Tendinous tissue properties after short and long-term functional overload: Differences between controls, 12 weeks and 4 years of resistance training

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ACTA PHYSIOLOGICA

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3

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14

15   **Short Title:**

16   Tendon adaptation to functional overload

17

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26   **Abstract**

27   **Aim:** The potential for tendinous tissues to adapt to functional overload, especially after

28   several years of exposure to heavy resistance training is largely unexplored. This study

29   compared the morphological and mechanical characteristics of the patellar tendon and knee-

30   extensor tendon-aponeurosis complex between young men exposed to long-term (4 years;

31   n=16), short-term (12 weeks; n=15) and no (untrained controls; n=39) functional overload in

32   the form of heavy resistance training. **Methods:** Patellar tendon cross-sectional area, vastus-

33   lateralis aponeurosis area and quadriceps femoris volume, plus patellar tendon stiffness and

34   Young's modulus, and tendon-aponeurosis complex stiffness, were quantified with MRI,

35   dynamometry and ultrasonography. **Results:** As expected long-term trained had greater

36   muscle strength and volume (+58% and +56% vs untrained, both P<0.001), as well as a

37   greater aponeurosis area (+17% vs untrained, P<0.01), but tendon cross-sectional area (mean

38   and regional) was not different between groups. Only long-term trained had reduced patellar

39   tendon elongation/strain over the whole force/stress range , whilst both short-term and long-

40   term overload groups had similarly greater stiffness/Young's modulus at high force/stress

41   (short-term +25/22%, and long-term +17/23% vs untrained; all P<0.05). Tendon-aponeurosis

42   complex stiffness was not different between groups (ANOVA, P = 0.149). **Conclusion:**

43   Despite large differences in muscle strength and size, years of resistance training did not

44   induce tendon hypertrophy. Both short-term and long-term overload, demonstrated similar

45   increases in high force mechanical and material stiffness, but reduced elongation/strain over

46   the whole force/stress range occurred only after years of overload, indicating a force/strain

47   specific time-course to these adaptations.

48

49   **Key Words:** Aponeurosis, Hypertrophy, Muscle, Resistance Training, Stiffness, Tendon

50

Introduction

52   Tendons are integral to *in vivo* neuromechanical function transmitting skeletal muscle

53   contractile force to the skeleton whilst also optimising the contractile conditions via their

54   viscoelastic properties.[1,2] The response of tendinous tissue to mechanical loading is of great

55   interest, since it may influence function[3,4,5,6] and be related to injury incidence.[7-9] Impaired

56   tendinous tissues properties are evident in older adults and patient groups[10-14] and are

57   associated with reduced muscle-tendon unit functional capacity.[3,5,11,15] Functional overload,

58   in the form of resistance exercise is widely recommended for improving musculo-skeletal

59   function of all adults,[16,17] including older individuals and patients (e.g. osteoarthritis;[18]).

60   However our understanding of how tendons alter their properties in response to short-term

61   (weeks-months) and especially long-term (years) loading is limited. Tendinous tissue may

62   exhibit morphological (cross-sectional area), mechanical (stiffness), and material (Young's

63   modulus) adaptations to functional overload,[19,20] however the magnitude and time-course of

64   these adaptations has not been clearly elucidated.

65

66   Whilst skeletal muscle tissue has been widely documented to undergo hypertrophy in

67   response to functional overload with resistance training,[21] the evidence for tendon

68   hypertrophy is equivocal; short-term resistance training studies have reported region specific

69   increases in tendon cross-sectional area[22-24] or no change.[25-27] Explanations for this

70   controversy could be the relatively slow turnover of collagenous tissues,[28,29] and thus changes

71   in tendon size within the first 14 weeks of resistance training that are on the threshold of what

72   can be accurately detected. A substantially longer exposure to functional overload may

73   provide sufficient time for the accumulation of new tissue and thus demonstrable tendon

74   hypertrophy. However, preliminary cross-sectional studies of long-term functional overload

75   vs untrained controls have used insufficient methods to dispel this conflict, reporting tendon

76   hypertrophy (low resolution ultrasound[30]) or no hypertrophy for 4 out of 5 tendon sites

77   (limited locations along the tendon with MRI[31]). The potential for aponeurosis hypertrophy in

78   response to resistance training has also had limited research attention.[32,33]

79

80   Short-term functional overload with resistance training (up to 14 weeks) utilising high load

81   contractions consistently increases 'free' tendon[22,24,34,35] and tendon-aponeurosis complex

82   [23,25,26,36-38] stiffness. The increased tendon stiffness after short-term resistance training is

83   typically ascribed to the approximately parallel increases in tendon Young's modulus

84   (material stiffness[24,35]) rather than substantive changes in tendon size, as mentioned above.

85   However, the potential for further changes in tissue mechanical and material stiffness after

86   long-term resistance training remains largely unexplored. Preliminary reports include no

87   difference in patellar tendon material stiffness between long-term resistance trained and

88   untrained men[30] and no additional changes in Achilles tendon-aponeurosis stiffness from 14

89   weeks to 18 months of resistance training in elderly women.[39] However, both these studies

90   assessed stiffness at different forces/stresses, which confounds comparable measurements of

91   the curvi-linear *in vivo* stress-strain relationship.[40,41]

92

93   Whilst existing data have been insufficient to confirm if functional overload results in

94   hypertrophy of tendinous tissues, and whether mechanical and material stiffness continues to

95   adapt with prolonged resistance training, we theorised that (i) the high tendinous tissue loads

96   consequent to the known adaptations of large increases in muscle strength and size after long-

97   term loading[21], and (ii) prolonged exposure to these high loads would trigger substantial

98   adaptive responses in the tendinous tissues, in order to constrain peak tissue strain within sub-

99   failure physiological limits[42,43]. The purpose of this study therefore was to compare the

100 morphological and mechanical properties of the patellar tendon (stiffness, Young's modulus,

101 CSA [mean and regional]) and quadriceps femoris tendon-aponeurosis complex (stiffness,

102 muscle volume, vastus lateralis aponeurosis area), between participants exposed to long-term

103 (4 years [LTT]) and short-term (12 weeks [STT]) resistance training and no (untrained

104 controls [UC]) functional overload. Specific hypotheses were that tendon characteristics

105 would be progressive according to the duration of overload exposure (controls<short-

106 term<long-term), and specifically that long-term overload (resistance training) would be

107 characterised by not only greater muscle size and strength, but also a larger tendon and

108 aponeurosis, higher tendon Young's modulus, as well as greater free tendon and tendon-

109 aponeurosis stiffness.

110

111 **Results**

112 **Group Characteristics**

113 Age, height and body mass were similar between UC and STT groups (P = 0.262, P = 0.488

114 and P = 0.465 respectively; Table 1), while LTT were younger, taller and had a larger body

115 mass than UC and STT (all P ≤ 0.003). Tendon-aponeurosis complex and patellar tendon

116 length were similar between UC and STT (P = 0.114 and P = 0.195), although LTT had

117 longer tissue lengths than both UC and STT (tendon-aponeurosis complex LTT +6.5% vs UC

118 and +9.2% vs STT; both P<0.001; patellar tendon length LTT +9.8% vs UC P = 0.006, and

119 +15.5% vs STT P = 0.022).

120

121 **Muscle-tendon unit size and strength**

122 Maximal voluntary torque (Figure 4a) differed between all three groups, being considerably

123 greater in LTT than UC (+58.1%, P < 0.001, ES = 2.90 "very large") and STT (+34.4%, P <

124 0.001, ES = 1.66 "large"). STT was also stronger than UC (+17.6%, P = 0.001, ES = 1.04

125 "moderate"). QUADSvol (Figure 4b) was considerably larger in LTT than UC (+55.7%, P <

6

126   0.001, ES = 3.55 "very large") and STT (+46.2%, P < 0.001, ES = 2.83 "very large"),

127   although SST was similar to UC (+7%, P = 0.179, ES = 0.42 "small"). Vastus lateralis

128   aponeurosis area (Figure 4c) was also larger in LTT than UC (+17.3%, P < 0.001, ES = 1.41

129   "large") and STT (+13.5%, P = 0.006, ES = 1.09 "moderate"), but STT was not different to

130   UC (+3.3%, P = 0.331, ES = "small"). In contrast, patellar tendon mean CSA (Figure 4d) was

131   similar between groups (ANOVA P = 0.169), and this was also the case for regional patellar

132   tendon CSA (proximal, middle, distal; ANOVA P > 0.141; Table 2), demonstrating no

133   overall or region specific hypertrophy.

