1   **TITLE**

2   Stretching of active muscle elicits chronic changes in multiple strain risk factors

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16 **RUNNING HEAD**

17 Stretching active muscle affects strain risk

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ABSTRACT

Introduction: The muscle stretch intensity imposed during 'flexibility' training influences the magnitude of joint range of motion (ROM) adaptation. Thus, stretching whilst the muscle is voluntarily activated was hypothesized to provide a greater stimulus than passive stretching. The effect of a 6-week program of stretch imposed on an isometrically-contracting muscle (i.e. qualitatively similar to isokinetic eccentric training) on muscle-tendon mechanics was therefore studied in 13 healthy human volunteers. **Methods:** Before and after the training program, dorsiflexion ROM, passive joint moment, and maximal isometric plantar flexor moment were recorded on an isokinetic dynamometer. Simultaneous real-time motion analysis and ultrasound imaging recorded gastrocnemius medialis muscle and Achilles tendon elongation. Training was performed twice weekly and consisted of five sets of 12 maximal isokinetic eccentric contractions at $10°·s^{-1}$. **Results:** Significant increases (*P<0.01*) in ROM (92.7% [14.7°]), peak passive moment (i.e. stretch tolerance; 136.2%), area under the passive moment curve (i.e. energy storage; 302.6%), and maximal isometric plantar flexor moment (51.3%) were observed after training. While no change in the slope of the passive moment curve (muscle-tendon stiffness) was detected (-1.5%; *P>0.05*), a significant increase in tendon stiffness (31.2%; *P<0.01*) and decrease in passive muscle stiffness (-14.6%; *P<0.05*) was observed. **Conclusion:** The substantial positive adaptation in multiple functional and physiological variables that are cited within the primary aetiology of muscle strain injury, including strength, ROM, muscle stiffness, and maximal energy storage, indicate that the stretching of active muscle might influence injury risk in addition to muscle function. The lack of change in muscle-tendon stiffness simultaneous with significant increases in tendon stiffness and decreases in passive muscle stiffness indicates that tissue-specific effects were elicited.

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51

52   **INTRODUCTION**

53   Muscular strength and joint range of motion (ROM), as well as the resistance to stretch

54   within the ROM (i.e. tissue stiffness), are important physical characteristics that influence the

55   capacity to perform athletic tasks and activities of daily living (26), impact muscle strain

56   injury risk (27,29,38,39), and are affected considerably with progression to older age (13).

57   With respect to muscle strain injury, muscle stretching exercises are commonly used to

58   increase ROM under the assumption that it will influence injury risk (24). Despite static

59   stretching being the most commonly used stretching mode, proprioceptive neuromuscular

60   facilitation (PNF) regularly produces greater increases in ROM (16). A common a form of

61   PNF is contract-relax (CR) stretching, where a brief (sometimes maximal) isometric

62   contraction is performed with the muscle in a highly-stretched position. However, using CR

63   stretching can be practically problematic as performing these contractions can be painful,

64   induce muscle damage, and requires partner assistance (16). Despite the efficacy of these

65   techniques to increase ROM, prospective studies often cite muscle strength (29) and active

66   muscle-tendon complex (MTC) stiffness (38) as strong predictors of muscle strain injury,

67   with the association between ROM and injury being less clear. Moreover, muscle strain

68   injuries usually occur within a normal ROM and may be load rather than muscle length

69   dependent (21). Thus, identifying strategies that enhance the muscle's ability to withstand

70   mechanical loading and absorb or dissipate strain energy may be more important than, or at

71   least of comparable importance to, improving the maximal passive elongation capacity of the

72   muscles (i.e. ROM).

73

74   Even when stretch-induced increases in ROM are considered useful, these increases are more

75   commonly associated with an increase in 'stretch tolerance' (i.e. a reduced stretch, discomfort

76   or pain perception at a given ROM) than a reduction in muscle stiffness (40) or increase in

77   muscle (5,36) or fascicle (5) length (i.e. tissue-related changes). Research using animal (rat)

78   models has indicated that passive static stretching does not provide an adequate myocellular

79   signalling response to promote mechanical or physiological adaptations within the

80   musculature (36). Thus, passive muscle stretching may not elicit the breadth of adaptations

81   required to either optimally improve ROM (or muscle extensibility) or alter important

82   mechanical or physiological characteristics that additionally influence muscle strain injury

83   risk. These issues may explain the limited and equivocal findings supporting the efficacy of

84   muscle stretching to reduce muscle strain injury incidence (24). Therefore, considering the

85   limited adaptive stimulus provided by passive (static) muscle stretching, identifying

86   alternative interventions that simultaneously influence multiple risk factors (e.g. muscle

87   strength, stiffness, energy storage/dissipation, and ROM) is of particular importance.

88

89   One avenue for research is the use of eccentric strength training as eccentric contractions

90   allow the highest level of volitional force to be produced. Importantly, higher-intensity

91   strength training tends to elicit greater muscle strength (14,33) and joint ROM (10)

92   adaptations, consequently eccentric training might best elicit the broad-ranging adaptations

93   required to reduce injury risk. In fact, eccentric training has been shown to elicit changes in

94   tendon stiffness (22) as well as provide relief from tendinopathy (1) and protection against

95   exercise-induced muscle damage (repeated-bout effect; 6, 28), which are often considered

96   within the aetiology of muscle strain injury (21,29). There appears also to be a consistent

97   increase in joint ROM following isotonic forms of eccentric training (for review see ref. 30)

98   that is comparable in magnitude to that achieved following similar-duration passive static

99   stretching programs (~6°; for review see ref. 32). It is not surprising therefore, that

100   reductions in the incidence of both new and recurrent muscle strain injuries have been

101   reported following the completion of eccentric training programs (31,34).

