Title: Individual variation in Achilles tendon morphology and geometry changes susceptibility to injury

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Abstract

The unique structure of the Achilles tendon, combining three smaller sub-tendons,

enhances movement efficiency by allowing individual control from connected

muscles. This requires compliant interfaces between sub-tendons, but compliance

decreases with age and may account for increased injury frequency. Current

understanding of sub-tendon sliding and its role in the whole Achilles tendon function

is limited. Here we show changing the degree of sliding greatly affects the tendon

20 mechanical behaviour. Our *in vitro* testing discovered distinct sub-tendon mechanical
 21 properties in keeping with their mechanical demands. *In silico* study based on
 22 measured properties, subject-specific tendon geometry, and modified sliding capacity
 23 demonstrated age-related displacement reduction similar to our *in vivo*
 24 ultrasonography measurements. Peak stress magnitude and distribution within the
 25 whole Achilles tendon is affected by individual tendon geometries, the sliding
 26 capacity between sub-tendons, and different muscle loading conditions. These results
 27 suggest clinical possibilities to identify patients at risk and design personalised
 28 rehabilitation protocols.

29

30 **Introduction**

31 The Achilles tendon is fundamental to human locomotion and its mechanical
 32 properties are an important contributor to athletic ability. Although the Achilles
 33 tendon is the strongest tendon in the human body and solely responsible for powering
 34 ankle extension in ‘push off’ (Finni et al., 1998), the Achilles tendon is often the
 35 Achilles heel in athletes and also in the general adult population. While tendon
 36 strength greater than the force generated by the associated muscles is sufficient, the
 37 stiffness of the tendon needs to be precisely matched to applied forces, to fulfil the
 38 specialised role of the Achilles tendon to store energy (Lichtwark and Wilson, 2007).

39 The timely release of elastic energy is important to facilitate forward body propulsion
 40 in the spring-like function of the Achilles tendon. The tendon spring operates
 41 optimally at relatively high strains as this increases the utilisation of elasticity
 42 (Lichtwark, 2005; Lichtwark and Wilson, 2006) but as a consequence, the risk of
 43 developing tendon injuries, such as chronic painful tendinopathy or acute tendon
 44 ruptures increases (Magnan et al., 2014; Martin et al., 2018). Allowing for a high
 45 degree of elongation during numerous cycles of loading and unloading is challenging
 46 for a biological material, particularly given the complex structure of the Achilles
 47 tendon.

48

49 The human Achilles tendon, unlike the majority of tendons, can be separated into
 50 three distinct ‘bundles’ instead of one uniform common tendon (Edama et al., 2015;
 51 Szaro et al., 2009). The bundles, termed Achilles sub-tendons (Handsfield et al., 2016),
 52 arise from the lateral and medial heads of the gastrocnemius and the soleus muscles.
 53 The fascicles (the primary load bearing structure of tendon) within each sub-tendon
 54 do not intertwine until reaching the insertion site on the calcaneus. Achilles tendon
 55 morphology is highly individualised with different geometries at both whole- and
 56 sub-tendon levels and different rotational angles of sub-tendons (Edama et al., 2015;
 57 Pękala et al., 2017). These anatomical features support the idea that each sub-tendon

58 should be regarded as an individual functional unit within the whole Achilles tendon
 59 in order to fully understand the internal force distribution. Current medical imaging
 60 modalities however have yet to develop sufficient resolution to accurately visualise
 61 the borders of sub-tendons in vivo. As a result, there is limited understanding of the
 62 relationship between tendon morphology, mechanical behaviour and injury risk and a
 63 lack of translation from anatomical knowledge to clinical treatment options.
 64
 65 Although retaining the separation of sub-tendons along the length of the Achilles
 66 tendon poses mechanical challenges, having individual control of three sub-tendons is
 67 likely to be beneficial. At the distal end of the muscle-tendon-bone unit the joints in
 68 the human foot and ankle are highly mobile to ensure firm contact with the ground in
 69 uneven terrains. Proximally, gastrocnemii and soleus muscles show distinct muscle
 70 fibre characteristics (Gollnick et al., 1974) and activate differently to serve their
 71 distinct functions in human movement (Cronin et al., 2013). These features
 72 corroborate the idea that having individual control of sub-tendons improves
 73 movement efficiency. Since tendons adapt to local mechanical stimuli (Birch, 2007),
 74 it is reasonable to assume that different sub-tendons (especially between gastrocnemii
 75 and soleus) have different material properties to fulfil individual muscle-tendon unit
 76 functions. Most importantly, for this optimised control to occur, the interface among