134

135   **Patellar tendon mechanical properties (Table 3)**

136   The patellar tendon force-elongation relationships (Figure 2a) indicated that patellar tendon

137   elongation at the highest common force level (4200 N, Figure 2b) of LTT was 13.5% less

138   than UC (2.6 ± 0.5 vs 3.0 ± 0.6 mm, P = 0.063, ES = 0.75 "moderate") and 15.4% lower than

139   STT (3.1 ± 0.6 mm, P = 0.048, ES = 0.86 "moderate"), indicating greater stiffness over the

140   whole force range up to 4200 N for LTT only, whereas STT and UC were similar (P = 0.698,

141   ES = 0.10 "trivial"). However, patellar tendon stiffness measured over a high force range

142   (3360-4200N; Figure 2c) was greater for SST (+24.5%, P = 0.0004, ES = 1.27 "large") and

143   LTT (+16.7%, P = 0.021, ES = 0.85 "moderate") than UC, though similar for LTT and STT

144   (P = 0.287, ES = 0.35 "small").

145

146   Patellar tendon stress-strain relationships (Figure 3a) revealed that at the common stress level

147   of 40 MPa (Figure 3b), patellar tendon strain of LTT (5.1 ± 1.0%) was 24.4% lower than UC

148   (6.4 ±1.4%, P = 0.008, ES = 1.30 "large") and 19.9% less than STT (6.7 ± 1.7%, P = 0.006,

149   ES = 0.97 "moderate"), indicating greater material stiffness over the whole stress range up to

150   40 MPa for LTT only, whereas STT and UC were very similar (P = 0.369, ES = 0.17

151   "trivial"). However, patellar tendon Young's modulus (Figure 3c) derived over a common

152   stress range (32-40 MPa) was greater for both SST (+21.9%, P = 0.003, ES = 1.00

153   "moderate") and LTT (+23.3%, P = 0.002, ES = 1.13 "moderate") than UC, but was very

154   similar for LTT and STT (P = 0.855, ES = 0.06 "trivial").

155

156   **Tendon-aponeurosis complex mechanical properties (Table 3)**

157   Force-elongation relationships for the tendon-aponeurosis complex (Figure 4a) showed that

158   at the common force level of 4200 N (Figure 4b), tendon-aponeurosis complex elongation

159   exhibited no main group effect (ANOVA, P = 0.375), indicating similar overall tendon-

160   aponeurosis complex elongation in both resistance trained and the untrained group. Likewise,

161   tendon-aponeurosis complex stiffness (Figure 4c) was not statistically different between

162   groups (ANOVA, P = 0.149).

163

164   **Discussion**

165   The present study compared the morphological, mechanical and material properties of the

166   patellar tendon and knee extensor tendon-aponeurosis complex between young men exposed

167   to long-term (4 years), short-term (12 weeks) and no (untrained controls) functional overload.

168   The main findings were that despite large differences in muscle strength and size, there were

169   modest differences in aponeurosis size, greater in LTT only, and no differences in patellar

170   tendon CSA. Only LTT had reduced elongation/strain over the whole force/stress range up to

171   4200 N/40 MPa, whilst both overload groups had greater patellar tendon stiffness/Young's

172   modulus at high force/stress than UC. Therefore short-term overload appears sufficient to

173   produce changes in high force mechanical and material stiffness, with no further adaptation

174   with prolonged exposure, but changes in elongation/strain over the whole force range

175   occurred only after years of overload (LTT). Contrary to these differences in tendon

176   mechanics, tendon-aponeurosis complex stiffness and strain were similar between all three

177   groups.

178

Tendon and aponeurosis size

180   The duration of overload showed progressive differences in muscle strength between the

181   groups (STT +18% and LTT +58% vs UC) and muscle size was also substantially greater

182   after regular long-term loading (LTT +56% vs UC). Despite these substantial differences, and

183   contrary to our hypothesis there were no group differences in patellar tendon mean or

184   regional CSA. The similar patellar tendon CSA of STT than UC might have been expected as

185   there is contrasting evidence for tendon hypertrophy after short-term resistance training (8-14

186   weeks), that has been attributed to limited region-specific hypertrophy and/or slow tendon

187   collagen turnover.[28,29] Nonetheless we hypothesised that LTT would exhibit greater tendon

188   hypertrophy, due to a combination of (i) their higher loading and stress/strain as a

189   consequence of their substantially greater strength, and (ii) prolonged regular exposure to 4

190   years of these high loads, that might provide sufficient stimulus for the disruption of tissue

191   homeostasis and time for an accumulation of tendon collagen.[44] However there were no

192   differences in tendon CSA between LTT and UC despite the substantial differences in muscle

193   strength and size, indicating that tendon size does not adapt in proportion to either muscle

194   size or strength/loading. Our study utilised MRI (regarded as the most accurate method[45,46])

195   to assess CSA along the full tendon length (typically 20 slices; capturing region specific

196   CSA), and images were acquired with sensitive spatial resolution (2 mm thick images, 0 mm

197   gap, pixel size 0.313 x 0.313 mm), as well as careful tendon segmentation performed on each

198   image by the same-blinded investigator. Moreover, with this procedure tendon CSA measures

199   demonstrated very good reliability (CV $\leq$ 3.5%), which provides confidence in the validity of

200   this data. Hitherto, cross-sectional studies of tendon size in long-term vs untrained

201 individuals present conflicting evidence from unconvincing methodologies (low resolution

202 ultrasound[30]; limited locations along the tendon with MRI[31]). A sole longitudinal study used

203 mixed low and high load training of older females, reporting short-term tendon hypertrophy

204 after 14 weeks, but no further long-term (1.5 years) changes, perhaps due to the surprising

205 lack of long-term strength improvements and thus limited overload.[39] In contrast our results

206 provides convincing evidence that long-term (4 years) exposure to high loads (+58% greater

207 strength and +56% greater size) is not a stimulus for tendon hypertrophy.

208

209 In support of our findings, there is evidence that functional overload via resistance training

210 does not stimulate *in vivo* tendon collagen synthesis,[47] nor increased concentration of

211 procollagen type 1 N-propetide, a biomarker of collagen synthesis, in the patellar tendon

212 peritendinous tissue, concomitant with no change in tendon CSA after short-term resistance

213 training.[27] In contrast, lower intensity higher volume loading, equivalent to endurance

214 training, might induce *in vivo* tendon collagen synthesis; increased peritendinous tissue

215 procollagen peptide levels,[48] and uptake of radio-labelled amino acids,[49] although this is not a

216 consistent finding.[50,51] Furthermore long term habitual exposure to endurance training or high

217 volumes of low-moderate loading have been found to induce greater tendon size: larger

218 tendon CSA in distance runners vs non-runners[52,53] and in dominant vs non-dominant limbs

219 after asymmetrical loading[54]. Therefore it may be that high volumes of low/moderate loading

220 may be the important stimulus for tendon hypertrophy, as chronic exposure to high load does

221 not appear to be a key stimulus.

