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Journal of Sport and Health Science

Original article

Multiscale hamstring muscle adaptations following 9 weeks of eccentric training

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Received 30 March 2024; revised 13 June 2024; accepted 28 June 2024
Available online 24 October 2024

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Abstract

Background: Eccentric training, such as Nordic hamstring exercise (NHE) training, is commonly used as a preventive measure for hamstring strains. Eccentric training is believed to induce lengthening of muscle fascicles and to be associated with the addition of sarcomeres in series within muscle fibers. However, the difficulty in measuring sarcomere adaptation in human muscles has severely limited information about the precise mechanisms of adaptation. This study addressed this limitation by measuring the multiscale hamstring muscle adaptations in response to 9 weeks of NHE training and 3 weeks of detraining.

Methods: Twelve participants completed 9 weeks of supervised NHE training, followed by a 3-week detraining period. We assessed biceps femoris long-head (BFlh) muscle fascicle length, sarcomere length, and serial sarcomere number in the central and distal regions of the muscle. Additionally, we measured muscle volume and eccentric strength at baseline, post-training, and post-detraining.

Results: NHE training over 9 weeks induced significant architectural and strength adaptations in the BFlh muscle. Fascicle length increased by 19% in the central muscle region and 33% in the distal muscle region. NHE also induced increases in serial sarcomere number (25% in the central region and 49% in the distal region). BFlh muscle volume increased by 8%, and knee flexion strength increased by 40% with training. Following 3 weeks of detraining, fascicle length decreased by 12% in the central region and 16% in the distal region along with reductions in serial sarcomere number.

Conclusion: Nine weeks of NHE training produced substantial, region-specific increases in BFlh muscle fascicle length, muscle volume, and force generation. The direct measurement of sarcomere lengths revealed that the increased fascicle length was accompanied by the addition of sarcomeres in series within the muscle fascicles.

Keywords: Nordic hamstring exercise; Muscle architecture; Adaptation

1. Introduction

Hamstring strains are the most prevalent cause of lost practice and match time in running-based sports.[1–5] These injuries not only disrupt training and match performance[6–8] but also carry significant physical, emotional, and financial consequences.[7,9] The high recurrence rate of hamstring injuries compounds these negative consequences.[2,10–12] While eccentric exercises have been proposed to mitigate hamstring injuries,[13–15] the precise structural and mechanical adaptations within the hamstring muscles remain poorly understood.

Hamstrings can undergo remarkable adaptations to eccentric training, including increases in fascicle length and muscle

Peer review under responsibility of Shanghai University of Sport.
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https://doi.org/10.1016/j.jshs.2024.100996
Cite this article: Andrews MH, et al. Multiscale hamstring muscle adaptations following 9 weeks of eccentric training, *J Sport Health Sci* 2025;14:100996

volume.[16–18] Although eccentric exercises, such as the Nordic hamstring exercise (NHE), modify muscle architecture at the macroscopic level,[16–18] the corresponding microscopic changes that occur and may protect against hamstring injuries remain less clear. Bridging this knowledge gap from the microscopic (sarcomere) to the macroscopic (fascicle) level is essential to enhance understanding of these commonly used exercises.

The addition of parallel sarcomeres in response to eccentric training is suggested to reduce the stress per sarcomere at a given level of muscle force, whereas the addition of serial sarcomeres may decrease the strain per sarcomere at a given level of muscle stretch.[19] An increase in the number of parallel sarcomeres is associated with greater cross-sectional area of muscle fibers, consequently enhancing maximum force.[20] A trending hypothesis proposes that eccentric training stimulates serial sarcomerogenesis, leading to the addition of sarcomeres in series and increases in hamstring fascicle length.[16,17,21,22] This addition of sarcomeres in series is thought to be induced by the large forces generated during muscle lengthening and may decrease strain per sarcomere at a given level of muscle stretch, as suggested above.[19,23,24] Thus, muscle fascicle elongation after eccentric training may stimulate the addition of serial sarcomeres to reduce the strain on each sarcomere and enhance force generation capacity in line with the sarcomere force-length relationship.[23,25]

Several studies have examined the time-course of sarcomere length and serial sarcomere number adaptations in animal models.[26–28] Only 1 study has examined *in vivo* adaptations of sarcomere length and serial sarcomere number in response to eccentric training in humans.[29] Using a novel microendoscopy technique,[30,31] this previous study measured the effects of 3 weeks of NHE on the initial adaptations of human hamstrings.[29] The study found sarcomere lengthening to be the primary source of fascicle lengthening, specifically in the distal region of the biceps femoris long-head (BFlh).[29] It is plausible that 3 weeks of training was too short to induce the addition of serial sarcomeres.[32] Given the short duration of this previous study, it is important to examine structural changes across the different levels of muscle organization of the hamstrings over a longer duration of eccentric training.

The training adaptations in hamstring muscles induced by eccentric training appear to undergo rapid reversal during periods of detraining.[18] Some evidence indicates that NHE training-induced adaptations in fascicle length tend to revert to baseline lengths after 4 weeks of detraining.[16,33,34] In contrast, strength gains are better maintained during these detraining periods despite a decrease in muscle mass.[16,34] This could be explained by maintenance of neural adaptations that contribute to maximum force production.[35] As both strength and fascicle length are predictors of hamstring injury risk,[36,37] understanding the mechanisms that contribute to the regression of architectural adaptations during detraining could inform strategies for mitigating the loss of potentially protective benefits acquired from training.

Building on our previous study revealing early increases in BFlh fascicle length following 3 weeks of NHE training with no concomitant increase in serial sarcomere number,[29] this study explored the effects of a longer training period, in which serial sarcomerogenesis may be more likely to occur.[26,27,32] Given the protective nature of eccentric training against hamstring injury risk,[15,38–40] a longer eccentric exercise program would be expected to induce an addition of sarcomeres in series within the BFlh muscle fascicles.[32] To explore these adaptations, this study investigated the impact of an NHE program over 9 weeks, followed by a 3-week detraining period. We examined the effects on BFlh fascicle length, sarcomere length and number, muscle volume, and knee flexor strength. We hypothesized that the 9-week intervention would result in increased fascicle length and the addition of sarcomeres in series. Based on our previous work,[29] we expected larger adaptations in the distal region of the BFlh muscle. Following detraining, we hypothesized that there would be a reduction in fascicle length and sarcomere number but not in eccentric strength.

2. Methods

2.1. Study design

A longitudinal follow-up intervention design was adopted to test the effect of 9 weeks of NHE training followed by 3 weeks of detraining (Fig. 1). Before beginning the training program, baseline measurements of passive fascicle length, sarcomere length, sarcomere number, muscle volume, and strength were recorded. These measures were repeated after completion of the 9-week training program and again after the 3-week detraining period. Passive fascicle length was measured after completion of Weeks 3 and 6 of the 9-week training intervention. Strength measures were recorded weekly throughout the NHE intervention and after the 3-week detraining.

