TITLE

Stretch imposed on active muscle elicits positive adaptations in strain risk factors and exercise-induced muscle damage

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RUNNING HEAD

Stretching active muscle and injury risks

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ABTRACT

Introduction: Stretching highly-contracted plantar flexor muscles (isokinetic eccentric contractions) results in beneficial adaptations in muscle strain risk factors; however its effects in other muscle groups, and on architectural characteristics and exercise-induced muscle damage (EIMD), are unknown. **Methods:** The influence of a 6-week knee extensor training programme was studied in 26 volunteers (13 control; 13 experimental). Before and after the training programme, passive and maximal isometric and eccentric knee extensor moments and range of motion (ROM) were recorded on an isokinetic dynamometer with simultaneous ultrasound imaging of vastus lateralis (VL). On a separate day, EIMD markers (creatine kinase [CK], delayed onset muscle soreness [DOMS]) were measured before and 24 h after a 20-min downhill run. The 6-week training programme was performed twice-weekly where five sets of 12 stretches (3 s per stretch) were imposed on maximally contracted knee extensor muscles. **Results:** Significant ($P<0.05$) increases in eccentric (29.5%) and isometric (17.4%) moments, ROM (5.2°), stretch tolerance (55.4%), elastic energy storage (73.0%), VL thickness (7.8%), pennation angle (9.0%), and tendon stiffness (8.7%) occurred. No change ($P>0.05$) in passive muscle-tendon stiffness (-9.4%) or resting fascicle length (-0.7%) occurred. The downhill run resulted in substantial DOMS and significant increase in CK concentration before the training programme (107.6%), however DOMS was eliminated from the knee extensors and a significantly smaller increase in CK (-70.0%) occurred post-training. **Conclusion:** Positive adaptations in functional and physiological variables confirm that imposing stretch on maximally contracted muscle provides beneficial adaptations likely to mitigate EIMD and injury risk and enhance functional performance.

Keywords: Active stretching; isokinetic eccentric training; range of motion; EIMD; tissue stiffness.

INTRODUCTION

Both muscle strength[1] and active muscle-tendon complex (MTC) stiffness[2] have been reported in prospective studies to be stronger predictors of muscle strain injury than joint range of motion (ROM).[3] However, passive static muscle stretching remains a common exercise in athletic environments,[4] probably in an attempt to increase ROM and reduce injury risk. The weaker association may result from chronic stretch-induced increases in ROM often being associated with increased stretch tolerance (i.e. a reduced stretch/pain perception at a given ROM) rather than a reduction in muscle stiffness[5] or increased muscle[6] or fascicle[7] length (i.e. tissue-related changes). As stored elastic energy (loading capacity) rather than tissue deformation is more closely associated with tissue failure,[8] the lack of substantive mechanical loading or physiological adaptation following passive muscle stretching might explain its limited beneficial effect on muscle strain injury incidence.[3,4] Furthermore, as muscle stiffness is also associated with greater symptoms of exercise-induced muscle damage (EIMD),[9] identifying suitable interventions that influence stiffness whilst enhancing the muscle's ability to generate force and absorb or dissipate strain energy, is likely more important than improving only the maximal passive elongation capacity of the muscles (i.e. ROM).

Research using animal (rat) models has confirmed that passive muscle stretching does not provide an adequate stimulus sufficient to promote myocellular signalling for substantial mechanical or physiological adaptation.[6] However, greater signalling[6] and preservation of sarcomere number[10] have been observed when stretch is forcibly imposed on active muscle, i.e. qualitatively similar to eccentric contractions. The superior adaptive potential of high-loading isotonic eccentric training (compared with isometric or concentric training) has been demonstrated repeatedly, with greater increases in muscle mass,[11] strength,[12] fascicle length,[13] and protection against EIMD[14,15] being reported. Furthermore, substantial reductions in new and recurrent muscle strain injury incidence have been reported following the completion of

isotonic eccentric training programmes,[16-18] with a threefold increase in the likelihood of injury when eccentric training is not performed.[19] Collectively, these data are indicative that greater tissue loading, evident from intense eccentric contractions (i.e. stretch imposed on highly active muscle), likely induces wide-ranging adaptations in mechanical and physiological properties that attenuates EIMD and provides a greater protective effect than passive static muscle stretching.

Despite providing greater tissue loading potential than concentric or isometric contractions, isotonic eccentric contractions result from reducing neural drive to enable a fixed external load to overcome internal muscle forces;[20] consequently loading potential often remains submaximal. However, isokinetic eccentric contractions are velocity- rather than load-dependent,[21] thus enabling maximal voluntary effort throughout the full ROM (i.e. not compromised by force-length properties or internal/external moment arms) and during every repetition (i.e. not limited by fatigue). Whilst greater loading potential may exist in isokinetic forms of eccentric contractions, it requires specialised equipment that is expensive and difficult to administer across a large cohort. Unsurprisingly, relatively limited data exist describing neural, architectural and mechanical adaptations,[22] and its impact on injury incidence. Nonetheless, a recent study confirmed the potential of isokinetic eccentric training (i.e. stretch forcibly imposed on maximally contracted muscle) to provide substantially greater improvements in plantar flexor ROM (~15°)[23] when compared to the expected changes reported in review articles following passive static stretching (~6°)[24] or isotonic eccentric (~6°)[25] training programmes. Furthermore, very large increases in strength (~50%), elastic energy storage (~300%), and tendon stiffness (~30%) were also observed.[23] Nonetheless, EIMD biomarkers and morphological (muscle mass) and architectural (pennation angle, fascicle length) adaptations were not measured, limiting our understanding of possible

adaptations that could elucidate the underlying mechanisms associated with reductions in EIMD and muscle strain injury risk.