222

223 In contrast to tendon size, LTT, but not STT, had a much larger VL aponeurosis area than UC

224 (+17%), demonstrating that aponeurosis size is responsive to the long-term functional

225 overload of the muscle-tendon unit via resistance training. This is coherent with the limited

226   previous reports of greater aponeurosis size post short-term resistance training and in well-

227   trained weightlifters vs untrained.[32,33] The greater aponeurosis area for LTT vs UC was

228   however substantially smaller than the difference in muscle size (+17 vs +56%), which may

229   be attributable to the greater rate of myofibrillar than connective tissue collagen synthesis in

230   response to resistance exercise.[28,55]

231

232   **Patellar Tendon Stiffness**

233   In the absence of group differences in patellar tendon CSA, our results for patellar tendon

234   stiffness were attributable to parallel changes in material stiffness, which is in accordance

235   with extensive literature indicating that enhanced material properties are the primary cause of

236   increased mechancial properties.[19,20] As expected, STT and LTT possessed greater high force

237   patellar tendon stiffness/Young's modulus than UC, which is in accordance with previous

238   short-term resistance training studies[22,24,30,34,35]: mean change after resistance training

239   ~27/22% for tendon stiffness/modulus.[20] Interestingly though LTT had no greater patellar

240   tendon stiffness/Young's modulus than STT.

241

242   However LTT did demonstrate lesser overall patellar tendon elongation/strain at a common

243   force/stress than STT and UC, and thus greater absolute and material stiffness over the whole

244   force range. Consequently short-term overload was sufficient to produce changes in high

245   force stiffness/Young's modulus, with no further adaptation after prolonged exposure, but

246   changes in elongation/strain over the whole force range occurred only after years of overload

247   (LTT). Given the identical elongation/strain at high forces/stress after STT and LTT, the

248   effect of lower elongation/strain over the whole force range after LTT could only be due to

249   greater stiffness/young's modulus at lower forces/stresses. Qualitatively this difference

250   appears to be due to greater resistance to strain at low stress levels (<10MPa), as the gradients

251 of the stress-strain relationships after the initial most compliant region of tendon deformation

252 were equivalent in STT and LTT. Overall, our data imply that the potential mechanisms

253 (changes to internal structure and/or composition[56,57]) underpinning an increased high stress

254 tendon modulus, after short-term loading, are likely saturated after 12 weeks leading to a

255 plateau in adaptation, where as low stress-specific material adaptations appeared to continue

256 as these were most pronounced after long-term functional overload. This stress specific time

257 course of tendon adaptation, with decreased strain at high stresses occurring first after short-

258 term loading, followed later by decreased strain at low-stresses after continued long-term

259 loading is a novel finding. More detailed longitudinal investigations are required to verify

260 these results and the mechanisms for this apparent stress specific time-course of adaptation.

261

262 The validity our findings are reinforced by the thorough measurements of elongation,

263 stiffness and strain; e.g. multiple contractions at a standardised loading rate, duplicate

264 measurement sessions, measurements at identical absolute forces. Representative data were

265 derived across two sessions to yield good inter-test reliability for all stiffness, elongation and

266 stress measurements (CV <10%). In particular our methods avoided the likely bias of higher

267 stiffness measurements for stronger individuals due to: contracting at a higher loading rate

268 during fixed duration ramp contractions; or measuring stiffness/young's modulus at different

269 absolute forces (i.e. relative forces) on the curvilinear force-elongation/stress-strain

270 relationship.

271

272 **Tendon-Aponeurosis Stiffness**

273 Surprisingly, there were no group differences in tendon-aponeurosis complex mechancial

274 properties despite the presence of much larger muscle and aponeurosis size for LTT. This

275 finding is in contrast to previous reports of greater knee-extensor tendon-aponeurosis

276   complex stiffness assessed with repeated measures pre and post short-term resistance

277   training.[26,36,37] It is possible that a cross-sectional design lacks the sensitivity to detect

278   relatively modest differences in tendon aponeurosis stiffness. Alternatively, previous studies

279   have commonly measured stiffness at different absolute forces pre and post training, which

280   may have accentuated the scale of this training adaptation. The measurement of tendon-

281   aponeurosis complex stiffness is also not considered to be as robust as that of 'free tendon'.[58]

282   This is because it may reflect not only tendon-aponeurosis deformation, but also the active

283   state of the muscle fibres parallel to the aponeurosis,[59] as well as the fact that single site

284   measures of aponeurosis deformation with 2-D imaging may provide only a crude index of

285   the stiffness of a 3-D structure. Further investigations could incorporate three-dimensional

286   imaging techniques (ultrasound[60,61] or MRI[62,63]) that can capture the complex bi-axial

287   deformation of the muscle and aponeurosis along the length of the tendon-aponeurosis

288   complex.

289

290   In summary, the greater quadriceps femoris strength and volume in LTT (+58 and +56% vs

291   UC) was associated with modest increases in vastus lateralis aponeurosis area (+17% vs UC),

292   but not matched by a larger patellar tendon cross-sectional area, indicating that long-term

293   functional overload via high force resistance training does not lead to extramuscular tendon

294   hypertrophy. Short- and long-term overload groups had similar, but greater patellar tendon

295   stiffness/Young's modulus at high force/stress than UC, but only LTT had reduced

296   elongation/strain over the whole force/stress range up to 4200 N/ 40 MPa that was

297   attributable to changes at lower force/stress. Therefore we found evidence for a stress specific

298   time-course of adaptation in the patellar tendon with short-term overload sufficient to

299   produce changes in high force mechanical and material stiffness, with no further adaptation

300   to prolonged exposure, but increased low stress stiffness only occurring after years of

301   overload (LTT).

302

303   **Materials and Methods**

304   **Participants**

305   Seventy young men provided written informed consent before completing this study, which

306   was approved by the Loughborough University Ethical advisory committee, and was

307   conducted according to the principles expressed in the Declaration of Helsinki. All

308   participants were healthy and free from musculoskeletal injury with no previous history of

309   tendon pathology. The untrained control group (UC, n = 39) had no lower body resistance

310   training experience for >18 months. The short-term trained group (STT, n = 15) were

311   measured post 12-weeks of supervised resistance training. The long-term trained (LTT, n =

312   16) group had 4.0 ± 0.8 (mean ± SD) years of systematic heavy-resistance training

313   experience (~3 x wk of quadriceps sessions; typical exercises were squat, lunge, step-up, leg

314   press). LTT participants typically reported some nutritional supplement consumption

315   (predominantly whey protein and creatine), although none declared illegal performance-

316   enhancing substance use.

317

318   **Experimental Design**

319   Participants visited the laboratory for a familiarisation session, (STT were familiarised pre-

320   training) and two duplicate measurement sessions that were averaged to improve the

321   reliability of the measurements. Participants were seated in a custom-built isometric strength-

322   testing chair and completed a series of maximal voluntary contractions (MVCs) and ramp

323   voluntary contractions of the knee extensors as well as knee flexor MVCs of the dominant leg

324   (preferred kicking leg). MVCs established maximal voluntary torque (MVT) and ramp

325   contractions were performed to permit tissue stiffness estimation. Knee joint torque was

14

326 recorded throughout contractions. Knee flexor surface electromyography was recorded

327 during knee flexor MVCs and knee extensor ramp contractions. All contractions were

328 performed with equivalent resting joint angle configurations. Ultrasound images of the vastus

329 lateralis and patellar tendon were recorded throughout the ramp contractions to assess tissue

330 elongation. Measurement sessions were performed at a consistent time of the day (± 2 hours),

331 separated by at least 2 days and started between 12:00–19:00 p.m. Participants were

332 instructed not to participate in strenuous physical activity, consume alcohol/refrain from

333 caffeine consumption in the 36/6 hours before measurement sessions. All participants were

334 instructed to maintain their habitual physical activity and diet throughout the study. For the

335 SST group, post-measurement sessions one and two took place 3-5 and 6-8 days following

336 the last training session. Magnetic resonance imaging (MRI) was performed to assess

337 quadriceps femoris muscle, vastus lateralis aponeurosis, and patellar tendon size. Participants

338 were instructed to refrain from strenuous physical activity in the 24 hours prior to the MRI

339 scan. For the STT group, MRI was conducted 2-3 days after the final training session and

340 prior to post measurement sessions.

