TITLE

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

AUTHORS

Anthony D. Kay[1], Dominic Richmond[1], Chris Talbot[1], Minas Mina[2], Anthony W. Baross[1] & Anthony J. Blazevich[3]

AFFILIATION

[1]Sport, Exercise and Life Sciences, The University of Northampton, Northampton, United Kingdom

[2]School of Sport, Outdoor and Exercise Science, University of Derby, Derbyshire, United Kingdom

[3]Centre for Exercise and Sports Science Research (CESSR), School of Exercise and Health Sciences, Edith Cowan University, Joondalup, Australia

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Stretching active muscle affects strain risk

CONTACT INFORMATION

Anthony D. Kay[1]

Sport, Exercise and Life Sciences, The University of Northampton, Boughton Green Road, Northampton, NN2 7AL, United Kingdom. Tel: +44 (0)1604 892577; Fax: +44 (0)1604 720636; Email: tony.kay@northampton.ac.uk

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 $10°\cdot s^{-1}$. **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.

Keywords: Isokinetic eccentric training, ROM, muscle stiffness, injury, ultrasound.

INTRODUCTION

Muscular strength and joint range of motion (ROM), as well as the resistance to stretch within the ROM (i.e. tissue stiffness), are important physical characteristics that influence the capacity to perform athletic tasks and activities of daily living (26), impact muscle strain injury risk (27,29,38,39), and are affected considerably with progression to older age (13). With respect to muscle strain injury, muscle stretching exercises are commonly used to increase ROM under the assumption that it will influence injury risk (24). Despite static stretching being the most commonly used stretching mode, proprioceptive neuromuscular facilitation (PNF) regularly produces greater increases in ROM (16). A common a form of PNF is contract-relax (CR) stretching, where a brief (sometimes maximal) isometric contraction is performed with the muscle in a highly-stretched position. However, using CR stretching can be practically problematic as performing these contractions can be painful, induce muscle damage, and requires partner assistance (16). Despite the efficacy of these techniques to increase ROM, prospective studies often cite muscle strength (29) and active muscle-tendon complex (MTC) stiffness (38) as strong predictors of muscle strain injury, with the association between ROM and injury being less clear. Moreover, muscle strain injuries usually occur within a normal ROM and may be load rather than muscle length dependent (21). Thus, identifying strategies that enhance the muscle's ability to withstand mechanical loading and absorb or dissipate strain energy may be more important than, or at least of comparable importance to, improving the maximal passive elongation capacity of the muscles (i.e. ROM).

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,

there are currently no data describing the effects of muscle stretching imposed on isometrically-contracting muscle in humans.

Given the above, the aim of the present study was to examine the influence of 6 weeks of training where stretch was applied to isometrically-contracting muscle. A distinction needs to be made between the CR stretching technique and the method of stretch employed in the present study, i.e. stretch applied to isometrically-contracted muscle. CR stretching involves passively lengthening a muscle and holding for a period of time (static stretch phase) before an isometric contraction is performed with the muscle remaining in a highly-stretched position (16). However, in the present study the muscle was maximally contracted (isometrically) at a short muscle length, with the maximal contraction being maintained as the muscle was stretched (lengthened) by the dynamometer (i.e. an isokinetic eccentric contraction). Outcome variables included dorsiflexion ROM, maximal passive joint moment at full volitional ROM (i.e. stretch tolerance), the slope of the passive moment curve (i.e. MTC stiffness), the area under the passive moment curve (i.e. potential elastic energy storage), passive gastrocnemius medialis (GM) muscle stiffness and active Achilles tendon stiffness, and maximal isometric plantar flexor moment. We tested the hypothesis that such training would result in significant increases in strength, ROM, stretch tolerance, energy storage, MTC stiffness and tendon stiffness, while simultaneously reducing passive muscle stiffness.

MATERIALS & METHODS

Subjects

Thirteen athletic males (collegiate soccer players, age = 20.0 ± 0.9 yr, height = 1.8 ± 0.1 m, 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 $m \cdot s^{-1}$) 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

at 0.087 rad·s^{-1} (5°·s^{-1}) to determine dorsiflexion ROM and peak passive moment (stretch tolerance) at full volitional ROM. Two minutes later the subjects performed two maximal isometric plantar flexor contractions (2 min rest between contractions) to determine maximal isometric joint moment (where >5% difference in maximal isometric moment was found between contractions, the subjects performed a third contraction). At least 48 h later the subjects commenced the 6-week training program (described below). Upon completion of the program, the subjects repeated the passive and active experimental trials 3-5 days after the final training session.

