TITLE

Impact of the Nordic hamstring and hip extension exercises on hamstring architecture and morphology: implications for injury prevention

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The architectural and morphological adaptations of the hamstrings in response to training with different exercises have not been explored. **PURPOSE:** To evaluate changes in biceps femoris long head (BF<sub>LH</sub>) fascicle length and hamstring muscle size following 10-weeks of Nordic hamstring exercise (NHE) or hip extension (HE) training. **METHODS:** Thirty recreationally active male athletes (age, 22.0 ± 3.6 years, height, 180.4 ± 7 cm, weight, 80.8 ± 11.1 kg) were allocated to one of three groups: 1) HE training (n=10), NHE training (n=10), or no training (CON) (n=10). BF<sub>LH</sub> fascicle length was assessed before, during (Week 5) and after the intervention with 2D-ultrasound. Hamstring muscle size was determined before and after training via magnetic resonance imaging. **RESULTS:** Compared to baseline, BF<sub>LH</sub> fascicles were lengthened in the NHE and HE groups at mid- (<i>d</i> = 1.12 – 1.39, <i>p</i> < 0.001) and post-<i>training</i> (<i>d</i> = 1.77 – 2.17, <i>p</i> < 0.001) but remained unchanged for the CON group (<i>d</i> = 0.20 – 0.31, <i>p</i> > 0.05). BF<sub>LH</sub> volume increased more for the HE than the NHE (<i>d</i> = 1.03, <i>p</i> = 0.037) and CON (<i>d</i> = 2.24, <i>p</i> < 0.001) groups. Compared to the CON group, both exercises induced significant increases in semitendinosus volume (<i>d</i> = 2.16 – 2.50, ≤ 0.002), however, only the HE group displayed increased semimembranosus volume (<i>d</i> = 1.57, <i>p</i> = 0.007). **CONCLUSION:** NHE and HE training both stimulate significant increases in BF<sub>LH</sub> fascicle length, however, HE training may be more effective for promoting hypertrophy in the BF<sub>LH</sub> and semimembranosus than the NHE.
What are the new findings?

- Hip extension and Nordic hamstring exercise training both promote the elongation of biceps femoris long head fascicles, and stimulate improvements in eccentric knee flexor strength.

- Hip extension training promotes more hypertrophy in the biceps femoris long head and semimembranosus than the Nordic hamstring exercise, which preferentially develops the semitendinosus and the short head of biceps femoris.
INTRODUCTION

Hamstring ‘tears’ are endemic in sports involving high-speed running and upwards of 80% of these injuries involve the biceps femoris long head (BF\textsubscript{LH}).\[1-4\] Hamstring strains represent the most common injury in athletics,\[5\] Australian Rules football,\[6 7\] and soccer\[8\] and as many as 30% reoccur within 12 months.\[9\] These findings highlight the need for improved hamstring injury prevention programs while also suggesting the possibility that these programs should specifically target the BF\textsubscript{LH}.

There has been significant interest in exploring the patterns of muscle activity in hamstring exercises,\[10-15\] however there is no research examining the architectural and morphological adaptations of these muscles to different exercise interventions. The Nordic hamstring exercise (NHE) has proven effective in increasing eccentric knee flexor strength\[16\] and reducing hamstring injuries\[17-19\] in soccer, although there is disagreement in the literature as to which hamstring muscles are most active during this exercise\[10 14 15 20\]. We have previously reported that the NHE preferentially activates the semitendinosus (ST),\[10 15\] however, we have also observed high levels of BF\textsubscript{LH} activity in this exercise\[15\] which suggests that it may still provide a powerful stimulus for adaptation within this most commonly injured muscle.\[1-4\] Eccentric exercise has been proposed to increase muscle fascicle lengths via sarcomerogenesis\[21 22\] and Timmins and colleagues\[23\] have recently observed such an adaptation after eccentric knee flexor training on an isokinetic dynamometer while also noting that concentric training caused fascicle shortening despite occurring at long muscle lengths. Furthermore, we have recently reported that soccer players with shorter BF\textsubscript{LH} fascicles (<10.56cm) were at fourfold greater risk of hamstring strain injury than players with longer fascicles.\[23\] Given the effectiveness of the predominantly eccentric NHE in hamstring injury prevention and rehabilitation,\[17-19\] it is of interest to examine the impact of this and alternative exercises on BF\textsubscript{LH} fascicle lengths and morphology.
We have recently observed that the 45° hip extension (HE) exercise resulted in more uniform activation of the two-joint hamstrings and greater BF<sub>LH</sub> activity than the NHE[15]. HE exercises are also performed at longer hamstring muscle lengths than the NHE and it has been suggested that this may make them more effective in hamstring injury prevention than the NHE.[24] However, HE and most other hamstring exercises are typically performed with both eccentric and concentric phases and it remains to be seen how the combination of contraction modes will affect fascicle length by comparison with an almost purely eccentric exercise like the NHE. Nevertheless, the greater activation of BF<sub>LH</sub> during HE[10 15] may provide a greater stimulus for hypertrophy, which might have implications for rehabilitation practices given observations of persistent atrophy in this muscle following injury.[25]