341

342 **Short-term trained (STT) Group: Training Intervention**

343 Training sessions were completed three times per week on the same apparatus and with

344 equivalent joint angles as used for measurement sessions. After a brief warm-up of sub-

345 maximal contractions of both legs, participants completed four sets of ten unilateral isometric

346 knee-extensor contractions of each leg, with sets alternating between dominant and non-

347 dominant legs until 4 sets per leg had been completed. Contractions were sustained at

348 75%MVT, with 2 s rest between each contraction. In order to control the torque rise and hold

349 times, participants were presented with a target torque trace 2 s before every contraction and

350 instructed to match this target, which increased torque linearly from rest to 75% MVT over 1

351 s before holding a plateau at 75%MVT for a further 3 s. MVCs were performed at the start of

352 each training week to re-establish MVT and prescribe training torques.

353

354 **Torque Measurement**

355 Participants were positioned in an isometric strength-testing chair with resting knee and hip

356 joint angles of ~115° and ~126° (180° = full extension), respectively. The resting joint angle

357 configurations were determined from digitisation of sagittal plane video during pilot work.

358 Adjustable straps were tightly fastened across the pelvis and shoulders to prevent extraneous

359 movement. An ankle strap (35 mm width reinforced canvas webbing) was placed ~15% of

360 tibial length (distance from lateral malleolus to knee joint space) above the medial malleolus,

361 and positioned perpendicular to the tibia and in series with a calibrated S-Beam strain gauge

362 (Force Logic, Berkshire, UK). The analogue force signal was amplified (x370; A50

363 amplifier, Force Logic UK) and sampled at 2,000 Hz using an A/D converter (Micro 1401;

364 CED, Cambridge, UK) and recorded with Spike 2 computer software (CED). In offline

365 analysis, force signals were low-pass filtered at 500 Hz using a fourth order zero-lag

366 Butterworth filter, gravity corrected by subtracting baseline force, and multiplied by lever

367 length, the distance from the knee joint space to the centre of the ankle strap, to calculate

368 torque values.

369

370 **Knee Flexor Electromyography (EMG)**

371 Surface EMG recordings over the biceps femoris (BF) and semitendinosus (ST) were made

372 with a wireless EMG system (Trigno; Delsys Inc, Boston, MA) were made during knee

373 flexor MVCs and knee extensor ramp contractions. Following preparation of the skin

374 (shaving, abrading and cleansing with alcohol) single differential Trigno standard EMG

375 sensors (1 cm inter electrode distance; Delsys Inc, Boston, MA) were attached over each

376   muscle using adhesive interfaces. Sensors were positioned parallel to the presumed frontal

377   plane orientation of the underlying muscle fibres at 45% of thigh length (distance from the

378   greater trochanter to the lateral knee joint space) measured from the popliteal crease. EMG

379   signals were amplified at source (x300; 20-450 Hz bandwidth) before further amplification

380   (overall effective gain x 909) and sampled at 2000 Hz via the same A/D converter and

381   computer software as the force signal, to enable data synchronization. In offline analysis,

382   EMG signals were corrected for the 48 ms delay inherent to the Trigno EMG system.

383

384   **Knee Extension and Flexion Maximal Voluntary Contractions**

385   Following a brief warm-up (3 s contractions at 50% [x3], 75% [x3] and 90% [x1] of

386   perceived maximal), participants performed 3-4 MVCs and were instructed to either 'push as

387   hard as possible' (knee extension) or 'pull as hard as possible' (knee flexion) for 3-5 s and

388   rest $\geq$ 30 s. A horizontal cursor indicating the greatest torque obtained within the session was

389   displayed for biofeedback and verbal encouragement was provided during all MVCs. The

390   highest instantaneous torque recorded during any MVC was defined as MVT. During knee

391   flexor MVCs EMG amplitude was calculated as the root mean square (RMS) of the filtered

392   EMG signal of the BF and ST over a 500 ms epoch at knee flexion MVT (250 ms either side)

393   and averaged across the two muscles to give knee flexor EMG_{MAX}.

394

395   **MRI measurement of Muscle Tendon Unit Morphology and Moment Arm**

396   T1-weighted MR (1.5 T Signa HDxt, GE) images of the dominant leg (thigh and knee) were

397   acquired in the supine position at a knee angle of 163° (due to constraints in knee coil size)

398   and analysed using OsiriX software (Version 6.0, Pixmeo, Geneva, Switzerland). Using a

399   receiver 8-channel whole body coil, axial images (time of repetition/time to echo 550/14,

400   image matrix 512 x 512, field of view 260 x 260 mm, pixel size 0.508 x 0.508 mm, slice

401   thickness 5 mm, inter-slice gap 0 mm) were acquired from the anterior superior iliac spine to

402   the knee joint space in two overlapping blocks. Oil filled capsules placed on the lateral side

403   of the thigh allowed alignment of the blocks during analysis. The quadriceps femoris (QF)

404   muscles (vastus lateralis [VL] vastus intermedius [VI], vastus medialis, and rectus femoris)

405   were manually outlined in every third image (i.e. every 1.5 cm) starting from the most

406   proximal image in which the muscle appeared. The volume of each muscle was calculated

407   using cubic spline interpolation (GraphPad Prism 6, GraphPad Software, Inc.). Total QF

408   volume (QUADSvol) was the sum of the individual muscle volumes.

409

410   As previously described, the deep aponeurosis of the vastus lateralis muscle was defined as

411   the visible dark black segment between the VL and VI muscles in the thigh MRI images.[32]

412   VL aponeurosis width was defined as the transverse length (cm) of the deep aponeurosis

413   (distinct black segment) between the vastus lateralis and vastus intermedius, traced manually

414   on every third image (i.e. every 1.5 cm), starting in the most distal image where the

415   aponeurosis was visible. Aponeurosis width measures were plotted against the longitudinal

416   aponeurosis length (distance between most proximal and distal image where the aponeurosis

417   was visible [cm]). The surface area of VL aponeurosis was calculated as the area under a

418   spline curve fitted to the aponeurosis width and length plot, and termed VL aponeurosis area

419   (Figure 5).

420

421   Immediately after thigh imaging, a lower extremity knee coil was used to acquire axial (time

422   of repetition/time to echo 510/14, image matrix 512 x 512, field of view 160 x 160 mm, pixel

423   size 0.313 x 0.313, slice thickness 2 mm, inter-slice gap 0 mm) and sagittal images (time of

424   repetition/time to echo 480/14, image matrix 512 x 512, field of view 160 x 160 mm, pixel

425   size 0.313 x 0.313, slice thickness 2 mm, inter-slice gap 0 mm) of the knee joint. Contiguous

426   axial images spanned patellar tendon length, which during analysis, were reconstructed to be

427   aligned perpendicular to the line of action of the patellar tendon: straight line from the

428   tendons posterior fibres insertion at the patellar apex to the posterior fibres tibial insertion.

429   Images spanned from 2 cm superior to the patellar apex to 2 cm inferior to the tendon tibial

430   insertion Patellar tendon CSA (mm^2) was measured on each contiguous image along the

431   tendons length (first image where the patellar was no longer visible to the last image before

432   the tibial insertion). Images, viewed in greyscale, were sharpened and the perimeter manually

433   outlined. A spline curve was fitted to the tendon CSA values from each image and the

434   average of the spline equated to mean patellar tendon CSA (patellar tendon mean CSA). The

435   average of the spline CSA's measured over proximal, middle and distal thirds was defined as

436   proximal, mid and distal patellar tendon region CSA. Sagittal plane images were used to

437   determined patellar tendon moment arm, the perpendicular distance from the patellar tendon

438   line of action to the tibio-femoral contact point, which was the midpoint of the distance

439   between the tibio-femoral contact points of the medial and lateral femoral condyles.