77 sub-tendons must allow a certain degree of sliding (Handsfield et al., 2017; Sun et al.,
78 2015), creating different internal displacement gradients. Optimal sliding of loaded
79 sub-tendons may help distribute stress to other unloaded sub-tendons reducing the risk
80 of overload injury (Maas and Finni, 2018), but sliding may also create localised shear
81 stress and strain at the interfaces, which is likely to be detrimental
82 (Kondratko-Mittnacht et al., 2015; Scott et al., 2015). The physiological advantages
83 and the consequences of excessive or reduced sub-tendon sliding are not understood,
84 and simple, straightforward interpretation should be avoided due to the complexity of
85 sub-tendon geometry.

86

87 Non-uniform displacement within the Achilles tendon has been widely demonstrated
88 under various static (Arndt et al., 2012; Bogaerts et al., 2017; Stenroth et al., 2019)
89 and dynamic (Slane and Thelen, 2014; Franz et al., 2015; Franz and Thelen, 2015;
90 Slane and Thelen, 2015) loading conditions. This non-uniformity decreases as people
91 age (Clark and Franz, 2020; Franz and Thelen, 2015; Slane and Thelen, 2015) and
92 after repair of a ruptured tendon (Beyer et al., 2018; Fröberg et al., 2017). There is
93 also a trend towards more uniform displacement in tendons showing signs of
94 pathological changes (Couppé et al., 2020). Reduced sub-tendon sliding therefore
95 seems to negatively correlate with tendon function. Further evidence for the

96 advantage of sliding within the tendon substructure of energy storing tendons comes
 97 from the equine superficial digital flexor tendons (SDFT). The SDFT is one of the
 98 most extreme examples of an energy-storing tendon, and widely used as a model to
 99 understand human Achilles tendon behaviour. Age-related reduction in sliding
 100 capacity has been reported at the fascicle level in the equine SDFT, and this reduction
 101 results from changes of both extracellular matrix composition and material properties
 102 (Thorpe et al., 2013, 2012), and is believed to negatively affect energy-storing
 103 capacity and increase risk of injuries. Most *in vivo* human studies of strain distribution
 104 have used ultrasound speckle tracking techniques to observe intra-tendinous
 105 movement. The main limitation of this technique is the limited plane of view and the
 106 lack of visualisation of twisted and highly variable internal sub-tendon boundaries.
 107 Although it is convenient to assume that the superficial part of the Achilles tendon is
 108 composed largely of fascicles arising from gastrocnemii and the deeper part from
 109 fascicles from the soleus, this likely to be a considerable source of error as the actual
 110 geometry differs between individuals. This limitation can be overcome by employing
 111 finite element analysis (FEA) on subject-specific tendon geometry (Hansen et al.,
 112 2017) to systematically study the complex interaction between interface sliding
 113 property (Handsfield et al., 2017) and the force output from the associated muscles
 114 bellies.

115

116 In this study we have conducted a series of multi-modality experiments to understand

117 sub-tendon behaviour within the Achilles tendon. First, we dissected five human

118 Achilles tendons into their sub-tendon components and performed *in vitro* mechanical

119 tests respectively in order to compare their material properties. Second, a further three

120 Achilles tendons were dissected and precise morphology and geometry recorded. *In*

121 *silico* models were generated using the measured geometries and material properties.

122 We used FEA to explore how different sliding properties affects sub-tendon

123 displacement and stress distribution and the variation between different models.

124 Particular interest was paid to the proximal soleus sub-tendon face since this location

125 is an anatomical landmark, which can be clearly observed and quantitatively

126 measured using traditional ultrasonography. Finally, we conducted an *in vivo* study

127 combining non-invasive muscular stimulation and ultrasonography to compare the

128 modelling results with experimental data, particularly with respect to the impact of

129 age-related decrease in non-uniform displacement within the Achilles tendon. These

130 results provide a new paradigm for understanding human Achilles tendon function

131 and its injury mechanism.