Therefore, the aim of the present study was to examine the influence of a 6-week training programme where stretch was forcibly imposed on maximally contracted knee extensor muscles (i.e. maximal isokinetic eccentric contractions). Outcome variables included knee flexion ROM, knee extensor stretch tolerance, elastic energy storage, MTC stiffness, active vastus lateralis (VL) tendon stiffness, VL muscle thickness, fascicle length and pennation angle, maximal isometric and eccentric knee extensor moments, serum creatine kinase (CK) concentration and delayed onset muscle soreness (DOMS). We tested the hypothesis that such training would result in significant increases in strength, ROM, stretch tolerance, energy storage, stiffness, muscle size, fascicle length and pennation angle, and reductions in EIMD markers (i.e. CK and DOMS).

MATERIALS AND METHODS

Subjects

Twenty-six recreationally active subjects (16 men, 10 women; age = 27.8 ± 8 y, height = 1.7 ± 0.1 m, mass = 70.0 ± 13.8 kg) with no recent history of lower limb injury or illness volunteered for the study after completing a pre-test medical questionnaire and providing written informed consent. Ethical approval was granted by the University of Northampton's Ethics Committee with the study completed in accordance with the Declaration of Helsinki.

Protocol

Overview

The subjects were randomly assigned to experimental or control groups (matched only by sex, i.e. 8 males and 5 females in both control and experimental groups) and familiarised with the

testing protocols one week prior to data collection. During initial data collection, the subjects completed a dynamometry-based trial (described later) to determine passive and maximal active knee extensor joint moments, knee flexion ROM, and VL muscle-tendon mechanical, morphological and architectural characteristics. Forty-eight hours later, the subjects completed a VO_{2max} trial (described later) to predict 70% VO_{2max} running velocity. Seventy-two hours later, the subjects completed a 20-min downhill run at 70% VO_{2max} running velocity to promote exercise-induced muscle damage (EIMD; described later) in the lower limb extensor muscle groups. Creatine kinase (CK) serum concentration and delayed onset muscle soreness (DOMS) were measured immediately before and 24 h after the downhill run. Following these initial trials, the subjects in the experimental group commenced the 6-week active stretch training programme (described later), while the control group continued with their normal physical activities. The dynamometry and downhill running trials were repeated at least 3 and 5 days, respectively, after the final training session. The reliability of these measures has been reported previously,[23] however the control group (which undertook the initial downhill run but did not perform the 6-week eccentric training programme) was included to determine whether six weeks was an adequate 'wash out' period from any residual protective 'repeat bout effect' from the initial downhill run.

Active and passive dynamometry-based trials

Subject positioning

The subjects performed a 5-min jogging warm-up on a Gaitway II S treadmill (H/P Cosmos, Nussdorf, Germany) at a self-selected pace (1.9 ± 0.2 m·s^{-1}) at the transition between walking and jogging. Subjects were then seated in the isokinetic dynamometer chair (Biodex System 3 Pro, IPRS, Suffolk, UK) with the hips at 85° and both knees at 90° (anatomical position for all joints = 0°). The right lateral femoral epicondyle was aligned with the centre of rotation of the dynamometer with non-elastic strappings across the shoulders, hips and ankles to minimise

movement in the dynamometer chair (see Figure 1A). One experienced analyst conducted all trials in order to remove inter-tester variability.

Muscle morphology and architecture

Real-time ultrasound imaging (Vivid I, General Electric, Bedford, UK) was used to record the vastus lateralis (VL) muscle at 28 Hz frame rate using a wide-band linear probe (8L-RS, General Electric) with a 39 mm wide field of view and coupling gel (Ultrasound gel, Dahlhausen, Cologne, Germany) between the probe and skin. The probe was placed equidistant from the lateral femoral epicondyle and greater trochanter and manipulated until the superficial and deep aponeuroses could be visualized to enable longitudinal imaging of the VL muscle. The probe was fixed to the skin with zinc-oxide adhesive tape to ensure consistent and accurate imaging of muscle. Measurements were taken at rest with the subject in a seated position (hip and knee at 90° flexion; see Figure 1A) with muscle thickness measured at the centre of the image from the superficial to deep aponeuroses with pennation angle calculated as the average of three clearly visible fascicles on the deep aponeurosis. As full fascicle length could not be visualised, standard trigonometry[26] was used to calculate fascicle length (l_f) using the average pennation angle (PA) from three fascicles and muscle thickness (MT) measurements with the following equation: $l_f = MT / \sin(PA)$.

Isometric and eccentric knee extensor moments

Two minutes later the subjects performed two warm-up submaximal ramped isometric contractions at 50% and 75% perceived maximal voluntary contraction (MVC) on the right limb with the knee flexed to 80°, followed by 2 maximal ramped isometric contractions with MVC reached ~3 s after contraction initiation and held for 2 s (60 s rest between contractions). Two minutes later, the subjects performed two submaximal (50% and 75% perceived MVC) and two maximal isokinetic eccentric knee extensor contractions (60 s rest between

contractions) on the right limb initiated from full extension (0°) through 90° of flexion at 0.522 rad·s^{-1} (30°·s^{-1}); eccentric moment was also measured at 80° of knee flexion to enable the isometric-to-eccentric ratios to be calculated. During isometric and eccentric MVC trials, the greater of the two isometric and two eccentric trials were used as measures of maximal isometric and eccentric joint moments, respectively. Where >5% difference in MVC was obtained, the subjects performed a third contraction.

Tendinous tissue stiffness

To measure stiffness of the distal tendinous tissue, ultrasound imaging was used to identify the insertion of a clearly visible fascicle on the deep aponeurosis, which was manually digitised (Vivid I, General Electric) during the ramped isometric MVC trials to enable changes in tendon (and aponeurosis) length to be calculated. Joint moment and ultrasound data were electronically synchronised using a 5-V ascending transistor-transistor logic (TTL) pulse that simultaneously placed a marker on the AcqKnowledge (v4.1, Biopac) software and ended the capture of ultrasound data (preceding 15 s of data). Tendon tissue stiffness was calculated as the change in knee extensor moment from 30-80%MVC divided by the change in tendon length (Nm·mm^{-1}) during the ramped isometric contraction trials. To ensure stiffness was calculated in the same region of the force-length curve, an identical force range equating to 30-80%MVC taken from the pre-training testing session (41.8 ± 2.7 Nm to 111.4 ± 7.3 Nm) was used in both pre- and post-training calculations (see Figure 2A). To confirm that the loading rate did not influence tendon stiffness, the time taken to increase active joint moment from 30% to 80% of MVC (i.e. the range over which tendon stiffness was calculated) was recorded. No significant difference (pre-training = 1.8 ± 0.1 s, post-training = 1.9 ± 0.1 s; $P > 0.05$) was observed indicating that similar strain rates were achieved.