440

441   **Ramp Contractions for Determination of Tissue Stiffness**

442   Tissue stiffness was derived from synchronous recordings of torque and tissue elongation

443   (see below, corrected for passive tissue displacement via video recording of knee joint

444   changes) during isometric knee extension ramp contractions. Participants completed two sub-

445   maximal (~75% MVT) practice ramp contractions prior to five maximal attempts with 90 s

446   rest between contractions. Prior to each ramp contraction participants were shown a target

447   torque-time trace on a computer monitor that increased at a constant gradient (50 $Nm.s^{-1}$

448   loading rate) from zero up to MVT. They were instructed to match the target trace as closely

449   as possible for as long as possible (i.e. up to MVT), and real-time torque was displayed over

450   the target torque-time trace for feedback. The preceding knee extensor MVCs and sub-

451 maximal contractions were considered sufficient to elicit tissue preconditioning. The three

452 most suitable ramp contractions, according to highest peak torque, the closeness to the target

453 loading rate and ultrasound image clarity, were analysed and measurements averaged across

454 these three contractions.

455

456 **Measurement of Tissue Elongation**

457 Video images from two ultrasound machines and one video camera were captured to obtain

458 tissue and knee joint displacements during ramp contractions. An ultrasound probe (7.5 MHz

459 linear array transducer, B-mode, scanning width 60mm and depth 50 mm; Toshiba Power

460 Vision 6000, SSA-370A: Otawara-Shi, Japan) was fitted into a custom made high-density

461 foam cast that was strapped to the lateral aspect of the thigh with the mid-point of the probe

462 positioned at ~50 % thigh length. The probe was aligned so the fascicles inserting into the

463 vastus lateralis (VL) muscle deep aponeurosis could be visualized at rest and during

464 contraction. An echo-absorptive marker (multiple layers of transpore medical tape) was

465 placed beneath the ultrasound probe to provide a reference for any probe movement over the

466 skin. Another ultrasound probe (5-10 MHz linear array transducer, B-mode, scanning width

467 92 mm and depth 65 mm, EUP-L53L; Hitachi EUB-8500) was fitted into a custom made

468 high-density foam cast that was held firmly over the anterior aspect of the knee with the

469 probe aligned longitudinal to the patellar tendon such that the patellar apex and insertion of

470 the posterior tendon fibres at the tibia could be visualized at rest and throughout the

471 contraction. The ultrasound machines were interfaced with the computer collecting torque

472 data in Spike 2 and the video feeds were recorded synchronously with torque using Spike 2

473 video capture at 25 Hz. During off-line analysis tissue elongation was tracked frame-by-

474 frame using public-domain (www.cabrillo.edu/~dbrown/tracker) semi-automatic video

475 analysis software: Tracker, version 4.86. The distance measured over the surface of the skin

between the echo-absorptive marker on the VL and the tibial tuberosity defined resting

tendon-aponeurosis complex length. VL fascicle deep aponeurosis cross point displacement

relative to the skin marker provided a measure of tendon-aponeurosis elongation. Patellar

tendon elongation was determined by the longitudinal displacement of the patella apex and

the tendon tibial insertion. The distal insertion of the patellar tendon was not monitored for

the purpose of estimating overall tendon-aponeurosis displacement. To enable correction of

tissue displacement due to joint angle changes during ramp contractions individual ratios of

tissue displacement relative to joint angular displacement (mm/°) were obtained from passive

movements (i.e. plotting the tissue displacement-knee joint angle relationship). This ratio was

used to determine tissue displacement resulting from knee angle change during ramp

contractions, which was subsequently subtracted from total measured displacement.

Corrections were only applied to aponeurosis displacement. Tendon elongation under passive

conditions was deemed negligible. Passive movements were conducted prior to the ramp

contractions. Participants were instructed to completely relax as their knee was moved

through 90 to 130°. During passive movements and ramp contractions, knee joint angle

(angle between visible markers placed on the greater trochanter, lateral knee joint space and

lateral malleolus) was derived from sagittal plane video recorded using a camera mounted on

a tripod positioned (1.5 m) perpendicular to the strength-testing chair. The video camera was

interfaced with a computer and recorded using spike 2 video capture at 25 Hz

(simultaneously with force, EMG, and ultrasound images during the ramp contractions) and

analysed via Tracker software.

Calculation of Tendon Force

Patellar tendon force was calculated by dividing external absolute knee extensor torque by

the patellar tendon moment arm length. Direct measures of moment arm where acquired at

501   rest from MRI images as indicated above (*MRI measurement*). Due to constraints in the size

502   of the knee coil, sagittal images were acquired in an extended knee position (~163°).

503   Moment arm length for any specific knee angle measured at rest or during ramp contraction

504   was estimated from previously published data fitted with a quadratic function[64] scaled to each

505   participant's measured moment arm length at 163°. Absolute internal knee extensor torque

506   was given by summing net knee extension torque and the estimated knee flexor co-

507   contraction torque. Antagonist knee flexor torque was estimated by expressing the average

508   knee flexor EMG amplitude (RMS 50 ms moving window) during ramp contractions relative

509   to the knee flexor EMG_{MAX} and multiplying by the knee flexor MVT (assuming a linear

510   relationship between EMG amplitude and torque). During analysis, torque and EMG

511   amplitude were down-sampled to 25 Hz to match the ultrasound video frequency.

512

513   **Calculation of Tissue Stiffness and Tendon Young's Modulus**

514   For each of the three best ramp contractions analysed, tendon-aponeurosis (corrected for

515   passive tissue displacement) and patellar tendon elongation was plotted against total tendon

516   force (corrected for antagonist force). Force-elongation plots were fitted with a second-order

517   polynomial. Tendon-aponeurosis and patellar tendon stiffness was calculated as the gradient

518   (Δ tendon force [N]/Δ elongation [mm]; $N.mm^{-1}$) of the respective force-strain curve over 80-

519   100% (3360-4200N) of an absolute tendon force (4200N) that corresponded to the lowest

520   common force level attained by all participants during ramp contractions. Tendon stress was

521   obtained by dividing tendon force by mean tendon CSA. Tendon strain was the percentage

522   tendon displacement relative to the resting tendon length. Resting PT length was defined as

523   the distance between the patella apex and tibial insertion as measured prior to the ramp

524   contractions. A patellar tendon stress-strain curve was plotted and patellar tendon Young's

525   modulus (GPa) calculated as the slope (Δ tendon stress [MPa]/Δ tendon strain [%]) of the

526   stress-strain curve derived over 80-100% of an absolute common stress (40 MPa). The

527   stiffness and Young's modulus measures derived from each of the three ramp contractions

528   analysed was averaged to give each individuals representative values.

529

530   **Reproducibility and Statistical Analysis**

531   The reproducibility of tendinous tissue measurements over the duplicate test sessions was

532   calculated for the whole cohort (test 1 vs test 2) as within participant co-efficient of variation

533   (CV_w, %; [SD/mean]*100): elongation [0-4200 N] of the patellar tendon (7.9%) and tendon-

534   aponeurosis complex (9.6%); stiffness [3360-4200 N] of the patellar tendon (9.9%) and

535   tendon-aponeurosis complex (8.6%); patellar tendon strain (8.0%) and Young's modulus

536   (9.0%). Patellar tendon CSA measurements were highly reproducible, as indicated by the co-

537   efficient of variation (CV_W) of repeat measurements 12 weeks apart for a sub-sample of the

538   untrained control group (n=14): mean (2.7%), proximal (3.0%), mid (3.1%) and distal

539   (3.5%).

540

541   Muscle strength and tissue mechancial/material properties measured during the duplicate

542   laboratory sessions were averaged to produce criterion values for statistical analysis. An a

543   priori significance level of P<0.05 was set for all statistical tests which were performed using

544   SPSS Version 20.0 (IBM Corp., Armonk, NY). Descriptive data are presented as mean ±

545   standard deviation (SD) and percentage differences in the group means are given in the text.