2.2. Sample size calculation

The initial sample size calculations were based on the expected variations in BFlh fascicle length resulting from the training intervention. The effect size was determined from our recent study,[29] in which we observed a 21% increase in BFlh fascicle length in the distal region following 3 weeks of eccentric training ($\eta_p^2 = 0.81$, approximately equivalent to a Cohen's *d* effect size of 2.06). Previous research has also reported similarly large effect sizes, typically with a Cohen's *d* effect size ranging between 2.5 and 3.5.[16–18]

To enhance our sample size calculation, we considered the effect of sarcomere length changes. In our previous study, we found a 17% increase in sarcomere length in the distal region of the BFlh muscle after training ($\eta_p^2 = 0.90$, approximately equivalent to a Cohen's *d* effect size of 3.0).[29] Recognizing the large effects of training on sarcomere length, the current study conservatively sets the effect size at half of what was observed in fascicle length in our previous research.[29] It was determined that a sample size of 6 participants was needed to provide a statistical power of 95% to detect an effect size of 1.0 for fascicle length changes, using 3 repeated measures while maintaining a significance level of $\alpha = 0.01$. To

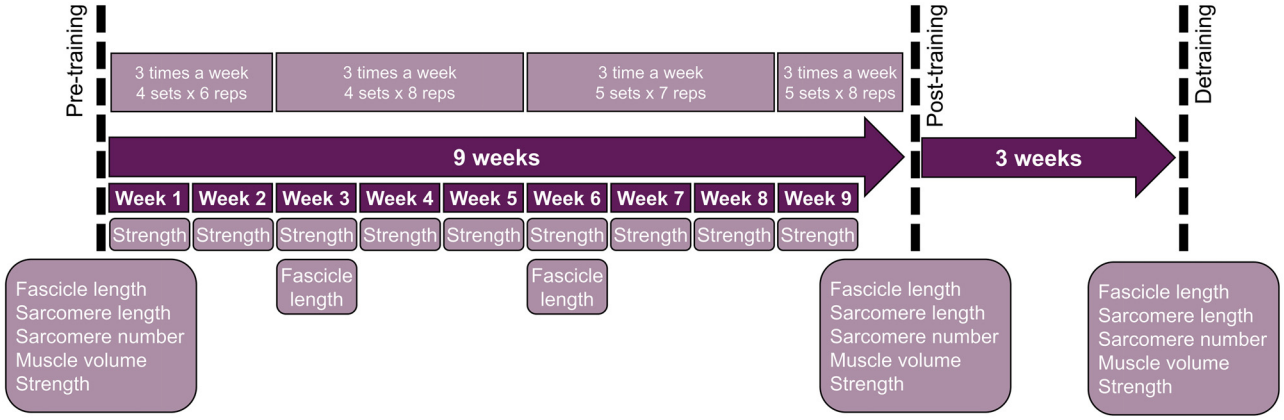


Fig. 1. Timeline of the study. Illustration of the key measurement phases and the 9-week Nordic hamstring exercise (NHE) training program, followed by a 3-week detraining period. reps = repetitions.

accommodate potential variability in usable measurements, particularly in the difficult-to-measure sarcomere lengths, we aimed to recruit a sample size twice that of the calculated minimum.

2.3. Participants

Twelve recreationally active participants (5 males: age = 27.6 ± 4.1 years, height = 1.87 ± 0.07 m, mass = 82.4 ± 7.5 kg; 7 females: age = 26.5 ± 3.6 year, height = 1.70 ± 0.04 m, mass = 64.9 ± 9.6 kg; mean ± SD) were recruited. The participants were free from lower limb injuries within the past 18 months and had not performed NHE training within the past 6 months. Each participant provided written informed consent before participation. The protocol was approved by the Stanford University Institutional Review Board (approval No. SQL 97942) and ratified by The University of Queensland Ethics Committee (approval No. 2022/HE001616).

2.4. NHE intervention

Participants performed 9 weeks of training with 3 sessions per week, consisting of 4−5 sets ranging from 6 to 8 repetitions (Fig. 1). Set and repetition range assignments were programmed to promote strength and hypertrophy. Based on extant studies, the total volume per week (72−120 repetitions) is considered sufficient to elicit muscle architectural changes.[16,41]

During the initial laboratory session, participants were familiarized with the NHE. They received instruction on proper technique and performed several practice repetitions. The instructions included kneeling on a padded board using a self-selected ankle angle with their ankles secured by straps. Starting from a kneeling position with the arms close to the chest, participants gradually leaned forward while attempting to maintain a consistent hip angle and controlled knee extension at approximately 18°/s. They continued lowering their bodies until they could no longer hold themselves up or their hands touched the floor. Catching themselves upon reaching the floor, participants used their arms to assist as they returned to the initial position to minimize concentric load as they prepared for the next repetition.

Given the importance of movement speed in the NHE,[42] participants performed the NHE at a controlled average speed of approximately 18°/s, which was dependent on the range of motion and controlled by a metronome set at a frequency of 60 beats per minute. This speed ensured that the time under tension for a full range NHE was approximately 5 s per repetition, which was monitored by the researcher (MHA) with feedback provided to participants. Participants self-selected a recovery period between repetitions, typically around 5 s, while a 2-min rest period between sets was overseen by the researcher (MHA). Subsequent training sessions, whether conducted in the laboratory or remotely, were supervised by the researcher (MHA). For remote training, participants used either a device (e.g., a barbell) or a partner to secure their ankles. A warm-up consisting of dynamic stretching and submaximal NHE repetitions preceded each session to prepare participants for the training session.

2.5. Strength

Eccentric hamstring strength during the NHE was measured using 2 uni-axial load cells (HT Sensor Technology, Shaanxi, China) attached to the ankles via straps, as previously described.[29] The NHE was performed in the same manner as during the training sessions. Before the strength testing, participants performed warm-up sets of 4 repetitions at 50% and 75% of maximum perceived effort. Following a 3-min rest period, participants performed a set of 4 repetitions at maximal effort with strong verbal encouragement. Eccentric hamstring strength was represented as peak knee flexor torque, calculated by multiplying peak knee force measured by the load cell (HT Sensor Technology) by the perpendicular distance from the line of action of force (at the ankle strap) to the approximate axis of rotation at the knee.

2.6. Passive fascicle length

Passive fascicle lengths of the BFlh muscle were measured with freehand 3D ultrasound. This technique extends the imaging field, capturing entire muscle fascicles across multiple regions, which overcomes the limitations of traditional

B-mode ultrasound.[43] The 3D ultrasound combines B-mode ultrasound (ArtUS EXT-1H beamformer and LF11-5H60-A3 transducer (60 mm); Telemed, Vilnius, Lithuania) with simultaneous 3D motion capture tracking (Optitrack; NaturalPoint, Corvallis, OR, USA). The ultrasound transducer had 4 reflective markers securely attached, enabling its position and orientation to be tracked in 3D space by the 4-camera motion analysis system (Optitrack).