102

103   Nonetheless, a number of issues impact on our decision as to how to optimise the use of

104   eccentric exercise, or to develop more effective intervention strategies. First, research in

105   animal (rat) models has shown significantly increased myocellular signalling (36) and

106   preservation of sarcomere number (35) when isometrically-contracting muscle is stretched as

107   compared to passive muscle stretching. However, stretch applied to isometrically-contracting

108   muscle (i.e. qualitatively similar to isokinetic eccentric exercise) is different in effect than the

109   performance of isotonic eccentric contractions (12), and it is not known how ROM is affected

110   by such training in humans, despite the positive findings in animal models. Second, whilst

111   gains in ROM and strength following heavy strength training are likely to be advantageous

112   for injury prevention, increases in muscle stiffness may increase muscle strain injury risk (38)

113   and post-exercise muscle damage (23). Thus, some adaptive responses to eccentric training

114   could be considered problematic from an injury reduction perspective. In this regard, few

115   studies have employed the requisite imaging techniques to determine the potentially

116   differential impacts of eccentric training on muscle versus tendon stiffness. Recent advances

117   in imaging technologies have revealed large increases in Achilles (2,3,18) and patellar tendon

118   (18,22) stiffness following traditional resistance training programs (i.e. cyclical bouts of

119   concentric-eccentric actions), yet eccentric training programs have differentially elicited no

120   change (19) or reductions (20) in whole MTC stiffness and increases (19) or no change in

121   tendon stiffness (20). These disparate tissue-specific responses provide tentative evidence of

122   distinct muscle and tendon adaptations following eccentric training. However, these

123   adaptations are yet to be fully described after isotonic eccentric training and, specifically,

124 there are currently no data describing the effects of muscle stretching imposed on

125 isometrically-contracting muscle in humans.

126

127 Given the above, the aim of the present study was to examine the influence of 6 weeks of

128 training where stretch was applied to isometrically-contracting muscle. A distinction needs

129 to be made between the CR stretching technique and the method of stretch employed in the

130 present study, i.e. stretch applied to isometrically-contracted muscle. CR stretching involves

131 passively lengthening a muscle and holding for a period of time (static stretch phase) before

132 an isometric contraction is performed with the muscle remaining in a highly-stretched

133 position (16). However, in the present study the muscle was maximally contracted

134 (isometrically) at a short muscle length, with the maximal contraction being maintained as the

135 muscle was stretched (lengthened) by the dynamometer (i.e. an isokinetic eccentric

136 contraction). Outcome variables included dorsiflexion ROM, maximal passive joint moment

137 at full volitional ROM (i.e. stretch tolerance), the slope of the passive moment curve (i.e.

138 MTC stiffness), the area under the passive moment curve (i.e. potential elastic energy

139 storage), passive gastrocnemius medialis (GM) muscle stiffness and active Achilles tendon

140 stiffness, and maximal isometric plantar flexor moment. We tested the hypothesis that such

141 training would result in significant increases in strength, ROM, stretch tolerance, energy

142 storage, MTC stiffness and tendon stiffness, while simultaneously reducing passive muscle

143 stiffness.

144

145 **MATERIALS & METHODS**

146 Subjects

147 Thirteen athletic males (collegiate soccer players, age = 20.0 ± 0.9 yr, height = 1.8 ± 0.1 m,

148 mass = 75.9 ± 8.5 kg) with no recent history (≥ 2 yr) of lower limb injury or illness

149 volunteered for the study after completing a pre-test medical questionnaire and providing

150 written and informed consent. Ethical approval was granted by The University of

151 Northampton's Ethics Committee, and the study was completed in accordance with the

152 Declaration of Helsinki.

153

154 Procedures

155 *Overview*

156 The subjects were fully familiarized with the experimental testing and training protocols one

157 week prior to pre-training data collection. They then visited the laboratory on two further

158 occasions under experimental conditions, once each before and after the training program,

159 and also visited the laboratory twice a week during the 6-week training period. During the

160 pre- and post-training experimental trials, the subjects performed a 5-min jogging warm-up

161 on a treadmill at a self-selected pace (1.9 ± 0.2 m·s^{-1}) at the transition between walking and

162 jogging and were then seated in the isokinetic dynamometer chair (Biodex System 3 Pro,

163 IPRS, Suffolk, UK) with the hip at 70°. The right knee was fully extended (0°) to ensure all

164 plantar flexor components contributed significantly to passive and active joint moments (7)

165 during active and passive trials. The foot was positioned in the dynamometer footplate with

166 the lateral malleolus aligned with the center of rotation of the dynamometer and the sole of

167 the foot perpendicular to the shank to ensure the ankle was in the anatomical position (0°).

168 To ensure valid and reliable passive moment data were obtained, non-elastic Velcro strapping

169 was used to minimize heel displacement from the dynamometer footplate; one experienced

170 analyst conducted all trials in order to remove inter-tester variability. Test re-test reliabilities

171 (ICC = 0.95, SE = 3.0%) of these methods in our laboratory have been reported previously

172 (16), further confirming the validity and reliability of the methods. Three passive joint

173 movements were then initiated from 20° plantar flexion through to full volitional dorsiflexion

7

174   at 0.087 rad·s^{-1} (5°·s^{-1}) to determine dorsiflexion ROM and peak passive moment (stretch

175   tolerance) at full volitional ROM. Two minutes later the subjects performed two maximal

176   isometric plantar flexor contractions (2 min rest between contractions) to determine maximal

177   isometric joint moment (where >5% difference in maximal isometric moment was found

178   between contractions, the subjects performed a third contraction).   At least 48 h later the

179   subjects commenced the 6-week training program (described below). Upon completion of

180   the program, the subjects repeated the passive and active experimental trials 3-5 days after

181   the final training session.

182

183   *Range of motion, passive moment and energy storage*

184   During the passive ROM trial, the subjects' foot was passively dorsiflexed through their full

185   ROM until they volitionally terminated the movement by pressing a hand-held release button

186   at the point of discomfort, a stretch intensity commonly used in ROM studies (16). The

187   movement velocity was chosen as it has been reported to be too slow to elicit a significant

188   myotatic stretch reflex response (25), thus we were confident that full volitional ROM was

189   reached and the moment data were considered reflective of the passive properties of the

190   plantar flexors. The passive dorsiflexion trial was repeated twice at 30-s intervals with

191   passive moment data being recorded from the third trial. The passive trials enabled ROM,

192   peak passive moment (stretch tolerance), the slope of the passive moment curve (MTC

193   stiffness), and the area under the passive moment curve (potential elastic energy storage) to

194   be calculated. Dorsiflexion ROM was calculated from the anatomical position (0°) to peak

195   dorsiflexion with peak passive moment measured within a 250-ms epoch at peak

196   dorsiflexion, while energy storage was calculated as the area under the passive moment curve

197   from the anatomical position to peak dorsiflexion (Nm^2). The slope of the passive moment

198   curve was calculated as the change in plantar flexor moment per change in joint angle

8

199 through the final 10° of dorsiflexion in the pre-stretching trials (Nm·°⁻¹), with identical joint

200 angles used in post-training analysis (16).

