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1 **Triceps surae muscle-tendon properties in older endurance- and sprint-**

2 **trained athletes**

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11   Running head: Muscle-tendon properties in older athletes

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Abstract

Previous studies have shown that aging is associated with alterations in muscle architecture and tendon properties. However, the possible influence of different types of regular exercise loading on muscle architecture and tendon properties in older adults is poorly understood. To address this, triceps surae muscle-tendon properties were examined in older male endurance (OE, n=10, age=74.0±2.8) and sprint runners (OS, n=10, age=74.4±2.8) with an average of 42 years of regular training experience, and compared to age-matched (OC, n=33, age=74.8±3.6) and young untrained controls (YC, n=18, age=23.7±2.0).

Compared to YC, Achilles tendon cross-sectional area (CSA) was 22% (p=0.022), 45 % (p=0.001) and 71% (p<0.001) larger in OC, OE and OS, respectively. Among older groups, OS had significantly larger tendon CSA compared to OC (p=0.033). No significant between-group differences were observed in Achilles tendon stiffness. In older groups, Young's modulus was 31-44% and maximal tendon stress 44-55% lower than in YC (p≤0.001). OE showed shorter soleus fascicle length than both OC (p<0.05) and YC (p<0.05).

These data suggest that long-term running does not counteract the previously reported age-related increase in tendon CSA, but instead, may have an additive effect. The greatest Achilles tendon CSA was observed in sprinters followed by endurance runners and older controls, suggesting that adaptation to running exercise is loading intensity dependent. Achilles tendon stiffness was maintained in older groups even though all older groups displayed larger tendon CSA and lower tendon Young's modulus. Shorter soleus muscle fascicles in older endurance runners may be an adaptation to life-long endurance running.

Keywords: Achilles tendon, mechanical properties, muscle architecture, aging, exercise

Introduction

Loss of muscle function with aging is associated with physical limitations and disability (40). Decline in muscle mass is undoubtedly an important contributor to the deterioration in muscle function with aging (16). However, longitudinal studies have shown a clear dissociation in loss of muscle function and cross-sectional area or mass with aging (9, 17), suggesting that other factors may also contribute to the age-related loss of muscle function. Muscle architecture and tendon mechanical properties greatly affect muscle performance (28, 51) and have been found to differ between young and old sedentary adults (36, 37, 44). Thus, age-related alterations in muscle architecture and tendon mechanical properties may partially explain the loss of muscle performance with age that occurs at a disproportionally faster rate than the decline in muscle mass.

Regular exercise is a key aspect supporting healthy aging. Indeed, it has been suggested that older athletes provide a model of exceptionally successful biological aging (46). For example, previous studies have shown that aged athletes with systematic exercise training habits exhibit much better cardiorespiratory, metabolic and bone health than their less active counterparts (22, 49). Regular exercise training, especially strength and sprint exercise, also helps to maintain muscle mass, function (21, 52) and composition (43), thus counteracting the age-related decline in functional performance typically observed in normal populations (9, 39).

In spite of several known beneficial effects of regular exercise on the musculoskeletal system in old age, little is known about the effects of regular participation in planned exercise on muscle architecture and tendon properties in older adults. Two previous studies have compared untrained older adults to older endurance runners. Firstly, Karamanidis and Arampatzis (19)

58 found that muscle architecture and tendon stiffness in medial gastrocnemius and vastus lateralis

59 were largely similar in older endurance runners compared to untrained older adults. The only

60 significant difference was greater medial gastrocnemius pennation angle in endurance runners.

61 Secondly, Couppé et al. (7) recently found that older endurance runners had a greater patella

62 tendon cross-sectional area but similar tendon stiffness compared to untrained peers. These

63 previous studies were conducted on endurance runners and thus knowledge of the long-term

64 effects of different types of exercise loading on muscle architecture and tendon properties in

65 older adults is missing.

66 Therefore, the aim of this study was to examine the association between different types of life-

67 long exercise and muscle-tendon properties by comparing muscle architecture and tendon

68 properties in older sprint and endurance runners to both age-matched and young untrained adults.

69 Triceps surae muscles were studied because of their important role in locomotion and because

70 they exhibit the greatest functional limitation of all lower limb muscle groups in older adults

71 during locomotion (24). Endurance running provides a model of high volume and moderate

72 intensity loading while sprint running represents a model of low volume but high intensity

73 loading of triceps surae muscles. The hypothesis was that older athletes with a life-long regular

74 running background would exhibit muscle fascicle length, pennation angle, muscle size, muscle

75 strength and tendon mechanical properties in the triceps surae muscle group that are more similar

76 to those of young adults than untrained older adults. In addition, based on previous cross-

77 sectional studies conducted in young adults (1, 2), it was hypothesized that sprint-trained older

78 athletes would be stronger, have stiffer Achilles tendons, lower pennation angle, and longer

79 muscle fascicles compared to endurance-trained older athletes.