During each imaging session, participants lay prone on a table with their hips and knees extended while their feet remained on the table surface, placing their ankles in a plantarflexed position. The scanning region of the BFlh muscle covered 20%−80% of the distance between the fibular head and the ischial tuberosity (Fig. 2). A trained researcher (MHA) moved the ultrasound transducer (Telemed) in a transverse orientation and at a constant speed and pressure between the defined regions of the BFlh muscle. We used 3D ultrasound software (Stradwin V6.0; Mechanical Engineering, Cambridge University, Cambridge, UK) to acquire multiple freehand ultrasound scans of the BFlh muscle, which were subsequently compiled into stacks of 2D B-mode ultrasound images to generate a 3D volume of the muscle. The 2 scans with the highest image quality, as determined by visual assessment of the clarity of fascicles, were selected from each imaging session for subsequent analysis.

Post-scanning, these ultrasound images were reconstructed to create a 3D volume of the muscle in the 3D ultrasound software (Stradwin: Mechanical Engineering, Cambridge University) according to the tracked position and orientation of the ultrasound transducer. From this 3D volume, a longitudinal 2D image of the regions of interest within the muscle was generated. This longitudinal image of the BFlh for architectural analysis was acquired by positioning a virtual muscle slice along the sagittal plane, parallel to the muscle fascicles.[29] Further details regarding this procedure can be found in Prager et al.[44] The central and distal regions of the BFlh muscle were determined based on the defined scanning region using the longitudinal muscle image (Fig. 2). In the 3D ultrasound software (Stradwin), the midpoint of each scan was identified, and 3 fascicles were selected in each region based on whether the majority of the fascicle was proximal or distal to that midpoint. In each region, fascicle length was determined by measuring 3 separate lengths using 6 points along each fascicle to account for curvature, extending from the superficial aponeurosis to the central aponeurosis. We have previously shown this to be a reliable method for obtaining fascicle length.[29] This process was repeated for 2 scans from each imaging session, with a single researcher (MHA) conducting all fascicle length measurements while remaining blinded to the participant and training status.

2.7. Sarcomere length and number

Second harmonic generation microendoscopy (Zebrascope; Zebra Medical Technology, Enspectra Health, Mountain View, CA, USA) was used to directly measure sarcomere length,

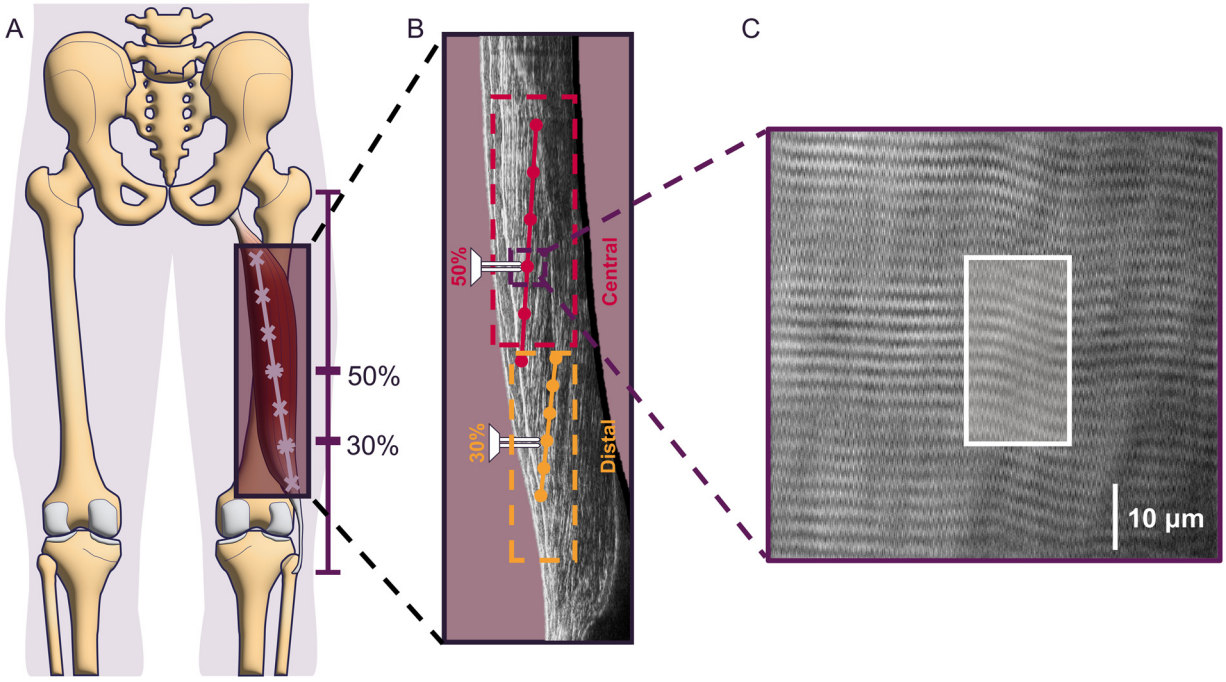


Fig. 2. Multiscale assessment of BFlh muscle fascicle length and sarcomere length. (A) Experimental configuration for BFlh fascicle and sarcomere assessment. The scanning region for 3D ultrasound fascicle imaging of the BFlh covers 20%−80% of the distance between the fibular head and the ischial tuberosity, with 30% and 50% landmarks denoting the insertion sites for sarcomere imaging. (B) BFlh architecture extracted from a 3D ultrasound scan. Solid lines with 6 points along each fascicle represent fascicle length measures in the central and distal regions. Sarcomere insertion sites at 30% and 50% are indicated by the needle probe icon. (C) Sarcomere image collected with second harmonic generation microendoscopy.[24,25] The boxed area represents the region of interest where Fourier transform analysis was applied to estimate sarcomere lengths. BFlh = biceps femoris long-head.

enabling the calculation of serial sarcomere number. Unlike previous approaches to measuring sarcomere length, which involve either surgical procedures or *in vitro* measures,[45,46] microendoscopy allows for direct and minimally invasive measurement of sarcomere length within living human muscle.[30,31]

The microendoscope probe (Zebrascope) consists of 2 needles: the first emits laser light and receives second harmonic generation images, while the other reflects the signal to increase signal strength.[31] To ensure consistent imaging of sites of sarcomeres in the distal (30%) and central (50%) regions of the BFlh, the microendoscope insertion sites were aligned with the previously identified scanning region for ultrasound (Fig. 2). B-mode ultrasound (Telemed) was used to verify the insertion position and orientation of the microendoscope needles in each region of BFlh. The microendoscope probe was rapidly inserted into the muscle tissue using a spring-loaded device, and a second harmonic generation microscope was then attached. The images collected appeared as repeating black and white bands and were sampled at 1.9 Hz as the researcher slowly moved the microendoscope needle through the muscle to collect images at different depths (−5 to 15 mm). A sequence of images from different muscle fibers was stored for subsequent analysis. Between imaging sessions, the needles were placed in a disinfecting solution.