201

202 *Maximal isometric moment and tendon stiffness*

203 Two minutes later, the subjects produced a ramped maximal isometric plantar flexor

204 contraction with the ankle placed in the anatomical position (0°), and with maximal joint

205 moment being reached ~3 s after contraction initiation and then held for 2 s (i.e. there was a

206 visible plateau in the moment trace). The ramped contraction enabled maximal isometric

207 plantar flexor moment to be determined and also enabled tendon deformation to be captured

208 using sonography (described later), which allowed tendon stiffness to be calculated when

209 combined with joint moment data (15). To ensure that the loading rate during the ramped

210 contraction did not influence tendon stiffness, the subjects repeated the ramped contractions

211 using visual feedback during the familiarization session until they could reliably achieve a

212 linear increase in joint moment and reach maximal voluntary contraction (MVC) after ~3 s.

213 In the pre- and post-training experimental trials, the time taken to increase active joint

214 moment from 50% to 90% of MVC (i.e. the range over which tendon stiffness was

215 calculated) was recorded; no significant difference (pre-training = 2.1 ± 0.1 s, post-training =

216 2.0 ± 0.1 s; *P > 0.05*) in the 50-90%MVC interval time was found, indicating that similar

217 strain rates were achieved. Joint moment and angle data were directed from the

218 dynamometer to a high level transducer (model HLT100C, Biopac, Goleta, CA) before

219 analog-to-digital conversion at a 2000-Hz sampling rate (model MP150 Data Acquisition,

220 Biopac). The data were then directed to a personal computer running AcqKnowledge

221 software (v4.1, Biopac) and filtered with a zero lag, 6-Hz Butterworth low-pass filter prior to

222 ROM and joint moments being determined.

223

Muscle and tendon length

Real-time motion analysis using four infrared digital cameras (ProReflex, Qualisys, Gothenburg, Sweden) operating Track Manager 3D software (v.2.0, Qualisys) recorded the movement of infrared reflective markers during the experimental trials. Using methods previously described to calculate Achilles tendon and GM muscle length (16), markers were placed over the origin of the medial head of the gastrocnemius at the medial femoral epicondyle (see Figure 1; *marker A*), on the distal edge of the ultrasound probe (*marker B*) positioned over the GM-Achilles muscle tendon junction (MTJ), and over the insertion of the Achilles at the calcaneus (*marker C*). Raw coordinate data were sampled at 100 Hz and smoothed using a 100-ms averaging window prior to the calculation of Achilles tendon and GM muscle lengths.

Real-time ultrasound imaging (Vivid I, General Electric, Bedford, UK) recorded the position (i.e. excursion) of the GM-Achilles MTJ (see Figure 2) at a 28-Hz frame rate using a wide-band linear probe (8L-RS, General Electric) with a 39 mm wide field of view. The probe was orientated to enable longitudinal imaging of the GM-Achilles MTJ and then manipulated until the superficial and deep GM aponeuroses could be visualized to enable triangulation of the GM-Achilles MTJ. The probe was then fixed to the skin with zinc-oxide adhesive tape to ensure consistent and accurate imaging of the MTJ (see Figure 2). The distance between the MTJ and distal edge of the ultrasound image (see Figure 2. D) was manually digitized (Vivid I, General Electric) during passive and active trials to enable changes in muscle and tendon length to be calculated.

Joint moment, motion analysis, and ultrasound data were electronically synchronized using a 5-V ascending transistor-transistor logic (TTL) pulse that simultaneously placed a pulse trace

10

249   on the AcqKnowledge (v4.1, Biopac) software and ended the capture of both motion analysis

250   and ultrasound data (preceding 15 s of data). Tendon length was calculated as the sum of the

251   distance from the position of the MTJ to the distal edge of the ultrasound image (using

252   ultrasound) and the distance between reflective *markers B* and *C* (using motion analysis), in a

253   method identical to that previously reported (16). Tendon stiffness was calculated as the

254   change in plantar flexor moment from 50-90%MVC divided by the change in tendon length

255   (Nm·mm^{-1}) during the isometric ramped contraction trials. Muscle length was calculated as

256   the distance between reflective *markers A* and *B* (using motion analysis) minus the distance

257   from the MTJ to the distal edge of the ultrasound image (using ultrasound). Passive muscle

258   stiffness was calculated as the change in moment through the final 10° of dorsiflexion during

259   the passive ROM trial (in the linear portion of the stress-strain) divided by the change in

260   muscle length (Nm·mm^{-1}); muscle stiffness was calculated post-training using identical

261   absolute joint angles.

262

263   *Active muscle stretching program*

264   Before each training session the subjects performed the standardized 5-min jogging warm-up

265   and were then seated in the dynamometer chair in identical positioning to that described

266   above (see Overview). Training was performed on both limbs twice weekly (at least 48 h

267   between sessions) for 6 weeks. For 5 sets of 12 repetitions, the subjects produced maximal

268   isometric contractions at 20° plantar flexion before the dynamometer dorsiflexed the ankle at

269   10°·s^{-1} though a 30º ROM through to 10° dorsiflexion with the subject maintaining maximal

270   effort throughout the repetition (i.e. a maximal voluntary isokinetic eccentric contraction).

271   This end-point is well within the maximum ROM of the ankle joint of the subjects and was

272   not difficult to achieve. After each repetition, the subjects relaxed and the footplate plantar

273   flexed the foot to the starting position at 30°·s^{-1}, providing a 1-s rest between contractions; 60

274   s of rest was provided between sets. Post-training testing was performed 3-5 days after the

275   final training session.

276

277   Data analysis

278   All data were analyzed using SPSS statistical software (version 20; LEAD Technologies,

279   Chicago, IL). Data are reported as mean ± SE, and Cohen's D was used to calculate effect

280   size (ES). Normal distribution for pre- and post-group data in all variables was assessed

281   using Kolmogorov-Smirnov and Shapiro-Wilk tests; no significant difference ($P > 0.05$) was

282   detected in any variable, indicating that all data sets were normally distributed. As several

283   variables were clearly related separate multiple analyses of variance (MANOVA) with

284   repeated measures were used to test for differences between pre- and post-training data in 1)

285   MTC stiffness (slope of the passive moment curve), passive GM muscle stiffness, and active

286   Achilles tendon stiffness, and 2) dorsiflexion ROM and peak passive moment (stretch

287   tolerance). Repeated measures t-tests were used to test for differences in maximal isometric

288   plantar flexor moment and the area under the passive moment curve (elastic potential

289   energy). Statistical significance for all tests was accepted at $P < 0.05$.

