

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

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TITLE

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3	and morphology: implications for injury prevention
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32 ABSTRACT

The architectural and morphological adaptations of the hamstrings in response to training with 33 different exercises have not been explored. PURPOSE: To evaluate changes in biceps femoris 34 long head (BF_{LH}) fascicle length and hamstring muscle size following 10-weeks of Nordic 35 hamstring exercise (NHE) or hip extension (HE) training. METHODS: Thirty recreationally 36 active male athletes (age, 22.0 ± 3.6 years, height, 180.4 ± 7 cm, weight, 80.8 ± 11.1 kg) were 37 allocated to one of three groups: 1) HE training (n=10), NHE training (n=10), or no training 38 (CON) (n=10). BF_{LH} fascicle length was assessed before, during (Week 5) and after the 39 intervention with 2D-ultrasound. Hamstring muscle size was determined before and after 40 training via magnetic resonance imaging. **RESULTS:** Compared to *baseline*, BF_{LH} fascicles 41 were lengthened in the NHE and HE groups at *mid*- (d = 1.12 - 1.39, p < 0.001) and *post*-42 training (d = 1.77 - 2.17, p < 0.001) but remained unchanged for the CON group (d = 0.20 - 1.00)43 44 0.31, p > 0.05). BF_{LH} volume increased more for the HE than the NHE (d = 1.03, p = 0.037) and CON (d = 2.24, p < 0.001) groups. Compared to the CON group, both exercises induced 45 46 significant increases in semitendinosus volume ($d = 2.16 - 2.50, \le 0.002$), however, only the HE group displayed increased semimembranosus volume (d= 1.57, p = 0.007). 47 **CONCLUSION:** NHE and HE training both stimulate significant increases in BF_{LH} fascicle 48 length, however, HE training may be more effective for promoting hypertrophy in the BF_{LH} 49 and semimembranosus than the NHE. 50

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56	What are the new findings?
57	• Hip extension and Nordic hamstring exercise training both promote the elongation of biceps femoris long head fascicles, and stimulate improvements in eccentric knee flexor
58	strength.
59	• 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.

60 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_{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_{LH}.

There has been significant interest in exploring the patterns of muscle activity in hamstring 67 exercises, [10-15] however there is no research examining the architectural and morphological 68 adaptations of these muscles to different exercise interventions. The Nordic hamstring exercise 69 70 (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 71 hamstring muscles are most active during this exercise [10 14 15 20]. We have previously 72 73 reported that the NHE preferentially activates the semitendinosus (ST),[10 15] however, we have also observed high levels of BF_{LH} activity in this exercise[15] which suggests that it may 74 still provide a powerful stimulus for adaptation within this most commonly injured muscle.[1-75 76 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 77 adaptation after eccentric knee flexor training on an isokinetic dynamometer while also noting 78 that concentric training caused fascicle shortening despite occurring at long muscle lengths. 79 Furthermore, we have recently reported that soccer players with shorter BF_{LH} fascicles 80 81 (<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 82 prevention and rehabilitation,[17-19] it is of interest to examine the impact of this and 83 84 alternative exercises on BF_{LH} fascicle lengths and morphology.

85 We have recently observed that the 45° hip extension (HE) exercise resulted in more uniform activation of the two-joint hamstrings and greater BF_{LH} activity than the NHE[15]. HE 86 exercises are also performed at longer hamstring muscle lengths than the NHE and it has been 87 88 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 89 90 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 91 92 the NHE. Nevertheless, the greater activation of BF_{LH} during HE[10 15] may provide a greater 93 stimulus for hypertrophy, which might have implications for rehabilitation practices given observations of persistent atrophy in this muscle following injury.[25] 94

The primary purpose of this study was to evaluate changes in BF_{LH} architecture and hamstring 95 muscle volume and anatomical cross-sectional area (ACSA) following 10-week resistance 96 97 training programs consisting exclusively of NHE or HE training. We tested the hypotheses that 1) HE training would stimulate greater increases in BF_{LH} fascicle length than the NHE, on the 98 99 basis of the suggestion that the 'elongation stress' in hamstring exercises may be an important 100 factor in triggering this adaptation[24]; 2) HE training would promote more BF_{LH} hypertrophy than the NHE; and 3) the NHE would result in more hypertrophy of the ST muscle than the HE 101 102 exercise.

