shear mechanics between sub-tendons. The equine DDFT and AL
91 have a separate origin and proximal portion but combine to form one, seemingly
92 homogeneous, structure in the more distal part. The DDFT lies just beneath (deep to) the
93 extensively studied SDFT and provides more of a support and positioning role during
94 locomotion compared to the SDFT [35]. The loading pattern differs between the two branches
95 of this structure. The DDFT originates from the deep digital flexor muscle and its loading
96 directly associates with muscular actions; the AL originates from the common palmar
97 ligament of the carpus, so mechanical behaviour is largely dictated by the metacarpo-
98 phalangeal joint angle [35]. Along the descent, the AL gradually flattens and wraps around
99 the DDFT at the mid-metacarpus level. A considerable degree of sliding is required at the
100 junction due to the different mechanical demands and different strain patterns during gait
101 cycles between structures [35]. Distally, the AL completely blends with the DDFT, forming
102 one elliptical adjoined tendon before passing through the metacarpo-phalangeal joint (Fig. 1).
103 Given this bifurcated proximal geometry and the different functional roles, the regional
104 mechanical properties are likely different between the two proximal ends and joined regions.

105 It has been demonstrated that the proximal DDFT is a stiffer structure than the proximal AL
 106 [35, 36], but no previous study has investigated the mechanical properties at the junction,
 107 where the two structures join. The junction is subjected to intermittent high asymmetrical
 108 loads transmitted from either the AL or DDFT, resulting in complex force interactions [35].
 109 The shear-related displacement of the less-strained contralateral side will be influenced by the
 110 properties of the inter-sub-structure matrix governing the force transmission and the relative
 111 sliding behaviour between the two structures [8, 33, 37]. Similarities therefore exist between
 112 the human Achilles tendon and the equine DDFT. Both tendons have a sub-tendon/ligament
 113 level structure; the difference between tendon and ligament is anatomical rather than a
 114 structural or functional feature. Both tendons function to receive and transmit force from
 115 different sub-structures and exhibit non-uniform displacements. The requirements of the
 116 matrix between Achilles sub-tendons and the matrix between the DDFT and AL are therefore
 117 very similar. We propose that studying the AL-DDFT mechanics will improve our knowledge
 118 of the sub-tendon level mechanical behaviour within the Achilles tendon.

119

120 This study aims to explore the mechanical response at the junction between the AL and
 121 DDFT and along the length of the tendon when subjected to asymmetrical loads at both whole
 122 tendon and fascicle levels. We hypothesise that the branched nature of this tendinous structure
 123 creates high displacement discrepancy at the junction during loading, which is transmitted to
 124 the more distal parts of the structure.

125

126 **2. Methods**

127 *2.1 Sample preparation*

128 In total 17 pairs of equine forelimbs (aged from 3 to 24 years) were collected at a commercial
 129 abattoir where horses are euthanised for reasons other than research. The Animal (Scientific
 130 Procedures) Act 1986, Schedule 2, does not define collection from these sources as scientific

131 procedures. The DDFT and AL between the carpus and metacarpo-phalangeal joint of each
132 forelimb were harvested within 24 hours and kept frozen at -80°C until further analysis. No
133 sign of tendon pathology was noticed during the harvest. We conveniently chose all the left
134 limbs for biomechanical tests, assuming no significant limb differences between left and right
135 sides.

136

137 2.2 Whole tendon biomechanical tests

138 On the test day, specimens (n=10) were thawed at room temperature before measuring the
139 CSA of different regions with the previously described method [38]. The CSA of free-AL and
140 free-DDFT were measured at a point 30 mm proximal to the junction between the AL and the
141 DDFT. The CSA of the joined region was measured at a point 30 mm distal to the junction
142 (Fig. 1).

143

144 Non-destructive quasi-static tests were conducted with sequential loading (AL then DDFT) of
145 two proximal free ends. Specimens were vertically mounted, distal part at the bottom and
146 proximal parts at the top, secured by cryoclamps in a screw-driven mechanical testing device
147 (Instron 5967, Instron, MA, US) with a 30 kN load cell. We first mounted the free-AL while
148 keeping the free-DDFT relaxed. After securing the specimen in the device, four markers
149 approximately 25 mm apart were placed directly on the surface along the midline at the
150 regions where the CSAs were measured on the free-AL and free-DDFT and both sides of the
151 joined AL and DDFT (Fig. 1, *black dots, schematic representation*). A preload of 100 N was
152 applied for 1 min, and the effective gauge length was measured between the two freeze lines.
153 The specimen was then preconditioned by applying a 5% strain for 20 cycles using a
154 triangular wave at 1 Hz frequency. After the last cycle, the specimen was returned to the slack
155 position before pulled to 10% strain at a speed of 12 mm/sec. We chose a high-speed loading
156 scenario to represent the *in vivo* physiological loading condition and the influence of

viscoelastic behaviour [39] on the mechanical response of the tissue. The free-AL of the specimen was dismounted, and the free-DDFT of the same specimen was then mounted and tested under an identical protocol. We chose to test the AL first as our pilot data showed that the AL is a more compliant structure and therefore the target strain of 10% is reached at a lower load with less chance of damage. Tested specimens were visually inspected after loading through the AL and showed no sign of structural disruption. Subsequently the force-displacement relationship was checked in the data files to ensure no reduction in gradient providing further evidence that the structure had not been damaged.