Materials and methods

Subjects

Male subjects were recruited in two age categories, one from 18 to 30 years old (untrained young controls, YC, n=18) and the other from 70 to 80 years old. The older cohort was recruited in three groups: untrained older controls (OC, n=33), older athletes competing in endurance running events (OE, n=10) and older athletes competing in sprint running events (OS, n=10).

Untrained young and older control groups were part of a Europe-wide collaborative study called MyoAge (34) and included in the current study to represent general populations of healthy young and older adults. We defined untrained as a person who may be recreationally active but is not training for, or participating in, competitive sport. YC were recruited from among university students using study advertisements via e-mail and bulletin boards. We excluded those who studied sport sciences as well as competitive athletes. OC were recruited from the University of the Third Age or from weekly community meetings of retired people. The aim was to recruit healthy older people who were socially active and free from comorbidity. Using telephone interviews, an equal number of sedentary and physically active (competitive athletes excluded) older subjects were recruited to obtain a representative sample of older people with varying physical activity levels. Sedentariness was defined as exercising for fitness and health one or fewer times per week. Physically active was defined as exercise three or more times per week (30 min or more with intensity sufficient to cause sweating or breathlessness). Results (44) and more detailed description of the recruitment (34) of YC and OC have been presented earlier.

Older athletes were recruited among the participants of the World Master Athletics Indoor Championships held in Jyväskylä, Finland in 2012. Twenty male athletes were recruited based

102 on the events they participated in during the championships. Ten subjects were recruited from

103 sprint running events (60 m, 60 m hurdles, 200 m, 400 m) and 10 were recruited from endurance

104 running events (3 km, ½ marathon and 8 km cross country running). Some subjects in the OS

105 and OE groups participated in several sprint or endurance running events, respectively. Mean

106 results of the subjects competing in the championships were: 60 m 9.13 ± 0.48 (sec, n=8), 60 m

107 hurdles 10.15 (sec, n=1), 200 m 30.64 ± 1.97 (sec, n=7), 400 m 1:13 ± 8 (min:sec, n=3), 3000 m

108 13:48 ± 60 (min:sec, n=4), ½ marathon 1:43:37 ± 11:34 (hr:min:sec, n=5) and 8 km cross

109 country running 44:19 ± 6:25 (min:sec, n=7). These results correspond to 8, 1, 11, 16, 22 and 16

110 % slower than the world record times for 75 year old men in 60 m, 60 m hurdles, 200 m, 400 m,

111 3000 m and ½ marathon respectively. Thus, the participating subjects can be considered to be

112 highly competitive athletes.

113 Subject exclusion criteria were Achilles tendon pain, history of Achilles tendon rupture or

114 surgery, pain in calf muscles during measurements, neurologic and progressive severe illnesses,

115 insulin treated diabetes, fracture within the previous year, immobilization for one week during

116 the last three months, daily use of painkillers, use of immunosuppressive drugs or anticoagulants,

117 or severe visual or hearing impairment.

118 The ethics committee of the Central Finland Health Care District approved the study. All

119 participants signed an informed consent prior to participating in the study and measurements

120 were conducted according to the standards set by the latest revision of the Declaration of

121 Helsinki.

Measurements

Training characteristics of OE and OS groups were assessed with self-reported questionnaire. The athletes were asked about their training history (yr.), overall training volume (h/wk.) and amount of endurance (km/wk.), sprint (sessions/wk.) and strength training (sessions/wk.) in their current normal training routines.

Laboratory measurements included assessment of triceps surae muscle architecture and size and Achilles tendon cross-sectional area and mechanical properties. The measurement procedures have been previously described in detail (44) but are briefly described below.

For the measurements of Achilles tendon and both gastrocnemius and soleus muscle architecture and size at rest, the subjects were lying prone facing down with ankle angle at 90°. Tendon cross-sectional area (mm^2) was measured from a B-mode ultrasound image taken 4 cm proximal from the proximal border of the calcaneal tubercle where the free Achilles tendon typically reaches its smallest cross-sectional area (38). Body mass normalized tendon cross-sectional area was calculated by dividing cross-sectional area by body mass$^{2/3}$ (20). Muscle architecture from medial gastrocnemius and soleus muscles was assessed from ultrasound images taken at 50% of medial gastrocnemius length and mid-muscle belly in the medial-lateral direction. Fascicle length (mm), pennation angle (°) and muscle thickness (mm) were measured from the images. In order to take into account between-subject differences in stature, fascicle length was normalized to tibia length. The combined anatomical cross-sectional area (cm^2) of medial and lateral gastrocnemius was measured from a panoramic B-mode ultrasound image taken at 50% of medial gastrocnemius length as a measure of the size of the gastrocnemius muscles. All measurements from ultrasound images were taken twice using an open source computer program

144   (ImageJ 1.44b, National Institutes of Health, US) and the mean was used for subsequent data

145   analysis.