132

133 **Results**

134 *Sub-tendons within the Achilles tendon have different mechanical properties*

135 Five (3 males and 2 females, aged 78 – 87 years) fresh frozen human Achilles tendon

136 specimens were carefully dissected, and individual sub-tendons were subjected to

137 quasi-static mechanical tests to failure. The soleus sub-tendon had the greatest

138 cross-sectional area (CSA), the highest failure force and maximal stiffness compared

139 to both gastrocnemii sub-tendons (Table 1, detailed statistical analysis results in

140 Supplementary Table 1).

141 Table 1. Mechanical testing results of three Achilles sub-tendons.

Specimen (age-sex)	CSA (mm ²)			Failure force (N)			Ultimate stress (MPa)			Ultimate strain (%)			Young's modulus (MPa)			Stiffness (N/mm)		
	LG	MG	S	LG	MG	S	LG	MG	S	LG	MG	S	LG	MG	S	LG	MG	S
69-M	13.6	14.5	39.2	325.0	402.2	1701.2	24.0	27.7	45.3	9.7	10.0	19.1	247.8	277.7	237.2	55.9	72.1	148.6
78-F	12.4	10.8	20.6	154.7	571.1	1155.6	12.5	52.6	56.2	11.6	8.9	17.5	107.7	598.4	320.8	22.6	113.0	127.9
84-F	5.7	10.7	30.9	503.6	621.8	1577.0	89.0	58.3	51.0	8.9	12.2	10.4	994.7	477.9	488.6	95.4	91.1	256.0
85-F	10.4	14.0	38.2	787.5	688.3	1090.9	75.8	49.0	28.6	11.9	10.6	15.8	635.4	461.3	181.2	112.9	108.2	118.3
87-M	4.6	17.0	35.2	301.6	647.7	1578.5	65.9	38.1	44.9	8.2	10.2	10.8	807.1	371.9	415.7	63.1	107.2	239.7
Mean	9.3*	13.4*	32.8	414.5*	586.2*	1420.6	53.4	45.1	45.2	10.1	10.4	14.7	558.5	437.4	328.7	70.0*	98.3*	178.1
SD	4.0	2.7	7.6	242.5	111.3	277.1	33.4	12.2	10.4	1.6	1.2	3.9	373.4	120.3	125.8	35.3	16.8	64.9

142 CSA: cross-sectional area, LG: lateral gastrocnemius, MG: medial gastrocnemius, S: soleus sub-tendon. *: significantly different from soleus

143 sub-tendon after post-hoc analysis (p<.017).

144

145 *Sub-tendons within the Achilles tendon have different geometries*

146 Another three Achilles tendons (age-sex: 54-M, 55-M, and 14-F - constructed into

147 Model 1, 2, and 3 respectively) were collected and dissected into sub-tendon

148 components. The precise morphology and geometry were recorded and used to create

149 3-D computer-aided design models. The tendons varied in CSA from 42.3 to 90.4

150 mm² and the geometry of their sub-tendons showed great individual variability (Fig.

151 1), as reported previously (Edama et al., 2015; Pękala et al., 2017). In the proximal

152 part of the tendon, the anterior portion was mostly occupied by the soleus sub-tendon

153 and the posterior portion by medial gastrocnemius on the medial aspect and lateral

154 gastrocnemius on the lateral aspect. Passing distally, the sub-tendons rotate in a lateral

155 direction and the longest tendon (Model 1, 70 mm) rotated the most since its lateral

156 gastrocnemius sub-tendon almost completely occupied the anterior surface. In the

157 shorter tendons (Models 2 and 3, 40 mm), at the distal end, the anterior portion was

158 occupied by the lateral gastrocnemius and soleus sub-tendons. The tendon used for

159 Model 3 was the thinnest (CSA: 42.3 mm²) while tendons used in Models 1 and 2

160 were substantially thicker (89.1 and 90.4 mm²). The models closely replicated the

161 original sub-tendon geometries, capturing individual variability.

162

Figure 1. Three-dimensional computer models of Achilles sub-tendons.

Sub-tendon displacement is affected by the degree of sliding but is geometry dependent

To study the effect of sub-tendon loading on the displacement of the proximal soleus face we ran a FEA of each model with the same axial tension (100N) in each sub-tendon but with different degrees of sliding between them – namely, frictionless, frictional (with friction coefficient 0.2, 0.4, 0.6, 0.8, and 1.0) and bonded (no sliding). When the soleus sub-tendon was loaded in isolation, assigning frictional contacts reduced the soleus displacement compared to the frictionless contact across all three models. When the medial gastrocnemius sub-tendon was loaded, soleus displacement increased with increasing friction. This trend was also observed when the lateral gastrocnemius was loaded, but interestingly only in Models 1 and 2 (Fig. 2).

Figure 2. Mean displacement of the proximal soleus face of three models with

different friction contacts when each sub-tendon was loaded in isolation.