Range of motion, passive moment and energy storage

During the passive ROM trial, the subjects' foot was passively dorsiflexed through their full ROM until they volitionally terminated the movement by pressing a hand-held release button at the point of discomfort, a stretch intensity commonly used in ROM studies (16). The movement velocity was chosen as it has been reported to be too slow to elicit a significant myotatic stretch reflex response (25), thus we were confident that full volitional ROM was reached and the moment data were considered reflective of the passive properties of the plantar flexors. The passive dorsiflexion trial was repeated twice at 30-s intervals with passive moment data being recorded from the third trial. The passive trials enabled ROM, peak passive moment (stretch tolerance), the slope of the passive moment curve (MTC stiffness), and the area under the passive moment curve (potential elastic energy storage) to be calculated. Dorsiflexion ROM was calculated from the anatomical position (0°) to peak dorsiflexion with peak passive moment measured within a 250-ms epoch at peak dorsiflexion, while energy storage was calculated as the area under the passive moment curve from the anatomical position to peak dorsiflexion (Nm^2). The slope of the passive moment curve was calculated as the change in plantar flexor moment per change in joint angle

199 through the final 10° of dorsiflexion in the pre-stretching trials ($Nm \cdot {}^{\circ -1}$), 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

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 ($N{\cdot}m{\cdot}mm^{-1}$) 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 ($N{\cdot}m{\cdot}mm^{-1}$); 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 $10°{\cdot}s^{-1}$ 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 $30°{\cdot}s^{-1}$, providing a 1-s rest between contractions; 60

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

Data analysis

All data were analyzed using SPSS statistical software (version 20; LEAD Technologies, Chicago, IL). Data are reported as mean ± SE, and Cohen's D was used to calculate effect size (ES). Normal distribution for pre- and post-group data in all variables was assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests; no significant difference ($P > 0.05$) was detected in any variable, indicating that all data sets were normally distributed. As several variables were clearly related separate multiple analyses of variance (MANOVA) with repeated measures were used to test for differences between pre- and post-training data in 1) MTC stiffness (slope of the passive moment curve), passive GM muscle stiffness, and active Achilles tendon stiffness, and 2) dorsiflexion ROM and peak passive moment (stretch tolerance). Repeated measures t-tests were used to test for differences in maximal isometric plantar flexor moment and the area under the passive moment curve (elastic potential energy). Statistical significance for all tests was accepted at $P < 0.05$.

Reliability

Intratester reliability for the manual digitization of MTJ excursion within the ultrasound images (n = 5) has been determined previously in our laboratory (15) by calculating the intraclass correlation coefficient (ICC) and coefficient of variation (CoV; expressed as a percentage of the mean). A high ICC (0.99) and low CoV (0.4%) were calculated; no significant difference was detected between mean values ($P > 0.05$). Test-retest reliability has also been determined previously (16) for peak isometric moment, peak passive moment (stretch tolerance), ROM, slope of the passive moment curve (MTC stiffness), muscle

stiffness and tendon stiffness. No significant difference was detected between test-retest mean values ($P > 0.05$) for any measure; intraclass correlation coefficients (ICC) were 0.89, 0.97, 0.97, 0.95, 0.80, and 0.96. Coefficients of variation and standard errors (expressed as a percentage of the mean) were 9.5% (SE = 2.3%), 7.8% (SE = 1.9%), 4.4% (SE = 1.1%), 12.4% (SE = 3.0%), 11.1% (SE = 2.7%), and 4.4% (SE = 1.1%), respectively.

Sample size

Effect sizes (Cohen's D) were calculated from mean changes in variables (strength, ROM, muscle and tendon stiffness, and peak passive moment) from previous studies employing similar methods (4,15,16). To ensure adequate statistical power for all analyses, power analysis was conducted for the variable with the smallest effect size (ROM; ES = 1.2) using the following parameters (power = 0.80, alpha = 0.05, effect size = 1.2, attrition = 20%). The analysis revealed that the initial sample size required for statistical power was 12, thus 16 subjects were recruited to account for possible data loss and subject attrition. Three subjects withdrew from the study with non-related injuries; statistical analyses were conducted on data sets for 13 subjects who completed the testing.