Range of motion and passive moment

After completing the active trials, the subjects were placed in a prone position on the dynamometer chair with the knees flexed to 90° and right lateral femoral epicondyle aligned with the centre of rotation of the dynamometer. Non-elastic strappings across the shoulders minimised movement (horizontal displacement) in the dynamometer chair (see Figure 1B). Three passive rotations (30 s rest between rotations) were initiated at 0.087 rad·s^{-1} (5°·s^{-1}) through their full knee flexion ROM until the subjects volitionally terminated the rotation at the point of discomfort, a stretch intensity commonly used in ROM studies.[27,28] As one of the aims was to determine the impact of the training on ROM, during familiarization any subjects whose knee flexion ROM was limited by anatomical structures (i.e. heels able to touch buttocks) were removed from the study. Joint moment and angle data were directed from the dynamometer to a high-level transducer (model HLT100C, Biopac, Goleta, CA) before analog-to-digital conversion at a 2000-Hz sampling rate (model MP150 Data Acquisition, Biopac). The data were directed to a personal computer running AcqKnowledge software (v4.1, Biopac) and filtered with a zero lag, 6-Hz Butterworth low-pass filter prior to ROM and joint moments being determined.

Passive moment data were recorded from the third ROM trial to ensure thixotropic properties of skeletal muscle did not influence joint moment data.[29] Peak passive moment (i.e. stretch tolerance) was measured within a 250-ms epoch at full volitional ROM. The area under the passive moment curve (i.e. elastic energy storage [Nm^2]) was measured from the inflexion point of the slope of the passive moment curve (126.0 ± 2.7°) to full volitional ROM to ensure moment data were reflective of tissue stiffness rather than slack length or limb weight.[30] The slope of the passive moment curve (i.e. MTC stiffness) was calculated as the change in knee extensor moment per change in joint angle through the final 10° of knee flexion in the pre-training trials (Nm·$^{\circ-1}$); identical joint angles were used in post-training analysis to ensure the

same region of the force-length curve was used to calculate passive tissue stiffness (see Figure 2B).

VO_{2max} and downhill running trials

Forty-eight hours later, the subjects performed a VO_{2max} trial on the Gaitway II S treadmill with O_2 consumption recorded using a Metalyzer 3B online breath-by-breath gas analyser (Cortex, Leipzig, Germany) using Cortex software (v.3.9.1). Starting at 1.39m·s^{-1} (5 km·h^{-1}) at 0° incline, velocity was increased by 2 km·h^{-1} every 3 min until volition exhaustion with VO_2 measured in the final 30 s of each stage. Regression analysis enabled the calculation of running velocity at 70% VO_{2max}, which was used in the downhill running trial. Seventy-two hours later, the subjects completed a 20-min downhill run on the treadmill (16% decline) at 2.5 ± 0.4 m·s^{-1} (i.e. ~70% VO_{2max}) to promote EIMD in the three lower limb extensor muscle groups (i.e. hip extensors, knee extensors, plantar flexors). Creatine kinase (CK) serum concentration and DOMS were measured before and 24 h after the run. To ensure CK serum concentrations were indicative of resting levels, subjects were instructed not to perform any vigorous exercise for 72 h prior to the downhill running trial. Before the run, the subjects' middle finger was sterilised using an alcohol wipe (Robinson Healthcare, Worksop, UK) and pricked by a Unistik 3 Extra lancet (Owen Mumford, Oxfordshire, UK). Manual pressure expressed blood from the finger that was collected in a 30 µl Microsafe capillary tube (SafeTec®, Pennsylvania, USA), transferred to a CK strip (Roche Diagnostics, Mannheim, Germany) and placed in a Reflotron Plus analyser (Roche Diagnostics) with serum CK concentration determined using reflectance photometry. CK was also measured 24 h later as initial pilot testing (5 subjects) revealed that CK concentration peaked at 24 h post-run. Pilot testing revealed poor reliability and validity of the visual analog scale (VAS) to subjectively determine DOMS (i.e. subjects reporting 6-9 on the VAS within the same trial, and subjects reporting 3-5 on the VAS for DOMS prior to any exercise); therefore subjects were asked to rank DOMS only for presence within, and

severity between, the three lower limb muscle groups (i.e. hip extensors, knee extensors, plantar flexors).

Six-week active stretch training programme

Before each training session the subjects in the experimental group performed the standardised 5-min jogging warm-up and were seated in the dynamometer chair in identical positioning to that described above (see active dynamometry-based trial above). Active stretch training was performed on both limbs, twice-weekly (at least 48 h between sessions) for six weeks. For 5 sets of 12 repetitions, a 3 s active stretch of the knee extensors was generated by the dynamometer initiated from 0° (full extension) to 90° flexion at $30°\cdot s^{-1}$ with the subjects producing a maximal contraction throughout the stretch (i.e. a maximal voluntary isokinetic eccentric contraction). During familiarization, maximal isometric moment was determined at full extension (initiation point of the stretch) with the dynamometer programmed to begin the stretch only when subjects reached 80%MVC to ensure subjects were at near-maximal effort prior to stretch initiation. After each repetition, the subjects relaxed and the dynamometer extended the knee to the starting position at a velocity of $90°\cdot s^{-1}$ providing a 1-s rest period between contractions; 60 s rest was provided between sets.