290

291   Reliability

292   Intratester reliability for the manual digitization of MTJ excursion within the ultrasound

293   images (n = 5) has been determined previously in our laboratory (15) by calculating the

294   intraclass correlation coefficient (ICC) and coefficient of variation (CoV; expressed as a

295   percentage of the mean). A high ICC (0.99) and low CoV (0.4%) were calculated; no

296   significant difference was detected between mean values ($P > 0.05$). Test-retest reliability

297   has also been determined previously (16) for peak isometric moment, peak passive moment

298   (stretch tolerance), ROM, slope of the passive moment curve (MTC stiffness), muscle

299   stiffness and tendon stiffness. No significant difference was detected between test-retest

300   mean values ($P > 0.05$) for any measure; intraclass correlation coefficients (ICC) were 0.89,

301   0.97, 0.97, 0.95, 0.80, and 0.96. Coefficients of variation and standard errors (expressed as a

302   percentage of the mean) were 9.5% (SE = 2.3%), 7.8% (SE = 1.9%), 4.4% (SE = 1.1%),

303   12.4% (SE = 3.0%), 11.1% (SE = 2.7%), and 4.4% (SE = 1.1%), respectively.

304

305   Sample size

306   Effect sizes (Cohen's D) were calculated from mean changes in variables (strength, ROM,

307   muscle and tendon stiffness, and peak passive moment) from previous studies employing

308   similar methods (4,15,16). To ensure adequate statistical power for all analyses, power

309   analysis was conducted for the variable with the smallest effect size (ROM; ES = 1.2) using

310   the following parameters (power = 0.80, alpha = 0.05, effect size = 1.2, attrition = 20%). The

311   analysis revealed that the initial sample size required for statistical power was 12, thus 16

312   subjects were recruited to account for possible data loss and subject attrition. Three subjects

313   withdrew from the study with non-related injuries; statistical analyses were conducted on data

314   sets for 13 subjects who completed the testing.

315

316   **RESULTS**

317   Range of motion, passive moment and energy storage

318   A significant increase in dorsiflexion ROM ($92.7 \pm 19.9\%$ [$14.7 \pm 2.0°$], ES = 1.78; $P < 0.01$)

319   was found after the training program. This very large increase in ROM was accompanied by

320   a significant increase peak passive moment ($136.2 \pm 30.2\%$, ES = 2.11; $P < 0.01$) measured

321   at full volitional ROM (i.e. stretch tolerance; see Figure 3). Similarly, a significant increase

322   ($302.6 \pm 95.8\%$, ES = 1.88; $P < 0.01$) in the area under the passive moment curve (i.e. energy

323   storage; see Figure 3) was detected after training. Significant correlations (see Figure 4) were

324 observed between the change in ROM and changes in stretch tolerance (r = 0.72; *P < 0.05*)

325 and energy storage (r = 0.59; *P < 0.05*). A significant correlation was also detected between

326 the changes in stretch tolerance and energy storage (r = 0.92; *P < 0.01*). These results are

327 indicative that changes in peak passive loading and total elastic energy storage are closely

328 associated with improvements in ROM.

329

330 MTC, muscle, and tendon stiffness

331 Passive moment was similar post-training at all dorsiflexion angles (see Figure 5. A) along

332 the joint moment-angle curve. No significant change (-1.5 ± 6.8%, ES = 0.05; *P > 0.05*) in

333 the slope of the passive moment curve (MTC stiffness) was observed during the passive

334 ROM trial following the training (see Figure 5. B). However, analysis of the ultrasound data

335 revealed a significant increase in tendon stiffness (31.2 ± 5.0%, ES = 1.10; *P < 0.01*) during

336 the maximal isometric plantar flexor trial (see Figure 6) and a significant reduction in GM

337 muscle stiffness (14.6 ± 4.3%, ES = 0.24; *P < 0.05*) during the passive ROM trial (see Figure

338 6) following the training. No correlation (*P > 0.05*) was observed between change in ROM

339 and changes in muscle (r = 0.17) or tendon (r = 0.08) stiffness.

340

341 Maximal isometric plantar flexor moment

342 A significant increase in maximal isometric plantar flexor moment (51.3 ± 7.5%, ES = 1.65;

343 *P < 0.01*) was observed following the 6-week training program, with joint moment increasing

344 substantially from pre- (136.2 ± 10.0 Nm) to post-training (200.5 ± 11.5 Nm). A significant

345 correlation was observed between changes in maximal isometric plantar flexor moment and

346 Achilles tendon stiffness (r = 0.62; *P < 0.05*). The substantial mean increase in isometric

347 moment and very large effect size clearly indicate a substantial and meaningful increase in

348 joint moment generating capacity.

14

349

DISCUSSION

The primary aim of the present study was to examine the impact of stretches imposed on isometrically-contracting muscle on ankle joint ROM, passive joint moment at full ROM, and muscle, tendon and whole MTC stiffness. In agreement with our hypothesis, a large increase in dorsiflexion ROM (~15°, ES = 1.78) and maximal plantar flexor torque (~51%, ES = 1.65) was detected. As very large effect sizes were calculated in the present study, both *a priori* and *post-hoc* analyses confirmed an adequate sample size was used to reach statistical power. A recent review (30) reported consistent increases in dorsiflexion ROM following lower-intensity isotonic eccentric exercise (i.e. standing heel drops) that were comparable to those elicited by passive static muscle stretching (~6°; for review see ref. 32). Whilst PNF stretching has been reported to achieve greater (~3°) increases in dorsiflexion ROM than static stretching (16), no systematic review has quantified the expected changes in ankle ROM. Therefore, the magnitude of change detected in the present study represents a 145% or ~2.5 fold greater increase in dorsiflexion ROM when compared to previous passive static stretch or lower-intensity isotonic eccentric training programs (30,32). These data are consistent with the findings of a greater extensibility of rat muscle obtained after training that imposed stretches on activated muscle (35,36), and suggest that the strategy may have marked effects in humans. Notably, passive (static) muscle stretching is usually performed daily and to the point of discomfort, whereas training in the present study was performed twice weekly through a well-tolerated, submaximal ROM (30° range, ~10° below initial full ROM). Thus, the present data are clearly indicative that stretching of a muscle while in a fully contracted state is substantively more effective than current passive static stretching practices (and isotonic eccentric exercise) for achieving clinically relevant chronic increases in ankle joint ROM.