146   For the measurement of Achilles tendon mechanical properties, the subjects were seated in a

147   custom built dynamometer with ankle angle at 90°, knee fully extended and hip at 60° of flexion

148   (full extension 0°). After a standardized warm-up, three maximal voluntary contractions (MVC)

149   lasting approximately 3 seconds were performed with strong verbal encouragement to measure

150   plantar flexion strength (Nm). The highest value obtained during MVC trials was used for

151   subsequent analysis. Warm-up and plantar flexion MVCs served to precondition the tendon

152   before the measurement of tendon mechanical properties (30). Achilles tendon mechanical

153   properties were measured from several isometric plantar flexion contractions up to a force level

154   of 80% of MVC. Tendon force was calculated by multiplying measured reaction force by the

155   ratio between Achilles tendon moment arm length and moment arm of the reaction force.

156   Achilles tendon moment arm was defined as the distance from the center of the Achilles tendon

157   to the outermost tip of the medial malleolus in the sagittal plane measured using a ruler. The

158   moment arm of the reaction force around the ankle joint was defined as the sagittal plane

159   distance between the outermost tip of the medial malleolus and the head of the first metatarsal.

160   Achilles tendon elongation (mm) was defined as the change in the distance between the proximal

161   border of the calcaneal tubercle and the medial gastrocnemius muscle-tendon junction. Changes

162   in the location of the calcaneal tubercle in the laboratory coordinate system were measured using

163   a potentiometer that measures heel lift from the dynamometer footplate. Medial gastrocnemius

164   muscle-tendon junction location in the laboratory coordinate system was measured with a

165   combination of B-mode ultrasonography and motion analysis. Ultrasound images of the muscle-

166   tendon junction were collected at 70 Hz and the location of the muscle tendon junction within the

167 image was defined by automatic tracking software (32). The location of the muscle-tendon

168 junction was converted to the laboratory coordinate system using video based motion capture of

169 the ultrasound probe. Two parameters that describe tendon mechanical properties were

170 calculated, tendon stiffness (N/mm) and Young's modulus (GPa). Tendon stiffness characterizes

171 mechanical properties of the tendon and is defined as the slope of the linear portion of the tendon

172 force-elongation curve. We calculated tendon stiffness as a linear fit to force-elongation data

173 from 10 to 80% MVC force since the curves were almost perfectly linear in this region (Fig. 1,

174 $r^2=0.999$ from linear fits to average force-elongation curves). Tendon Young's modulus is the

175 slope of the linear portion of the tendon stress-strain curve and represents tendon stiffness

176 normalized to tendon dimensions. Young's modulus describes the mechanical properties of the

177 material from which a tendon is composed. To derive Young's modulus, Achilles tendon stress

178 (Pa) was calculated by dividing Achilles tendon force (N) by tendon cross-sectional area (m^2)

179 and strain (%) was calculated by dividing elongation (mm) by initial tendon length (mm).

180 Young's modulus was calculated as a linear fit to force-elongation data from 10 to 80% MVC

181 force.

182 **Statistical analyses**

183 Due to inadequate image quality, soleus muscle architecture data were excluded for two subjects

184 from the OS group and three from the OC group, while medial gastrocnemius muscle

185 architecture data were excluded for one subject from OE, and gastrocnemius cross-sectional area

186 data from one subject from OC. Data were first checked for normality with Shapiro-Wilk test

187 and for homogeneity of variance with Levene's test. Differences in muscle and tendon properties

188 between the groups were tested using single factor analysis of variance and Tukey-Kramer post

189 hoc test. Games-Howell post hoc test was used when inhomogeneous variances between the

190   groups were observed and Kruskal-Wallis test with Bonferroni correction for non-normally

191   distributed variables. Differences in training characteristics between OE and OS were tested

192   using Mann-Whitney U-test. The level of statistical significance was set at $\alpha = 0.05$ for all tests.

193   Statistical analyses were performed using IBM SPSS Statistics (version 20.0.0.2). Standardized

194   mean differences between YC and groups of older adults were calculated for main results of the

195   study (Table 2 and 3) as a measure of effect sizes using Hedges' g including a correction for

196   small sample bias (12).

197   **Results**

198   Subject characteristics and training status for the older athletes are reported in Table 1. Older

199   adults in the three different groups were matched for age, height and body mass. YC were

200   significantly taller than OC ($p<0.001$). OC had significantly greater BMI compared to YC

201   ($p=0.006$) and OE ($p=0.009$). Significantly lower plantar flexion strength was found in OC (34%,

202    $p=0.001$) and OE (42%, $p<0.001$) compared to YC but not in OS compared to YC ($p=0.077$). OE

203   and OS groups did not differ in years of training, hours of training per week, or number of

204   strength training sessions per week. Endurance training measured in distance was 8 times greater

205   in OE in comparison with OS ($p<0.001$), and OS did 3 times more sprint training sessions per

206   week than OE ($p=0.006$).