RESULTS

Range of motion, passive moment and energy storage

A significant increase in dorsiflexion ROM (92.7 ± 19.9% [14.7 ± 2.0°], ES = 1.78; $P < 0.01$) was found after the training program. This very large increase in ROM was accompanied by a significant increase peak passive moment (136.2 ± 30.2%, ES = 2.11; $P < 0.01$) measured at full volitional ROM (i.e. stretch tolerance; see Figure 3). Similarly, a significant increase (302.6 ± 95.8%, ES = 1.88; $P < 0.01$) in the area under the passive moment curve (i.e. energy storage; see Figure 3) was detected after training. Significant correlations (see Figure 4) were

observed between the change in ROM and changes in stretch tolerance (r = 0.72; $P < 0.05$) and energy storage (r = 0.59; $P < 0.05$). A significant correlation was also detected between the changes in stretch tolerance and energy storage (r = 0.92; $P < 0.01$). These results are indicative that changes in peak passive loading and total elastic energy storage are closely associated with improvements in ROM.

MTC, muscle, and tendon stiffness

Passive moment was similar post-training at all dorsiflexion angles (see Figure 5. A) along the joint moment-angle curve. No significant change (-1.5 ± 6.8%, ES = 0.05; $P > 0.05$) in the slope of the passive moment curve (MTC stiffness) was observed during the passive ROM trial following the training (see Figure 5. B). However, analysis of the ultrasound data revealed a significant increase in tendon stiffness (31.2 ± 5.0%, ES = 1.10; $P < 0.01$) during the maximal isometric plantar flexor trial (see Figure 6) and a significant reduction in GM muscle stiffness (14.6 ± 4.3%, ES = 0.24; $P < 0.05$) during the passive ROM trial (see Figure 6) following the training. No correlation ($P > 0.05$) was observed between change in ROM and changes in muscle (r = 0.17) or tendon (r = 0.08) stiffness.

Maximal isometric plantar flexor moment

A significant increase in maximal isometric plantar flexor moment (51.3 ± 7.5%, ES = 1.65; $P < 0.01$) was observed following the 6-week training program, with joint moment increasing substantially from pre- (136.2 ± 10.0 Nm) to post-training (200.5 ± 11.5 Nm). A significant correlation was observed between changes in maximal isometric plantar flexor moment and Achilles tendon stiffness (r = 0.62; $P < 0.05$). The substantial mean increase in isometric moment and very large effect size clearly indicate a substantial and meaningful increase in joint moment generating capacity.

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

375 Historically, muscle stretching exercises have been used to increase ROM partly with the aim
376 of reducing muscle injury risk, yet equivocal and limited benefits have been reported (24) so
377 the efficacy of muscle stretching programs has been questioned. However, recent studies
378 have reported that isotonic eccentric training reduced (31) or even eliminated (34) the
379 incidence of new and recurrent muscle strain injuries, although possible underlying
380 mechanisms associated with this benefit were not examined. No studies have reported the
381 effects of muscle stretch imposed during muscle contraction, i.e. similar to maximal
382 isokinetic eccentric exercise, on injury incidence. Despite no change in MTC stiffness (i.e.
383 slope of passive moment curve) being detected in the present study (~2%), a significant
384 increase in active Achilles tendon stiffness (~31%, ES = 1.10) and a decrease in passive GM
385 muscle stiffness (~15%, ES = 0.24) were revealed when joint moment data were examined in
386 conjunction with sonographic data. These disparate results may be explained as the decrease
387 in muscle and concomitant increase in tendon stiffness resulting in a lack of overall change in
388 MTC stiffness. These findings are consistent with previous passive, static stretching research
389 where no change in MTC stiffness was detected despite greater fascicle and whole muscle
390 lengthening (5). The findings are also consistent with studies imposing isotonic eccentric
391 training, where MTC stiffness was unchanged despite a significant increase in tendon
392 stiffness being observed (19). Nonetheless, it should be noted that shorter-duration, lower-
393 intensity isotonic eccentric training elicited a reduction in passive MTC stiffness without
394 detectable changes in tendon stiffness (20). Cumulatively, these findings are consistent with
395 the present study and indicate that reductions in passive muscle stiffness may occur following
396 passive stretch and stretch imposed on active muscles (e.g. isotonic and isokinetic eccentric
397 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; $N{\cdot}m{\cdot}mm^{-1}$), 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; $N{\cdot}m^{-2}$ 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