165

2.3 Calculation

Global force and displacement data were recorded by the mechanical testing machine at 100 Hz. During the test, marker movements were captured at 30 Hz by two video cameras, one facing each side of the specimen. The pixel movements of the four markers (~25 mm apart) were captured in each video frame and the inter-marker distances were calculated. The regional displacement was then calculated by subtracting the initial inter-marker distances. This regional displacement was then synchronised, using external auditory cues generated by the mechanical testing machine, with force data to plot the regional force-displacement relationship and the linear region was used for calculating stiffness. Regional stress (force / regional CSA) and regional strain (difference in the regional displacement / initial length) were then further calculated and the linear region of the stress-strain relationship was used for calculating Young's modulus of the free-AL and free-DDFT. An in-house MATLAB (R2020, MathWorks, MA) code was used for video processing, regional displacement measurement, offline synchronisation, and calculations.

180

2.4 Fascicle level mechanical testing

A further seven tendons were used for asymmetric IFM and inter-sub-bundle matrix testing.

183 The peritendinous tissues were carefully removed and the junction of AL and DDFT was
 184 visually identified (Fig. 1, *yellow triangles*). For the inter-sub-bundle matrix isolation, a
 185 fascicle pair (one fascicle from the AL and one from the DDFT) was visually tracked and
 186 separated from proximal (~5 mm above the junction) to distal, retaining the inter-sub-bundle
 187 matrix that binds the AL and DDFT together (Fig. 1, *bottom left*). For the IFM of AL and
 188 DDFT, the fascicle pairs were selected at the same junction region of the inter-sub-bundle
 189 separation, but with sufficient distance from the inter-sub-bundle matrix (e.g. Fig. 1, *bottom*
 190 *right black arrows*). Fascicle pairs were approximately 30 mm in length and each fascicle was
 191 cut leaving a 10 mm interfascicular or inter-sub-bundle matrix for testing [27] (Fig. 2). We
 192 could only separate the inter-sub-bundle matrix in the periphery due to the difficulty in
 193 tracking fascicle origins at the core. However, previous studies on other equine tendons found
 194 no significant difference in mechanical properties between fascicles isolated from the core or
 195 the periphery [27].

196

197 The intact ends of the fascicles were mounted in a mechanical testing machine (Instron E1000)
 198 with a 250 N dynamic load cell (to an accuracy of 0.5% of 1 N and measurement uncertainty
 199 less than one-third of the permissible error. 2527-131 Dynacell, Instron.) and custom-made
 200 jagged grips. Grip to grip distance was 20 mm (Fig. 2). A preload (0.02 N, as displayed on the
 201 mechanical testing machine) was applied and the specimen was preconditioned with 10 cycles
 202 of sine waves between 0 to 0.5 mm. The structure was then allowed to slacken before being
 203 pulled to failure at 1 mm/s [28]. Force and displacement were recorded at 100 Hz during the
 204 test. To normalise data for comparison, the force-displacement relationship was displayed by
 205 plotting each 10% of maximal displacement against force. The stiffness was calculated as the
 206 slope of the linear region of the force-displacement relationship of each specimen.
 207 Additionally, single fascicles (~30 mm in length) from AL and DDFT (n=7) were dissected
 208 and mechanically tested using an identical testing protocol as the IFM, but with load applied
 209 in tension instead of shear (Fig. 2).

210

211 *2.5 Statistical analysis*

212 Statistical analysis was performed using SPSS (v. 26, IBM, NY, US) with two-tailed
213 significant level set at 0.05. A one-way ANOVA with Bonferroni post-hoc adjustment was
214 performed to compare the CSA of three regions. Paired t-tests were performed to compare
215 means of mechanical testing results between free-AL and free-DDFT, between joined-AL and
216 joined-DDFT, and between AL and DDFT fascicles. Strain and stiffness were also compared
217 between free and joined region within the AL or the DDFT. One-way ANOVAs with
218 Bonferroni post-hoc adjustment were conducted for comparing tendon level displacements
219 when loaded in isolation (e.g. free-DDFT, joined-DDFT, and joined-AL when DDFT was
220 loaded) and also interfascicular mechanical properties (AL IFM, DDFT IFM, and inter-sub-
221 bundle matrix).

222

223 **3. Results**

224 *3.1 Tendon level mechanical properties*

225 The CSA (n=10) of the joined region ($159 \pm 30 \text{ mm}^2$) was significantly larger than the free
226 ends of both AL ($96 \pm 32 \text{ mm}^2$) and DDFT ($89 \pm 28 \text{ mm}^2$) but smaller than the combined CSAs.
227 The mechanical testing results of the tendon level (n=7) and fascicle level (n=7) are
228 summarised in Tables 1-3. The mechanical testing data from 3 limbs were discarded due to
229 poor image quality or clamp slippage during the whole tendon testing.