207   Achilles tendon cross-sectional area was 22, 45 and 71% larger in OC ($p=0.022$), OE ($p=0.001$)

208   and OS ($p<0.001$) compared to YC, respectively (Table 2). Tendon cross-sectional area in OS

209   was significantly larger than in OC ($p=0.033$). Body mass normalized tendon cross-sectional area

210   yielded similar results to the unnormalized values. No statistically significant differences were

211 observed between the groups in Achilles tendon stiffness (Figure 1) but Young's modulus was

212 31, 35, and 44% smaller in OC (p<0.001), OE (p=0.001) and OS (p<0.001) in comparison to YC,

213 respectively. Maximal tendon force during MVC was significantly lower in OC (35%, p<0.001)

214 and OE (38%, p<0.001) but not in OS (p=0.156) compared to YC. Average tendon stress during

215 MVC was greater in YC than the older groups (p<0.001). Tendon elongation at 80% MVC was

216 significantly greater in YC compared to OC (p=0.014) but the difference did not reach statistical

217 significance in OE (p=0.114) or OS (p=0.352). However, effect sizes between YC and OE and

218 OS were greater than the effect size between YC and OC. The groups did not differ significantly

219 in tendon strain at 80% MVC.

220 Results of soleus and gastrocnemius muscle architecture and size, as well as plantar flexion

221 muscle strength, are presented in Table 3. Soleus fascicle length was significantly shorter in OE

222 compared to YC (absolute p=0.014, normalized p=0.002) and also compared to OC (absolute

223 p=0.047, normalized p<0.001). No significant differences were found in soleus pennation angle

224 or muscle thickness. Medial gastrocnemius fascicle length and pennation angle did not differ

225 between the groups. In OC, medial gastrocnemius muscle thickness was significantly smaller in

226 contrast to YC (p=0.043) and gastrocnemius cross-sectional area was significantly smaller in

227 contrast to YC (p=0.011) and OS (p=0.011).

228 **Discussion**

229 We examined selected triceps surae muscle-tendon properties of two differently trained groups

230 of older athletes with an average of 42 years of regular running training, and compared them to

231 untrained age-matched older and young adults. The main findings of the study were that Achilles

232   tendon cross-sectional area was significantly larger in all older adult groups than young adults,

233   and in older sprinters compared to age-matched untrained older adults, while there were no

234   statistically significant group differences in Achilles tendon stiffness. The greater tendon cross-

235   sectional area was also reflected in tendon Young's modulus and tendon average tensile stress

236   during maximal isometric force production, both of which were significantly lower in all older

237   groups compared to young untrained adults. Only minor differences were observed in triceps

238   surae muscle architecture, the most important being significantly shorter fascicle length in soleus

239   muscle in older endurance runners. The current study adds new insight into possible effects of

240   exercise loading on muscle and tendon structure and function in older age. The novelty of the

241   current study is that measurements of triceps surae muscle architecture and Achilles tendon

242   properties were made from top-level older athletes that included both endurance and sprint

243   runners.

244   *Achilles tendon properties*

245   To the best of our knowledge, this is the first study to show greater Achilles tendon cross-

246   sectional area in older adults with a regular exercise training background. Contradicting our

247   hypothesis, the results suggest that long-term exercise did not counteract the age-related increase

248   in Achilles tendon cross-sectional area. Previous cross-sectional studies suggest that Achilles

249   tendon cross-sectional area increases in response to both long-term exercise loading (20, 33) and

250   normal aging (31, 44). The present results suggest that the Achilles tendon responds to regular

251   loading by increasing cross-sectional area in an intensity-dependent manner. Moreover, the

252   increase in cross-sectional area appears to be additive to the increase due to normal aging. This

253   finding supports recent findings by Couppé et al. (7), who showed that regular endurance

254 running was associated with larger patella tendon cross-sectional area in both young and older

255 adults.

256 A possible explanation for ageing and exercise training to be associated with larger tendon cross-

257 sectional area is that tendon hypertrophy is needed to compensate for an age-related decrease in

258 mechanical properties of the tendon collagen structure. Another possible explanation is that

259 greater tendon cross-sectional area in older adults is observed as a consequence of intratendinous

260 accumulation of lipids or water. These two possible mechanisms are not exclusive and could

261 together explain the observed results. The following paragraphs introduce these proposed

262 explanations in more detail.