The analysis of sarcomere lengths was performed using the sequence of images and following the previously described process.[29,47] This involved applying a Gaussian filter to the image to subtract noise and using a Fourier transform to calculate the strongest frequency spectrum across the image between 1.5 μm and 5.0 μm, encompassing a physiologically feasible range of sarcomere lengths.[48] A region on the sharpest fiber image was selected for analysis, and the Fourier transform was applied across that region of interest to estimate the average sarcomere length. Given the novelty of the method, a limited supply of microendoscope needles was available for sarcomere length measurements. Each subject was allocated a dedicated needle for sanitary reasons. However, degradation of the needles with sterilization between sessions limited the number of sarcomere measurements. To account for variability in sarcomere length in a particular muscle region, multiple measures were taken from different fibers, with a mean of 6.1 measures collected for each region and session (range 2−26). This variability in the number of measurements arises from the challenge of imaging sarcomeres *in vivo*. In some subjects, many different fibers produced clear images, whereas in others, only a few fibers yielded high-quality images. During image analysis, as many feasible images as possible were selected for each region, session, and participant, based on the clarity of the repeating sarcomere structure and signals that fell within the sarcomere length range, while sequential images likely representing the same fiber were ignored. Sarcomere length measurements were acquired from 9 participants in the central region and 6 participants in the distal region at pre-training; from 9 and 7 participants, respectively, at post-training; and from 5 and 4 participants after detraining. All sarcomere length measurements were conducted by a researcher who was blinded to the participant and condition. The serial sarcomere number was estimated by the equation:

$$\text{Serial sarcomere number} = \frac{\text{Mean passive fascicle length}}{\text{Mean passive sarcomere length}}$$

2.8. Muscle volume

Magnetic resonance imaging (MRI) was performed on a 3 Tesla MR scanner (3.0T GE SIGNA Premier; GE Healthcare, Chicago, IL, USA) to assess BFlh volume. Eleven of the 12 subjects underwent MRI scans placed in a feet-first prone position, with their hip, knees, and ankles extended. A 21-channel blanket air coil placed on their thigh was used for signal reception. The entire thigh region was imaged as 2 scan volumes: a superior and an inferior volume with a small region of overlap between the 2 scan volumes. This extended the field of view and mitigated the signal drop-off in slices farther away from the scanner isocenter.

The scan protocol for each volume consisted of a 6-point Dixon sequence (repetition time/echo time = 12.96/6.14 ms, number of echoes = 6, flip angle = 5 °, matrix = 252 × 252, field of view: 22.5−45.0 cm, slice thickness = 6 mm, number of slices: ∼40, scan time: ∼3 min). Dixon sequence can provide separate fat and water images. The post-processing step involved stitching the superior and the inferior scan volumes together to generate a single volume of the thigh region. Using 3DSlicer (Version 5.0.3, http://www.slicer.org),[49] BFlh boundary was manually outlined on each slice of this single volume by an experienced musculoskeletal MRI researcher (APS) blinded to the participant and condition. For consistency in measurements across all participants and visits, BFlh muscle volume was computed ranging from the superior image slice where the muscle belly was first visible to the inferior MR slice where the patella was first visible. To maintain consistency with other measurements, the muscle volumes from the right side were considered for analysis.

2.9. Statistical analysis

Linear mixed effects models (LMMs) were used to assess the effects of the 9-week NHE training and 3-week detraining on passive fascicle length, serial sarcomere number, muscle volume, and eccentric knee flexor torque. LMMs offer a useful analytical approach by handling repeated measures, individual variability, and unbalanced data, allowing for an examination of all trials and accounting for subject-specific variability in baseline muscle properties, training, and detraining effects.[50,51]

In the primary analysis, LMMs were performed on the main outcome measures. Fixed effects included “Time” and “Region”, whereas participant number was considered a random effect. Time encompassed the 3 time points (pre-training, post-training, and detraining). Region was defined as central or distal, for the fascicle and sarcomere analysis. For the assessment of muscle volume analysis, time was the sole fixed effect. The sarcomere data from the detraining phase were excluded from the statistical analysis because only 5

subjects were able to provide microendoscopy images for the central region and 4 subjects for the distal region, as the degradation of microendoscope needles with sterilization prevented accurate measurements for other subjects.

To provide a deeper understanding of the temporal changes in the measured variables, supplementary analyses were conducted with a focus on passive fascicle length (measured every 3 weeks) and eccentric knee flexor torque (measured weekly). Additional analyses using LMMs were performed, with the same fixed and random effects. The time variable was extended to accommodate additional time points.

Each LMM was fitted using the restricted maximum likelihood method. Then the determination of p values for the fixed effects was achieved through Satterthwaite's method[52] and presented in a Type III analysis of variance table. Effect sizes are reported as partial *eta* squared (η_p^2). Effects of 0.01, 0.06, and 0.14 were considered to be small, medium, and large, respectively.[53] Pairwise comparisons with Tukey's corrections were applied where significant main or interaction effects of muscle architecture and strength variables were found to determine where any differences occurred. The Shapiro−Wilk test was used to assess the normality of the residuals. In instances where normality assumptions were violated, outliers were identified and excluded using Cook's distance method, with a threshold of 2.5 times the SDs. All statistical tests were performed in RStudio (Version 2023.6.1.524; Posit Software, PBC, Boston, MA, USA) with an α set at $p < 0.05$.

3. Results

3.1. Effects of Nordic eccentric exercise training

Eccentric training of the hamstrings over 9 weeks significantly increased BFlh muscle fascicle length, serial sarcomere number, volume, and knee flexion torque (Fig. 3). There was no statistically significant change observed in sarcomere length ($p = 0.083$), and the effect size was small ($\eta_p^2 = 0.01$) compared to the significant effect of eccentric training on fascicle length ($\eta_p^2 = 0.68$, $p < 0.001$), number of sarcomeres in series ($\eta_p^2 = 0.85$, $p < 0.001$), muscle volume ($\eta_p^2 = 0.66$, $p < 0.001$), and knee flexion torque ($\eta_p^2 = 0.64$, $p < 0.001$).

3.2. Effects of training on regional fascicle and sarcomere adaptations

Adaptations in the fascicle lengths differed between regions in response to the training intervention. A significant interaction effect between Region and Time for fascicle length indicated a more pronounced training effect in the distal region than in the central region ($p < 0.001$; Fig. 3B). In the central region, the initial 3 weeks of training displayed no significant changes in fascicle length ($p = 0.999$), followed by a significant increase in fascicle length at the end of the training period ($p < 0.001$). The distal region displayed increasing fascicle length throughout the NHE training program ($p < 0.001$).