230

231 When the AL was loaded, the machine recorded lower maximal force (global) compared to
232 the DDFT loading condition. The regional displacement and strain (measured by cameras)
233 showed significant regional differences between free-AL, joined-AL, and joined DDFT,
234 which receives load only through shear. The stiffness of the free-AL was significantly lower

235 than the joined-AL when the AL was directly loaded (Table 1).

236

237 When the DDFT was loaded in isolation, the displacement, strain, and stiffness were similar
238 between the free- and joined-DDFT, contrary to the AL loading condition (Table 2).

239 Comparing the two loading conditions revealed that the free-DDFT was a stiffer structure
240 than the free-AL and had a higher material stiffness (Fig. 3). Interestingly, when comparing
241 the displacement of the contralateral joined regions, the force transmittance pattern was
242 different between AL and DDFT loading conditions. When the DDFT was directly loaded,
243 the joined-AL remained stationary and started to elongate only after a substantial force was
244 imposed on the DDFT (Fig. 4, *right*). When the AL was directly loaded, the joined-DDFT
245 displaced proportionally to the joined-AL strain (Fig. 4, *left*). Maximal displacement
246 discrepancy at the junction was approximately 3.0 mm (DDFT loaded, 3920 N) and 1.7 mm
247 (AL loaded, 4192 N).

248

249 *3.2 Fascicle level mechanical properties*

250 AL and DDFT fascicles exhibited similar failure force and maximal displacement (Table 3)
251 and their mechanical properties were not significantly different. The averaged AL IFM
252 appeared to have a slightly higher failure force and stiffness than the DDFT IFM and the
253 inter-sub-bundle matrix, but the differences were not statistically significant (Table 3). The
254 relationship between load and displacement however differs significantly; at 20% and 30% of
255 normalised displacement levels, AL IFM exhibited significantly higher force than the inter-
256 sub-bundle matrix (Fig. 5). At higher normalised displacement levels differences were not
257 significant.

258

259 **4. Discussion**

260 Our results demonstrate that this equine tendon model exhibits large displacement
261 discrepancy at the junction when subjected to ~~asymmetry~~ loads from different branches as
262 expected. Interestingly, the results confirm our hypothesis and demonstrate that this
263 discrepancy is maintained in the more distal parts of the structure where the AL and DDFT
264 appear to be tightly bound. Since similar structural and functional features exist between the
265 human Achilles tendon and this equine tendon model, exploiting this new tendon model may
266 help us understand the mechanisms behind the complex mechanical behaviour at the sub-
267 tendon level [30].

268

269 The mechanical properties obtained from the free-AL and free-DDFT are comparable with
270 previous studies on isolated AL [40] and DDFT [35] and, similar to previous reports,
271 demonstrated high individual variability at the tendon level [29, 40] and fascicle level [28, 29],
272 reflecting the nature variation between animals. The higher material stiffness of the DDFT
273 fulfils its mechanical role of positioning distal joints in flexion during galloping, a function
274 similar to the equine digital extensor tendons and the human tibialis anterior tendon. The
275 differences in stiffness between the free-DDFT and the free-AL was not reflected at the
276 fascicle level, at least at the joined region. Future research is warranted to elucidate the origin
277 of the difference in mechanical properties of the AL and DDFT, such as the inter-structure
278 matrix composition or the ratio between IFM and fascicles. Interestingly, in our study the
279 palmar and dorsal aspects of the joined AL and DDFT structure behaved differently compared
280 to the proximal free regions. The regional strain of the joined-AL was significantly lower than
281 the free-AL, suggesting a large proportion of force transmitted through shear-loaded alone to
282 the contralateral joined-DDFT, the regional strain of which was lower but closely related to
283 the joined-AL. On the contrary, the regional strain of free- and joined-DDFT were almost
284 identical when loaded in isolation, suggesting a more homogeneous structure throughout the
285 DDFT and a lower force transmitting capacity at the junction compared to force transmission

286 from the AL to the DDFT. The adjacent joined-AL displaced surprisingly little even when the
287 DDFT was subjected to a substantial level of force (~4000 N).

288

289 This force transmission discrepancy at the junction between different loading conditions can
290 be partially explained by the mechanical properties of DDFT and AL. To a first
291 approximation they can be considered as springs in parallel. However, in a classical parallel
292 spring system, the displacements of joined-DDFT and joined-AL would be the same. Another
293 explanation of this unique behaviour at the junction is the different geometry between the AL
294 and DDFT. The AL gradually flattens and wraps around the DDFT when descending, forming
295 a C-shape structure surrounding the O-shape DDFT at the junction. (Fig. 1, *upper row insets*).

296 Due to Poisson's effect, the C-shape AL when loaded could exert compressive force on the
297 DDFT, transmitting force through the inter-sub-bundle matrix resulting in displacement of the
298 joined-DDFT. When load is applied through the DDFT however, the decreasing CSA of the
299 O-shape DDFT when loaded may make it less likely to exert force on the joined-AL.