Displacement data were normalised to frictionless contact conditions to observe the

relative displacement change and therefore the impact of increasing cohesion between

the sub-tendons, as may occur with ageing (Fig. 3). When the soleus was loaded, all

three models showed a similar decrease in soleus displacement as friction increases.

In the gastrocnemii loaded conditions, a substantial increase in soleus displacement

was observed in Model 1 and a lesser increase was observed in Model 2, suggesting

geometries affect force transmission across sub-tendons when interface properties

change.

Figure 3. Normalised (to frictionless, coefficient = 0) proximal soleus face

displacement of different friction contacts when each sub-tendon was loaded in

isolation.

Reduced sliding decreases mean stress of the whole tendon but not peak stress

All models showed decreased mean von Mises stress for frictional and bonded

contacts compared to frictionless contact (Fig. 4, *upper row*); however, the peak von

Mises stress, a prediction of material yielding, differed among all three models (Fig. 4

lower row). As friction increased, the peak stress intensity of Model 1 decreased but

Models 2 and 3 fluctuated with different sub-tendon loading conditions. For bonded

contact conditions all models showed lower peak stress compared to the frictionless

and frictional contact conditions, but the size of the decrease varied greatly between

models. Furthermore, changing the contact properties, in addition to affecting the

intensity of peak stress, also changed the region where the peak stress occurred (Fig.

5). Due to the large geometry differences across the three models, no clear pattern in

the change of peak stress location is seen.

208

209

Figure 4. Mean normalised (*upper row*) and peak (*lower row*) von Mises stress of the

whole Achilles tendon for different friction coefficients when each sub-tendon was

loaded in isolation.

213

Figure 5. Change in peak stress location when shifting from frictionless (upper row)

to frictional (lower row) contact is geometry dependent. LG: lateral gastrocnemius,

MG: medial gastrocnemius, S: soleus sub-tendon. View planes: Ant.: anterior, Pos.:

posterior, Lat.: lateral, Med.: medial, Int.: internal view with the covering sub-tendon

removed.

In vivo age-related displacement decline can be explained by modelling results

We further explored whether our modelling results (Fig. 3) reflects *in vivo* age-related

decline in displacement non-uniformity. Each muscle was electrically stimulated and

the resulting soleus musculotendinous junction displacements were recorded and

normalised to their relative muscular displacements. The older group of participants,

aged 52 – 67 years (n=7) demonstrated significantly lower normalised soleus junction

displacement compared to the younger group (20 – 29 years, n=9) for soleus stimulation (Fig. 6, *left*). This displacement negatively correlates with age ($r(15) = .610$, $p < .001$; Fig. 6, *right*), showing an identical trend as the modelling result when the soleus sub-tendon was loaded in isolation (Fig. 3, *right*). We found no detectable differences in soleus junction displacement between groups for both gastrocnemii stimulation conditions.

Figure 6. Group differences in normalised soleus junction displacement during different stimulation trials (*left*) and scatter plot of participant age against soleus junction displacement during soleus stimulation trial (*right*).

Discussion

Our results demonstrate that the mechanical behaviour of the Achilles tendon is highly complex and affected by a combination of factors including different sub-tendon mechanical properties, individual tendon morphology and geometry, and age-related

243 changes in sub-tendon cohesion. We suggest that radical changes are needed to the
244 way in which Achilles tendon mechanics are studied and also in the way in which we
245 think about factors leading to the development of tendon injuries and rehabilitation.
246
247 Different mechanical properties between sub-tendons most likely relate to the
248 different properties of their associated muscle bellies. As a result of high mass and
249 relatively short muscle fibre length, the soleus muscle has the greatest force
250 generating capacity and can produce over half of the total ankle plantar flexion torque
251 (Dayton, 2017). Reflecting this large difference in force generating capacity between
252 gastrocnemii and soleus, the soleus sub-tendon had the greatest CSA and highest
253 failure force and stiffness among the three sub-tendons. Adjusting stiffness could
254 therefore be an adaptation strategy to reduce sub-tendon displacement differences and
255 maintain whole Achilles tendon integrity during muscle contractions. Our results
256 suggest that material properties may also be tuned to specific requirements as the
257 soleus sub-tendon had a higher ultimate strain and a lower Young's modulus, although
258 with the relatively small group size in this study the differences were not significant.
259 Our measured Young's modulus of sub-tendons was approximately half of that
260 previously reported for the whole Achilles tendon (Wren et al., 2001). This lower
261 value for material stiffness of the sub-units compared to the whole structure may be