The primary purpose of this study was to evaluate changes in BF<sub>LH</sub> architecture and hamstring muscle volume and anatomical cross-sectional area (ACSA) following 10-week resistance training programs consisting exclusively of NHE or HE training. We tested the hypotheses that 1) HE training would stimulate greater increases in BF<sub>LH</sub> fascicle length than the NHE, on the basis of the suggestion that the ‘elongation stress’ in hamstring exercises may be an important factor in triggering this adaptation[24]; 2) HE training would promote more BF<sub>LH</sub> hypertrophy than the NHE; and 3) the NHE would result in more hypertrophy of the ST muscle than the HE exercise.
METHODS

Participants

Thirty recreationally active males (age, 22.0± 3.6 years, height, 180.4 ± 7 cm, weight, 80.8 ± 
11.1 kg) provided written informed consent to participate in this study. Participants 
were free from soft tissue and orthopaedic injuries to the trunk, hips and lower limbs and had 
no known history of hamstring strain, anterior cruciate ligament or other traumatic knee injury. 
Before enrolment in the study, all participants completed a cardiovascular screening 
questionnaire and a standard MRI questionnaire to ensure it was safe for them to enter the 
magnetic field. This study was approved by the Queensland University of Technology Human 
Research Ethics Committee and the University of Queensland Medical Research Ethics 
Committee.

Study design

This longitudinal training study was conducted between April and June, 2015. Approximately 
one week before the intervention commenced, participants underwent MR and 2D ultrasound 
imaging of their posterior thighs to determine hamstring muscle size and BF\textsubscript{LH} architecture, 
respectively. After scanning, all participants were familiarised with the NHE and 45° HE 
exercise and subsequently underwent strength assessments on each exercise. After all of the 
pre-training assessments had been completed, participants were allocated to one of three 
groups: NHE, HE or control (CON). Allocation of participants to groups was performed on the 
basis of baseline BF\textsubscript{LH} fascicle lengths to ensure that groups did not differ in this parameter 
prior to commencement of the study. Of the three participants with the longest fascicles, the 
first (with the longest fascicles) was allocated randomly to one of the three groups and then the 
second was allocated at random to one of the remaining two groups and the third allocated to 
the remaining group. This process was repeated for the participants with the 4\textsuperscript{th} to 6\textsuperscript{th} longest
fascicles, the 7th to 9th longest fascicles and so forth until each group had 10 participants. The NHE and HE groups completed a 10-week progressive strength training program consisting exclusively of their allocated exercise (Table 1). The CON group were advised to continue their regular physical activity levels but not to engage in any resistance training for the lower body. At the beginning of every training session, participants in both training groups reported their level of perceived soreness in the posterior thigh using a 1-10 numeric pain rating scale. All CON participants were required to report to the laboratory at least once per week. For all participants, BF\textsubscript{LH} architecture was re-assessed 5 weeks into the intervention and within 5 days of the final training session. MRI scans were acquired for all participants <7 days after the final training session. Strength testing was conducted after all imaging had been completed.

**Training intervention**

*Nordic hamstring exercise (NHE)*

An illustration of the NHE can be found in Figure 1a (see also video supplement). Participants knelt on a padded board, with the ankles secured immediately superior to the lateral malleolus by individual ankle braces which were attached to uniaxial load cells. The ankle braces and load cells were secured to a pivot which allowed the force generated by the knee flexors to be measured through the long axis of the load cells. From the initial kneeling position with their ankles secured in yokes, arms on the chest and hips extended, participants lowered their bodies as slowly as possible to a prone position.[10] Participants performed only the lowering (eccentric) portion of the exercise and were instructed to use their arms and flex at the hips and knees to push back into the starting position so as to minimise concentric knee flexor activity. When participants developed sufficient strength to completely stop the movement in the final 10-20° of the range of motion, they were required to hold a weight plate (range = 2.5kg to
20kg) to their chest (centred to the xiphoid process) to ensure the exercise was still of supramaximal intensity. Participants were provided with 3min of rest between each set.

*Hip extension exercise (HE)*

Participants were positioned in a 45° hip extension machine (BodySolid, IL, USA) with their trunk erect and hip joints extended and superior to the level of support pad (Figure 1b; see also video supplement). The ankle of the exercised limb was ‘hooked’ under an ankle pad and the unexercised limb was allowed to rest above its ankle restraint. Participants held one or more circular weight plate(s) to the chest (centred to the xiphoid process) and were instructed to flex their hip until they reached a point approximately 90° from the starting position. Once participants had reached this position they were instructed to return to the starting position by extending their hip, while keeping their trunk in a rigid neutral position throughout. Both limbs were trained in alternating fashion; after completing a set on one limb participants rested for 30s before training the opposite limb, and then recovered for 3min before the next set. The load held to the chest in week 1 represented 60-70% of the estimated 1-RM and was progressively increased throughout the training period whenever the prescribed repetitions and sets could be completed with appropriate technique (Table 2).

**Hamstring training program**

Participants in both intervention groups completed a progressive intensity training program consisting of 20 supervised exercise sessions (2 per week) over the 10 week period (Tables 1 & 2). Each session was followed by at least 48 hours of recovery and participants were
prohibited from engaging in any other resistance training for the lower body. The training program was based on the approximate loads, repetitions and sets employed in previous interventions using the NHE,[16-18] although the volume (number of repetitions) was reduced in the final two weeks to accommodate increases in exercise intensity. All sessions were conducted in the same laboratory, employed the same exercise equipment and were supervised by the same investigators (MNB and SJD) to ensure consistency of procedures.