Data Analysis

All data were analysed using SPSS statistical software (version 22; IBM, Chicago, IL) and reported as mean ± SE; Cohen's D was used to calculate effect size (ES). Normal distribution for pre- and post-training data in all variables was assessed using Shapiro-Wilk tests. As several variables were clearly related, separate two-way (2 × 2) multivariate analyses of variance (MANOVA) were used to test for differences between condition (control vs. experimental) and time (pre- vs. post-training) in (i) isometric and eccentric strength, (ii) passive MTC stiffness (slope of the passive moment curve) and active tendon stiffness, and

(iii) knee flexion ROM, peak passive moment (stretch tolerance) and the area under the passive moment curve (elastic energy storage). A two-way ANOVA was used to test for differences in CK concentration between condition (control vs. experimental) and time (pre- vs. post-training). Normal distribution was determined for absolute change score data in all variables using Shapiro-Wilk tests. Pearson's product moment correlation coefficients (*r*) were computed to quantify the relationship between the changes in all variables. Statistical significance for all tests was accepted at $P < 0.05$.

Sample size

Effect sizes (Cohen's D) were calculated from mean changes in variables (strength [ES = 1.65], hypertrophy [ES = 1.58], creatine kinase [ES = 3.95], ROM [ES = 1.78], tendon stiffness [ES = 1.10], elastic energy storage [ES = 1.88], and peak passive moment [ES = 2.11]) from previous studies employing similar interventions.[23,31,32] To ensure adequate statistical power for all analyses, power analysis was conducted for tendon stiffness (i.e. the variable with the smallest effect size) using the following parameters (variable = tendon stiffness, power = 0.80, alpha = 0.05, effect size = 1.1, attrition = 20%). The analysis revealed that the total sample size required for statistical power was 22, thus 28 subjects were initially recruited to account for possible attrition. Two subjects withdrew from the study with unrelated injuries prior to the completion of the study, with statistical analyses conducted on the complete data sets for 26 subjects.

RESULTS

No significant difference ($P > 0.05$) was found between control and experimental groups in any pre-training measure, indicating that the groups were similar at baseline. In the control group (see Table 1), no significant difference ($P > 0.05$) was found after six weeks in knee flexion ROM (-0.7 ± 1.5°), peak passive moment (-2.0 ± 1.9%), slope of the moment curve (-

1.6 ± 8.9%), area under the moment curve (-1.0 ± 4.0%), maximal isometric (-6.3 ± 4.8%) and eccentric (5.0 ± 2.8%) knee extensor moments, VL tendon stiffness (3.4 ± 6.8%), muscle thickness (-0.1 ± 0.3%), pennation angle (0.2 ± 0.3°) and fascicle length (-0.9 ± 2.2%). Significant increases in CK concentration were found in the control group 24 h after the downhill running trials performed before (pre-run = 142.5 ± 26.0 µl, post-run = 272.4 ± 73.8 µl; increase = 115.4 ± 50.7% [ES = 0.70]) and after (pre-run = 137.4 ± 20.6 µl, post-run = 298.4 ± 64.6 µl; increase = 120.5 ± 36.3% [ES = 0.80]) the 6-week training programme. However, no significant difference ($P > 0.05$) in the increase in CK, or in 24 h post-run CK concentrations, was detected between pre- and post-training trials indicating that six weeks was an adequate 'wash out' period as similar markers of muscle damage occurred after both trials. This was further confirmed by substantial DOMS reported in all three muscle groups 24 h after both pre- and post-training downhill running trials.

In the experimental group, significant ($P < 0.05$) increases in knee flexion ROM (5.2 ± 1.5°; ES = 0.98), peak passive moment (i.e. stretch tolerance, 55.4 ± 19.4%; ES = 0.76), and area under the moment curve (i.e. elastic energy storage, 73.0 ± 23.7%; ES = 0.78) occurred, while a non-significant reduction ($P > 0.05$) in the slope of the moment curve (i.e. MTC stiffness, -9.4 ± 5.9%; ES = 0.35) was detected (see Table 1). Significant correlations (see Figure 3) were detected between the change in ROM and changes in stretch tolerance (r = 0.76; $P < 0.01$) and elastic energy storage (r = 0.63; $P < 0.05$), and between the changes in elastic energy storage and stretch tolerance (r = 0.97; $P < 0.01$).

Significant ($P < 0.05$) increases in maximal isometric (17.4 ± 7.9%; ES = 0.58) and eccentric (29.5 ± 15.8%; ES = 0.73) knee extensor moments were detected after the 6-week training programme (see Figure 4). The greater increases in eccentric strength resulted in a significant ($P < 0.05$) decrease in the isometric-to-eccentric strength ratio (pre-training = 1:1.3, post-

training = 1:1.4; ES = 0.24). Significant ($P < 0.05$) increases in VL tendon stiffness (8.7 ± 2.8%; ES = 0.74), muscle thickness (7.8 ± 2.1%; ES = 1.01) and pennation angle (9.0 ± 2.8% [1.1 ± 0.3°]; ES = 0.88) were also observed; no change in fascicle length (-0.7 ± 0.9%; ES = 0.23) was detected (see Table 1). A significant correlation was observed between the change in pennation angle and change in muscle thickness (r = 0.97; $P < 0.01$). No significant correlations were observed between any other measures (r = 0.12-0.49; $P > 0.05$).

Twenty-four hours after the downhill running trials, significant ($P < 0.05$) increases in CK concentration were detected before (pre-run = 124.5 ± 18.6 µl, post-run = 253.0 ± 41.2 µl; increase = 107.6 ± 16.0% [ES = 1.45]) and after (pre-run = 131.3 ± 23.0 µl, post-run = 186.6 ± 34.1 µl; increase = 46.0 ± 17.7% [ES = 0.66]) the 6-week training programme (see Figure 5). However, the increase in CK concentration after the 6-week programme was significantly lower than the increase detected before the training programme (-70.4 ± 33.5%; ES = 0.70). Before the 6-week training programme, all subjects reported substantial DOMS 24 h after the downhill run in the hip extensors, knee extensors and plantar flexors (DOMS was reported as most severe in the knee extensor group). After the training programme, DOMS was reported as absent (10 subjects) or minimal (3 subjects) in the knee extensors (i.e. trained muscle group), whereas DOMS in the hip extensors and plantar flexors (untrained muscle groups) remained high.