262 an artefact of dissection and mechanical testing in isolation, as has been seen

263 previously when testing tendon fascicles isolated from whole tendons (Thorpe et al.,

264 2012). The inconsistency may also be a result of differences in the age groups studied,

265 as the study of whole Achilles tendons (Wren et al., 2001) found a negative

266 correlation between material properties and age and had significantly more younger

267 specimens comparing to our relatively old samples. It is generally agreed, but

268 inconclusive, that the *in vivo* modulus and strength of Achilles tendon decreases with

269 age (Svensson et al., 2016); however, studies have yet to measure the interface

270 property and sub-tendon morphology when studying individual mechanical properties,

271 ignoring two important confounding factors as our modelling result demonstrated.

272

273 Our modelling results provide insight into the impact of different sub-tendon shapes,

274 orientations, and material properties on sub-tendon sliding. Furthermore, our results

275 highlight the importance of understanding sub-tendon sliding when considering the

276 non-uniform displacement of Achilles tendon during movements (Handsfield et al.,

277 2017). We were able to study this by assigning frictional contacts and varying the

278 friction coefficient in our FEA to represent more closely the physiological sub-tendon

279 behaviour and changes across the lifespan, instead of the dichotomy between

280 frictionless sliding and completely bound conditions as has been applied previously

281 (Handsfield et al., 2017). We arbitrarily assigned identical coefficient across three
282 interfaces, but the true sliding capacity between sub-tendons and the rate of
283 age-related decline could be different. An increase in friction in our model, mimicking
284 the age-related reduction in sliding under physiological loads (Thorpe et al., 2013),
285 greatly affected the sub-tendon displacement and stress distribution. This was highly
286 dependent on geometry and varied between the three models, suggesting the rotation
287 and twist angle of the whole Achilles and sub-tendons affects how they interact. When
288 increasing the friction between sub-tendons, we would expect to see an increase in the
289 influence of individual muscle belly contractions on adjacent sub-tendons. In keeping
290 with this, the degree of soleus displacement due to gastrocnemius applied load mainly
291 increased but this was much more evident in the longest model with the greatest
292 sub-tendon twist (Model 1) and least in the shorter model with the smallest CSA
293 (Model 3). A greater transfer of load between the gastrocnemius and soleus would be
294 expected to result in reduced soleus displacement when the soleus muscle alone
295 applies force. All three morphologies studied showed reduced soleus displacement
296 with simulated soleus contraction as the coefficient of friction was increased.
297 However, contrary to the above, our studies showed the least reduction in Model 1
298 and the greatest reduction in Model 3. These results highlight the complexity of the
299 tendon and suggest that sub-tendon twist and rotation results in unequal distribution of

strain along the length of the tendon.

Similarly, when friction increased, the effect on peak stress intensity differed between the Models. In the most twisted model (Model 1), an increase in friction reduced the peak stress intensity while in the other models peak stress intensity fluctuated under different muscle loading conditions. These results strongly suggest that the impact of age-related changes to the stiffness of the interface depends on individual Achilles tendon morphology and balance of muscle strength. Load sharing, such as between adjacent muscle-tendon units (Maas and Finni, 2018) and between fascicles (Thorpe et al., 2013), has been proposed as a strategy to minimise stress and lower the risk of injury. As we demonstrate here however, the reduction of the whole tendon stress does not necessarily translate to a reduction in the peak stress (Fig. 4), which is more important when considering the strength and integrity of a material under load. The high stress region during loading could represent a mechanical weak point that may later develop pathological changes. It has been demonstrated that stress distribution within the Achilles tendon is geometry dependent and, interestingly, changing the material properties had minimal effect on tendon stress distribution (Hansen et al., 2017). In addition, our results further imply that certain types of tendon geometry have a higher injury risk factor and may predispose to age-related injury. Our study is

319 limited to three different morphologies, but it is likely that the range of morphologies
320 is far greater than that modelled here, and this would result in an even more disparate
321 strain and stress distributions.