374

Historically, muscle stretching exercises have been used to increase ROM partly with the aim of reducing muscle injury risk, yet equivocal and limited benefits have been reported (24) so the efficacy of muscle stretching programs has been questioned. However, recent studies have reported that isotonic eccentric training reduced (31) or even eliminated (34) the incidence of new and recurrent muscle strain injuries, although possible underlying mechanisms associated with this benefit were not examined. No studies have reported the effects of muscle stretch imposed during muscle contraction, i.e. similar to maximal isokinetic eccentric exercise, on injury incidence. Despite no change in MTC stiffness (i.e. slope of passive moment curve) being detected in the present study (~2%), a significant increase in active Achilles tendon stiffness (~31%, ES = 1.10) and a decrease in passive GM muscle stiffness (~15%, ES = 0.24) were revealed when joint moment data were examined in conjunction with sonographic data. These disparate results may be explained as the decrease in muscle and concomitant increase in tendon stiffness resulting in a lack of overall change in MTC stiffness. These findings are consistent with previous passive, static stretching research where no change in MTC stiffness was detected despite greater fascicle and whole muscle lengthening (5). The findings are also consistent with studies imposing isotonic eccentric training, where MTC stiffness was unchanged despite a significant increase in tendon stiffness being observed (19). Nonetheless, it should be noted that shorter-duration, lower-intensity isotonic eccentric training elicited a reduction in passive MTC stiffness without detectable changes in tendon stiffness (20). Cumulatively, these findings are consistent with the present study and indicate that reductions in passive muscle stiffness may occur following passive stretch and stretch imposed on active muscles (e.g. isotonic and isokinetic eccentric exercise).

398

399   In the present study, muscle stiffness was calculated as the change in tissue length per load

400   change during the passive ROM trials (i.e. the stress-strain curve; Nm·mm^{-1}), which provides

401   an estimate of the stiffness of whole muscle-tendon structure rather than tissue-specific

402   stiffness. However, this method of calculating stiffness may limit our understanding of the

403   mechanisms underpinning changes in stiffness. Examining cross-sectional area in addition to

404   tissue strain provides an estimate of muscle tissue modulus (i.e. Young's Modulus; N·m^{-2} or

405   Pa) and therefore, whether any changes are likely attributable to structural (e.g. cross-

406   sectional area) or mechanical (e.g. collagen synthesis) adaptations (for review see ref. 17).

407   Nonetheless, increased muscle volume has commonly been reported following similar

408   durations of high-intensity isotonic eccentric training (4,9). Importantly, an increase in

409   muscle cross-sectional area should increase muscle stiffness when calculated using the stress-

410   strain model, accordingly the reduction in muscle stiffness reported in the present study likely

411   underestimates the change in muscle tissue-specific stiffness (i.e. Young's Modulus). While

412   muscle stiffness was clearly decreased in the present study, a large increase in tendon

413   stiffness was observed. Previous studies imposing isometric (2,3) and isotonic eccentric

414   (11,18) training have reported significant increases in tendon stiffness similar to the increases

415   detected in present study. While lower intensity isotonic eccentric exercise (standing body

416   weight) resulted in no change in Achilles tendon stiffness after six weeks (20), a similar study

417   reported a significant increase (~22%) after 14 weeks (11). Similarly, while no change in

418   tendon stiffness was detected after 14 weeks of isometric exercise at 55% MVC, a significant

419   increase was detected after training at 95% MVC (2). However, more intense eccentric

420   training (120% concentric MVC) induced significant increases in Achilles tendon stiffness

421   (~18%) after only seven weeks (8), with larger increases (~30%) reported after 12 weeks

422   (18). Collectively, these data are indicative that adaptations in tendon stiffness depend on

423   both loading intensity and program duration, with isokinetic eccentric exercise providing

424 more effective and efficient increases in tendon stiffness than isometric or isotonic eccentric

425 exercises.

426

427 In the present study the substantial increase in ROM was associated with further adaptations

428 likely being beneficial to strain injury risk, including very large increases in peak passive

429 moment at full volitional ROM (i.e. stretch tolerance; ~136%, ES = 2.11) and in the area

430 under the passive moment curve (i.e. elastic potential energy storage; ~300%, ES = 1.88).

431 Furthermore, significant correlations were observed between the changes in ROM and

432 changes in both stretch tolerance (r = 0.72) and energy storage (r = 0.59). During passive

433 tissue lengthening in isolated muscle preparations, elastic potential energy has been

434 associated with the onset of strain injury (21) whereas no association was found for muscle

435 length. Importantly, stiffer tissue is often less extensible (i.e. reduced deformation capacity)

436 and its ability to store and dissipate strain energy limited when compared with more

437 compliant tissue. This inability to deform and store energy has been suggested to increase

438 muscle strain injury risk in stretch-shortening cycle exercise (for review see ref. 39). In the

439 present study, the substantial increase in ROM, elastic energy storage potential and peak

440 passive loading are suggestive that the muscle was able to tolerate substantially greater

441 loading and deformation, which likely translates into a protective effect against muscle strain

442 injury. Furthermore, maximal isometric joint moment also increased substantially (~51%, ES

443 = 1.65) indicating that the training performed in the present study (qualitatively similar to

444 isokinetic eccentric exercise) enhanced the muscle's ability to generate force and tolerate

445 loading. Thus, applying stretch to maximally activated muscle generates substantial

446 beneficial changes in several measures associated with muscle strain injury risk including

447 muscle stiffness (38), muscle strength (29), energy storage (21), and ROM (37). These

448 considerable and concomitant adaptations in multiple important risk factors may highlight the

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449 possible underlying functional and mechanical changes associated with the efficacy of

450 eccentric exercise to reduce injury incidence (31,34), although to date no study has examined

451 the impact of the present training methodology on injury incidence.

452

453 In summary, the concomitant increases in ROM and strength observed in the present study

454 are important findings which represent substantially greater improvements than those

455 previously reported after traditional stretching or resistance training practices. Crucially, the

456 singular imposition of muscle stretch through a submaximal ROM on isometrically-

457 contracting muscle resulted in substantial beneficial adaptations in multiple muscle strain

458 injury risk factors, including ROM, muscle stiffness, muscular strength, and peak loading

459 capacity (stretch tolerance and elastic energy storage). These findings may have significant

460 practical implications, because training similar to that used in the present study can be

461 applied in the clinical/rehabilitation setting by use of isokinetic training practices or in the

462 field by the use of partner-assisted stretches imposed with the muscles contracted.

463 Randomized controlled trials are needed to compare the influence of this training to standard

464 (isotonic) training and static muscle stretching programs as the magnitude of adaptations

465 achieved in the present study following twice-weekly training are likely capable of achieving

466 substantially greater, and more efficient, increases in several outcome measures crucial in

467 both athletic and clinical populations.