**Table 1. Training program variables for both the Nordic hamstring and hip extension training groups**

<table>
<thead>
<tr>
<th>Week</th>
<th>Frequency</th>
<th>Sets</th>
<th>Repetitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
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</tr>
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<td>2</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>5-8</td>
<td>2</td>
<td>5</td>
<td>8-10</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

**Table 2. Application of progressive overload for both the Nordic hamstring and hip extension training groups**

<table>
<thead>
<tr>
<th>Training Intensity (Load)</th>
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</thead>
<tbody>
<tr>
<td><strong>Nordic Hamstring exercise</strong></td>
</tr>
<tr>
<td>Load was added to the chest in increments of 2.5kg when participants developed sufficient strength to stop at the end of the range of motion.</td>
</tr>
<tr>
<td>60-70% of 1-RM</td>
</tr>
<tr>
<td>All exercise was completed at maximal intensity of effort. Loads were progressively increased when</td>
</tr>
</tbody>
</table>
Strength assessments

Before and <7 days after the intervention, all participants underwent an assessment of their maximal eccentric knee-flexor strength during three repetitions of the NHE, and their 3-repetition maximum (3-RM) strength on the 45° hip extension machine. All strength tests were conducted by the same investigators (MNB, SJD and AJS) with tests completed at approximately the same time of day before and after the intervention.

Nordic eccentric strength test

The assessment of eccentric knee flexor force using the NHE has been reported previously.[3 4 23 26] Participants completed a single warm-up set of 5 submaximal repetitions followed, 1 minute later, by a set of 3 maximal repetitions of the bilateral NHE. Eccentric strength was determined for each leg from the highest of 3 peak forces produced during the 3 repetitions of the NHE and was reported in absolute terms (N).

Hip extension strength test

All strength assessments on the 45° hip extension machine were conducted unilaterally. Participants initially warmed up by performing 8-10 repetitions on each leg using body weight only. Subsequently, loads held to the chest were progressively increased until investigators determined the maximal load that could be lifted three times. At least 2min of rest was provided between sets.

BF1,H architecture assessment
BF<sub>LH</sub> fascicle length was determined from ultrasound images taken along the longitudinal axis of the muscle belly utilising a two-dimensional, B-mode ultrasound (frequency, 12MHz; depth, 8cm; field of view, 14 x 47mm) (GE Healthcare Vivid-i, Wauwatosa, U.S.A). Participants were positioned prone on a plinth with their hips in neutral and knees fully extended, while images were acquired from a point midway between the ischial tuberosity and the knee joint fold, parallel to the presumed orientation of BF<sub>LH</sub> fascicles. After the scanning site was determined, the distance of the site from various anatomical landmarks were recorded to ensure its reproducibility for future testing sessions. These landmarks included the ischial tuberosity, head of the fibula and the posterior knee joint fold at the mid-point between BF and ST tendon. On subsequent visits the scanning site was determined and marked on the skin and then confirmed by replicated landmark distance measures. Images were obtained from both limbs following at least five minutes of inactivity. To gather ultrasound images, the linear array ultrasound probe, with a layer of conductive gel was placed on the skin over the scanning site, aligned longitudinally and perpendicular to the posterior thigh. Care was taken to ensure minimal pressure was placed on the skin by the probe as this may influence the accuracy of the measures.[27] The orientation of the probe was manipulated slightly by the sonographer (RGT) if the superficial and intermediate aponeuroses were not parallel.

Ultrasound images were analysed using MicroDicom software (Version 0.7.8, Bulgaria). For each image, 6 points were digitised as described by Blazevich and colleagues.[28] Following the digitising process, muscle thickness was defined as the distance between the superficial and intermediate aponeuroses of the BF<sub>LH</sub>. A fascicle of interest was outlined and marked on the image (Figure 2). Fascicle length was determined as the length of the outlined fascicle between aponeuroses and was reported in absolute terms (cm). As the entire fascicles were not visible in the probe’s field of view, their lengths were estimated using the following equation:[28 29]
$FL = \sin(AA + 90^\circ) \times \frac{MT}{\sin(180^\circ - (AA + 180^\circ - PA))}$.

Where $FL$=fascicle length, $AA$=aponeurosis angle, $MT$=muscle thickness and $PA$=pennation angle.

All images were collected and analysed by the same investigator (RGT) who was blinded to training group allocation. The assessment of BF$_{LH}$ architecture using the aforementioned procedures by this investigator (RGT) is highly reliable (intraclass correlations $>0.90$).[30]

Muscle volumes and anatomical cross-sectional area assessment All MRI scans were performed using a 3-Tesla (Siemens TrioTim, Germany) imaging system with a spinal coil. The participant was positioned supine in the magnet bore with the knees fully extended and hips in neutral, and straps were placed around both limbs to prevent any undesired movement. Contiguous T1-weighted axial MR images (transverse relaxation time: 750ms; echo time: 12ms; field of view: 400mm; slice thickness: 10mm; interslice distance: 0mm) were taken of both limbs beginning at the iliac crest and finishing distal to the tibial condyles. A localiser adjustment (20s) was applied prior to the acquisition of T1-weighted images to standardise the field of view. In addition, to minimise any inhomogeneity in MR images caused by dielectric resonances at 3T, a post-processing (B1) filter was applied to all scans.[31] The total scan duration was 3min 39sec.