322

323 The results of our study suggest that using the traditional anatomical landmark (soleus
324 musculotendinous junction, the start of the free Achilles tendon) to measure *in vivo*
325 tendon mechanical properties (Kongsgaard et al., 2011) is problematic, especially
326 when studying aged or repaired Achilles tendons. The displacement of the soleus
327 musculotendinous junction is used in *in vivo* studies to calculate tendon strain and
328 stiffness; however, our study has shown that displacement is not equivalent nor
329 proportional to whole tendon strain when comparing tendons with different
330 morphologies and sub-tendon arrangements. In low friction conditions, simulating a
331 young person's tendon, the displacement of the soleus junction is predominately from
332 the soleus muscle-(sub-)tendon unit displacement; in high friction conditions,
333 mimicking aged tendons, the displacement of the soleus junction is the combination
334 of both the decreasing trend from soleus and the increasing influence from
335 gastrocnemii muscle-tendon units. Moreover, the exact geometry and mechanical
336 properties of each sub-tendon among individuals are unknown and each associated
337 muscle exhibits different age and injury related compositional (Csapo et al., 2014) and

structural (Stenroth et al., 2012) changes. Together, we suggest that measuring ‘whole’ tendon properties is insufficient when investigating such tendons. Previous studies have shown that repaired Achilles tendons show higher displacements when loaded compared to healthy tendons, despite being thicker and transmitting lower forces (Geremia et al., 2015; Wang et al., 2013). Considering that those sub-tendons are surgically bound together and show uniformed displacements (Beyer et al., 2018; Fröberg et al., 2017), the actual decline in material properties post-surgery may be even greater than reported since sub-tendons exhibit much lower displacement when bound, given the same force and material properties.

In our *in vivo* studies, we found a negative relationship between participant age and the electrical-induced soleus junction displacements, and this relationship supported our FEA results when assigning frictional contacts, reinforcing the belief that the matrix between sub-tendons stiffens with age. No statistically significant difference was detected between young and old groups in both gastrocnemii stimulation trials. Our modelling results suggested that gastrocnemii-induced soleus displacement is generally low and, importantly, depends on the geometry, which we were unable to measure in our *in vivo* studies. Furthermore, the gastrocnemii have longer tendinous parts that have complex interaction with the underlying soleus muscle and

aponeurosis (Finni et al., 2003; Magnusson et al., 2003), and the CSA of gastrocnemii sub-tendons are smaller and may have less influence on the larger soleus sub-tendon. The simplification of our *in vivo* study may therefore overlook certain age-related features in muscle-tendon units and surrounding structures that may potentially affect our results. To the best of our knowledge, no study has investigated whether material properties of sub-tendons reduce with age or whether the reduction is similar among three sub-tendons; thus, we cannot conclude that the measured age-related decline in displacement is solely from the reduction of interface sliding capacity without the influence from altered tendon material properties. Our use of minimal electrical intensity during stimulation prevented co-contraction of other muscles, but the expected contraction force was low and less than most physiological loading scenarios. Overall, our *in vivo* method provides a relatively simple, low-cost setup that is able to detect *in vivo* sub-tendon sliding capacity. In future studies combining this method with other techniques such as ultrasound speckle tracking, shear-wave elastography (Lima et al., 2018), skin advance-glycation end-products measurements (Couppé et al., 2014), or Raman spectroscopy (Yin et al., 2020) may enable non-invasive measurement of ‘tendon age’ which is a valuable but currently unobtainable clinical parameter in studying tendon injuries.

Conclusions

In conclusion, our study provides novel concepts and improved understanding of Achilles tendon mechanical behaviour. The different mechanical properties of Achilles sub-tendons could originate from various structural or compositional adaptations (Birch, 2007). Understanding both the extracellular matrix composition and mechanical properties of sub-tendons will provide a better understanding of how sub-tendons adapt to different functional demands. Our results suggest that using the musculotendinous junction displacement to measure Achilles tendon strain *in vivo* should be done with caution and should not be used to compare tendons from different individuals without regard for tendon morphological variations. Differences in the transfer of force between sub-tendons with different geometrical arrangements suggest that some tendon morphology types have a higher risk of injury and that sites within the tendon most prone to injury will vary between individuals. Therefore, personalised intervention or prevention regimes to reduce tendon injuries, particularly with advancing age, informed by improved imaging modalities delineating individual tendon geometry are warranted. Our results also suggest that tailoring rehabilitation to influence muscle force balance would be beneficial in an individualised rehabilitation approach, which might for example be achieved by varying combinations of different types of muscle loading exercise and strategically positioned joint angles to facilitate

or reduce certain sub-tendon movements. Our study has provided new insights into tendon mechanics and could inspire new, potentially personalised, treatment and prevention strategies to revolutionise current management of Achilles tendon health, injury prevention and rehabilitation.