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FIGURES & CAPTIONS

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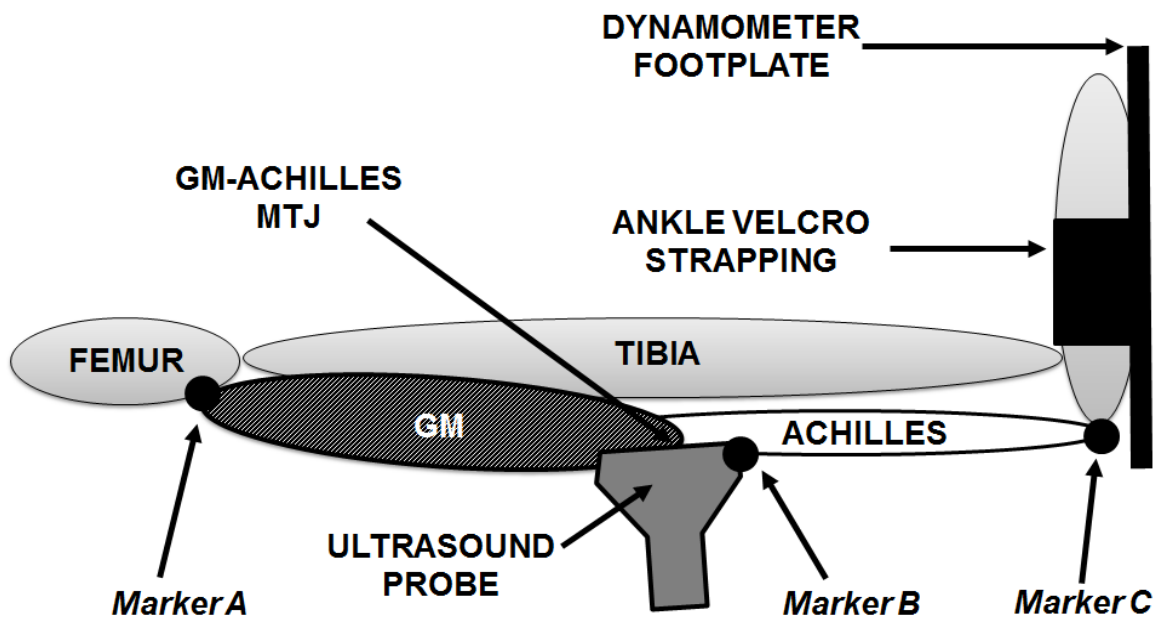
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598 **Figure 1.** Schematic depicting motion analysis reflective marker placements and ultrasound

599 probe positioning. Gastrocnemius medialis (GM) muscle length was estimated from the

600 distance between reflective markers placed over the origin of the GM muscle on the medial

601 femoral epicondyle (*marker A*) and the distal edge of the ultrasound probe (*marker B*), which

602 was positioned over the GM-Achilles muscle-tendon junction (MTJ). Achilles tendon length

603 was estimated from the distance between reflective markers placed over the distal edge of the

604 ultrasound probe (*marker B*) and the insertion of the Achilles on the calcaneus (*marker C*).

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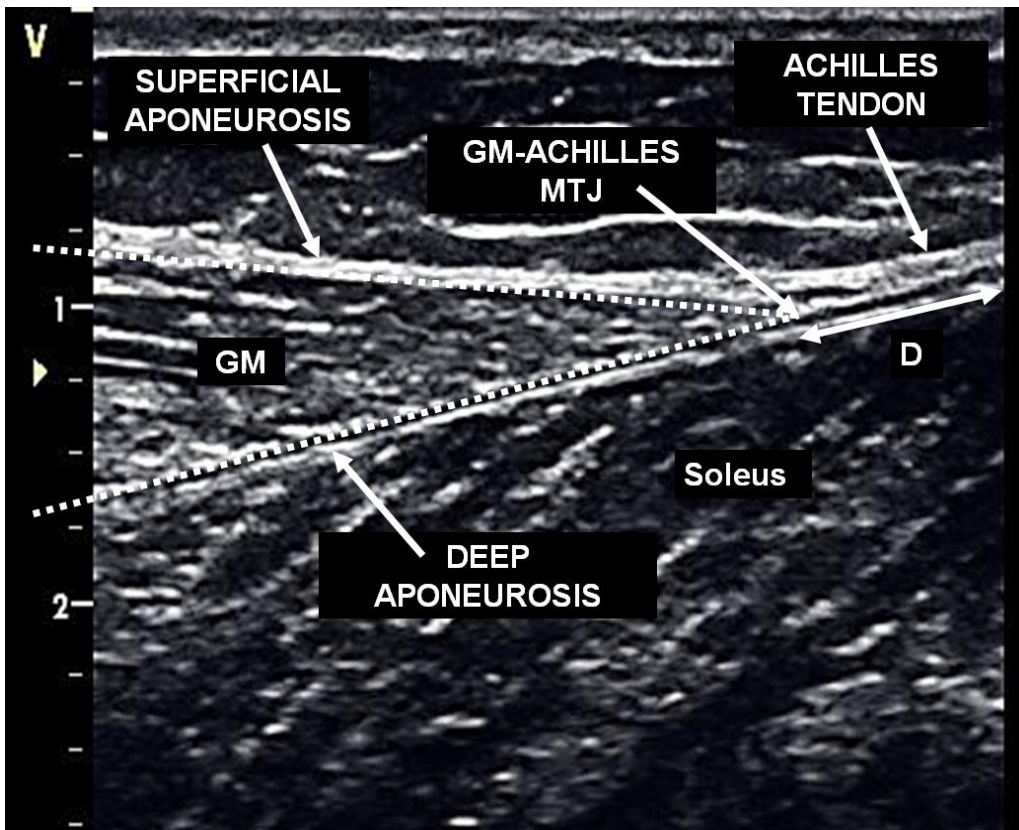
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607 **Figure 2.** Ultrasound image of the GM-Achilles MTJ. Real-time ultrasound imaging

608 recorded the position and displacement of the gastrocnemius medialis (GM)-Achilles muscle-

609 tendon junction (MTJ) during passive and active trials. The MTJ was identified as the point

610 where the superficial and deep GM aponeuroses merged with the Achilles tendon.

611 Displacement of the MTJ from the distal edge of the image (D) was synchronized with

612 motion analysis data to calculate GM muscle and Achilles tendon lengths.