546   The influence of group (UC, STT, LTT) on all muscle and tendinous tissue variables was

547   examined by univariate ANOVA. Main group effects were followed by least significant

548   difference (LSD) post-hoc paired comparisons to delineate between group differences; Holm-

549   Bonferroni corrections were applied to LSD P-values, and between group Hedges g effect

550 size (ES) was calculated.[65] Effect size magnitude was classified as <0.2= "trivial"; 0.2-0.6 =

551 "small"; >0-6-1.2 = "moderate"; >1.2-2.0 = "large"; >2.0 = "very large".[66]

552

Competing Interests

557 None to declare

560

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743

744

Physiological Relevance

The potential capacity for tendinous tissue to adapt to long-term functional overload via heavy resistance training is unclear. Our cross-sectional data show that short-term (12 weeks) and long-term (~ 4 years) resistance trained males had similar patellar tendon mechanical and material (Young's modulus) stiffness, with both resistance-trained groups having stiffer tendons that untrained controls. In contrary, neither resistance-trained group had larger tendon size. Furthermore, no differences in tendon-aponeurosis complex stiffness were observed between groups, despite substantially greater muscle strength and size in the long-term resistance trained group, which was also accompanied by a larger muscle-aponeurosis size.

Table 1. Descriptive characteristics of the participants.

	UC	STT	LTT	ANOVA (P)
Sample size, n =	39	15	16	
Age, years	25 ± 2	25 ± 2	22 ± 2	<0.001
Height, cm	176 ± 6	175 ± 8	183 ± 6	0.001
Body mass, kg	72 ± 9	70 ± 9	90 ± 10	<0.001
Tendon-aponeurosis complex length, mm	336 ± 16	328 ± 17	358 ± 18	<0.001
Patellar tendon length, mm	47.7 ± 5.5	45.1 ± 5.5	52.1 ± 5.9	0.005
Patellar tendon moment arm, mm	43.8 ± 2.7	44.8 ± 3.1	45.8 ± 2.5	0.054

770   Data are mean ± SD.

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Table 2. Regional patellar tendon cross-sectional area (mm^2).

Region	UC (n=39)	STT (n=15)	LTT (n=16)	ANOVA (P)
Proximal	93.0 ± 9.9	92.0 ± 13.5	98.4 ± 13.1	0.216
Mid	104.4 ± 12.6	97.0 ± 14.3	104.9 ± 13.3	0.146
Distal	110.4 ± 17.9	101.6 ± 14.3	112.1 ± 15.7	0.141

Data are mean ± SD.

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805 **Table 3.** Summary of group differences in strength (maximal voluntary torque, MVT), muscle tendon

806 unit size and tissue (tendon-aponeurosis complex and patellar tendon [PT]) stiffness between long-

807 term resistance trained (LTT), short-term resistance trained (STT) and untrained control (UC) groups.

Function	
MVT, Nm	LTT > STT > UC
Muscle-Tendon Unit size	
QUADSvol, cm^3	LTT > STT & UC
VL Aponeurosis Area, cm^2	LTT > STT & UC
PT CSA, mm^2	-
Indices of Tissue Stiffness	
PT elongation (0-4200 N), mm	LTT < STT & UC
PT stiffness (3360-4200 N), N.mm^{-1}	LTT & STT > UC
PT strain (0-40 MPa), %	LTT < STT & UC
PT Young's modulus (32-40 MPa), GPa	LTT & STT > UC
Tendon-aponeurosis elongation (0-4200 N), mm	-
Tendon-aponeurosis stiffness (3360-4200 N), N.mm^{-1}	-

808   Significant group differences groups: greater (>) or less (<) than. No

809   difference (-). PT CSA: both mean and regional CSA measures.

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819   **Figure Legends**

820   **Figure 1.** Group comparisons: Isometric knee extension maximal voluntary torque (a),

821   Quadriceps femoris muscle volume (QUADSvol, b), Vastus Lateralis (VL) aponeurosis area

822   (c) and Patellar tendon mean cross-sectional area (CSA, d) for untrained control (UC, n =

823   39), short-term resistance trained (STT, n = 15) and long-term resistance trained (LTT, n =

824   16) groups. Data are mean ± SD. Bold numbers are between groups hedges g effect size.

825   Post-hoc tests: Least significant difference Holm-Bonferroni corrected P-values. *P<0.05,

826   †P<0.01, ‡P<0.001.

827

828   **Figure 2. (a)** Relationships between patellar tendon force (N) and elongation (mm) in the

829   untrained control (UC, n = 37), short-term resistance trained (STT, n = 15) and long-term

830   resistance trained (LTT, n = 15) groups. Curves show the group mean relationship. Data

831   points correspond to within group average values for the elongation at 10% intervals of group

832   mean maximal voluntary tendon force, plotted up to 80% (highest common level achieved

833   during ramp contractions). Error bars indicate the within-group standard deviation for force

834   (y-axis bar) and elongation (x-axis bar). **(b) And (c)** Group comparisons of the patellar

835   tendon elongation at the common force level of 4200 N, and patellar tendon stiffness

836   (gradient of curves in **a** over 80-100% of the highest common force level [3360-4200 N]).

837   Bars are mean ± SD. Bold numbers are the between groups hedges g effect size. Post-hoc

838   tests: Least significant difference Holm-Bonferroni corrected P-values. *P<0.05, ‡P<0.001.

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840   **Figure 3. (a)** Relationships between patellar tendon stress (MPa [$N.mm^2$]) and strain (%) in

841   the untrained control (UC, n = 37), short-term resistance trained (STT, n = 15) and long-term

842   resistance trained (LTT, n = 15) groups. Curves show the group mean relationship. Data

843   points correspond to within group average values for the strain at 10% intervals of group

844 mean maximal voluntary tendon stress, plotted up to 80% (highest common level achieved

845 during ramp contractions). Error bars indicate the within-group standard deviation for stress

846 (y-axis bar) and strain (x-axis bar). **(b) And (c)** Group comparisons of the PT strain at the

847 common stress level of 40 MPa, and PT Young's modulus (gradient of curves in **a** over 80-

848 100% common stress level [32-40 MPa]). Bars are mean ± SD. Bold numbers are the

849 between groups hedges g effect size. Post-hoc tests: Least significant difference Holm-

850 Bonferroni corrected P-values. †P<0.01.

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852 **Figure 4. (a)** Relationships between patellar tendon force (N) and tendon-aponeurosis

853 complex elongation (mm) in the untrained control (UC, n = 37), short-term resistance trained

854 (STT, n = 14) and long-term resistance trained (LTT, n = 16) groups. Curves show the group

855 mean relationship. Data points correspond to within group average values for the strain at

856 10% intervals of group mean maximal voluntary tendon force, plotted up to 80% (highest

857 common level achieved during ramp contractions). Error bars indicate the within-group

858 standard deviation for force (y-axis bar) and strain (x-axis bar). **(b) And (c)** Group

859 comparisons of the tendon-aponeurosis complex elongation at the common force level of

860 4200 N, and tendon-aponeurosis complex stiffness (gradient of curves in **a** over 80-100%

861 common force level [3360-4200 N]). Bars are mean ± SD.

862

863 **Figure 5**. Measurement of the surface area of the vastus lateralis deep aponeurosis. On axial

864 magnetic resonance images at 1.5 cm intervals along the longitudinal aponeurosis length:

865 distance from the most distal image to the most proximal where the aponeurosis was visible

866 (a), the transverse length of the distinct visible black segment between the vastus lateralis

867 (VL) and vastus intermedius (VI) muscles was traced manually and defined as aponeurosis

868 width (b). The aponeurosis width measures on each image were plotted against aponeurosis

869   length and the area under a cubic spline curve fitted through the data points defined the VL

870   aponeurosis area (c).