Materials and Methods

Separation of Achilles sub-tendons

The tendon specimens (total n=8) used in this study were collected from the Vesalius Clinical Training Centre, University of Bristol (REC 08/H0724/34) and from UCL/UCLH Biobank for Studying Health and Disease (HTA license number 12055) with Local R&D approval (Ref: 11/0464). Fresh-frozen specimens (from the Vesalius Clinical Training Centre, n=5) were used for mechanical testing and fresh specimens (from UCL/UCLH Biobank, n=3) were used for morphology measurements and computer model construction. Only tendons showing no signs of injury or disease were included in the study. Sub-tendons were separated as described in the study by Szaro *et al* (2009)(Szaro et al., 2009). For the three specimens used for model construction, a longitudinal reference line (from the mid-point of calcaneal insertion site to mid-point between the two gastrocnemii bellies) was drawn before separation as a guide to align tendon cross-sections (see below). Exterior moulds were created to

414 minimise geometry distortion during separation (Goodship and Birch, 2005).

415

416 *Measurement of sub-tendon mechanical properties*

417 The CSA of each sub-tendon was measured (ImageJ, v1.51) at the same level as the

418 thinnest region (~10 to 40 mm proximal to insertion) of the whole Achilles tendon.

419 Each sub-tendon was tested using a mechanical testing system (5967, Instron,

420 Norwood, MA, US) with a linear electric motor and a 3kN load cell. After secured

421 into cryoclamps with 60 mm gauge length, a pre-load was applied (10 N for

422 gastrocnemii and 20 N for soleus) and then 20 cycles of pre-conditioning (triangular

423 wave to 5% strain) was applied. The load was removed to allow the specimen to go

424 slack (4 mm) before pulling to failure (rate: 0.75mm/s). Stiffness was calculated by

425 dividing maximal force by maximal displacement.

426

427 *Construction of Achilles sub-tendon computer models*

428 After separation, the three sub-tendons were carefully assembled and fitted into the

429 original exterior mould. Cross-sections (10 mm apart) were cut through the mould and

430 the sub-tendons from proximal to distal, starting from the soleus musculotendinous

431 junction. These cross-sections were then photographed together with a ruler (1 mm

432 accuracy) using a 12-megapixel camera yielding a resolution of 0.014 mm/pixel.

Since the fascicles intertwined at the insertion, we could only confidently separate sub-tendons to a level approximately 10 mm proximal to the calcaneus, thus the reconstructed models end at this point.

Three separate Achilles sub-tendon models were created in a web-based computer aided design platform (Onshape, PTC, Boston, MA, US). First, each sub-tendon cross-section was delineated (perimeter, then internal borders) and aligned according to the longitudinal reference line. The CSA differences between the drawn sub-tendon area and the photographic measurements were less than 1 mm². Next, the cross-sections belonging to each sub-tendon were constructed from distal to proximal, creating three solid parts. The models constructed were inspected to ensure no penetration or separation between each part before conducting simulations.

Finite element analysis

A commercially available FEA software (ANSYS v19.0, Ansys Inc., Canonsburg, PA, US) was used for mesh generation and static stress analysis. Each sub-tendon was modelled as a hyperelastic, non-linear, neo-Hookean material (Handsfield et al., 2017; Hansen et al., 2017) with the initial modulus (Lat. G: 226.7 MPa, Med. G: 143.2 MPa, Soleus: 103.1 MPa) obtained by curve-fitting the *in vitro* axial tensile testing results.

Sub-tendons were meshed into quadratic 10-node tetrahedral solid elements [TET10].

A mesh convergence test was conducted using static stress analysis with criteria of 1% for each model. Maximal element edge length for each model was 0.8, 0.5, and 0.4 mm respectively and yielded 258402, 195286, and 223423 elements, respectively.

The contact faces between sub-tendon pairs were manually selected and assigned different contact properties: frictionless, frictional (with coefficient 0.2, 0.4, 0.6, 0.8, and 1.0) and bonded. Surface-to-surface translational joints, with no restriction along the longitudinal axis while limiting transverse plane separation, were assigned at the contact faces to prevent sub-tendon separation. Fixed displacement at distal faces (to mimic the fixed calcaneus insertion) and individual 100 N linear ramped tensile forces at the proximal face of each sub-tendon (to mimic muscular pull) were applied.

In vivo muscular stimulation study – participants

This study was approved by the University College London Research Ethics Committee (ref. 1487/1001). All participants were well informed and provided consent in accordance with the Declaration of Helsinki. Participants were assigned into two groups according to their age (Young: 20 to 30 years, n=9; Old: over 50 years, n=7). The exclusion criteria were having any previous diagnosed Achilles tendon injuries and any systematic disease that may affect muscle-tendon functions.