300 Although this theory alone does not explain why the joined-AL eventually displaced when
301 high loads were imposed on the DDFT. Understanding how the mechanical properties and
302 geometry of sub-components within one tendon structure affects mechanical behaviour will
303 be a breakthrough in understanding Achilles tendon mechanics since a similar mechanical
304 property differences and geometry feature (C-shape gastrocnemii surrounds the soleus sub-
305 tendon) exists in Achilles sub-tendons [8, 10-12]. A third explanation is that the orientation of
306 load bearing fibres in the inter-sub-bundle matrix is such that they come under tension much
307 later when loaded through the DDFT than when loaded through the AL resulting in a
308 difference in timing of force transmission between the two structures.

309

310 Fourthly, the relative mechanical properties of the interfascicular and inter-bundle matrix
311 could also contribute to this unique behaviour seen at the junction. Given the large differences

in the force-displacement relationship between the AL IFM and inter-sub-bundle matrix, a near-failure force for the inter-sub-bundle matrix could only result in approximately half of the displacement in the AL IFM. On the contrary, since the force-displacement relationships between DDFT IFM and inter-sub-bundle matrix were similar and both were more compliant and weaker than the AL IFM, a fully loaded AL could result in a substantial displacement of the DDFT, showing a more synchronised displacement between the shear-loaded joined-DDFT and the directly loaded joined-AL. However, these inter-fascicular differences in mechanical properties were only statistically significant at initial displacements (<30%). This may be due to the small sample size, mixed ages, and the inherent variation between individual animals. We were not able to measure the thickness or CSA of the inter-fascicular matrices or to maintain identical IFM quantity (volume) during our test. Although we did not find significant correlation between age and measured tendon and fascicle level mechanical properties for both AL and DDFT, ageing has been proposed to have a significant influence on the IFM tensile mechanical properties [27, 28] and fascicle and fibre level viscosity [41]. A greater adhesion of inter-sub-bundle matrix would drastically affect force transmission at the AL-DDFT junction. Future studies may benefit from obtaining a larger sample size with a wide age range to detect potential fascicle IFM and inter-sub-bundle matrix level differences. Our measured fascicle level mechanical properties were similar to those reported for the SDFT [27, 28], and these results also imply that interfascicular differences have a greater influence on whole structure mechanical properties than the fascicle differences. Additional research is warranted to explore whether fascicle sliding also plays an important role in the DDFT during loading as there are important differences in function and composition between the SDFT and DDFT [35, 42]. We could only confidently dissect out fascicles at the periphery due to the difficulty in tracing the fascicular origin at the core. Previous studies however suggest that the fascicle and IFM mechanical properties are similar between at the core and the periphery in other equine tendons [27]. We only reported the force-displacement relationship since we were unable to accurately measure the CSA of the tested fascicles. The

339 actual material strength of fascicles is supposedly greater than the IFM [31]. Future study
340 could also investigate the influence from the lower hierarchies (fibril or fibre level) on the
341 fascicle and sub-tendon level mechanical behaviours [39, 43-46].

342

343 In some specimens, an initial negative displacement of the joined-AL was observed when the
344 DDFT was loaded. This apparent negative displacement could result from an error from our
345 image-based measurement. The highest negative displacement measured under 4000 N was -
346 0.017 mm (maximal positive displacement: 0.022 mm). We believe this potential
347 measurement error did not significantly affect the reported outcome. Alternatively, the
348 negative displacement may result from a bending of the structure when load is initially
349 applied to the contralateral side. It is also worth noting that macroscale strain does not fully
350 represent the microscale strain [47]. Therefore, our measured external regional strain may not
351 fully represent the internal strain of the highly heterogenous tendon structure.

352

353 Our mechanical testing results can give an insight into why injury to the AL is more prevalent
354 than injury to the DDFT [48, 49]. During galloping, the AL is taut rapidly when the carpal
355 joint moves from flexion to extension while the DDFT remains taut continuously. Taken
356 together that the AL is less stiff than the DDFT and the free-AL is more compliant than the
357 joined-AL, the rapid and high-intensity loading imposed on the whole heterogeneous AL
358 structure may produce localised high strains proximal to the junction and high shear strain at
359 the junction. Indeed, injury to the AL proper (free-AL region) is more frequently reported
360 than injuries to the free-DDFT or below the junction level [48]. It is also worth noting that
361 adhesion between AL and DDFT is sometimes reported with AL desmitis [48]. Adhesion at
362 the junction may be a reaction to injury and may interrupt the unique mechanical response in
363 this tendon complex, but the exact consequence is unknown.