A significant regional effect on serial sarcomere number indicated that there were more serial sarcomeres in the central region compared to the distal region ($p < 0.001$, Fig. 3B). However, there was no interaction effect between Region and Time ($p = 0.339$). This indicates that the increase in serial sarcomere number in response to NHE training was consistent across both central and distal regions despite the inherent nonuniformity in the number of serial sarcomeres.

3.3. Effects of detraining on muscle architecture and strength

Fascicle length decreased from the end of training to the end of detraining ($p < 0.001$) but remained longer than pre-training lengths ($p < 0.001$). Fig. 3 presents the limited available data on sarcomere length and calculated serial sarcomere number, but the statistical power is insufficient to determine significant changes in either variable. Muscle volume was maintained after detraining ($p = 0.270$). A decrease in eccentric strength was observed after the detraining phase ($p = 0.006$), although eccentric strength remained higher than pre-training levels ($p < 0.001$).

4. Discussion

This study demonstrates that 9 weeks of NHE training induced serial sarcomerogenesis in the BFlh, as evidenced by a significant increase in fascicle length with a corresponding increase in the calculated number of sarcomeres in series. The increased fascicle length, muscle hypertrophy, and enhanced strength induced by the 9-week training regime could plausibly provide protection against strain injuries.

4.1. Muscle architecture and strength adaptations to training and detraining

BFlh fascicle length increased after 9 weeks of NHE training, which is in line with the findings of our previous 3-week NHE intervention study.[29] This elongation of muscle fascicles has been proposed as a primary protective mechanism associated with eccentric exercise against hamstring injury risk.[22] Our previous work showed that early fascicle length adaptations (3 weeks) to eccentric training appear to result from the stretching of sarcomeres.[29] This increase in sarcomere length would presumably afford no reduction in injury risk because sarcomeres are likely to be more easily overstretched. Our new data suggest that this stretching of sarcomeres, as we demonstrated after 3 weeks of NHE training,[29] might provide a stimulus for serial sarcomerogenesis with continued training. The timeline for serial sarcomerogenesis appears to align with the overall temporal pattern of muscle hypertrophy, where increases in cross-sectional area become more prominent after 3 weeks of training.[54,55] The present study provides evidence for the addition of sarcomeres in series as a longer-term adaptation to eccentric training. This suggests that with longer muscle fascicles due to serial sarcomere addition, at a given level of force, each sarcomere would be stretched less. Serial sarcomerogenesis could plausibly protect muscle fibers from overstretching, as has been previously suggested.[56,57]

Regional differences in fascicle length were evident, with greater adaptations in the distal region compared to the central