Muscle volumes and anatomical cross-sectional areas (ACSAs) of the BF$_{LH}$ and short head (BF$_{SH}$), semitendinosus (ST) and semimembranosus (SM) muscles were determined for both limbs using manual segmentation. Muscle boundaries were identified and traced on each image in which the desired structure was present using image analysis software (Sante Dicom Viewer...
Volumes were determined for each muscle by multiplying the summed CSAs (from all the slices containing the muscle of interest) by the slice thickness. ACSA was determined by locating the 10mm slice with the greatest CSA and averaging this along with the two slices immediately cranial and caudal (five slices). All traces (pre- and post-training) were completed by the same investigator (MNB) who was blinded to participant identity and training group in all post-testing.

Statistical analysis

All statistical analyses were performed using SPSS version 22.0.0.1 (IBM Corporation, Chicago, IL). Repeated measures split plot ANOVAs were used to determine training-induced changes in BF\textsubscript{LH} architecture, hamstring muscle volumes and ACSA, strength, and ratings of perceived soreness, for each group. For the analysis of BF\textsubscript{LH} fascicle length, the within-subject variable was \textit{time} (baseline, mid-training, and post-training) and the between-subject variable was \textit{group} (HE, NHE, CON). Because BF\textsubscript{LH} architecture did not differ between limbs (dominant vs non-dominant) at any time point (p>0.05), the left and right limbs were averaged to provide a single value for each participant. To determine differences in the percentage change in hamstring muscle volume and ACSA between groups, the within-subject variable was \textit{muscle} (BF\textsubscript{LH}, BF\textsubscript{SH}, ST, and SM) and the between-subject variable was \textit{group} (HE, NHE, CON). To explore changes in Nordic and 45° hip extension strength the within-subject variable was \textit{time} (baseline and post-training) and the between-subject variable was \textit{group} (HE, NHE, CON). Lastly, to determine if ratings of perceived soreness changed over time, or differed between training groups, within-subject variable was \textit{time} (weeks 1-10) and the between-subject variable was \textit{group} (HE, NHE, CON). For all analyses, when a significant main effect
was detected, post hoc independent t tests with Bonferroni corrections were used to determine which comparisons differed. For all analyses, the mean differences were reported with their 95% confidence intervals (CIs), and where appropriate, Cohen’s $d$ was reported as a measure of the effect size.

**Sample size**

*A priori* sample size estimates were based on anticipated differences in BF$_{LH}$ fascicle length following the training intervention. A sample size of 10 in each group was calculated to provide sufficient statistical power (80%) to detect an effect size of 1.0 for the difference in fascicle length changes between training groups, with $p<0.05$. 

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RESULTS

No significant differences were observed in age, height or body mass between the three groups (p > 0.05) (Table 3). Compliance rates were excellent for both training groups (HE: 100%; NHE: 99.5%).

Table 3. Participant characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Mass (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE</td>
<td>23.1±4.1</td>
<td>180±6.3</td>
<td>81.6±9.7</td>
</tr>
<tr>
<td>NHE</td>
<td>21.6±3.2</td>
<td>182.8±8.7</td>
<td>85.0±10.9</td>
</tr>
<tr>
<td>CON</td>
<td>21.3±3.7</td>
<td>178.5±5.4</td>
<td>75.9±11.8</td>
</tr>
</tbody>
</table>

Biceps femoris long head (BF\textsubscript{LH}) fascicle length

Between-group comparisons

A significant group x time interaction was observed for fascicle length during the training period (p<0.001) (Figure 4). No significant differences were observed between training groups at either baseline (d = 0.15), mid- (d = 0.49) or post-training points (d = 0.80) (all p > 0.05).

However, the NHE group displayed significantly longer fascicles than the CON group at mid- (mean difference = 1.50cm, 95% CI = 0.58 to 2.41cm, d = 1.64, p = 0.001) and post-training (mean difference = 2.40cm, 95% CI = 1.28 to 3.53cm, d = 2.19, p < 0.001). Similarly, the HE group exhibited significantly longer fascicles than the CON group at mid- (mean difference = 1.14cm, 95% CI = 0.22 to 2.05cm, d = 1.52, p = 0.011) and post-training (mean difference = 1.63cm, 95% CI = 0.51 to 2.76cm, d = 1.84, p = 0.003).
Within-group comparisons

Post hoc analyses revealed that BF<sub>LH</sub> fascicle length increased significantly from baseline in the NHE group at mid- (mean difference = 1.23 cm, 95% CI = 0.84 to 1.63 cm, $d = 1.39$, $p < 0.001$) and post-training (mean difference = 2.22 cm, 95% CI = 1.74 to 2.69 cm, $d = 2.17$, $p < 0.001$). The HE group also displayed significantly lengthened fascicles at mid- (mean difference = 0.75 cm, 95% CI = 0.35 to 1.15 cm, $d = 1.12$, $p < 0.001$) and post-training (mean difference = 1.33 cm, 95% CI = -0.86 to 1.80 cm, $d = 1.77$, $p < 0.001$). However, the CON group remained unchanged relative to baseline values at all time points ($p > 0.05$, $d = 0.20 – 0.31$).