104 **METHODS**

105 Participants

Thirty recreationally active males (age, 22.0 ± 3.6 years, height, 180.4 ± 7 cm, weight, $80.8\pm$ 106 11.1 kg) provided written informed consent to participate in this study. Participants 107 were free from soft tissue and orthopaedic injuries to the trunk, hips and lower limbs and had 108 109 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 110 questionnaire and a standard MRI questionnaire to ensure it was safe for them to enter the 111 magnetic field. This study was approved by the Queensland University of Technology Human 112 Research Ethics Committee and the University of Queensland Medical Research Ethics 113 Committee. 114

115 Study design

116 This longitudinal training study was conducted between April and June, 2015. Approximately one week before the intervention commenced, participants underwent MR and 2D ultrasound 117 imaging of their posterior thighs to determine hamstring muscle size and BF_{LH} architecture, 118 respectively. After scanning, all participants were familiarised with the NHE and 45° HE 119 exercise and subsequently underwent strength assessments on each exercise. After all of the 120 pre-training assessments had been completed, participants were allocated to one of three 121 groups: NHE, HE or control (CON). Allocation of participants to groups was performed on the 122 basis of *baseline* BF_{LH} fascicle lengths to ensure that groups did not differ in this parameter 123 124 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 125 second was allocated at random to one of the remaining two groups and the third allocated to 126 the remaining group. This process was repeated for the participants with the 4th to 6th longest 127

fascicles, the 7th to 9th longest fascicles and so forth until each group had 10 participants. The 128 NHE and HE groups completed a 10-week progressive strength training program consisting 129 exclusively of their allocated exercise (Table 1). The CON group were advised to continue 130 131 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 132 their level of perceived soreness in the posterior thigh using a 1-10 numeric pain rating scale. 133 134 All CON participants were required to report to the laboratory at least once per week. For all participants, BF_{LH} architecture was re-assessed 5 weeks into the intervention and within 5 days 135 136 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. 137

138 Training intervention

139 Nordic hamstring exercise (NHE)

An illustration of the NHE can be found in Figure 1a (see also video supplement). Participants 140 knelt on a padded board, with the ankles secured immediately superior to the lateral malleolus 141 by individual ankle braces which were attached to uniaxial load cells. The ankle braces and 142 load cells were secured to a pivot which allowed the force generated by the knee flexors to be 143 144 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 145 146 as slowly as possible to a prone position.[10] Participants performed only the lowering 147 (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. 148 When participants developed sufficient strength to completely stop the movement in the final 149 150 $10-20^{\circ}$ of the range of motion, they were required to hold a weight plate (range = 2.5kg to

151 20kg) to their chest (centred to the xiphoid process) to ensure the exercise was still of152 supramaximal intensity. Participants were provided with 3min of rest between each set.

153 *Hip extension exercise (HE)*

Participants were positioned in a 45° hip extension machine (BodySolid, IL, USA) with their 154 trunk erect and hip joints extended and superior to the level of support pad (Figure 1b; see also 155 video supplement). The ankle of the exercised limb was 'hooked' under an ankle pad and the 156 unexercised limb was allowed to rest above its ankle restraint. Participants held one or more 157 circular weight plate(s) to the chest (centred to the xiphoid process) and were instructed to flex 158 their hip until they reached a point approximately 90° from the starting position. Once 159 participants had reached this position they were instructed to return to the starting position by 160 161 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 162 30s before training the opposite limb, and then recovered for 3min before the next set. The load 163 164 held to the chest in week 1 represented 60-70% of the estimated 1-RM and was progressively 165 increased throughout the training period whenever the prescribed repetitions and sets could be completed with appropriate technique (Table 2). 166

167

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INSERT FIGURE 1

169

170 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

Week	Frequency	Sets	Repetitions
1	2	2	6
2	2	3	6
3	2	4	8
4	2	4	10
5-8	2	5	8-10
9	2	6	6
10	2	5	5

182

183 Table 2. Application of progressive overload for both the Nordic hamstring and hip

184 extension training groups

	Training Intensity (Load)		
Week	Nordic Hamstring exercise	Hip extension exercise	
1 2 3 4 5-8 9	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.	60-70% of 1-RM 70-80% of 1-RM All exercise was completed at maximal intensity of effort. Loads were progressively increased when	

	10	desired repetitions and sets were
		achieved.
185		

187 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 3repetition 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.