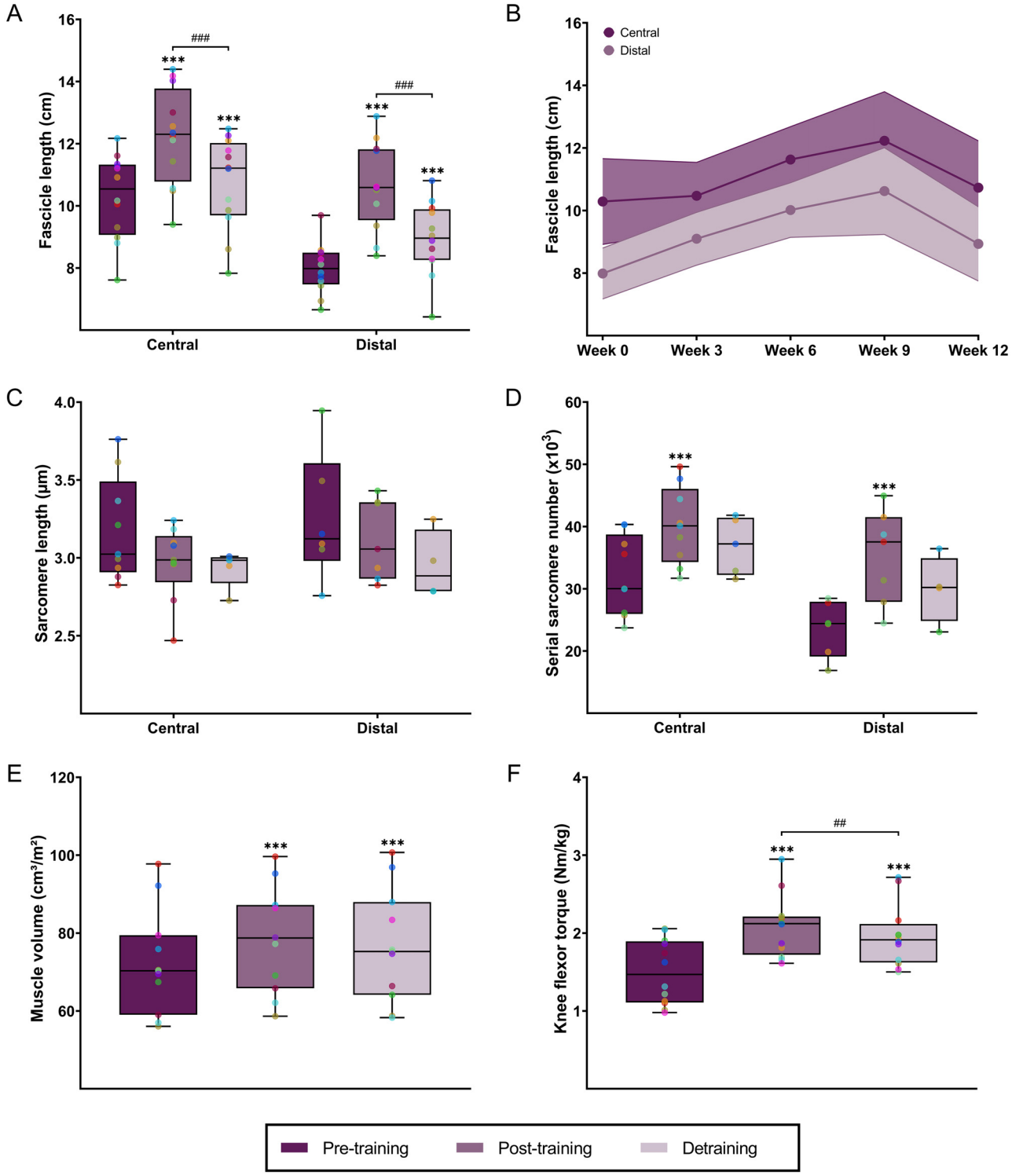


Fig. 3. (A) BFlh muscle fascicle length substantially increased after NHE training, later decreasing during detraining but remaining longer than pre-training ($F_{,415} = 442$, $\eta_p^2 = 0.68$, $p < 0.001$). Fascicle length was longer in the central region compared to the distal region ($F_{1,415} = 871$, $\eta_p^2 = 0.68$, $p < 0.001$). (B) Temporal progression of fascicle length revealed distinct region-specific adaptations ($F_{2,415} = 10$, $\eta_p^2 = 0.05$, $p < 0.001$) with no significant changes in the central region during the initial 3 weeks of training, followed by a substantial increase throughout the training period, whereas the distal region consistently displayed an increase in fascicle length throughout NHE training. (C) Passive sarcomere length shows no significant changes post-training and conservation after detraining ($F_{1,254} = 3$, $\eta_p^2 = 0.01$, $p = 0.083$). Inherent nonuniformity among sarcomere lengths was present ($F_{1,257} = 5$, $p = 0.027$, $\eta_p^2 = 0.02$), with varied adaptations between regions with training ($F_{1,256} = 6$, $\eta_p^2 = 0.02$, $p = 0.017$). (D) NHE training program stimulated sarcomerogenesis ($F_{1,19} = 105$, $\eta_p^2 = 0.85$, $p < 0.001$) with a slight decrease in sarcomere number after detraining. There were more serial sarcomeres in the central region ($F_{1,19} = 56$, $\eta_p^2 = 0.75$, $p < 0.001$) but no significant interaction effect ($F_{1,19} = 1$, $\eta_p^2 = 0.05$, $p = 0.339$). (E) BFlh hypertrophy with NHE training ($F_{2,20} = 20$, $\eta_p^2 = 0.66$, $p < 0.001$), maintained during detraining. Muscle volume has been normalized to height squared. (F) Increased eccentric strength occurred with training ($F_{2,129} = 113$, $\eta_p^2 = 0.64$, $p < 0.001$), remaining higher than pre-training levels even with detraining. Knee flexor torque was normalized to body mass. The main effects summarized in brackets include *F*-statistic values, degrees of freedom, *p* values, and effect size (η_p^2) categorized as small (0.01), medium (0.06), and large (0.14). ** $p < 0.01$, *** $p < 0.001$ indicate significant differences between pre-training and post-training. $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ indicate significant differences between post-training and detraining. Colored dots represent individual participant averages. BFlh = biceps femoris long-head; NHE = Nordic hamstring exercise.

region. The uneven distribution of hamstring injuries,[58] particularly in high-strain areas,[59] underscores the importance of understanding region-specific adaptations to eccentric training. Heterogeneity in muscle fiber strain during eccentric exercise[60,61] may explain the observed regional adaptations. As muscle fibers lengthen during force generation, it is possible that some regions experience greater fascicle and sarcomere stretch than others.[62] We hypothesized that larger strains in the shorter distal fascicles would drive greater increases in fascicle length in the distal region observed in this and our prior study.[29]

With detraining, there was a reversal of adaptation of fascicle length in the BFlh muscle. We also observed a decrease in strength after 3 weeks of detraining, yet strength remained higher than pre-training, despite regression of some architectural features.

4.2. Implications for hamstring injury mitigation programs

The initial increase of passive fascicle length within the BFlh muscle following 3 weeks of NHE training appears to primarily result from sarcomere lengthening.[29] This stretching of sarcomeres precedes the later addition of sarcomeres in series, a longer-term adaptation seen after 9 weeks of NHE training in the present study. The subsequent serial sarcomerogenesis potentially allows sarcomeres to regain a beneficial length for force production.[57,63] Concurrently, significant hamstring hypertrophy offers the potential to mitigate the risk of hamstring injuries. Hypertrophy, often associated with the addition of sarcomeres in parallel, not only lessens the stress per sarcomere at a given muscle force level but also increases the force-generating capacity of each muscle fiber.[20,64] The reversal of muscle architectural adaptations during detraining underscores that it is important to sustain a consistent training stimulus for these potentially protective hamstring muscle adaptations to be maintained.

4.3. Limitations

Several limitations of this study point to potential directions for future research. The absence of a control group limits our ability to isolate the effects of training and detraining from other potential factors. The modest sample size warrants caution when interpreting the results, particularly regarding the possibility of overlooking small effects. Additionally, the limited data on sarcomere lengths after detraining limit our understanding of the microscopic changes to muscle following the cessation of training stimuli.

Our focus on the BFlh muscle constrains the extrapolation of our results to other muscles within the hamstrings. Furthermore, while our findings provide insights on the adaptations induced by NHE training and detraining, the direct link between these changes and injury risk requires additional investigation.

Another limitation is our focus on passive fascicles and sarcomeres. Although measuring active sarcomere length in humans *in vivo* remains infeasible, investigating active fascicle lengthening provides avenues for future research to make inferences about sarcomere length ranges during eccentric contractions. Addressing these limitations in future research could provide a more comprehensive understanding of hamstring muscle adaptations and their implications for injury prevention and performance enhancement.

5. Conclusion

This study provides insight into how the macrostructure and microstructure of the BFlh muscle adapt over an eccentric exercise program. Collectively, these results suggest that serial sarcomerogenesis accompanies the increase in fascicle length after 9 weeks of eccentric exercise training. These adaptations have the potential to reduce the risk of overstretching sarcomeres during running and to help protect the hamstring muscles from injury.

Authors' contributions

MHA, APS, and RDG were responsible for data collection and analysis. All authors participated in the conception and design of the study, the interpretation of results, and manuscript writing. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Chat Generative Pre-Trained Transformer (ChatGPT; OpenAI, San Francisco, CA, USA) to enhance readability and language, aiding in formulating and structuring content. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Data availability statement

The data that support the findings of this study are openly available in the UQ eSpace at. Andrews M, Anoosha Pai S, Gurchiek R, et al. Multiscale adaptations in hamstring muscles publication dataset. The University of Queensland; 2024. 10.48610/a38a05d.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

We extend our gratitude to all participants who generously volunteered for the study. Special appreciation goes to Tyler Friedrich of Stanford Athletics and Tim Hanway of Simmons University for their contributions to the development of the NHE training protocol, along with Martino Franchi for discussions around the protocol. Our sincere thanks are extended to Gabriel Sanchez from Enspectra Health for providing technical support with data collection. Additionally, we gratefully acknowledge the members of the Neuromuscular Biomechanics Lab at Stanford University for their assistance in data

collection. This work was supported by the Australian Research Council Discovery Project (DP200101476), and in part by National Institute of Health grants (R01 AR077604, RO1 EB002524, RO1 AR079431, and P41 EB02706), Stanford Graduate Fellowship, The University of Queensland Graduate Scholarship, National Health and Medical Research Council of Australia Fellowship (#1194937), and by Wu Tsai Human Performance Alliance at Stanford University and the Joe and Clara Tsai Foundation.

Supplementary materials

Supplementary materials associated with this article can be found in the online version at doi:10.1016/j.jshs.2024.100996.