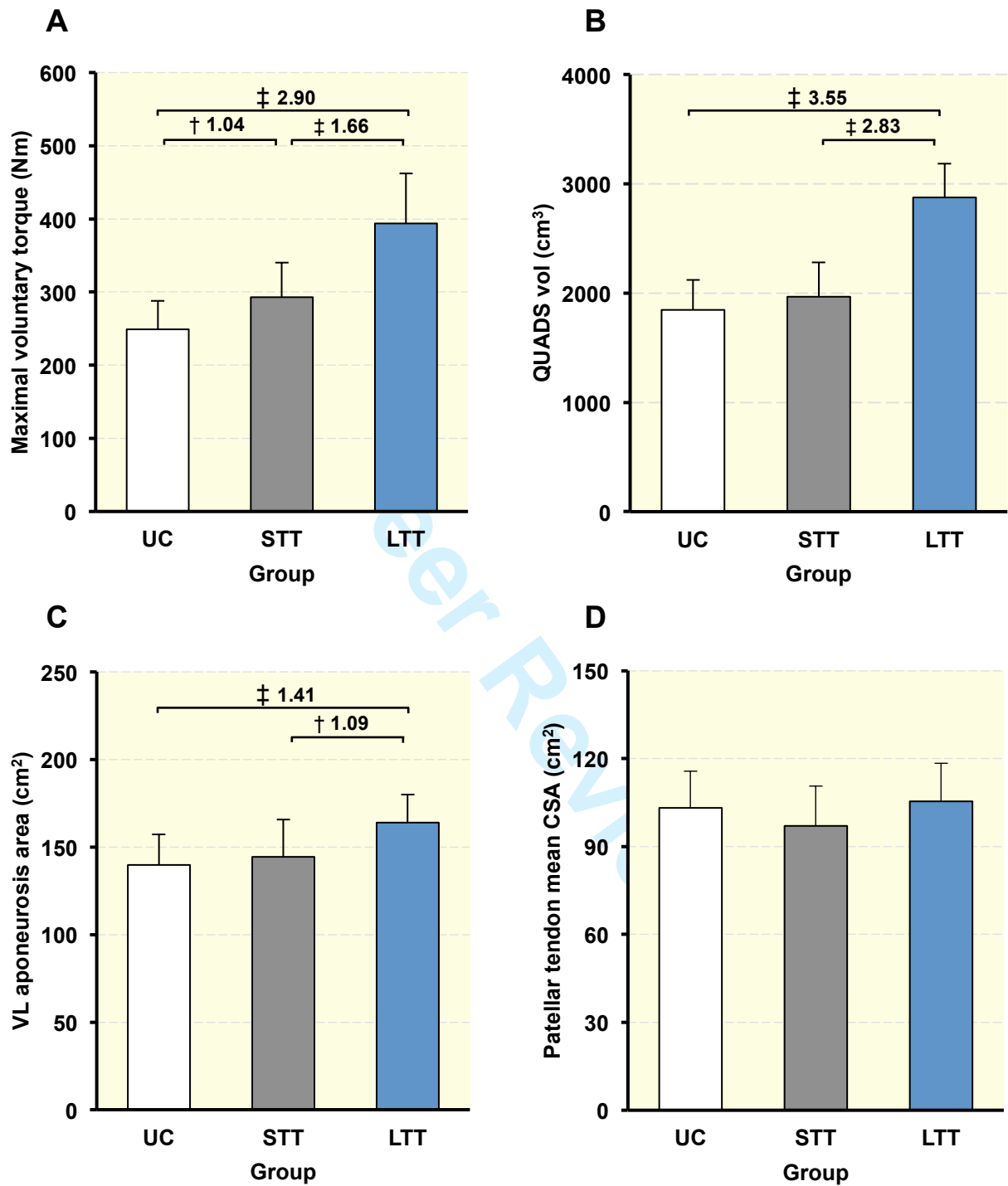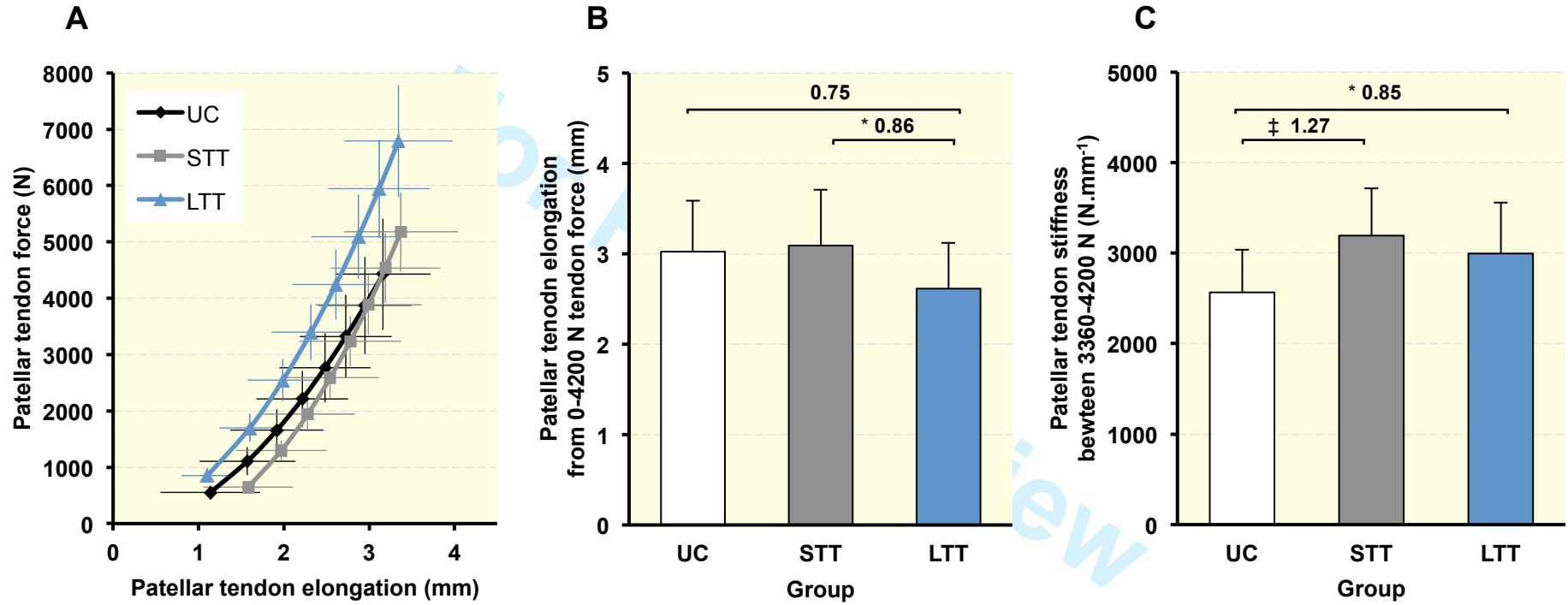**Figure 1.**

Figure 2.

Figure 3.