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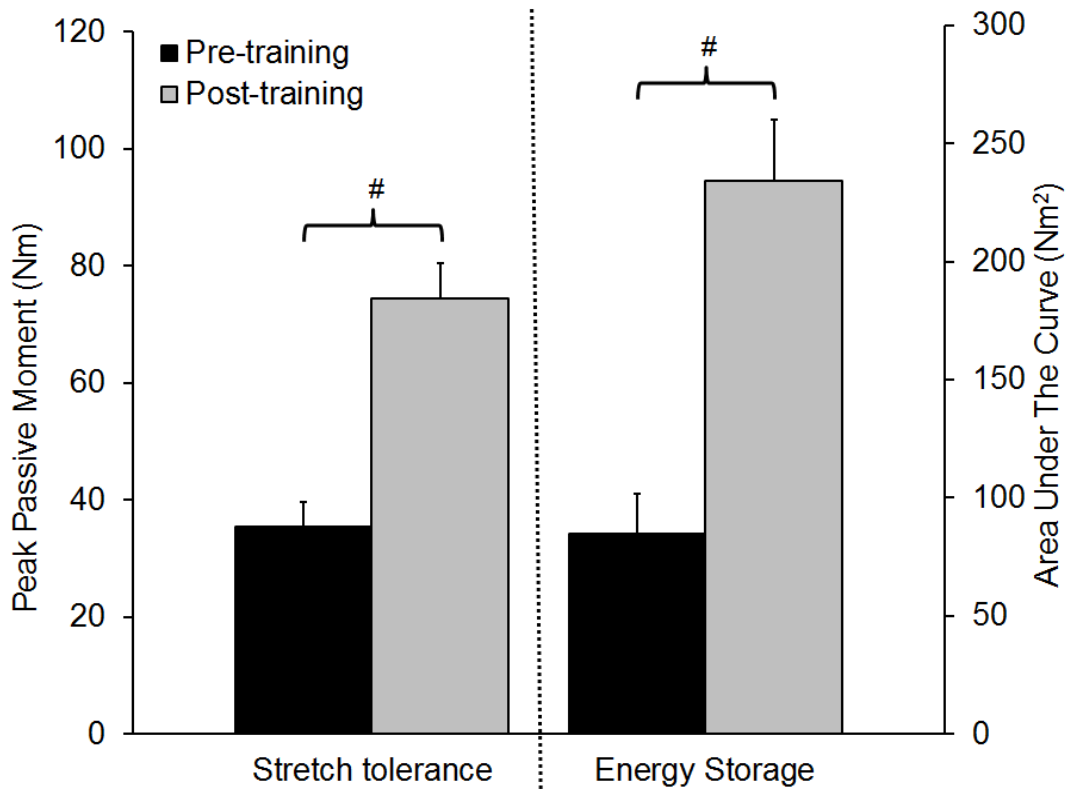
Figure 3. Peak passive moment and the area under the passive moment curve pre- and post-training. A significant increase in peak passive moment (i.e. stretch tolerance; $136.2 \pm 30.2\%$) was found with peak passive moment increasing substantially from pre- (35.4 ± 4.2 Nm) to post-training (74.5 ± 5.9 Nm). Similarly, a significant increase in the area under the passive moment curve (i.e. energy storage; $302.6 \pm 95.8\%$) was with the area increasing substantially from pre- (84.7 ± 16.9 Nm^2) to post-training (234.0 ± 26.1 Nm^2). #Significant to *P < 0.01*.

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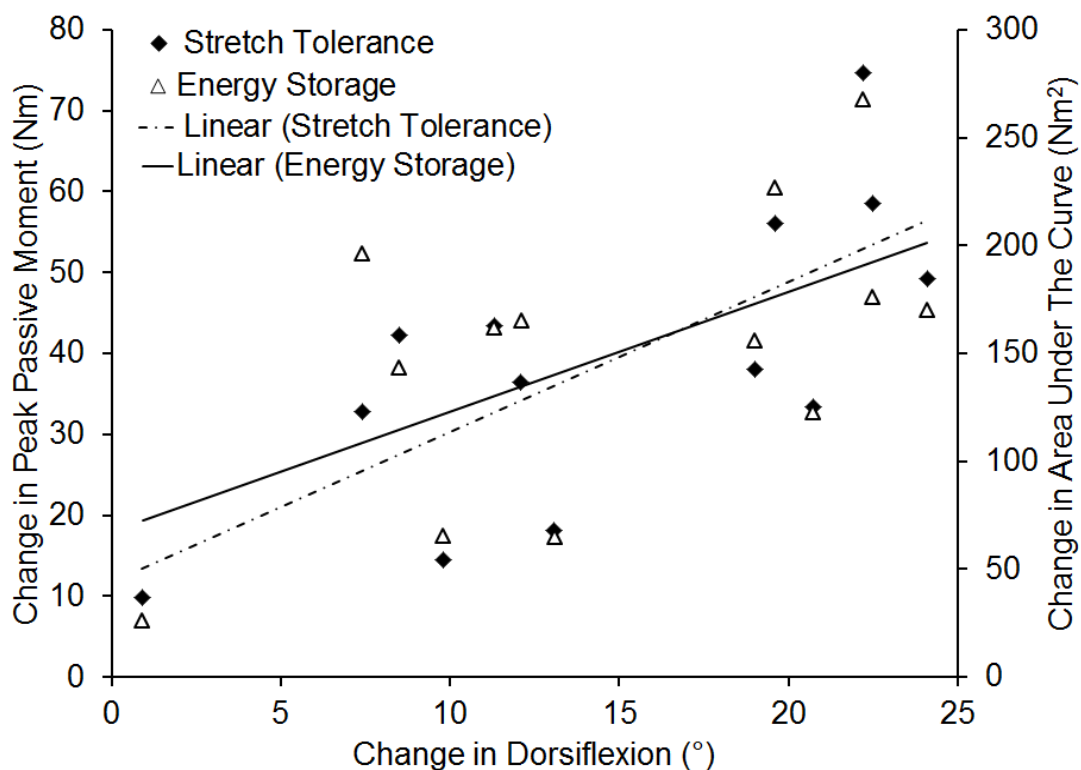
Figure 4. Correlations between change in ROM (pre-to-post intervention) and changes in peak passive joint moment and elastic energy storage. Significant correlations were found between the change in range of motion (ROM) and changes in peak passive moment (i.e. stretch tolerance [r = 0.72; *P < 0.05*]) and the area under the passive moment curve (i.e. elastic energy storage [r = 0.59; *P < 0.05*]) after training.