364

365 The human Achilles tendon is also likely to receive considerable shear forces and it has been
366 suggested that high shear between Achilles sub-tendons is a risk factor for developing
367 tendinopathy ([50-52] and review [53]). The soleus muscle-tendon unit constantly activates
368 during bipedal movements. It is composed of mainly type I fibres and has a high fatigue
369 resistance to allow continuous support of body weight during stance and walking.
370 Gastrocnemii have shorter muscle bellies and longer tendinous parts compared to the soleus
371 and are involved during explosive movements such as jumping or at the push-off phase
372 during late stance [19, 20]. Moreover, the length of gastrocnemii is affected by both knee and
373 ankle joint angle while the length of soleus, a one-joint muscle, is dictated only by the ankle
374 joint angle. These anatomic and functional differences of human triceps surae muscles can
375 create large displacement discrepancy within the Achilles tendon, with the highest potential
376 difference between the soleus and gastrocnemii sub-tendons. Younger Achilles tendons
377 exhibit more non-uniformity in sub-tendon displacements compared to aged tendons [15, 16];
378 therefore, shear strains at the interfaces should be greater in younger than aged Achilles
379 tendons. In support, one study suggested that longitudinal tears within the Achilles tendon are
380 an under-reported pathology prevalent in young, elite-level athletes [51]. Elite athletes are
381 also subjected to high training doses and participate in competitive sports, both of which are
382 likely to increase the chance of asymmetrical loadings by either rapidly changing knee and
383 ankle positions or developing training-induced fatigue to the gastrocnemii. Our proposed
384 equine tendon model can help researchers to systematically study the shear-related
385 mechanical responses at the interfaces between structures and understand how tendons adapt
386 their extracellular matrix composition and organisation to withstand this high internal
387 displacement discrepancy. Understanding these responses will facilitate the development of
388 novel prevention and treatment strategies targeting Achilles tendon injuries.

389

390 **5. Conclusion**

391 The AL-DDFT tendon complex shows distinct structural and regional mechanical properties
 392 and exhibits large displacement discrepancy at and below the junction when ~~subjecting to~~
 393 ~~asymmetrical~~ loaded through difference branches. This tendon model can be studied
 394 macroscopically using conventional mechanical testing devices at both whole tendon and
 395 fascicle level, providing tendon researchers an easy-access model to study intra-tendinous
 396 shear mechanics, such as the shear between human soleus and gastrocnemii sub-tendons. The
 397 relatively low injury rate of the DDFT compared to the SDFT, despite having high internal
 398 displacement discrepancy, may suggest a specialised adaptation strategy exists at the junction.
 399 Studying the mechanism behind these shear-related mechanical responses and the adaptation
 400 strategy can improve our understanding of human Achilles tendon which, in a manner similar
 401 to the AL-DDFT, is constantly subjected to ~~asymmetrical~~ loads from different origins and
 402 exhibits non-uniform displacements during activities.

403

404 **Acknowledgements**

405 N.Y. thanks the generosity of the Taiwanese government in providing a scholarship for his
 406 PhD studies.

407 **Disclosures**

408 The authors declare no conflict of interests.

409

410 **References**

- 411 [1] C. Hopkins, S.C. Fu, E. Chua, X. Hu, C. Rolf, V.M. Mattila, L. Qin, P.S. Yung, K.M.
 412 Chan, Critical review on the socio-economic impact of tendinopathy, Asia Pac J Sports Med
 413 Arthrosc Rehabil Technol 4 (2016) 9-20.
- 414 [2] B. Magnan, M. Bondi, S. Pierantoni, E. Samaila, The pathogenesis of Achilles
 415 tendinopathy: a systematic review, Foot Ankle Surg 20(3) (2014) 154-9.

- 416 [3] N.L. Millar, K.G. Silbernagel, K. Thorborg, P.D. Kirwan, L.M. Galatz, G.D. Abrams,
417 G.A.C. Murrell, I.B. McInnes, S.A. Rodeo, Tendinopathy, *Nat Rev Dis Primers* 7(1) (2021) 1.
- 418 [4] V.B. Shim, J.W. Fernandez, P.B. Gamage, C. Regnery, D.W. Smith, B.S. Gardiner, D.G.
419 Lloyd, T.F. Besier, Subject-specific finite element analysis to characterize the influence of
420 geometry and material properties in Achilles tendon rupture, *J Biomech* 47(15) (2014) 3598-
421 604.
- 422 [5] W. Hansen, V.B. Shim, S. Obst, D.G. Lloyd, R. Newsham-West, R.S. Barrett, Achilles
423 tendon stress is more sensitive to subject-specific geometry than subject-specific material
424 properties: A finite element analysis, *J Biomech* 56 (2017) 26-31.
- 425 [6] P.H.T. Tran, N.M. Malmgaard-Clausen, R.S. Pugaard, R.B. Svensson, J.D. Nybing, P.
426 Hansen, P. Schjerling, A.H. Zinglensen, C. Coupe, M. Boesen, S.P. Magnusson, M. Kjaer,
427 Early development of tendinopathy in humans: Sequence of pathological changes in structure
428 and tissue turnover signaling, *FASEB J* 34(1) (2020) 776-788.
- 429 [7] A. Del Buono, F. Oliva, L. Osti, N. Maffulli, Metalloproteases and tendinopathy, *Muscles*
430 *Ligaments Tendons J* 3(1) (2013) 51-7.
- 431 [8] N.H. Yin, P. Fromme, I. McCarthy, H.L. Birch, Individual variation in Achilles tendon
432 morphology and geometry changes susceptibility to injury, *elife* 10 (2021) e63204.
- 433 [9] F.G. Parsons, On the Morphology of the Tendo-Achillis, *J Anat Physiol* 28(Pt 4) (1894)
434 414-8.
- 435 [10] P. Szaro, G. Witkowski, R. Smigielski, P. Krajewski, B. Ciszek, Fascicles of the adult
436 human Achilles tendon - an anatomical study, *Ann Anat* 191(6) (2009) 586-93.
- 437 [11] M. Edama, M. Kubo, H. Onishi, T. Takabayashi, T. Inai, E. Yokoyama, W. Hiroshi, N.
438 Satoshi, I. Kageyama, The twisted structure of the human Achilles tendon, *Scandinavian*
439 *Journal of Medicine & Science in Sports* 25(5) (2015) e497-e503.
- 440 [12] M. Edama, M. Kubo, H. Onishi, T. Takabayashi, E. Yokoyama, T. Inai, H. Watanabe, S.