193 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).

199 *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.

BFLH architecture assessment

206 BF_{LH} 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, 207 8cm; field of view, 14 x 47mm) (GE Healthcare Vivid-i, Wauwatosa, U.S.A). Participants were 208 209 positioned prone on a plinth with their hips in neutral and knees fully extended, while images 210 were acquired from a point midway between the ischial tuberosity and the knee joint fold, parallel to the presumed orientation of BF_{LH} fascicles. After the scanning site was determined, 211 212 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, 213 214 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 215 confirmed by replicated landmark distance measures. Images were obtained from both limbs 216 217 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, 218 aligned longitudinally and perpendicular to the posterior thigh. Care was taken to ensure 219 minimal pressure was placed on the skin by the probe as this may influence the accuracy of the 220 measures.[27] The orientation of the probe was manipulated slightly by the sonographer (RGT) 221 if the superficial and intermediate aponeuroses were not parallel. 222

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_{LH} . 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] 231 Where FL=fascicle length, AA=aponeurosis angle, MT=muscle thickness and PA=pennation 232 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]

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230

237

INSERT FIGURE 2

Muscle volumes and anatomical cross-sectional area assessment All MRI scans were 238 performed using a 3-Tesla (Siemens TrioTim, Germany) imaging system with a spinal coil. 239 240 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. 241 Contiguous T1-weighted axial MR images (transverse relaxation time: 750ms; echo time: 242 12ms; field of view: 400mm; slice thickness: 10mm; interslice distance: 0mm) were taken of 243 both limbs beginning at the iliac crest and finishing distal to the tibial condyles. A localiser 244 adjustment (20s) was applied prior to the acquisition of T1-weighted images to standardise the 245 field of view. In addition, to minimise any inhomogeneity in MR images caused by dielectric 246 resonances at 3T, a post-processing (B1) filter was applied to all scans.[31] The total scan 247 duration was 3min 39sec. 248

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 and Editor, Cornell University) (Figure 3). Volumes were determined for each muscle by
multiplying the summed CSAs (from all the slices containing the muscle of interest) by the
slice thickness.[25] 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.

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- 260

INSERT FIGURE 3

261 Statistical analysis

All statistical analyses were performed using SPSS version 22.0.0.1 (IBM Corporation, 262 Chicago, IL). Repeated measures split plot ANOVAs were used to determine training-induced 263 changes in BF_{LH} architecture, hamstring muscle volumes and ACSA, strength, and ratings of 264 265 perceived soreness, for each group. For the analysis of BF_{LH} fascicle length, the within-subject variable was time (baseline, mid-training, and post-training) and the between-subject variable 266 was group (HE, NHE, CON). Because BF_{LH} architecture did not differ between limbs 267 268 (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 269 change in hamstring muscle volume and ACSA between groups, the within-subject variable 270 was muscle (BFLH, BFSH, ST, and SM) and the between-subject variable was group (HE, NHE, 271 CON). To explore changes in Nordic and 45° hip extension strength the within-subject variable 272 was time (baseline and post-training) and the between-subject variable was group (HE, NHE, 273 CON). Lastly, to determine if ratings of perceived soreness changed over time, or differed 274 between training groups, within-subject variable was time (weeks 1-10) and the between-275 subject variable was group (HE, NHE, CON) For all analyses, when a significant main effect 276

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.

281 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.

286

288 **RESULTS**

289

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%).