Hamstring muscle volumes

Between-group comparisons

A significant main effect was detected for the muscle x group interaction for hamstring muscle volume changes ($p < 0.001$) (Figure 5). BF<sub>LH</sub> volume increased significantly more in the HE than the NHE (mean difference = 6.72%, 95% CI = 0.32 to 13.11%, $d = 1.03$, $p = 0.037$) and CON groups (mean difference = 12.10%, 95% CI = 5.71 to 18.50%, $d = 2.24$, $p < 0.001$), and a smaller nonsignificant difference was observed between the NHE and CON groups (mean difference = 5.39%, 95% CI = -1.01 to 11.78%, $d = 1.13$, $p = 0.122$) (Figure 5). BF<sub>SH</sub> volume increased more in the HE (mean difference = 8.51%, 95% CI = 0.17 to 16.85%, $d = 1.49$, $p = 0.044$) and NHE groups (mean difference = 15.29%, 95% CI = 6.95 to 23.63%, $d = 2.09$, $p < 0.001$) than in the CON group. Both the NHE (mean difference = 21.21%, 95% CI = 11.55 to 30.88%, $d = 2.50$, $p < 0.001$) and HE (mean difference = 14.32%, 95% CI = 4.65 to 23.98%, $d = 2.16$, $p = 0.002$) training groups exhibited a greater increase in ST volume than the CON group. However, no significant difference in ST volume change was noted between NHE and
HE groups (mean difference = 6.90%, 95% CI = -2.77 to 16.56%, d = 0.69, p = 0.239). The percentage change in volume for the SM was significantly greater for the HE group than for CON (mean difference = 8.95%, 95% CI = 2.21 to 15.69%, 1.57, p = 0.007), while no difference was observed between the NHE and CON group changes (mean difference = 3.38%, 95% CI = -3.36 to 10.12%, d = 0.68, p = 0.636) for this muscle.

Within-group comparisons

HE training stimulated a greater increase in volume for the ST than the BFSH (mean difference = 5.61%, 95% CI = 1.12% to 10.10%, d = 0.71, p = 0.009). No other significant between-muscle differences were noted for volume changes after HE training (p=0.054 – 0.999 for all pairwise comparisons) or in the CON group (p > 1.000). After NHE training, ST volume increased more than BFSH (mean difference = 15.28%, 95% CI = 10.69 to 19.87%, d = 3.54, p<0.001) and SM (mean difference = 16.06%, 95% CI = 10.96 to 21.16%, d = 3.53, p<0.001). Similarly, in the NHE group the percentage change in volume was greater for the BFSH than the BFSH (mean difference = 9.56%, 95% CI = 4.30 to 14.80%, d = 1.18, p <0.001) and SM (mean difference = 10.33%, 95% CI = 5.33 to 15.34%, d = 1.26, p < 0.001).

Hamstring muscle anatomical cross-sectional area (ACSA)

Between-group comparisons

A significant main effect was detected for the muscle x group interaction (p < 0.001) (Figure 6). The percentage change in BFSH ACSA was greater in the HE training group than in the NHE (mean difference = 5.24%, 95% CI = 0.061 to 10.41, d = 0.98, p = 0.047) and CON groups (mean difference = 8.90%, 95% CI = 3.73 to 14.07%, d = 1.94, p < 0.001), while no
difference was observed between the NHE and CON groups (mean difference = 3.67%, 95% CI = -1.51 to 8.84%, $d = 1.07$, $p = 0.245$) (Figure 6). BF$_{SH}$ ACSA increased significantly more in the NHE than the CON group (mean difference = 13.26%, 95% CI = 4.98 to 21.54%, $d = 1.97$, $p = 0.001$), while no difference was observed between changes exhibited by the HE and CON groups for this muscle (mean difference = 5.69%, 95% CI = -2.59 to 0.70%, $d = 0.90$, $p = 0.273$). The percentage change in ST ACSA was significantly greater in the NHE (mean difference = 17.60%, 95% CI = 7.60 to 27.61%, $d = 2.17$, $p < 0.001$) and HE (mean difference = 15.16%, 95% CI = 5.15 to 25.17%, $d = 1.95$, $p = 0.002$) groups than the CON group, however no significant difference was noted between changes in the NHE and HE groups (mean difference = 2.4%, 95% CI = -7.57 to 12.45%, $d = 0.24$, $p = 1.000$). The percentage increase in SM ACSA was greater in the HE than the CON group (mean difference = 7.19%, 95% CI = 1.21 to 13.18%, $d = 1.34$, $p = 0.015$), but was not significantly greater in NHE than CON (mean difference = 2.02%, 95% CI = -3.97 to 8.01%, $d = 0.49$, $p = 1.000$). No significant difference in SM ACSA change was noted between the HE and NHE groups (main difference = 5.17%, 95% CI = -8.2 to 11.16%, $d = 0.85$, $p = 0.109$).

Within-group comparisons

After HE training, the change in ACSA observed for the ST was significantly greater than the BF$_{LH}$ (mean difference = 6.46, 95% CI = 0.84 to 12.10%, $d = 0.78$, $p = 0.017$), BF$_{SH}$ (mean difference = 9.98%, 95% CI = 4.25 to 15.71%, $d = 1.09$, $p < 0.001$) and SM (mean difference = 6.73%, 95% CI = 1.54 to 11.92%, $d = 0.78$, $p = 0.006$). No other significant pairwise between-muscle differences in ACSA change were noted after HE training (all $p > 0.05$). After NHE training, the change in ACSA was greater for BF$_{SH}$ than BF$_{LH}$ (mean difference = 9.30%, 95% CI = 3.47 to 15.12%, $d = 1.34$, $p = 0.001$) and SM (mean difference = 9.50%, 95% CI = 4.92 to 14.08, $d = 1.33$, $p < 0.001$), while ST ACSA increased more than BF$_{LH}$ (mean difference
= 14.14%, 95% CI = 8.52 to 19.76%, \(d = 1.76, p < 0.001\) and SM (mean difference = 14.35%, 95% CI = 9.15 to 19.54%, \(d = 1.75, p < 0.001\)).