DISCUSSION

The primary aim of the present study was to examine the impact of a 6-week, twice-weekly training programme of stretches imposed on highly-contracted knee extensor muscles (i.e. maximal isokinetic eccentric contractions) on multiple functional and physiological variables cited within the primary aetiology of muscle strain injury and commonly associated with EIMD. In agreement with our hypothesis, substantial increases in knee flexion ROM (~5°; ES

= 0.98), passive knee extensor torque at full ROM (i.e. stretch tolerance, ~55%; ES = 0.76), and area under the moment-angle curve (i.e. passive elastic energy storage, ~73%; ES = 0.78) were detected after the training programme. These findings are consistent with previous studies examining the impact of active muscle stretching imposed on other muscle groups (i.e. plantar flexors) in both human[23] and animal[6,10] models. Substantially greater increases in ROM, elastic energy storage and stretch tolerance have recently been reported following active muscle stretching[23] when compared to passive stretch practices.[24,28] Furthermore, substantial reductions in both new and recurrent muscle strain injuries have been reported following isotonic eccentric training routines,[16-18] using similar loading strategies to the present study. Collectively, these findings indicate that stretching of a muscle while in a fully contracted state is substantively more effective than current passive static stretching practices for achieving clinically relevant changes in passive mechanical properties commonly associated with muscle strain injury.

The substantial increase in elastic energy storage and ROM detected in the present study are suggestive that the muscle was able to tolerate substantially greater loading and deformation, respectively. Furthermore, reviews have suggested that individuals with muscles unable to effectively deform and store energy were likely more susceptible to muscle strain injury risk in stretch-shortening cycle exercise,[33] with stiffer individuals also exhibiting greater symptoms of EIMD following eccentric exercise.[9] In the present study, similar increases in creatine kinase concentration and DOMS occurred in the control group following pre- and post-training downhill running trials. However, in the experimental group, a significant reduction in the increase in creatine kinase concentration (~70%; ES = 0.70) and elimination of DOMS from the knee extensors (i.e. the trained muscle group) occurred after the training programme. Thus, we are confident that (i) an adequate 'wash-out' period from the initial downhill run was imposed and that (ii) changes in EIMD markers in the experimental group were attributable to

the 6-week training programme. While an increase in active tendon tissue stiffness (~9%; ES = 0.74) was observed in the present study, no significant change in passive whole muscle-tendon complex (MTC) stiffness was detected (~-9%; ES = 0.35). These apparent tissue-specific changes are consistent with previous studies examining the effects of identical isokinetic (plantar flexors)[23] and similar isotonic[34] eccentric training programmes. The increases in tendon stiffness however, are expected to be of functional benefit in a variety of movement tasks and can be considered a beneficial outcome of the training. A possible limitation of the present study was that motion analysis was not used to correct for potential knee extension during the "isometric" contraction. A greater movement of the muscle-tendon junction resulting from knee extension would likely result in tendon stiffness being underestimated, therefore the tendon absolute stiffness values should be considered with this limitation in mind. Nonetheless, as stiffness was determined within an identical force range during pre- and post-training testing sessions (i.e. 30-80%MVC calculated from pre-training testing), the error is expected to be similar across tests and could not explain the increase in active tendon stiffness detected post-training. Collectively, these data are indicative of a protective effect of the active stretching training programme, consistent with previous reports of a protective 'repeat bout effect' following similar intense eccentric (active muscle lengthening) contractions.[14]

A significant increase in muscle thickness (~8%; ES = 1.01) occurred, which is consistent with previous studies following isokinetic[35] and isotonic[36] eccentric training, and pennation angle (9%; ES = 0.88) was increased, which is also a common finding.[35,37] Indeed, the increases in muscle thickness and pennation angle were strongly correlated (r = 0.97), indicating that the increase in muscle thickness was predominantly associated with changes in pennation angle following the training. However, no change in fascicle length was detected (<1%: ES = 0.23), which contrasts with some studies showing that isotonic eccentric training can significantly

increase fascicle length.[13] Nonetheless, a lack of fascicle length change has been reported after isokinetic training even though a significant increase was observed after isotonic training in the same study (~11%).[38] It is also pertinent that VL fascicle length changes after 10 weeks of isokinetic knee extensor eccentric training (30 sessions) were relatively small (~3%, p=0.056),[35] especially considering that 30 sessions were performed in that study (compared to 12 sessions in the current study). A possible limitation of the study was that morphological and architectural measurements were taken with the knee flexed to 90°, however any change in passive tension at this joint angle that may have influenced these measurements was not recorded. Nonetheless, the lack of change in MTC stiffness (as measured using the stress/strain curve) provides some evidence changes in passive tension were negligible. Collectively, these data are suggestive of a lesser change in fascicle length in response to isokinetic than isotonic/isoinertial eccentric training.