293

294 Table 3. Participant characteristics

295

Group	Age (years)	Height (cm)	Mass (kg)	
HE	23.1±4.1	180±6.3	81.6±9.7	-
NHE	21.6±3.2	182.8±8.7	85.0±10.9	
CON	21.3±3.7	178.5±5.4	75.9±11.8	

296

297

298 Biceps femoris long head (BF_{LH}) fascicle length

299 Between-group comparisons

A significant group x time interaction was observed for fascicle length during the training 300 period (p<0.001) (Figure 4). No significant differences were observed between training groups 301 at either baseline (d = 0.15), mid- (d = 0.49) or post-training points (d = 0.80) (all p > 0.05). 302 However, the NHE group displayed significantly longer fascicles than the CON group at mid-303 304 (mean difference = 1.50 cm, 95% CI = 0.58 to 2.41 cm, 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 305 group exhibited significantly longer fascicles than the CON group at *mid*- (mean difference = 306 307 1.14cm, 95% CI = 0.22 to 2.05cm, d = 1.52, p = 0.011) and *post-training* (mean difference = 308 1.63cm, 95% CI = 0.51 to 2.76cm, d = 1.84, p = 0.003).

309 *Within-group comparisons*

Post hoc analyses revealed that BF_{LH} fascicle length increased significantly from *baseline* in the NHE group at *mid*- (mean difference = 1.23cm, 95% CI = 0.84 to 1.63cm, *d* = 1.39, p < 0.001) and *post-training* (mean difference = 2.22cm, 95% CI = 1.74 to 2.69cm, *d* = 2.17, p < 0.001). The HE group also displayed significantly lengthened fascicles at *mid*- (mean difference = 0.75cm, 95% CI = 0.35 to 1.15cm, *d* = 1.12, p < 0.001) and *post-training* (mean difference = 1.33cm, 95% CI = -0.86 to 1.80cm, *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).

317

INSERT FIGURE 4

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319 Hamstring muscle volumes

320 Between-group comparisons

321 A significant main effect was detected for the *muscle x group* interaction for hamstring muscle volume changes (p < 0.001) (Figure 5). BF_{LH} volume increased significantly more in the HE 322 than the NHE (mean difference = 6.72%, 95% CI = 0.32 to 13.11%, d = 1.03, p = 0.037) and 323 CON groups (mean difference = 12.10%, 95% CI = 5.71 to 18.50%, d = 2.24, p < 0.001), and 324 a smaller nonsignificant difference was observed between the NHE and CON groups (mean 325 difference = 5.39%, 95% CI = -1.01 to 11.78%, d = 1.13, p = 0.122) (Figure 5). BF_{SH} volume 326 increased more in the HE (mean difference = 8.51%, 95% CI = 0.17 to 16.85%, d = 1.49, p = 327 0.044) and NHE groups (mean difference = 15.29%, 95% CI = 6.95 to 23.63%, d = 2.09, p < 328 0.001) than in the CON group. Both the NHE (mean difference = 21.21%, 95% CI = 11.55 to 329 30.88%, d = 2.50, p < 0.001) and HE (mean difference = 14.32\%, 95% CI = 4.65 to 23.98\%, 330 d = 2.16, p = 0.002) training groups exhibited a greater increase in ST volume than the CON 331 332 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.

338 Within-group comparisons

HE training stimulated a greater increase in volume for the ST than the BF_{SH} (mean difference 339 = 5.61%, 95% CI = 1.12% to 10.10%, d = 0.71, p = 0.009). No other significant between-340 muscle differences were noted for volume changes after HE training (p=0.054 - 0.999) for all 341 pairwise comparisons) or in the CON group (p > 1.000). After NHE training, ST volume 342 increased more than BF_{LH} (mean difference = 15.28%, 95% CI = 10.69 to 19.87%, d = 3.54, 343 p<0.001) and SM (mean difference = 16.06%, 95% CI = 10.96 to 21.16%, d = 3.53, p<0.001). 344 Similarly, in the NHE group the percentage change in volume was greater for the BF_{SH} than 345 the BF_{LH} (mean difference = 9.56%, 95% CI = 4.30 to 14.80%, d = 1.18, p <0.001) and SM 346 (mean difference = 10.33%, 95% CI = 5.33 to 15.34%, d = 1.26, p < 0.001). 347