A significant group x time interaction effect was observed for the Nordic eccentric strength test (\(p < 0.001\)) (Figure 7). Post hoc t tests demonstrated that the NHE (mean difference = 97.38N, 95% CI = 65.51 to 129.26N, \(d = 2.36, p < 0.001\)) and HE (mean difference = 110.47N, 95% CI = 76.87 to 144.07N, \(d = 1.26, p < 0.001\)) groups were significantly stronger at post-training compared to baseline while the CON group did not change (mean difference = 8.91N, 95% CI = -42.51 to 24.69N, \(d = 0.14, p = 0.590\)). No groups differed at baseline (\(p > 0.461\)), however, at post-training the NHE (mean difference = 123.436N, 95% CI = 39.93 to 206.93N, \(d = 2.07, p = 0.003\)) and HE (mean difference = 94.27N, 95% CI = 8.60 to 179.94N, \(d = 1.14, p = 0.028\)) groups were both significantly stronger than the CON group. No significant difference was observed between training groups at post-training (mean difference = 29.16N, 95% CI = -54.34 to 112.66N, \(d = 0.41, p = 0.999\)).
Hip extension strength test

A significant group x time interaction effect was also observed for 3-RM strength as assessed during the 45° HE strength test (p < 0.001) (Figure 8). Post hoc analyses demonstrated that the HE (mean difference = 41.00kg, 95% CI = 35.97 to 46.03kg, d = 4.59, p < 0.001) and NHE groups (mean difference = 26.00kg, 95% CI = 20.97 to 31.03kg, d = 2.36, p < 0.001) improved significantly from baseline whereas the CON group did not change (mean difference = 3.50kg, 95% CI = -1.53 to 8.53kg, d = 0.33, p = 0.165). No groups differed significantly at baseline (p > 0.091) however at post-training, both the HE (mean difference = 43.50kg, 95% CI = 30.93 to 56.07kg, d = 4.21, p < 0.001) and NHE groups (mean difference = 32.0kg, 95% CI = 19.43 to 44.57kg, d = 2.66, p < 0.001) were significantly stronger than CON. Post-training, no significant difference was observed between training groups (mean difference = 11.50kg, 95% CI = -1.07 to 24.07kg, d = 1.09, p = 0.082).

Perceived soreness

No significant group x time interaction effect (p = 0.397) was detected for ratings of perceived soreness throughout the intervention (Figure 9). The average soreness measures reported across the 10-week training period were 2.2 ± 0.4 (mean ± SE) for the NHE group and 2.3 ± 0.5 for the HE group.
DISCUSSION

This study is the first to explore the architectural and morphological adaptations of the hamstrings in response to different strength training exercises. These data suggest that both the HE and NHE stimulate significant increases in BF\textsubscript{LH} fascicle length and, contrary to our hypothesis, that the longer muscle lengths encountered in the HE exercise do not result in greater lengthening of fascicles than are observed after NHE training. As hypothesised, HE training appears to elicit more hypertrophy in the BF\textsubscript{LH} than does the NHE; while contrary to our hypothesis, the NHE was not significantly more effective at increasing ST volume or cross sectional area than the HE. Both exercises resulted in significant strength increases which were similarly evident in the NHE and HE strength tests.

Fascicle lengthening is one possible mechanism by which the NHE[17-19] and other eccentric or long length hamstring exercises[22] protect muscles from injury. We have recently shown, prospectively, that professional soccer players with fascicles <10.56cm were ~4 times more likely to suffer a hamstring strain than athletes with longer fascicles and that the probability of injury was reduced by ~74% for every 0.5cm increase in fascicle length.[23] In the current study, participants increased their fascicle lengths from ~10.6cm prior to training, to 12.8 and 12.0cm in the NHE and HE groups, respectively, which would likely result in large reductions in hamstring injury risk.

Despite its success in reducing hamstring strain injuries, the adoption of the NHE in elite European soccer has been reported to be poor with only ~11% of Norwegian premier league and UEFA teams deemed to have adequately implemented the NHE programs that have proven effective in randomised controlled trials[17-19]. Some conditioning coaches and researchers[24] believe that the exercise does not challenge the hamstrings at sufficient lengths to optimise injury prevention benefits. However, this study shows, for the first time, that the limited excursion of the hamstrings during the NHE does not prevent the exercise from
increasing BF<sub>LH</sub> fascicle length. Indeed, the exercise resulted in greater fascicle lengthening than the HE, although the current study lacked the statistical power to distinguish between the two. Together with observations that long length concentric hamstring training can shorten muscle fascicles,[33] the current findings are consistent with the possibility that the combination of concentric and eccentric contractions somewhat dampens the elongation of BF<sub>LH</sub> fascicles. The advantage of the NHE may be its almost purely eccentric or eccentrically-biased nature. Further work is needed to clarify whether eccentrically-biased or purely eccentric HE exercise may yield greater improvements in BF<sub>LH</sub> fascicle length than the combined concentric and eccentric contraction modes used in this investigation.