The change in pennation angle may have important functional implications where potentially greater fascicle rotation during muscle stretch,[39] may limit fascicle strain. However, subject positioning prevented ultrasound imaging during passive trials to determine these changes. Nonetheless, the effect of concomitant changes in both muscle shape (size) and pennation can be explored through the use of a simple 2-D model, e.g. of a 40-cm long muscle elongating by 2 cm (see Data, Supplemental Digital Content 1, which models architectural changes during muscle elongation). At pre-training in the current study, fascicles would have rotated from 12.5° to 11.9°, thickness reduced from 27.3 cm to 26.6 mm (i.e. the muscle CSA is reduced during elongation), and fascicle length increased from 126.1 mm to 129.0 mm during the stretch. The change in fascicle length was therefore 2.9 mm or 2.3%. At post-training the fascicles would have rotated from 13.6° to 13.0, thickness reduced from 29.3 to 26.6 mm, and fascicle length increased from 124.6 to 127.6 mm during the stretch. The change in fascicle

length would therefore be 3.0 mm or 2.4%, which is almost identical to pre-training. Thus, although fascicle length did not appreciably change with the training, fascicle lengthening (strain) during stretch is unlikely to be different even though other architectural parameters were changed significantly. Because VL imaging during stretch was not possible in the present study (subject positioning prevented an ultrasound probe being placed in the middle of the muscle belly), fascicle strain was not directly measured. Therefore, direct examinations during dynamic actions (where changes in intramuscular pressure will influence the fascicle length-to-muscle length change ratio)[39] are required to fully quantify the effect of active muscle stretch training on muscle-tendon function during dynamic activities.

Whilst mechanical properties, including elastic energy storage, ROM and stiffness have been associated with injury risk,[3,8] prospective studies have reported stronger associations with functional characteristics including eccentric muscle strength.[1,40] In addition to the substantial changes in passive mechanical properties detected in the present study, large increases in isometric (~17%; ES = 0.58) and eccentric (~29%; ES = 0.73) strength, and a small decrease in the isometric-to-eccentric strength ratio (~10%; ES = 0.24), were also observed. The magnitude of strength changes combined with the greater increase in eccentric than isometric strength are consistent with previous studies examining the impact of isotonic forms of eccentric-only training.[11] While the increase in eccentric muscle strength is functionally important for improving athletic task performance and for reducing muscle strain injury risk,[1,40] individuals are thought to remain susceptible when a high isometric-to-eccentric strength ratio exists (i.e. similar isometric-to-eccentric force capacities), regardless of absolute strength.[40] As the magnitude of eccentric loading during stretch shortening cycle (SSC) movements is dictated somewhat by the magnitude of prior concentric (propulsive) force, the lower ratio would likely ensure that the ability to develop propulsive force was substantially lower than the ability of the muscle to absorb the loading energy during the subsequent

eccentric phase. As eccentric-only training is commonly reported in reviews to generate the greatest increase in eccentric strength compared to other contraction modes,[11] exposure to eccentric-only training should not only provide the greatest improvements in eccentric strength but is likely to effectively decrease the isometric-to-eccentric strength ratio, which may minimise muscle strain injury risk and incidence.[40] Thus, whilst muscular strength is clearly associated with injury risk, imposing generic strength training may not be as effective as eccentric-only training (i.e. stretches imposed on highly-active muscle) for reducing injury risk, and its use as part of an overall training and injury prevention regimen will likely result in more effective (and time efficient) beneficial adaptations.

PERSPECTIVE

The concomitant and substantial adaptations in multiple functional, physiological and mechanical characteristics highlight the likely adaptations following eccentric training routines that may enhance our understanding of the previously reported reductions in muscle damage[14,15] and both new and recurrent muscle strain injuries[16-18] following these training routines. The potential for significant practical outcomes exists as training similar to that used in the present study can be applied within the clinical rehabilitative environment by use of isokinetic training practices or in applied athletic environments by the use of partner-assisted stretches imposed with the muscles in a highly active state. Randomised, controlled trials are needed in the future to compare the influence of this training to standard (isotonic) eccentric training, as the magnitude and rate of adaptations achieved in multiple outcome measures commonly associated with injury risk in the present study have substantial implications for current preventative training routines and injury incidence.

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TABLE AND FIGURE CAPTIONS

Table 1. Mechanical and architectural measurements during pre- and post-training assessments in the control and experimental groups (Mean ± SEM).

Measurements	Pre-training	Post-training	Effect Size
Knee Flexion ROM (°)	147.7 ± 2.6	*152.9 ± 2.7	0.98
Elastic Energy Potential (Nm^2)	152.4 ± 33.1	*222.6 ± 43.0	0.78
Stretch Tolerance (Nm)	96.2 ± 17.0	*133.4 ± 21.5	0.76
MTC Stiffness (Nm·°)	5.1 ± 1.1	4.7 ± 1.2	0.35
VL Tendon Stiffness ($Nm \cdot mm^{-1}$)	5.1 ± 0.3	*5.5 ± 0.4	0.78
VL Fascicle Length (mm)	127.4 ± 6.3	126.3 ± 6.1	0.23
VL Pennation Angle (°)	12.5 ± 0.4	*13.6 ± 0.4	0.88
VL Thickness (mm)	27.3 ± 1.1	*29.3 ± 1.1	1.01

Acronyms: ROM: range of motion, MTC; muscle-tendon complex, VL: vastus lateralis, *: significantly different to pre-training ($P < 0.05$).