References

1. Diemer WM, Winters M, Tol JL, Pas HIMFL, Moen MH. Incidence of acute hamstring injuries in soccer: A systematic review of 13 studies involving more than 3800 athletes with 2 million sport exposure hours. *J Orthop Sports Phys Ther* 2021;**51**:27–36.
2. Ekstrand J, Bengtsson H, Waldén M, Davison M, Khan KM, Hägglund M. Hamstring injury rates have increased during recent seasons and now constitute 24% of all injuries in men's professional football: The UEFA Elite Club injury study from 2001/02 to 2021/22. *Br J Sports Med* 2023;**57**:292–8.
3. Lu Y, Pareek A, Lavoie-Gagne OZ, et al. Machine learning for predicting lower extremity muscle strain in National Basketball Association athletes. *Orthop J Sports Med* 2022;**10**:23259671221111742. doi:10.1177/23259671221111742.
4. Smith NA, Franettovich Smith MM, Bourne MN, Barrett RS, Hides JA. A prospective study of risk factors for hamstring injury in Australian Football League players. *J Sports Sci* 2021;**39**:1395–401.
5. Zachazewski J, Silvers H, Li B, Pohlig R, Ahmad C, Mandelbaum B. Prevalence of hamstring injuries in summer league baseball players. *Int J Sports Phys Ther* 2019;**14**:885–97.
6. Eftekhari A, Cogan C, Pandya N, Feeley B. Hamstring injury epidemiology in the National Basketball Association over a five-year period. *Muscles, Ligaments Tendons J* 2022;**12**:79–93.
7. Hägglund M, Waldén M, Magnusson H, Kristenson K, Bengtsson Hk, Ekstrand J. Injuries affect team performance negatively in professional football: An 11-year follow-up of the UEFA Champions League injury study. *Br J Sports Med* 2013;**47**:738–42.
8. Verrall GM, Kalairajah Y, Slavotinek JP, Spriggins AJ. Assessment of player performance following return to sport after hamstring muscle strain injury. *J Sci Med Sport* 2006;**9**:87–90.
9. Hickey J, Shield AJ, Williams MD, Opar DA. The financial cost of hamstring strain injuries in the Australian Football League. *Br J Sports Med* 2014;**48**:729–30.
10. de Visser HM, Reijman M, Heijboer MP, Bos PK. Risk factors of recurrent hamstring injuries: A systematic review. *Br J Sports Med* 2012;**46**:124–30.
11. Green B, Bourne MN, van Dyk N, Pizzari T. Recalibrating the risk of hamstring strain injury (HSI): A 2020 systematic review and meta-analysis of risk factors for index and recurrent hamstring strain injury in sport. *Br J Sports Med* 2020;**54**:1081–8.
12. Opar DA, Williams MD, Shield AJ. Hamstring strain injuries: Factors that lead to injury and re-injury. *Sports Med* 2012;**42**:209–26.
13. Askling C, Karlsson J, Thorstensson A. Hamstring injury occurrence in elite soccer players after preseason strength training with eccentric overload. *Scand J Med Sci Sports* 2003;**13**:244–50.
14. Rudisill SS, Varady NH, Kucharik MP, Eberlin CT, Martin SD. Evidence-based hamstring injury prevention and risk factor management: A systematic review and meta-analysis of randomized controlled trials. *Am J Sports Med* 2023;**51**:1927–42.
15. van der Horst N, Smits DW, Petersen J, Goedhart EA, Backx FJG. The preventive effect of the Nordic hamstring exercise on hamstring injuries in amateur soccer players: A randomized controlled trial. *Am J Sports Med* 2015;**43**:1316–23.
16. Presland JD, Timmins RG, Bourne MN, Williams MD, Opar DA. The effect of Nordic hamstring exercise training volume on biceps femoris long head architectural adaptation. *Scand J Med Sci Sports* 2018;**28**:1775–83.
17. Ribeiro-Alvares JB, Marques VB, Vaz MA, Baroni BM. Four weeks of Nordic hamstring exercise reduce muscle injury risk factors in young adults. *J Strength Cond Res* 2018;**32**:1254–62.
18. Timmins RG, Ruddy JD, Presland J, et al. Architectural changes of the biceps femoris long head after concentric or eccentric training. *Med Sci Sports Exerc* 2016;**48**:499–508.
19. Kruse A, Rivares C, Weide G, Tilp M, Jaspers RT. Stimuli for adaptations in muscle length and the length range of active force exertion—A narrative review. *Front Physiol* 2021;**12**: 742034. doi:10.3389/fphys.2021.742034.
20. Aagaard P, Andersen JL, Dyhre-Poulsen P, et al. A mechanism for increased contractile strength of human pennate muscle in response to strength training: Changes in muscle architecture. *J Physiol* 2001;**534**:613–23.
21. Bourne MN, Timmins RG, Opar DA, et al. An evidence-based framework for strengthening exercises to prevent hamstring injury. *Sports Med* 2017;**48**:251–67.
22. Timmins RG, Shield AJ, Williams MD, Lorenzen C, Opar DA. Architectural adaptations of muscle to training and injury: A narrative review outlining the contributions by fascicle length, pennation angle and muscle thickness. *Br J Sports Med* 2016;**50**:1467–72.
23. Herring SW, Grimm AF, Grimm BR. Regulation of sarcomere number in skeletal muscle: A comparison of hypotheses. *Muscle Nerve* 1984;**7**:161–73.
24. Hinks A, Franchi MV, Power GA. The influence of longitudinal muscle fascicle growth on mechanical function. *J Appl Physiol (1985)* 2022;**133**:87–103.
25. Burkholder TJ, Lieber RL. Sarcomere number adaptation after retinaculum transection in adult mice. *J Exp Biol* 1998;**201**:309–16.
26. Barnett JG, Holly RG, Ashmore CR. Stretch-induced growth in chicken wing muscles: Biochemical and morphological characterization. *Am J Physiol* 1980;**239**:39–46.
27. Hinks A, Jacob K, Mashouri P, et al. Influence of weighted downhill running training on serial sarcomere number and work loop performance in the rat soleus. *Biol Open* 2022;**11**: bio059491. doi:10.1242/bio.059491.
28. Matano T, Tamai K, Kurokawa T. Adaptation of skeletal muscle in limb lengthening: A light diffraction study on the sarcomere length in situ. *J Orthop Res* 1994;**12**:193–6.
29. Pincheira PA, Boswell MA, Franchi MV, Delp SL, Lichtwark GA. Biceps femoris long head sarcomere and fascicle length adaptations after 3 weeks of eccentric exercise training. *J Sport Health Sci* 2022;**11**:43–9.
30. Llewellyn ME, Barretto RPJ, Delp SL, Schnitzer MJ. Minimally invasive high-speed imaging of sarcomere contractile dynamics in mice and humans. *Nature* 2008;**454**:784–8.
31. Sanchez GN, Sinha S, Liske H, et al. *In vivo* imaging of human sarcomere twitch dynamics in individual motor units. *Neuron* 2015;**88**:1109–20.
32. Herzog W, dBFontana H. Does eccentric exercise stimulate sarcomerogenesis? *J Sport Health Sci* 2022;**11**:40–2.
33. Alonso-Fernandez D, Docampo-Blanco P, Martinez-Fernandez J. Changes in muscle architecture of biceps femoris induced by eccentric strength training with Nordic hamstring exercise. *Scand J Med Sci Sports* 2018;**28**:88–94.
34. Pollard CW, Opar DA, Williams MD, Bourne MN, Timmins RG. Razor hamstring curl and Nordic hamstring exercise architectural adaptations: Impact of exercise selection and intensity. *Scand J Med Sci Sports* 2019;**29**:706–15.
35. Kubo K, Ikebukuro T, Yata H, Tsunoda N, Kanehisa H. Time course of changes in muscle and tendon properties during strength training and detraining. *J Strength Cond Res* 2010;**24**:322–31.