441 Nashimoto, I. Kageyama, Structure of the Achilles tendon at the insertion on the calcaneal
442 tuberosity, *J Anat* 229(5) (2016) 610-614.

443 [13] G.G. Handsfield, L.C. Slane, H.R.C. Screen, Nomenclature of the tendon hierarchy: An
444 overview of inconsistent terminology and a proposed size-based naming scheme with
445 terminology for multi-muscle tendons, *J Biomech* 49(13) (2016) 3122-3124.

446 [14] W.H. Clark, J.R. Franz, Triceps surae muscle-subtendon interaction differs between
447 young and older adults, *Connect Tissue Res* 61(1) (2020) 104-113.

448 [15] J.R. Franz, D.G. Thelen, Depth-dependent variations in Achilles tendon deformations
449 with age are associated with reduced plantarflexor performance during walking, *J Appl*
450 *Physiol* (1985) 119(3) (2015) 242-9.

451 [16] L.C. Slane, D.G. Thelen, Achilles tendon displacement patterns during passive stretch
452 and eccentric loading are altered in middle-aged adults, *Med Eng Phys* 37(7) (2015) 712-6.

453 [17] R. Beyer, A.S. Agergaard, S.P. Magnusson, R.B. Svensson, Speckle tracking in healthy
454 and surgically repaired human Achilles tendons at different knee angles-A validation using
455 implanted tantalum beads, *Translational Sports Medicine* 1(2) (2018) 79-88.

456 [18] P.A. Pękala, B.M. Henry, A. Ochała, P. Kopacz, G. Tatoń, A. Młyniec, J.A. Walocha,
457 K.A. Tomaszewski, The twisted structure of the Achilles tendon unraveled: A detailed
458 quantitative and qualitative anatomical investigation, *Scandinavian Journal of Medicine &*
459 *Science in Sports* 27(12) (2017) 1705-1715.

460 [19] S. Angin, İ. Demirbüken, Ankle and foot complex, *Comparative Kinesiology of the*
461 *Human Body* 2020, pp. 411-439.

462 [20] N.E. Akalan, S. Angin, Kinesiology of the human gait, *Comparative Kinesiology of the*
463 *Human Body* 2020, pp. 499-525.

464 [21] A. Arndt, A.S. Bengtsson, M. Peolsson, A. Thorstensson, T. Movin, Non-uniform
465 displacement within the Achilles tendon during passive ankle joint motion, *Knee Surg Sports*

466 Traumatol Arthrosc 20(9) (2012) 1868-74.

467 [22] S. Bogaerts, C. De Brito Carvalho, L. Scheys, K. Desloovere, J. D'Hooge, F. Maes, P.

468 Suetens, K. Peers, Evaluation of tissue displacement and regional strain in the Achilles tendon

469 using quantitative high-frequency ultrasound, PLoS One 12(7) (2017) e0181364.

470 [23] L. Stenroth, D. Thelen, J. Franz, Biplanar ultrasound investigation of in vivo Achilles

471 tendon displacement non-uniformity, Transl Sports Med 2(2) (2019) 73-81.

472 [24] L.C. Slane, D.G. Thelen, Non-uniform displacements within the Achilles tendon

473 observed during passive and eccentric loading, J Biomech 47(12) (2014) 2831-5.

474 [25] J.R. Franz, L.C. Slane, K. Rasske, D.G. Thelen, Non-uniform in vivo deformations of the

475 human Achilles tendon during walking, Gait Posture 41(1) (2015) 192-7.

476 [26] A. Froberg, A.S. Cisse, M. Larsson, M. Martensson, M. Peolsson, T. Movin, A. Arndt,

477 Altered patterns of displacement within the Achilles tendon following surgical repair, Knee

478 Surg Sports Traumatol Arthrosc 25(6) (2017) 1857-1865.

479 [27] C.T. Thorpe, C.P. Udeze, H.L. Birch, P.D. Clegg, H.R. Screen, Specialization of tendon

480 mechanical properties results from interfascicular differences, J R Soc Interface 9(76) (2012)

481 3108-17.

482 [28] C.T. Thorpe, M.S.C. Godinho, G.P. Riley, H.L. Birch, P.D. Clegg, H.R.C. Screen, The

483 interfascicular matrix enables fascicle sliding and recovery in tendon, and behaves more

484 elastically in energy storing tendons, J Mech Behav Biomed Mater 52 (2015) 85-94.

485 [29] C.T. Thorpe, C.P. Udeze, H.L. Birch, P.D. Clegg, H.R. Screen, Capacity for sliding

486 between tendon fascicles decreases with ageing in injury prone equine tendons: a possible

487 mechanism for age-related tendinopathy?, Eur Cell Mater 25 (2013) 48-60.