471

472 *Experiment procedures*

473 The participants were prone on an examination bed with feet firmly step against the
474 wall (~90°). Three pairs of 50 mm diameter electrodes were placed on the motor
475 points of the three muscles bellies (Botter et al., 2011; Kim et al., 2005). The skin was
476 cleaned with alcohol swaps and shaved if necessary to ensure minimal intensity for
477 inducing muscular contraction and minimising discomfort. Each muscle was
478 stimulated using a commercially available transcutaneous electrical neuromuscular
479 stimulation device with 30Hz, 300µs pulse train and an on-off cycle of 8 seconds (4
480 sec on and 4 sec off) for two minutes to induce tetanic contractions. Before the
481 stimulation, an ultrasound probe (2-8MHz, 9L-D, LOGIQ S8, GE Healthcare,
482 Buckinghamshire, UK) was placed at the musculotendinous junction of the three
483 muscles to ensure proper image quality. During the stimulation, the intensity
484 increased gradually (to ~20mA) until visible displacement of the stimulated muscle
485 (e.g. medial gastrocnemius) was recorded while at the same time no visible
486 contraction of other muscles (e.g. lateral gastrocnemius and soleus) was observed,
487 before moving the probe to the soleus musculotendinous junction to measure its
488 displacement (Fig. 7). At least three consecutive contractions were recorded and
489 stored for offline analyses. Randomised sequence and ample rest between

stimulations were used to reduce artefacts.

Figure 7. Simplified sagittal plane view of triceps surae muscle-tendon units and corresponding ultrasound measurement locations (*left*) and sequences of measurement during each stimulation trial (*right*). MG/LG: medial or lateral gastrocnemius, SOL: soleus. MTJ: musculotendinous junction.

Ultrasound image analysis

The analysis was performed in a semi-automatic way using an in-house MATLAB code (R2019a, MathWorks, Natick, MA, US) utilising the Computer Vision Toolbox add-on for tracking displacements and correcting movement artefacts. The peak soleus musculotendinous junction displacement of each stimulation trial was tracked and normalised by its contracted muscular displacements (for gastrocnemii: musculotendinous junction, for soleus: one distal muscle fascicle-aponeurosis junction; Fig. 7, *left*), assuming the muscular displacement affects only by stimulation

intensity while sub-tendon displacement affects by interface sliding capacity. We arbitrarily chose the distal soleus muscle fascicle-aponeurosis junction as the reference point (Muramatsu et al., 2001) since no clear anatomical landmark lies within the soleus muscle.

Statistical analysis

Statistical analysis was performed using SPSS (v26, IBM, Armonk, NY, US). Kruskal-Wallis H tests were used for comparing mechanical properties among sub-tendons and Mann-Whitney U tests were used for post-hoc analysis. *In vivo* soleus junction displacement between groups were compared using Mann-Whitney U tests, and Pearson's correlation coefficient was calculated for accessing relationship between displacement and participant age. Level of significance was set at 0.05, two tailed.

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525 **Author contributions**

526 Conceptualisation, N.Y., I.M., H.B.; data collection, N.Y.; validation, P.F., I.M., H.B.;
527 writing – original draft, N.Y.; review and editing, P.F., I.M., H.B. All authors have
528 read and agreed to the published version of the manuscript.

529 **Competing interests.**

530 The authors declare no conflicts of interest.

531

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 699

700 Supplementary Table 1. Statistical analysis outcome of three sub-tendon mechanical
701 properties.

	Kruskal-Wallis test	Pairwise Mann-Whitney U test		
		LG – MG	LG – S	MG – S
Cross-sectional area				
Test statistics	10.820	-1.776	-2.611	-2.611
DOF	2.00			
p-value	.004	.095	.008	.008
Failure force				
Test statistics	10.220	-1.358	-2.611	-2.611
DOF	2.00			
p-value	.006	.222	.008	.008
Ultimate stress				
Test statistics	.380			
DOF	2.00			
p-value	.827			
Ultimate strain				
Test statistics	4.994			
DOF	2.00			
p-value	.082			
Young's modulus				
Test statistics	1.580			
DOF	2.00			
p-value	.454			
Maximal stiffness				
Test statistics	10.220	-1.358	-2.611	-2.611
DOF	2.00			
p-value	.006	.222	.008	.008

702