36. Bourne MN, Opar DA, Williams MD, Shield AJ. Eccentric knee flexor strength and risk of hamstring injuries in rugby union: A prospective study. *Am J Sports Med* 2015;**43**:2663–70.
37. Timmins RG, Bourne MN, Shield AJ, Williams MD, Lorenzen C, Opar DA. Short biceps femoris fascicles and eccentric knee flexor weakness increase the risk of hamstring injury in elite football (soccer): A prospective cohort study. *Br J Sports Med* 2016;**50**:1524–35.
38. Arnason A, Andersen TE, Holme I, Engebretsen L, Bahr R. Prevention of hamstring strains in elite soccer: An intervention study. *Scand J Med Sci Sports* 2008;**18**:40–8.
39. Petersen J, Thorborg K, Nielsen MB, Budtz-Jørgensen E, Hölmich P. Preventive effect of eccentric training on acute hamstring injuries in men's soccer: A cluster-randomized controlled trial. *Am J Sports Med* 2011;**39**:2296–303.
40. Seagrave 3rd RA, Perez L, McQueeney S, Toby EB, Key V, Nelson JD. Preventive effects of eccentric training on acute hamstring muscle injury in professional baseball. *Orthop J Sports Med* 2014;**2**: 2325967114535351. doi:10.1177/2325967114535351.
41. Bourne MN, Williams MD, Opar DA, Al Najjar A, Kerr GK, Shield AJ. Impact of exercise selection on hamstring muscle activation. *Br J Sports Med* 2017;**51**:1021–8.
42. Augustsson J, Alt T, Andersson H. Speed matters in Nordic hamstring exercise: Higher peak knee flexor force during fast stretch-shortening variant compared to standard slow eccentric execution in elite athletes. *Sports(Basel)* 2023;**11**:130. doi:10.3390/sports11070130.
43. Franchi MV, Raiteri BJ, Longo S, Sinha S, Narici MV, Csapo R. Muscle architecture assessment: Strengths, shortcomings and new frontiers of *in vivo* imaging techniques. *Ultrasound Med Biol* 2018;**44**:2492–504.
44. Prager R, Gee A, Treece G, Berman L. Freehand 3D ultrasound without voxels: Volume measurement and visualisation using the stradx system. *Ultrasonics* 2002;**40**:109–15.
45. Goulding D, Bullard B, Gautel M. A survey of *in situ* sarcomere extension in mouse skeletal muscle. *J Muscle Res Cell Motil* 1997;**18**:465–72.
46. Lieber RL, Loren GJ, Friden J. *In vivo* measurement of human wrist extensor muscle sarcomere length changes. *J Neurophysiol* 1994;**71**:874–81.
47. Lichtwark GA, Farris DJ, Chen X, Hodges PW, Delp SL. Microendoscopy reveals positive correlation in multiscale length changes and variable sarcomere lengths across different regions of human muscle. *J Appl Physiol (1985)* 2018;**125**:1812–20.
48. Burkholder TJ, Lieber RL. Sarcomere length operating range of vertebrate muscles during movement. *J Exp Biol* 2001;**204**:1529–36.
49. Fedorov A, Beichel R, Kalpathy-Cramer J, et al. 3D Slicer as an image computing platform for the quantitative imaging network. *Magn Reson Imaging* 2012;**30**:1323–41.
50. Brown VA. An introduction to linear mixed-effects modeling in R. *Adv Methods Pract Psychol Sci* 2021;**4**:251524592096035. doi:10.1177/2515245920960351.
51. Oberg AL, Mahoney DW. Linear mixed effects models. In: Ambrosius WT, editor. *Topics in biostatistics*. Totowa, NJ: Humana Press; 2007.p.213–34.
52. Kuznetsova A, Brockhoff PB, Christensen RHB. Lmertest package: Tests in linear mixed effects models. *J Stat Softw* 2017;**82**:1–26.
53. Cohen J. *Statistical power analysis for the behavioral sciences*. 2nd ed. Mahwah, NJ: Lawrence Erlbaum Associates; 1988.
54. Moritani T, deVries HA. Neural factors *versus* hypertrophy in the time course of muscle strength gain. *Am J Phys Med* 1979;**58**:115–30.
55. Seynnes OR, de Boer M, Narici MV. Early skeletal muscle hypertrophy and architectural changes in response to high-intensity resistance training. *J Appl Physiol(1985)* 2007;**102**:368–73.
56. Lynn R, Talbot JA, Morgan DL. Differences in rat skeletal muscles after incline and decline running. *J Appl Physiol(1985)* 1998;**85**:98–104.
57. Morgan DL, Proske U. Popping sarcomere hypothesis explains stretch-induced muscle damage. *Clin Exp Pharmacol Physiol* 2004;**31**:541–5.
58. Askling CM, Tengvar M, Saartok T, Thorstensson A. Acute first-time hamstring strains during high-speed running: A longitudinal study including clinical and magnetic resonance imaging findings. *Am J Sports Med* 2007;**35**:197–206.
59. Fiorentino NM, Blemker SS. Musculotendon variability influences tissue strains experienced by the biceps femoris long head muscle during high-speed running. *J Biomech* 2014;**47**:3325–33.
60. Benford J, Hughes J, Waldron M, Theis N. Concentric *versus* eccentric training: Effect on muscle strength, regional morphology, and architecture. *Transl Sports Med* 2021;**4**:46–55.
61. Hedayatpour N, Falla D. Non-uniform muscle adaptations to eccentric exercise and the implications for training and sport. *J Electromyogr Kinesiol* 2011;**22**:329–33.
62. Azizi E, Deslauriers AR. Regional heterogeneity in muscle fiber strain: The role of fiber architecture. *Front Physiol* 2014;**5**:303. doi:10.3389/fphys.2014.00303.
63. Morgan DL. New insights into the behavior of muscle during active lengthening. *Biophys J* 1990;**57**:209–21.
64. Lieber RL, Fridén J. Functional and clinical significance of skeletal muscle architecture. *Muscle Nerve* 2000;**23**:1647–66.