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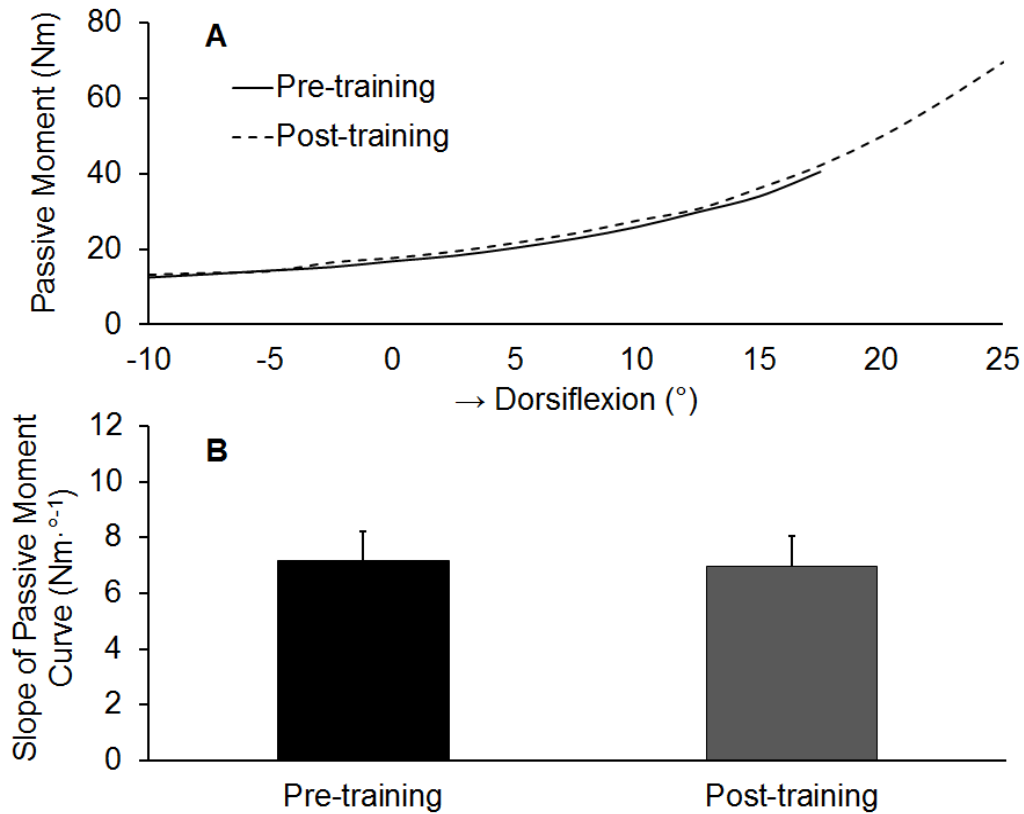
Figure 5. Passive plantar flexor moment pre- and post-training. Passive moment was similar post-training at all dorsiflexion angles (A) along the joint moment-angle curve (one subject's data depicted). No significant difference in the slope of the passive moment curve (B; i.e. whole muscle-tendon complex [MTC] stiffness) was found after training ($-1.5 \pm 6.8\%$) with MTC stiffness being similar at pre- (7.2 ± 1.1 Nm·$^{\circ-1}$) and post-training (7.0 ± 1.1 Nm·$^{\circ-1}$).
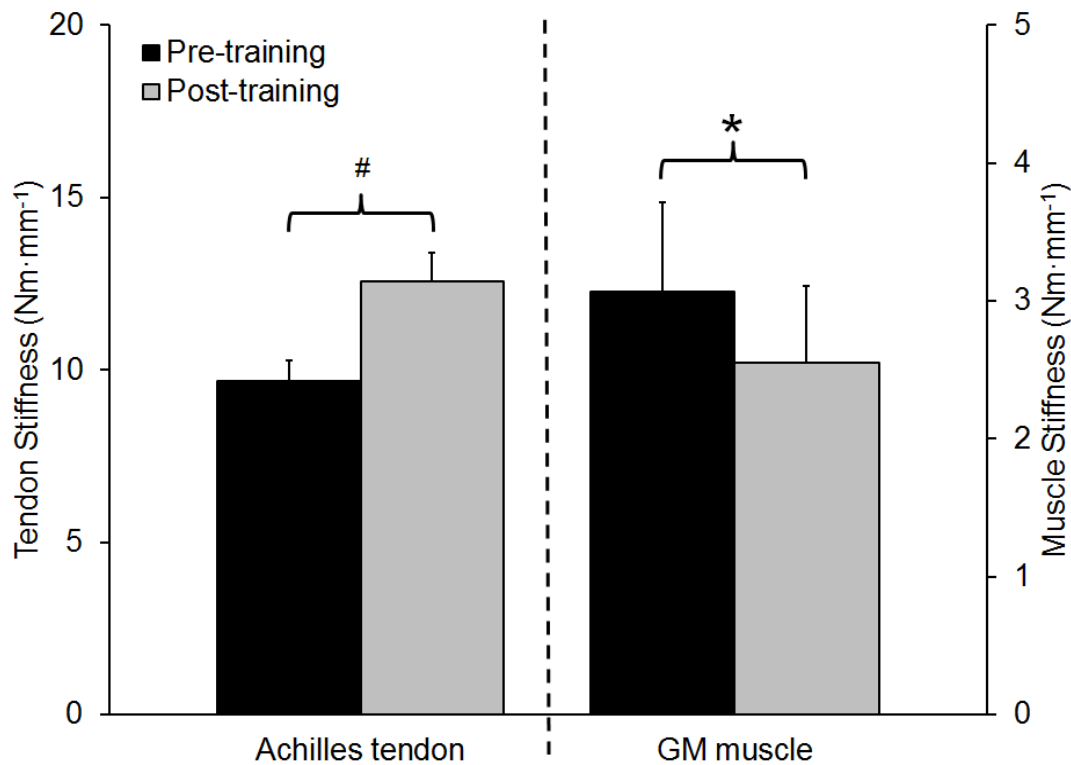
Figure 6. Achilles tendon stiffness and gastrocnemius medialis (GM) muscle stiffness pre- and post-training. A significant increase in tendon stiffness ($31.2 \pm 5.0\%$) was found as tendon stiffness increased substantially from pre- (9.7 ± 0.6 Nm·mm^{-1}) to post-training (12.6 ± 0.8 Nm·mm^{-1}). A significant decrease in muscle stiffness ($14.6 \pm 4.3\%$) was found as muscle stiffness decreased substantially from pre- (3.1 ± 0.7 Nm·mm^{-1}) to post-training (2.6 ± 0.6 Nm·mm^{-1}). *Significant to $P < 0.05$, #Significant to $P < 0.01$.