263 In animal models, aging has been linked with an increase in type V collagen and a greater

264 proportion of small collagen fibrils, which probably contribute to concurrently observed reduced

265 ultimate tensile stress (10, 48). Greater tendon cross-sectional area in older adults could be due to

266 a necessary adaptation to reduce maximal tendon stress to safe levels for older tendons that

267 possibly have reduced ultimate tensile stress. To reduce the stress to a safe level, cross-sectional

268 area must be proportional to maximal force acting on the tendon, thus explaining the greater

269 cross-sectional area in older sprint runners compared to older untrained adults observed in the

270 current study.

271 Greater tendon cross-sectional area in older adults could also serve to maintain sufficient

272 stiffness, which could be important both for protecting the tendon from strain-induced damage

273 and for muscle function. A possible age-related reduction in stiffness of tendon collagen

274 structure may be partly compensated by an age-related increase in collagen cross-links,

275 especially in advanced glycation end product cross-links (6), which stabilize collagen structure

276 and may increase tendon stiffness. Life-long endurance running has been shown to be associated

277 with lower advanced glycation end product cross-link density (7). If older athletes in the current

278 study had a lower density of collagen cross-links, this could explain the requirement for older

279 athletes to have even greater tendon cross-sectional area compared to untrained older adults, in

280 order to maintain tendon stiffness with aging.

281 Based on current knowledge of tendon adaptation, loading intensity is the main factor

282 determining adaptations in tendon mechanical properties (5). Thus, it seems unlikely that sprint-

283 trained older athletes would have the lowest Achilles tendon Young's modulus among the groups

284 in the current study. A possible explanation could be that larger tendon cross-sectional area in

285 older adults is not an adaptation to lowered tendon Young's modulus. Instead it could be due to

286 accumulation of tendon subcomponents that do not markedly affect tendon mechanical behavior.

287 These could include extracellular lipid deposits and proteoglycans and glycosaminoglycans that

288 attract water. Extracellular lipid deposits within tendon have been associated with aging (14) and

289 this could be common to all older adults irrespective of exercise training. On the other hand,

290 production of proteoglycans and glycosaminoglycans could be increased with exercise training

291 induced tendon loading (15). This would explain the observed lower Young's modulus and stress

292 of the tendon in older adults in the present study, and also explains why greater tendon cross-

293 sectional area was not related to greater tendon stiffness.

294 Within- and between-operator reliability of Achilles tendon cross-sectional area measurement

295 using ultrasound imaging has been reported to be good (11, 50). In the current study, duplicate

296 analysis of tendon cross-sectional area images produced intraclass correlation 0.989 and typical

297 error 2.1%. However, validity of tendon cross-sectional area measurement using ultrasound

298 imaging is not known, thus the results should be interpreted with some caution. Future studies

299　should try to replicate the findings of the current study, preferably using magnetic resonance

300　imaging, which allows measurements of tendon cross-sectional area along the whole tendon.

301　More research examining tendon composition and collagen structure in older adults is also

302　warranted to explain the mechanisms behind changes in tendon cross-sectional area.

303　In contrast to our hypothesis that life-long running would mitigate age-related changes in tendon

304　mechanical properties, we found that Young's modulus was significantly lower in older

305　compared to young adults irrespective of training status, with no significant differences between

306　the older groups. There were also no significant between-group differences in initial tendon

307　length or tendon stiffness. Thus, the lower Young's modulus in older compared to young adults

308　can be attributed mainly to the larger tendon cross-sectional area in older adults.

309　It should be noted that a toe-region with a lower slope of the tendon force-elongation curve at

310　low forces or stresses was not observed (Figure 1). We think that the reason for highly linear

311　force-elongation/stress-strain curves is initial force acting on the Achilles tendon at a 90° ankle

312　angle, and the fact that we calculated the curve starting from 10% MVC force. Lack of toe-

313　region has also been previously observed for Achilles tendon in vivo when elongation is

314　measured from the medial gastrocnemius muscle-tendon junction (27), as done in the present

315　study.

316　To summarize the findings regarding tendon mechanical properties, Young's modulus of the

317　Achilles tendon was significantly lower in older compared to young adults, irrespective of

318　training status. Despite this, Achilles tendon stiffness was conserved in all groups of older adults.

319　Thus, the lower muscle strength, greater tendon cross-sectional area and conserved tendon

320　stiffness resulted in reduced maximal tendon stress and strain in older adults. Reduced tendon

321 stress and strain could be a necessary mechanism to decrease the probability of tendon injury, as

322 aging may decrease tendon fascicle sliding that possibly leads to greater loading of the fascicles

323 themselves (47). A functional consequence of similar Achilles tendon stiffness but lower muscle

324 strength in older compared to young adults is a limited maximal capacity for elastic energy

325 storage and subsequent utilization during locomotion. This may contribute to the reported greater

326 metabolic cost of transport in older compared to young adults (35).