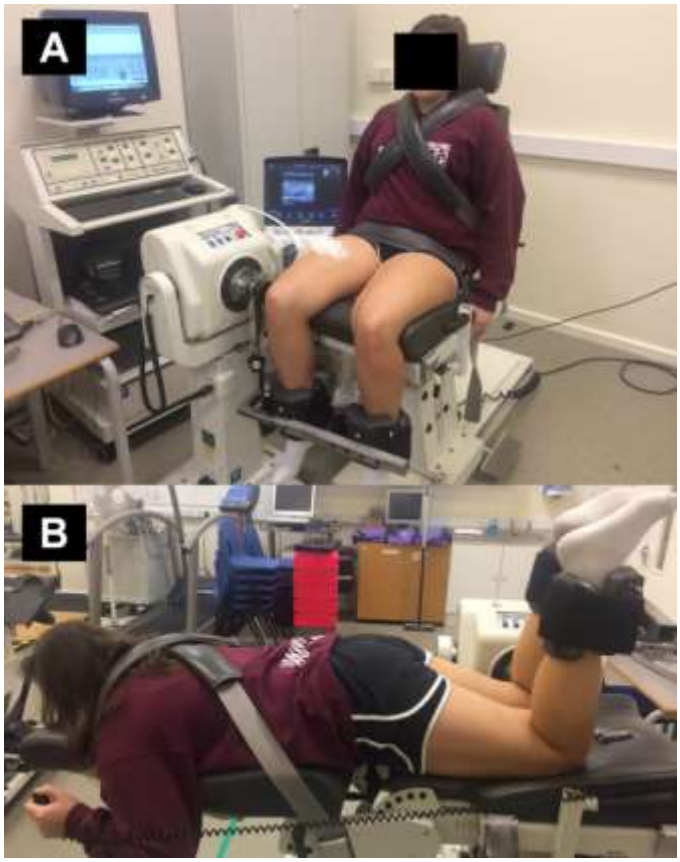


Figure 1. Subject positioning in the dynamometer during active and passive trials. During the active isometric and eccentric trials (A), subjects were seated with the hips flexed to 85° and knees flexed to 90° with non-elastic strappings across the shoulders, hips and right thigh to prevent unwanted movement. During the passive range of motion trials (B), subjects were placed in a prone position with the knees flexed to 90° with non-elastic strappings across the shoulders to prevent unwanted movement.

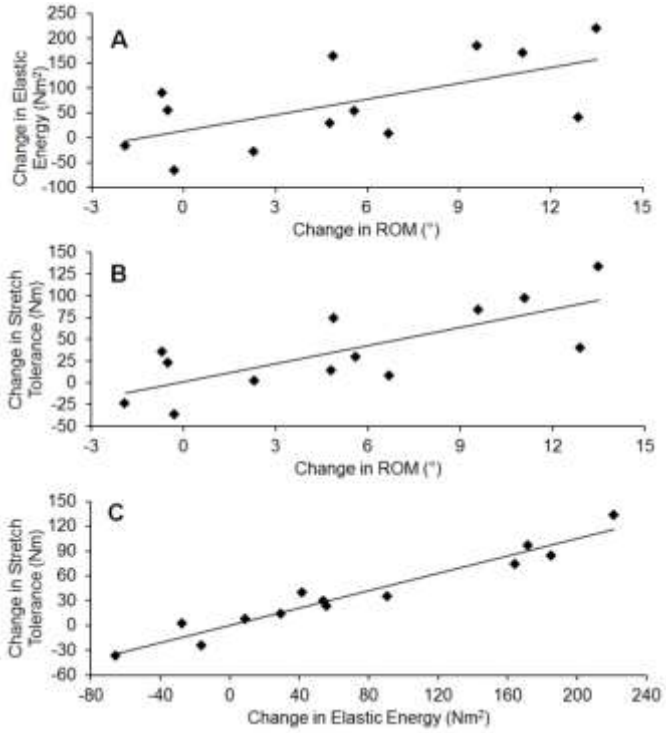


Figure 2. Active tendinous tissue elongation and passive moment measured during active and passive trials pre- and post-training. Exemplar data from an experimental group subject depicting ultrasound tendon elongation (A) and passive moment (B). Tendon stiffness was calculated through the force range from the pre-training active trials equating to 30-80% maximum voluntary contraction (MVC). Passive muscle-tendon complex (MTC) stiffness was calculated through the final 10° of knee flexion range of motion (ROM) from the pre-training trials.

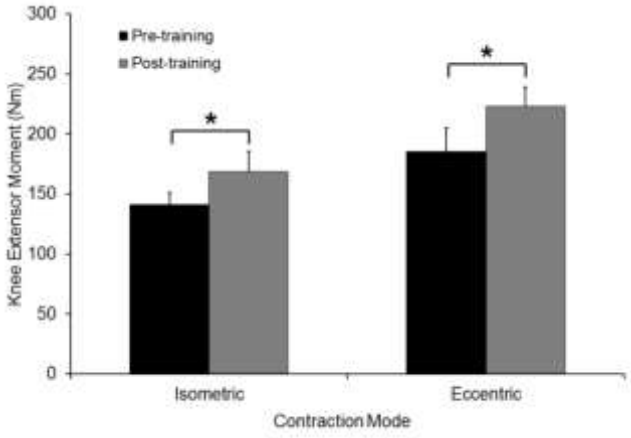


Figure 3. Correlations between changes in range of motion (ROM), stretch tolerance and elastic energy storage. Significant correlations were found after six weeks of training between the change in ROM and changes in stretch tolerance ([A] r = 0.76; $P < 0.01$) and elastic energy storage ([B] r = 0.63; $P < 0.05$), and between the changes in elastic energy storage and stretch tolerance ([C] r = 0.97; $P < 0.01$).

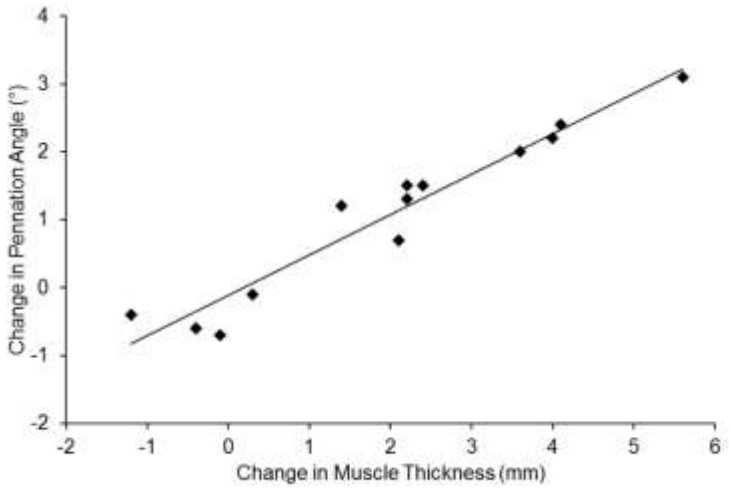


Figure 4. Mean and individual isometric and isokinetic eccentric knee extensor moments during pre- and post-training assessments. Significant increases in peak isometric (17.4 ± 7.9%; ES = 0.58) and eccentric (29.5 ± 15.8%; ES = 0.73) knee extensor moments were detected after the 6-week training programme. *Significant to $P < 0.05$.