Observations of increased fascicle length following eccentric hamstring exercise are largely consistent with existing literature. For example, Potier and colleagues[32] reported a 34% increase in BF<sub>LH</sub> fascicle length following eight weeks of eccentric leg curl exercise, while Timmins and colleagues[33] reported a 16% increase in BF<sub>LH</sub> fascicle length after six weeks of eccentric training on an isokinetic dynamometer.[33] These adaptations most likely result from the addition of in-series sarcomeres, as has been shown to occur within the rat vastus intermedius muscle after five days of downhill running.[34] It has been proposed that this increase in serial sarcomeres accounts for both a rightward shift in a muscle’s force-length relationship,[35] while also reducing its susceptibility to damage.[21 22] However, it is also at least theoretically possible that fascicle lengthening occurs as a result of increased tendon or aponeurotic stiffness and further research is needed to clarify the precise mechanism(s) responsible for these architectural changes.

To the authors’ knowledge, this is the first study to explore the morphological adaptations of the hamstrings to different strengthening exercises. These data suggest that the NHE and HE exercises induce heterogeneous patterns of hamstring muscle hypertrophy, with the former preferentially stimulating ST and BF<sub>SH</sub> growth and the latter resulting in significantly more
hypertrophy of the BF\textsubscript{LH} and more homogenous growth of all two-joint hamstring muscles. We have previously noted transient T2 relaxation time changes after 50 repetitions of each of these exercises that almost exactly fit this pattern\cite{15}, so it appears that the acute changes observed via functional MRI match quite well with the hypertrophic effects observed after 10 weeks of training. However, neither muscle volume nor ACSA have been identified as risk factors for hamstring strain injury, so the exact significance of these findings is unknown. Indeed, we have previously reported that BF\textsubscript{LH} muscle thickness measured via ultrasound is not a risk factor for hamstring injury in elite soccer\cite{23}. Nevertheless, BF\textsubscript{LH} muscle atrophy has been noted as long as 5-23 months after injury in recreational athletes\cite{25}, so unilateral HE exercises may prove more beneficial than the NHE at redressing this deficit in rehabilitation. Interestingly, reduced muscle volumes of the ST have been observed 12-72 months after anterior cruciate ligament injury\cite{36} and the results of the current investigation suggest that the NHE may be valuable in rehabilitation of this injury.

Hamstring strengthening is an important component of injury prevention strategies\cite{24,37,38}. Indeed, several large scale interventions employing the NHE have shown ~65% reductions in hamstring strain injury rates in soccer\cite{17-19} and recent prospective findings in elite Australian football\cite{3} and soccer\cite{23} suggest that eccentric strength improvements like those reported here and previously\cite{16} are at least partly responsible for these protective benefits. For example, elite athletes in these sports who generated less than 279\text{N} (Australian football) and 337\text{N} (soccer) of knee flexor force at the ankles during the NHE strength test were ~4 times more likely to sustain hamstring injuries than stronger counterparts\cite{3,23}. In this study, our recreational level athletes were able to generate, on average, 460\text{N} and 431\text{N} after 10 weeks of NHE and HE training, respectively, making them substantially stronger than these elite Australian football\cite{3} and soccer players\cite{23}. Significant improvements in 3-RM HE strength were also observed for both training groups, which suggests that hamstring strengthening, at
least in recreationally trained athletes, is not highly specific to the chosen exercise. While the
benefits of high levels of HE strength remain unclear from the perspective of injury prevention,
the observed effects of HE training on BF_{LH} fascicle lengths and eccentric knee flexor strength
suggest the potential for this exercise to reduce injury risk. Future intervention studies
analogous to those employing the NHE previously,[17-19 39] are needed to clarify whether
HE training is effective in reducing hamstring strain injuries, however, access to exercise
equipment (ie., a 45° HE machine) may be a limiting factor in designing such studies. It is also
noteworthy that strength improvements can be achieved with very modest levels of hamstring
muscle soreness when training is appropriately structured and progressively overloaded. These
observations are in agreement with Mjolsnes and colleagues[16] who have previously reported
very limited muscle soreness with a gradual increase in NHE volume.

The authors acknowledge that there are some limitations associated with the current study.
Firstly, muscle architecture was only assessed in the BF_{LH} and it may not be appropriate to
generalise these findings to other knee flexors, given that each hamstring muscle displays
unique architectural characteristics.[40] Further, the assessment of fascicle length using two-
dimensional ultrasound requires some degree of estimation, because the entire length of the
BF_{LH} fascicles are not visible in ultrasound images. While the estimation equation used in this
study has been validated against cadaveric samples,[29] there is still the potential for error, and
future studies employing extended field of view ultrasound methods may be needed to
completely eliminate this. Lastly, all of the athletes in this study were recreational level males
of a similar age, and it remains to be seen if these results are applicable to other populations.
However, our participants were, on average, as strong as elite Australian football players[3]
and stronger than professional soccer players[23] at the start of the study. Furthermore, our
cohort displayed average fascicle lengths before training that were within one standard
deviation of the values reported in elite soccer players previously,[23] so it is unlikely that they
were unrepresentative of higher-level athletes, in these parameters at least.