327 *Triceps surae muscle architecture, size and strength*

328 The present data also suggest that, in general, muscle architecture is not greatly different in older

329 habitual runners in contrast to both untrained older or young adults. Soleus fascicle length was

330 found to be significantly shorter in endurance-trained older adults than young and older

331 untrained adults. Although somewhat speculative, it may be that shorter fascicles observed in

332 long-term endurance runners in the current study are due to adaptation that improves the

333 efficiency of force production in locomotion. Soleus has short muscle fascicles compared to

334 tendon length (51). Consequently, soleus muscle operates mainly as a force rather than a power

335 producer in locomotion (4). Thus, as this muscle does not need to produce large amounts of

336 work, short fascicles may decrease the energy cost of force production due to lower activated

337 muscle volume per unit of force output compared to longer fascicles (29). We recently observed

338 that shorter fascicle length in soleus and gastrocnemius was associated with better mobility in

339 older adults (45), further supporting the suggestion that shorter soleus fascicle length in older

340 endurance runners may be an adaptive response to life-long exercise training.

341 Another finding of the present study is that long-term endurance running was not associated with

342 greater strength or size of triceps surae muscles compared to untrained older controls. Plantar

343　flexion strength and maximal tendon force in endurance-trained older adults was significantly

344　lower in comparison to young adults. Moreover, the effect sizes for the difference in

345　gastrocnemius thickness and cross-sectional area were comparable to those between young and

346　older controls, which were also statistically significant. Taken together, these results suggest that

347　endurance running is not a sufficient stimulus for maintenance of muscle mass and size with

348　aging.

349　In contrast, the current data suggest that high intensity loading due to sprint training may be an

350　effective stimulus to counteract the age-related decline in both muscle mass and strength in

351　triceps surae muscles. We observed that gastrocnemius muscle cross-sectional area was

352　significantly larger in sprint-trained older adults compared to untrained older controls. In

353　addition, plantar flexion strength and maximal tendon force were not significantly different from

354　those of young controls, with about half the effect size as in endurance-trained older adults

355　compared to young controls. These findings are supported by previous studies in young adults in

356　which sprint running but not endurance running was associated with greater muscle strength and

357　size in triceps surae muscles (18, 23). It may be that the beneficial effects of sprint training

358　preferentially target gastrocnemius muscle, which contains more fast twitch muscle fibers than

359　soleus (13).

360　*Methodological considerations*

361　The strengths of the current study are that the world-class older athletes measured in the present

362　study had a life-long physical activity background and had performed many decades of regular

363　exercise training. In addition, both the trained and untrained older adults were over 70 years old

364　and thus can be assumed to be affected by primary biological aging.

365   Limitations of the present study include the cross-sectional study design which does not allow

366   conclusions about cause-effect relationships that a longitudinal study design may allow. Cross-

367   sectional studies can be affected by selection bias. It is possible that subjects with favorable

368   muscle-tendon properties for endurance or sprint running were more likely to participate in such

369   activities. However, we did not observe differences between older trained and untrained subjects

370   in genetically determined variables such as Achilles tendon moment arm, forefoot length or

371   Achilles tendon length, all of which are related to running performance (3, 25, 26, 42). This

372   suggests that selection bias caused by genetic predisposition towards favorable musculoskeletal

373   properties for running did not considerably affect our data, although the possibility of selection

374   bias cannot be completely excluded. Another limitation of the current study is the small sample

375   size. However, it was not possible to obtain a larger sample of older athletes from the highest

376   performance level.

377   *Conclusions*

378   The current findings suggest that triceps surae muscle size, architecture, strength and tendon

379   stiffness are relatively unaffected by long-term running training in older adults. The reason for

380   this finding may be that the triceps surae muscle group is highly loaded in daily activities and

381   thus training produces only a small relative overload to this muscle group. Considering the

382   unparalleled physical performance of the older athletes in the present study, it appears that the

383   measured triceps surae muscle-tendon properties are not the key determining factors in their

384   physical performance. However, relatively high individual variation in these properties suggests

385   that a well-functioning muscle-tendon unit may be achieved via different combinations of muscle

386   and tendon properties. In addition, it is likely that in the current study there were differences

387   between the groups in factors that were not measured but that affect physical performance. These

388 include muscle fiber type, composition, molecular level modifications in contractile proteins and

389 neural activation (8, 21, 41). To further elucidate the importance of muscle architecture and

390 tendon mechanical properties for physical performance, future studies should investigate how

391 aging and physical loading affect muscle-tendon interaction during locomotion.

392 In conclusion, our data suggest that long-term physical loading induced by either endurance or

393 sprint running does not have a significant effect on Achilles tendon stiffness in older adults.