488 [30] D. Patel, E. Spiesz, C.T. Thorpe, H. Birch, G. Riley, P. Clegg, H. Screen, Energy storing

489 and positional human tendons: mechanics and changes with ageing, The Grey Area – Age and

490 the Extracellular Matrix, 2016.

- 491 [31] O.A. Komolafe, T.C. Doehring, Fascicle-scale loading and failure behavior of the
492 Achilles tendon, *J Biomech Eng* 132(2) (2010) 021004.
- 493 [32] B.T. Haraldsson, P. Aagaard, K. Qvortrup, J. Bojsen-Moller, M. Krogsgaard, S.
494 Koskinen, M. Kjaer, S.P. Magnusson, Lateral force transmission between human tendon
495 fascicles, *Matrix Biol* 27(2) (2008) 86-95.
- 496 [33] C.C. Gains, J.C. Correia, G.C. Baan, W. Noort, H.R.C. Screen, H. Maas, Force
497 Transmission Between the Gastrocnemius and Soleus Sub-Tendons of the Achilles Tendon in
498 Rat, *Frontiers in Bioengineering and Biotechnology*, 2020, pp. 1-15.
- 499 [34] K.D. Budras, W.O. Sack, S. Rock, A. Wünsche, E. Henschel, *Anatomy of the Horse: An*
500 *Illustrated Text*, Wiley2003.
- 501 [35] J.-M. Denoix, Functional Anatomy of Tendons and Ligaments in the Distal Limbs
502 (Manus and Pes), *Veterinary Clinics of North America: Equine Practice* 10(2) (1994) 273-322.
- 503 [36] M.D. Swanstrom, S.M. Stover, M. Hubbard, D.A. Hawkins, Determination of passive
504 mechanical properties of the superficial and deep digital flexor muscle-ligament-tendon
505 complexes in the forelimbs of horses, *American Journal of Veterinary Research*, 2004, pp.
506 188-197.
- 507 [37] T. Finni, M. Bernabei, G.C. Baan, W. Noort, C. Tijs, H. Maas, Non-uniform
508 displacement and strain between the soleus and gastrocnemius subtendons of rat Achilles
509 tendon, *Scand J Med Sci Sports* 28(3) (2018) 1009-1017.
- 510 [38] A.E. Goodship, H.L. Birch, Cross sectional area measurement of tendon and ligament in
511 vitro: a simple, rapid, non-destructive technique, *J Biomech* 38(3) (2005) 605-8.
- 512 [39] N. Karathanasopoulos, G. Arampatzis, J.F. Ganghoffer, Unravelling the viscoelastic,
513 buffer-like mechanical behavior of tendons: A numerical quantitative study at the fibril-fiber
514 scale, *J Mech Behav Biomed Mater* 90 (2019) 256-263.
- 515 [40] C.K. Becker, H.H. Savelberg, A. Barneveld, In vitro mechanical properties of the

516 accessory ligament of the deep digital flexor tendon in horses in relation to age, *Equine Vet J*
517 26(6) (1994) 454-9.

518 [41] N. Karathanasopoulos, J.F. Ganghoffer, Investigating the Effect of Aging on the
519 Viscosity of Tendon Fascicles and Fibers, *Front Bioeng Biotechnol* 7(107) (2019) 107.

520 [42] H.L. Birch, J.V. Bailey, A.J. Bailey, A.E. Goodship, Age-related changes to the
521 molecular and cellular components of equine flexor tendons, *Equine Vet J* 31(5) (1999) 391-6.

522 [43] S.P. Reese, S.A. Maas, J.A. Weiss, Micromechanical models of helical superstructures in
523 ligament and tendon fibers predict large Poisson's ratios, *J Biomech* 43(7) (2010) 1394-400.

524 [44] C.T. Thorpe, C. Klemm, G.P. Riley, H.L. Birch, P.D. Clegg, H.R. Screen, Helical sub-
525 structures in energy-storing tendons provide a possible mechanism for efficient energy
526 storage and return, *Acta Biomater* 9(8) (2013) 7948-56.

527 [45] T. Shearer, C.T. Thorpe, H.R.C. Screen, The relative compliance of energy-storing
528 tendons may be due to the helical fibril arrangement of their fascicles, *J R Soc Interface*
529 14(133) (2017).

530 [46] N. Karathanasopoulos, J.F. Ganghoffer, Exploiting Viscoelastic Experimental
531 Observations and Numerical Simulations to Infer Biomimetic Artificial Tendon Fiber Designs,
532 *Front Bioeng Biotechnol* 7(85) (2019) 85.

533 [47] S.P. Reese, B.J. Ellis, J.A. Weiss, Micromechanical model of a surrogate for collagenous
534 soft tissues: development, validation and analysis of mesoscale size effects, *Biomech Model*
535 *Mechanobiol* 12(6) (2013) 1195-204.

536 [48] S.J. Dyson, Desmitis of the accessory ligament of the deep digital flexor tendon: 27 cases
537 (1986-1990), *Equine Vet J* 23(6) (1991) 438-44.