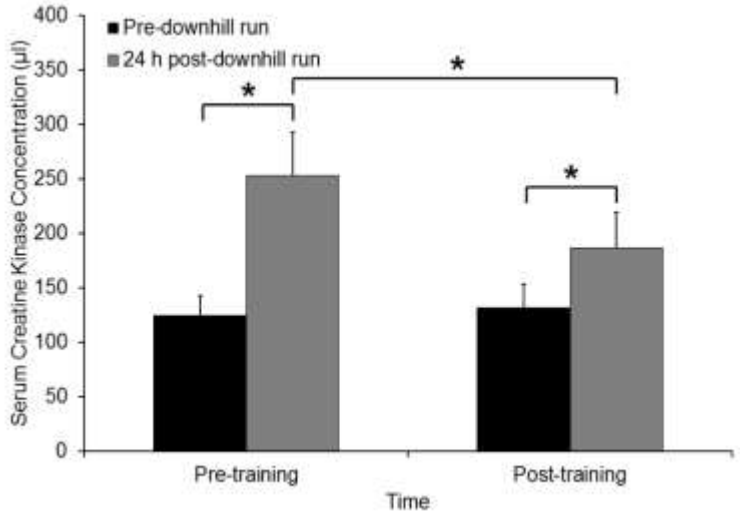
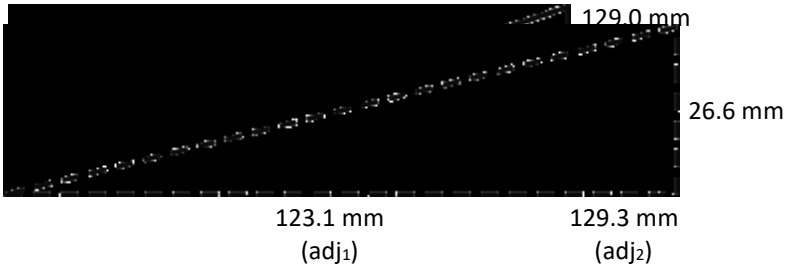


Figure 5. Mean and individual creatine kinsase (CK) concentration before and 24 h after the downhill running trials during pre- and post-training assessments. Significant ($P < 0.05$) increases in CK concentration occurred before (107.6 ± 16.0%; ES = 1.45) and after (46.0 ± 17.7%; ES = 0.66) the 6-week training programme. The increase in CK concentration was significantly lower after the 6-week programme (-70.4 ± 33.5%; ES = 0.70). *Significant to $P < 0.05$.

Supplemental Digital Content 1. Text and figures modelling fascicle strain during muscle elongation using pre- and post-training morphological and architectural data. pdf.

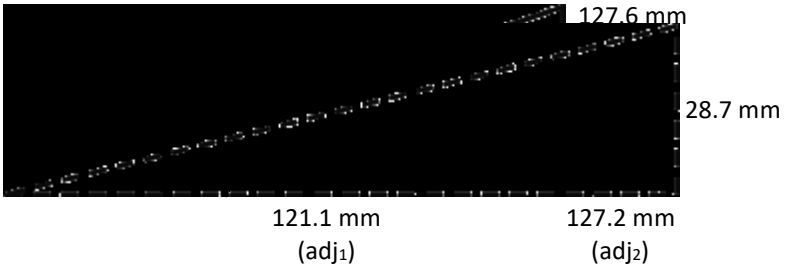
Take a muscle of 400-mm length, with a thickness of 27.3 mm and fascicle angle of 12.5° at pre-training (data taken from Table 1). Fascicle length (27.3/sin12.5) will be 126.1 mm and the muscle length encompassed by the fascicle will be 123.1 mm (Supplementary Figure 1; solid triangle).



Supplementary Figure 1. Pre-training architectural changes during muscle lengthening (adjacent side 1; adj_1, adjacent side 2; adj_2).

When stretched by 20 mm (from adj_1 to adj_2; dashed triangle) the fascicle will lengthen by 6.2 mm (123.1/400 = 30.1%) to 129.3 mm and the muscle thickness will be reduced in order to maintain volume (or thickness in this model) and thus become 26.6 mm (assuming muscle is cylindrical, muscle volume = 234021 mm^3, and the muscle thickness at muscle length of 420 mm = $2\left(\sqrt{234021/420\pi}\right) = 26.6\,mm$, given that volume = $\pi r^2 l$). The fascicle will rotate from 12.5° to 11.9° ($\sin^{-1}$[26.6/129.3]). The new fascicle length is therefore 129.0 mm (26.6/sin11.9), which is a strain of 2.3%.

After training in the current study the muscle had thickness of 29.3 mm and fascicle angle of 13.6°. Fascicle length will thus be 124.6 mm (29.3/sin13.6) and the muscle length encompassed by the fascicle (adj_1) will be 121.1 mm (Supplementary Figure 2; solid triangle).



Supplementary Figure 2. Post-training architectural changes during muscle lengthening (adjacent side 1; adj_1, adjacent side 2; adj_2).

When stretched by 20 mm (from adj_1 to adj_2) the fascicle will lengthen by 6.1 mm (121.1/400 × 20) to 127.2 mm and the muscle thickness will be reduced to 28.7 mm (assuming cylindrical muscle, muscle volume = 271409 mm^3, the muscle thickness at muscle length of 420 mm = $2\left(\sqrt{271409/420\pi}\right) = 28.7\ mm$). The fascicle will rotate to 13.0° ($\sin^{-1}$[28.7/127.2]). The new fascicle length is therefore 127.6 mm (28.7/sin13.0), which is a strain of 2.4%.

Based on this model, the fascicle strain is very similar between pre- and post-training.