394 However, the loading patterns associated with sprint and endurance training in older age both

395 appear to increase Achilles tendon cross-sectional area in an intensity dependent manner.

396 Furthermore, the present results suggest that sprint running but not endurance running may

397 mitigate age-related loss of muscle mass and strength in triceps surae muscles. On the other

398 hand, endurance training in older age may alter muscle architecture in a way that is beneficial for

399 movement economy.

Grants

400

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553

554   Figure legend

555   FIGURE 1. Mean Achilles tendon force-elongation (upper) and stress-strain (lower)

556   relationships for young controls (YC), older controls (OC), older endurance runners (OE), and

557   older sprint runners (OS). Lines are linear fits and represent Achilles tendon stiffness and

558   Young's modulus, respectively. Values are calculated at 10% MVC increments from 10 to 80%

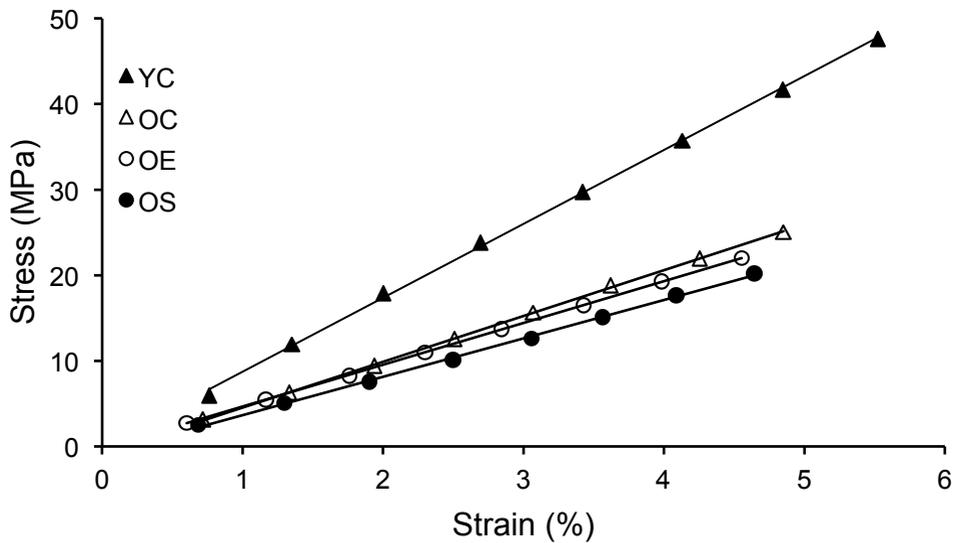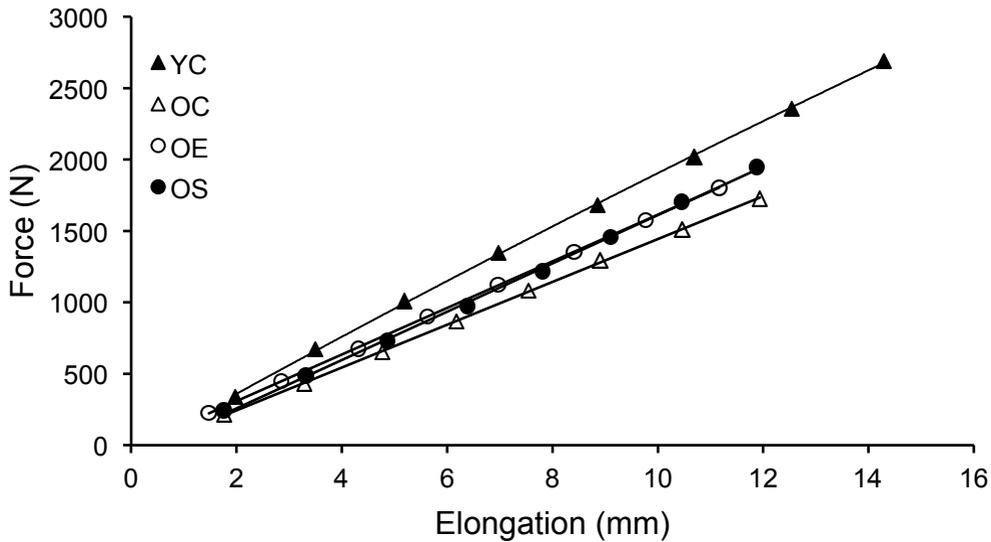559   MVC. Standard deviations are omitted for clarity.