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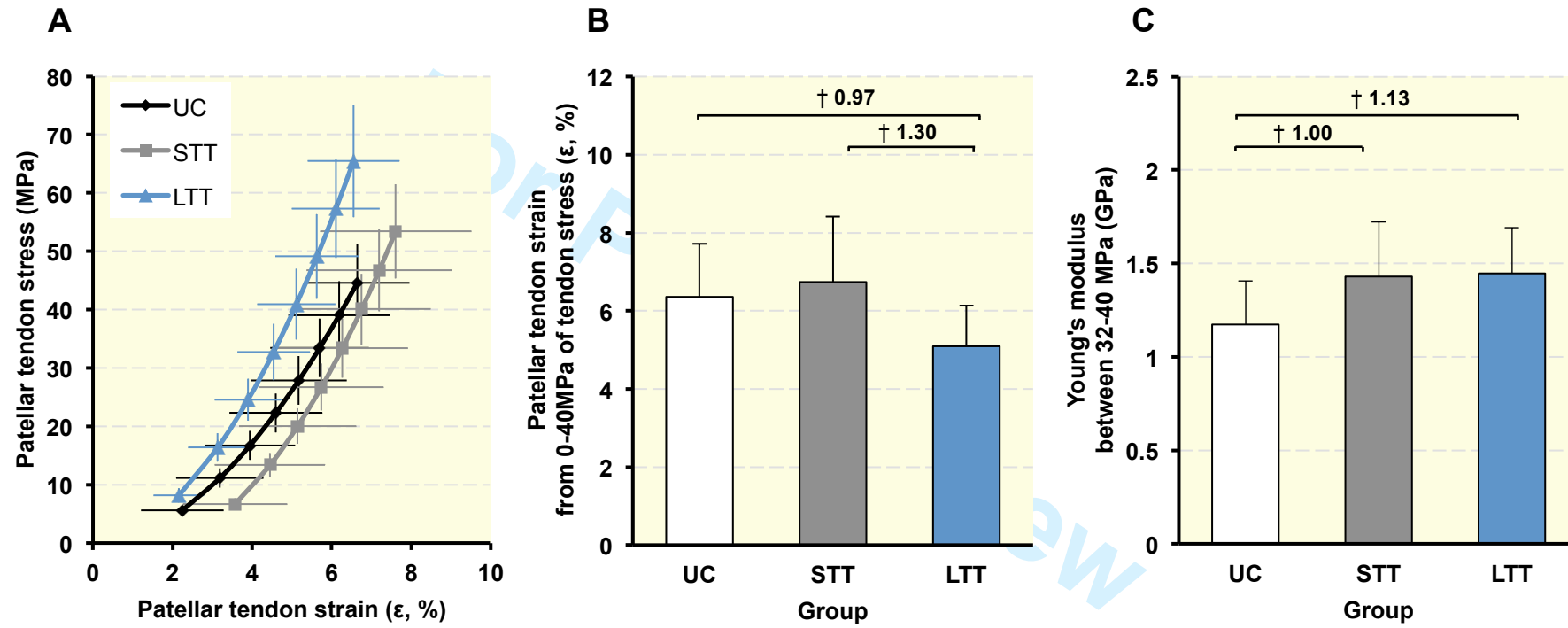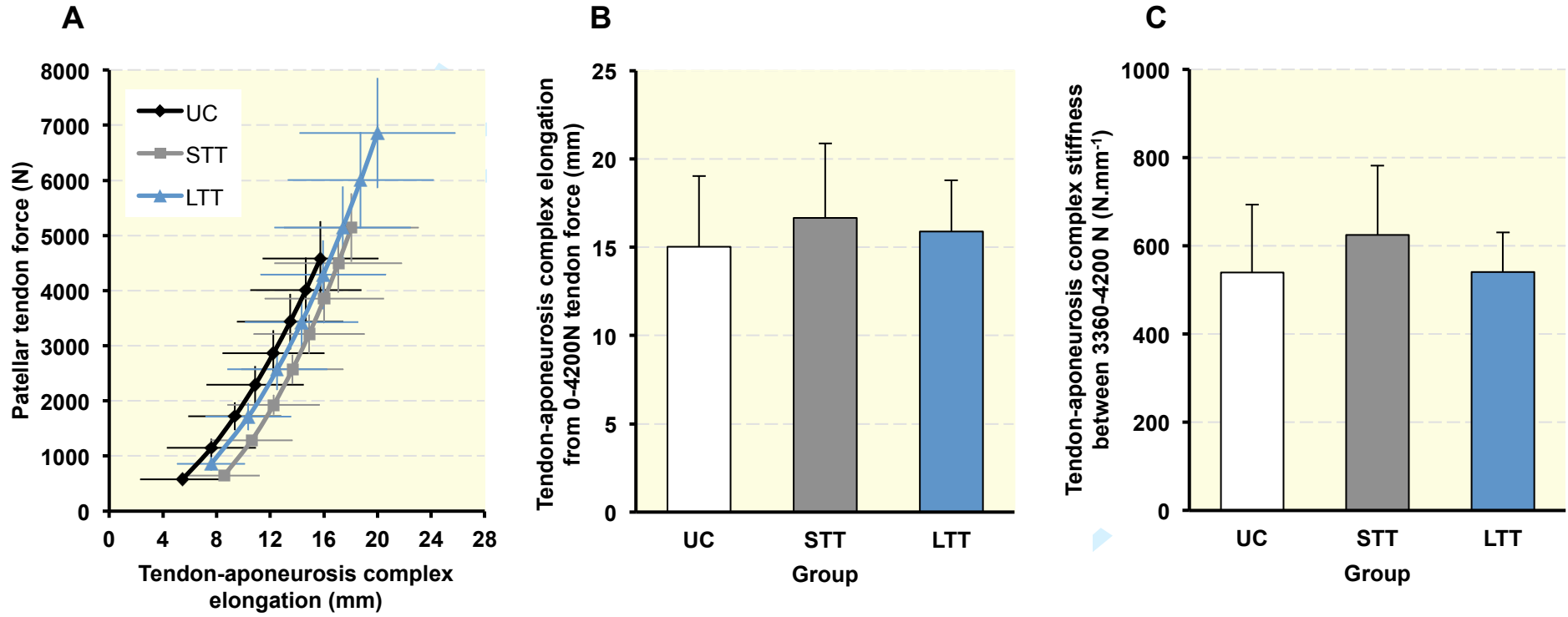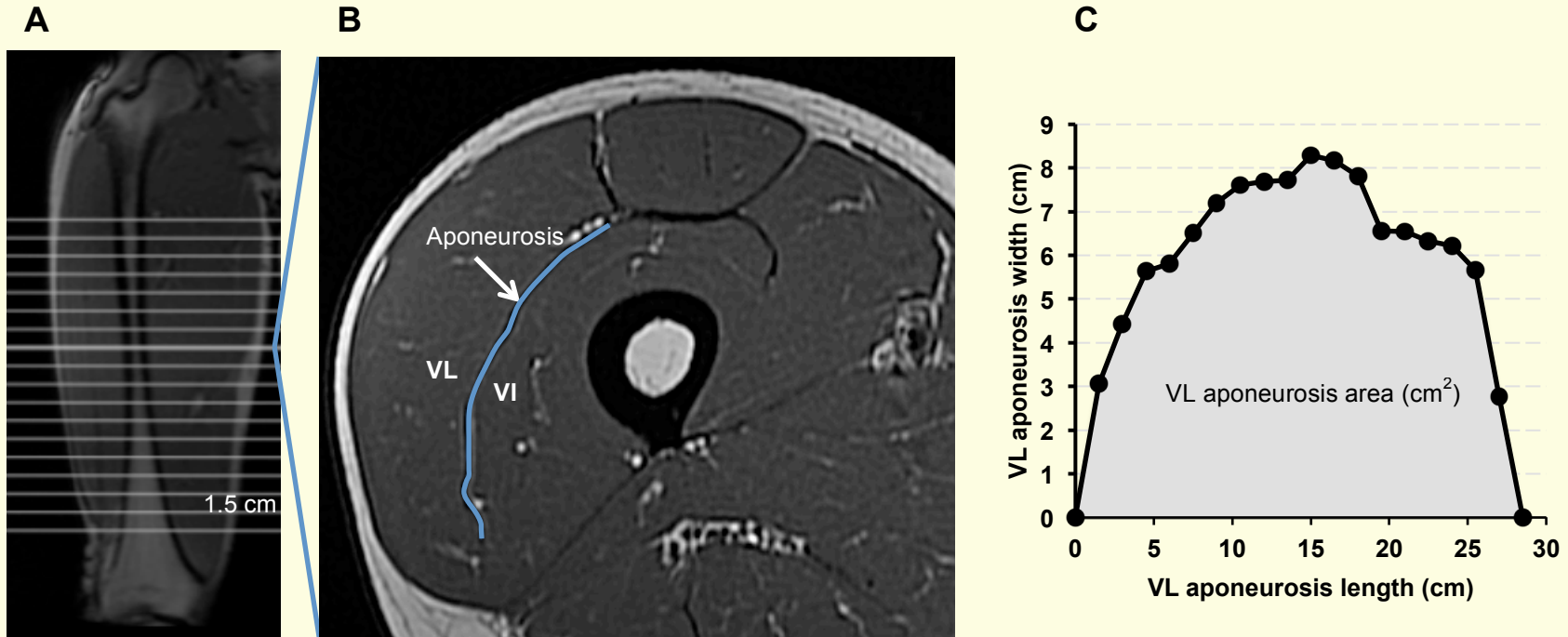40
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Figure 4.

A, B, C

Figure 5.

A

B

Aponeurosis

VL

VI

1.5 cm

C

VL aponeurosis area (cm^2)

VL aponeurosis width (cm)

VL aponeurosis length (cm)