538 [49] C.T. Thorpe, P.D. Clegg, H.L. Birch, A review of tendon injury: why is the equine
539 superficial digital flexor tendon most at risk?, *Equine Vet J* 42(2) (2010) 174-80.

- 540 [50] R. Kinugasa, T. Oda, T. Komatsu, V.R. Edgerton, S. Sinha, Interaponeurosis shear strain
541 modulates behavior of myotendinous junction of the human triceps surae, *Physiol Rep* 1(6)
542 (2013) e00147.
- 543 [51] O. Chan, S. Morton, M. Pritchard, T. Parkes, P. Malliaras, T. Crisp, N. Padhiar, N.
544 Maffulli, J. King, D. Morrissey, Intratendinous tears of the Achilles tendon - a new pathology?
545 Analysis of a large 4-year cohort, *Muscles Ligaments Tendons J* 7(1) (2017) 53-61.
- 546 [52] J. Kondratko-Mittnacht, R. Lakes, R. Vanderby, Jr., Shear loads induce cellular damage
547 in tendon fascicles, *J Biomech* 48(12) (2015) 3299-305.
- 548 [53] A. Scott, L.J. Backman, C. Speed, Tendinopathy: Update on Pathophysiology, *J Orthop*
549 *Sports Phys Ther* 45(11) (2015) 833-41.
- 550

551 Table 1. Mechanical testing results when load was applied through the AL

	Global	Regional		
		Free-AL	Joined-AL	Joined-DDFT
Maximal force (N)	4320±1684			
Displacement (mm)	13±1.8	5.0±1.4 ^{a, b}	3.6±1.5 ^b	1.9±0.8
Stress (MPa)		44.8±15.4 ^c		
Strain (%)	10±0.0	6.8±2.0 ^{a, b, c}	4.0±1.3 ^b	1.9±0.8
Young's modulus (MPa)		639±244 ^c		
Stiffness (N/mm)		841±327 ^c	1247±836	

552 Data are shown as mean ± SD (n=7). a: Significant difference from joined-AL. b: Significant
553 difference from joined-DDFT. c: Significant difference from the same location as DDFT
554 (Table 2).

555

556 Table 2. Mechanical testing results when load was applied through the DDFT.

	Global	Regional		
		Free-DDFT	Joined-DDFT	Joined-AL
Maximal force (N)	5370±2108			
Displacement (mm)	14±2.4	4.1±1.4 ^b	4.2±1.1 ^b	1.2±1.5
Stress (MPa)		60.7±26.9 ^c		
Strain (%)	10±0.0	5.3±1.5 ^{b, c}	4.9±1.8 ^b	1.4±1.8
Young's modulus (MPa)		1158±459 ^c		
Stiffness (N/mm)		1387±639 ^c	1224±486	

557 Data are shown as mean ± SD (n=7). a: Significant difference from joined-DDFT. b:

558 Significant difference from joined-AL. c: Significant difference from the same location as AL

559 (Table 1).

560

Table 3. Mechanical properties of fascicles and Interfascicular matrices

	Interfascicular matrices			Fascicles	
	AL	DDFT	Inter-sub-bundle	AL	DDFT
Failure force (N)	2.2±1.1	1.4±0.8	1.1±0.8	5.3±0.9	4.9±2.5
Failure strain (%)				7.5±5.5	7.5±4.5
Maximal displacement (mm)	1.9±0.7	1.7±0.5	1.8±0.7	1.5±1.1	1.5±0.9
Stiffness (N/strain%)				0.7±0.2	0.7±0.6
Stiffness (N/mm)	1.6±0.7	1.3±0.7	0.9±0.6	4.3±2.3	3.2±2.2

Data are shown as mean ± SD (n=7). N.B displacement for interfascicular matrix is given in mm due to un-defined effective gauge length.

565

566 Figure 1. The deep digital flexor tendon (DDFT) and its accessory ligament (AL) and the
 567 inter-sub-bundle matrix (*lower left*). Yellow triangles: the junction between two structures.
 568 Blue lines: levels measuring cross-sectional area. Upper row inlets: cross-sections at
 569 corresponding longitudinal regions. Black arrows: tendon/ligament fascicles.

570

571

Figure 2. Diagram of tendon level (*left*) and fascicle level (*right*) mechanical testing. The IFM was subjected to shear loading during testing and the fascicles to tensional loading [27, 28].

576

577 Figure 3. Averaged (n=7) stress-strain relationship of free-AL and free-DDFT. Error bars

578 represent standard deviation and shown at one side for clear visualisation.

579

580

581 Figure 4. Averaged (n=7) force-displacement relationship when AL (*left*) and DDFT (*right*)
582 were loaded in isolation. The contralateral side (joined-DDFT when AL loaded, joined-AL
583 when DDFT loaded; *closed triangles*) was shear loaded alone. Asterisks: significant
584 difference in maximal displacement. Error bars represent standard deviation of regional
585 displacement and are shown at one side for clear visualisation.

586

587

588 Figure 5. Relationship between force and displacement (*left*) and between force and
589 normalised displacement (*right*) of interfascicular matrices (n=7). Error bars are not shown in
590 the force-displacement curves (*left*) for better visual presentation of the data. Asterisks:
591 significant difference after post-hoc analysis ($p<0.017$).