TABLE 1. Subject characteristics and training status of older athletes

	YC	OC	OE	OS
Number of subject	18	33	10	10
Age (yr.)	23.7 ± 2.0	74.8 ± 3.6**	74.0 ± 2.8**	74.4 ± 2.8**
Height (cm)	181 ± 6	173 ± 5**	175 ±7	176 ± 7
Body mass (kg)	75.4 ± 9.0	76.1 ± 7.7	69.9 ± 6.9	74.3 ± 7.1
BMI (kgm^{-2})	23.1 ± 2.6	25.4 ± 2.4**††	22.7 ± 1.6	24.1 ± 1.9
Plantar flexion strength (Nm)	199 ± 56	132 ± 21**	116 ± 25**	153 ± 39
Years of training			39.4 ± 20.9	44.7 ± 19.7
Hours of training per week			6.8 ±3.3	6.2 ± 2.6
Endurance training per week (km)			55.2 ± 8.8	6.5 ± 2.8††
Sprint training sessions per week			0.8 ± 0.7	2.3 ± 1.2††
Strength training sessions per week			0.5 ± 0.2	0.9 ± 0.2

Values are expressed as mean ± SD. * significantly different from YC, † significantly different from OE, **/†† $p<0.01$. YC young controls, OC older controls, OE older endurance runners, OS older sprint runners.

TABLE 2. Achilles tendon cross-sectional area and mechanical properties

	YC	OC	OE	OS
Cross-sectional area (mm^2)	56.5 ± 9.6	69.0 ± 12.2 (-1.05)* ‡	82.0 ± 19.8 (-1.69)**	96.5 ± 24.9 (-2.26)**
Stiffness (N/mm)	186 ± 37	164 ± 47 (0.49)	172 ± 39 (0.34)	166 ± 35 (0.51)
Young's modulus (GPa)	0.86 ± 0.20	0.59 ± 0.17 (1.46)**	0.56 ± 0.22 (1.40)**	0.48 ± 0.19 (1.85)**
Max. tendon force (kN)	3.4 ± 0.9	2.2 ± 0.6 (1.58)**	2.1 ± 0.4 (1.64)**	2.6 ± 0.8 (0.80)
Max. tendon stress (MPa)	59.3 ± 14.9	33.1 ± 9.0 (2.22)**	26.5 ± 8.3 (2.36)**	30.1 ± 14.3 (1.86)**
Elongation at 80 % MVC (mm)	14.3 ± 2.5	11.9 ± 6.4 (0.42)*	11.2 ± 4.4 (0.88)	11.9 ± 4.2 (0.70)
Strain at 80 % MVC (%)	5.6 ± 1.5	4.8 ± 2.2 (0.42)	4.5 ± 1.8 (0.66)	4.7 ± 1.7 (0.56)

Values are expressed as mean ± SD (effect size compared to YC). * significantly different from YC, ‡ significantly different from OS, */‡ $p<0.05$, ** $p<0.01$. YC young controls, OC older controls, OE older endurance runners, OS older sprint runners, MG medial gastrocnemius.

TABLE 3. Muscle architecture and size

	YC	OC	OE	OS
Soleus fascicle length (mm)	40.6 ± 8.8	38.6 ± 7.6 (0.24) †	31.2 ± 3.9 (1.18)*	35.3 ± 8.3 (0.57)
Normalized soleus fascicle length (mm/mm)	0.102 ± 0.021	0.100 ± 0.021 (0.11) ††	0.073 ± 0.008 (1.55)**	0.083 ± 0.022 (0.83)
Soleus pennation angle (°)	21.0 ± 5.7	21.2 ± 4.0 (-0.05)	23.7 ± 5.3 (-0.46)	21.6 ± 8.3 (-0,08)
Soleus thickness (mm)	14.3 ± 2.6	13.1 ± 2.7 (0.44)	13.4 ± 2.7 (0.33)	12.8 ± 3.7 (0.49)
MG fascicle length (mm)	47.7 ± 6.6	45.0 ± 7.6 (0.35)	45.3 ± 6.5 (0.34)	47.7 ± 7.0 (0.00)
Normalized MG fascicle length (mm/mm)	0.121 ± 0.018	0.117 ± 0.022 (0.17)	0.108 ± 0.015 (0.71)	0.111 ± 0.021 (0.46)
MG pennation angle (°)	24.8 ± 4.0	24.4 ± 4.2 (0.09)	23.3 ± 4.8 (0.34)	24.1 ± 3.5 (0.18)
MG thickness (mm)	20.1 ± 2.5	17.7 ± 3.2 (0.77)*	17.2 ± 3.6 (0.94)	18.6 ± 2.7 (0.55)
Gastrocnemius CSA (cm^2)	24.2 ± 4.5	20.1 ± 4.5 (0.89)* ‡	20.9 ± 3.4 (0.73)	25.1 ± 4.4 (-0.19)

Values are expressed as mean ± SD (effect size compared to YC). * significantly different from YC, † significantly different from OE, ‡ significantly different from OS, */†/‡ $p<0.05$, **/†† $p<0.01$. YC young controls, OC older controls, OE older endurance runners, OS older sprint runners.