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

In the present study the substantial increase in ROM was associated with further adaptations likely being beneficial to strain injury risk, including very large increases in peak passive moment at full volitional ROM (i.e. stretch tolerance; ~136%, ES = 2.11) and in the area under the passive moment curve (i.e. elastic potential energy storage; ~300%, ES = 1.88). Furthermore, significant correlations were observed between the changes in ROM and changes in both stretch tolerance (r = 0.72) and energy storage (r = 0.59). During passive tissue lengthening in isolated muscle preparations, elastic potential energy has been associated with the onset of strain injury (21) whereas no association was found for muscle length. Importantly, stiffer tissue is often less extensible (i.e. reduced deformation capacity) and its ability to store and dissipate strain energy limited when compared with more compliant tissue. This inability to deform and store energy has been suggested to increase muscle strain injury risk in stretch-shortening cycle exercise (for review see ref. 39). In the present study, the substantial increase in ROM, elastic energy storage potential and peak passive loading are suggestive that the muscle was able to tolerate substantially greater loading and deformation, which likely translates into a protective effect against muscle strain injury. Furthermore, maximal isometric joint moment also increased substantially (~51%, ES = 1.65) indicating that the training performed in the present study (qualitatively similar to isokinetic eccentric exercise) enhanced the muscle's ability to generate force and tolerate loading. Thus, applying stretch to maximally activated muscle generates substantial beneficial changes in several measures associated with muscle strain injury risk including muscle stiffness (38), muscle strength (29), energy storage (21), and ROM (37). These considerable and concomitant adaptations in multiple important risk factors may highlight the

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.
468
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595 **FIGURE CAPTIONS**

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597 **Figure 1.** Schematic depicting motion analysis reflective marker placements and ultrasound
598 probe positioning. Gastrocnemius medialis (GM) muscle length was estimated from the
599 distance between reflective markers placed over the origin of the GM muscle on the medial
600 femoral epicondyle (*marker A*) and the distal edge of the ultrasound probe (*marker B*), which
601 was positioned over the GM-Achilles muscle-tendon junction (MTJ). Achilles tendon length
602 was estimated from the distance between reflective markers placed over the distal edge of the
603 ultrasound probe (*marker B*) and the insertion of the Achilles on the calcaneus (*marker C*).

604

605 **Figure 2.** Ultrasound image of the GM-Achilles MTJ. Real-time ultrasound imaging
606 recorded the position and displacement of the gastrocnemius medialis (GM)-Achilles muscle-
607 tendon junction (MTJ) during passive and active trials. The MTJ was identified as the point
608 where the superficial and deep GM aponeuroses merged with the Achilles tendon.
609 Displacement of the MTJ from the distal edge of the image (D) was synchronized with
610 motion analysis data to calculate GM muscle and Achilles tendon lengths.

611

612 **Figure 3.** Peak passive moment and the area under the passive moment curve pre- and post-
613 training. A significant increase in peak passive moment (i.e. stretch tolerance; 136.2 ±
614 30.2%) was found with peak passive moment increasing substantially from pre- (35.4 ± 4.2
615 Nm) to post-training (74.5 ± 5.9 Nm). Similarly, a significant increase in the area under the
616 passive moment curve (i.e. energy storage; 302.6 ± 95.8%) was with the area increasing
617 substantially from pre- (84.7 ± 16.9 Nm^2) to post-training (234.0 ± 26.1 Nm^2). #Significant to
618 $P < 0.01$.

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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.

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 ± 6.8%) with MTC stiffness being similar at pre- (7.2 ± 1.1 $Nm \cdot {}^{\circ -1}$) and post-training (7.0 ± 1.1 $Nm \cdot {}^{\circ -1}$).

Figure 6. Achilles tendon stiffness and gastrocnemius medialis (GM) muscle stiffness pre- and post-training. A significant increase in tendon stiffness (31.2 ± 5.0%) was found as tendon stiffness increased substantially from pre- (9.7 ± 0.6 $Nm \cdot mm^{-1}$) to post-training (12.6 ± 0.8 $Nm \cdot mm^{-1}$). A significant decrease in muscle stiffness (14.6 ± 4.3%) was found as muscle stiffness decreased substantially from pre- (3.1 ± 0.7 $Nm \cdot mm^{-1}$) to post-training (2.6 ± 0.6 $Nm \cdot mm^{-1}$). *Significant to $P < 0.05$, #Significant to $P < 0.01$.