This is the first study to demonstrate that training with different exercises elicits unique
architectural and morphological adaptations within the hamstring muscle group. We have
provided evidence to suggest that both HE and NHE training are effective in lengthening BF_{LH}
fascicles and that the greater excursion involved in the HE does not result in greater increases
in fascicle length. However, HE training appears to be more effective for promoting
hypertrophy in the commonly injured BF_{LH} than the NHE, which preferentially develops the
ST and BF_{SH} muscles. HE and NHE had very similar effects on ST volume and cross-sectional
area. These data may help to explain the mechanism(s) by which the NHE confers injury
preventive benefits and also provide compelling evidence to warrant the further exploration of
HE-oriented exercises in hamstring strain injury prevention protocols. Future prospective
studies are needed to ascertain whether HE training interventions are effective in reducing the
incidence of hamstring strain injury in sport and whether or not the combination of HE and
NHE training is more effective than the NHE alone.

How might it impact upon clinical practice in the future?

- Hip extension and Nordic hamstring exercise training are both effective in lengthening
  biceps femoris long head fascicles, and in promoting improvements in eccentric knee
  flexor strength, which may significantly reduce the risk of hamstring strain injury
- Hip extension exercise may be more useful than the Nordic hamstring exercise for
  stimulating hypertrophy in the commonly injured biceps femoris long head
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CONTRIBUTORS

MB was the principle investigator and was involved with study design, recruitment, analysis and manuscript write up. SD, RT were involved in data collection. MW, DO, GK and TS were involved with the study design, analysis and manuscript preparation. AA was involved in MRI data acquisition. All authors had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY DECLARATION

The lead author* (MB) affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained. * = The manuscript’s guarantor.

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DATA SHARING

Consent was not obtained for data sharing but the presented data are anonymised and risk of identification is low.

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COMPETING INTERESTS

None declared. All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that (1) the Queensland Academy of Sport’s Centre of Excellence for Applied Sports Science Research funded this study; (2) MB, SD, RT, MW, DO, GK, AA and TS have no relationships with companies that might have an interest in the submitted work in the previous 3 years; (3) their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and (4) MB, SD, RT, MW, DO, GK, AA and TS have no non-financial interests that may be relevant to the submitted work.

ETHICAL CLEARANCE

All participants provided written, informed consent for this study, which was approved by the Queensland University of Technology Human Research Ethics Committee and the University of Queensland Medical Research Ethics Committee.

Figure legends
Figure 1. (a) The Nordic hamstring exercise (NHE) and (b) the 45° hip extension (HE) exercise, progressive from left to right.

Figure 2. A two-dimensional ultrasound image of the biceps femoris long head (BF_{LH}), taken along the longitudinal axis of the posterior thigh. From these images, it is possible to determine the superficial and intermediate aponeuroses, muscle thickness, and angle of the fascicle in relation to the aponeurosis. Estimates of fascicle length can then be made via trigonometry using muscle thickness and pennation angle.

Figure 3. T1-weighted image (transverse relaxation time = 750ms; echo time = 12ms, slice thickness = 10mm), depicting the regions of interest for each hamstring muscle. The right side of the image corresponds to the participant’s left side as per radiology convention. BF_{LH}, biceps femoris long head; BF_{SH}, biceps femoris short head; ST, semitendinosus; SM, semimembranosus.

Figure 4. Biceps femoris long head (BF_{LH}) fascicle lengths before (baseline), during (mid-training) and after (post-training) the intervention period for the hip extension (HE), Nordic hamstring exercise (NHE) and control (CON) groups. Fascicle length is expressed in absolute terms (cm) with error bars depicting standard error (SE). * indicates p<0.05 compared to baseline (week 0). ** signifies p<0.001 compared to baseline. # indicates p<0.05 compared to the control group.

Figure 5. Percentage change in volume (cm³) for each hamstring muscle after the intervention. Values are expressed as a mean percentage change compared to the values at baseline with error bars representing standard error (SE). For all pairwise comparisons between groups, * indicates p<0.05 and ** signifies that p<0.001. BF_{LH}, biceps femoris long head; BF_{SH}, biceps femoris short head; ST, semitendinosus; SM, semimembranosus.
Figure 6. Percentage change in anatomical cross sectional area (ACSA) (cm$^2$) for each hamstring muscle after the intervention. Values are expressed as a mean percentage change compared to the values at baseline with error bars representing standard error (SE). For all pairwise comparisons between groups, * indicates p<0.05 and ** signifies that p<0.001. BF$_{LH}$, biceps femoris long head; BF$_{SH}$, biceps femoris short head; ST, semitendinosus; SM, semimembranosus.

Figure 7. Eccentric knee flexor force measured during the Nordic strength test before (baseline) and after (post-training) the intervention period for the hip extension (HE), Nordic hamstring exercise (NHE) and control (CON) groups. Force is reported in absolute terms (N) with error bars depicting standard error (SE). * indicates p<0.001 compared to baseline (week 0). # signifies p<0.05 compared to the control group.

Figure 8. Hip extension three-repetition maximum (3RM) before (baseline) and after (post-training) the intervention period for the hip extension (HE), Nordic hamstring exercise (NHE) and control (CON) groups. Force is reported in absolute terms (kg) with error bars depicting standard error (SE). ** indicates p<0.001 compared to baseline (week 0). # signifies p<0.001 compared to the control group.

Figure 9. Mean (± standard error) weekly soreness measured using a numeric pain rating scale (1-10) at the beginning of each training session.
References