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INSERT FIGURE 5

350

351 Hamstring muscle anatomical cross-sectional area (ACSA)

352 Between-group comparisons

A significant main effect was detected for the *muscle x group* interaction (p < 0.001) (Figure 6). The percentage change in BF_{LH} 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 357 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 358 in the NHE than the CON group (mean difference = 13.26%, 95% CI = 4.98 to 21.54%, d =359 360 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 361 = 0.273). The percentage change in ST ACSA was significantly greater in the NHE (mean 362 difference = 17.60%, 95% CI = 7.60 to 27.61%, *d* = 2.17, p < 0.001) and HE (mean difference 363 = 15.16%, 95% CI = 5.15 to 25.17%, d = 1.95, p = 0.002) groups than the CON group, however 364 365 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 366 in SM ACSA was greater in the HE than the CON group (mean difference = 7.19%, 95% CI = 367 368 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 369 in SM ACSA change was noted between the HE and NHE groups (main difference = 5.17%, 370 371 95% CI = -8.2 to 11.16%, d = 0.85, p = 0.109).

372 Within-group comparisons

After HE training, the change in ACSA observed for the ST was significantly greater than the 373 BF_{LH} (mean difference = 6.46, 95% CI = 0.84 to 12.10%, d = 0.78, p = 0.017), BF_{SH} (mean 374 375 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 376 between-muscle differences in ACSA change were noted after HE training (all p > 0.05). After 377 NHE training, the change in ACSA was greater for BF_{SH} than BF_{LH} (mean difference = 9.30%, 378 95% CI = 3.47 to 15.12%, d = 1.34, p = 0.001) and SM (mean difference = 9.50%, 95% CI = 379 380 4.92 to 14.08, d = 1.33, p < 0.001), while ST ACSA increased more than BF_{LH} (mean difference

381	= 14.14%, 95% CI = 8.52 to 19.76%, $d = 1.76$, p < 0.001) and SM (mean difference = 14.35%,
382	95% CI = 9.15 to 19.54%, <i>d</i> = 1.75, p < 0.001).
383	
384	
385	INSERT FIGURE 6
386	
387	
388	
389	Strength
390	Nordic eccentric strength test
391	A significant group x time interaction effect was observed for the Nordic eccentric strength test
392	(p < 0.001) (Figure 7). Post hoc t tests demonstrated that the NHE (mean difference = 97.38N,
393	95% CI = 65.51 to 129.26N, <i>d</i> = 2.36, p < 0.001) and HE (mean difference = 110.47N, 95%
394	CI = 76.87 to 144.07N, $d = 1.26$, p < 0.001) groups were significantly stronger at <i>post-training</i>
395	compared to <i>baseline</i> while the CON group did not change (mean difference = 8.91N, 95% CI
396	= -42.51to 24.69N, $d = 0.14$, p = 0.590). No groups differed at <i>baseline</i> (p > 0.461), however,
397	at <i>post-training</i> the NHE (mean difference = $123.436N$, 95% CI = 39.93 to $206.93N$, $d = 2.07$,
398	p = 0.003) and HE (mean difference = 94.27N, 95% CI = 8.60 to 179.94N, $d = 1.14$, $p = 0.028$)
399	groups were both significantly stronger than the CON group. No significant difference was
400	observed between training groups at <i>post-training</i> (mean difference = 29.16N, 95% CI = -54.34
401	to 112.66N, <i>d</i> = 0.41, p = 0.999).

INSERT FIGURE 7

A significant group x time interaction effect was also observed for 3-RM strength as assessed 406 during the 45° HE strength test (p < 0.001) (Figure 8). Post hoc analyses demonstrated that the 407 HE (mean difference = 41.00kg, 95% CI = 35.97 to 46.03kg, d = 4.59, p < 0.001) and NHE 408 groups (mean difference = 26.00kg, 95% CI = 20.97 to 31.03kg, d = 2.36, p < 0.001) improved 409 significantly from *baseline* whereas the CON group did not change (mean difference = 3.50kg, 410 411 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) 412 413 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 414 significant difference was observed between training groups (mean difference = 11.50kg, 95% 415 416 CI = -1.07 to 24.07kg, d = 1.09, p = 0.082).

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- INSERT FIGURE 8
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421 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 \pm SE) for the NHE group and 2.3 ± 0.5 for the HE group.

- 427 INSERT FIGURE 9 428
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432 **DISCUSSION**

This study is the first to explore the architectural and morphological adaptations of the 433 hamstrings in response to different strength training exercises. These data suggest that both the 434 HE and NHE stimulate significant increases in BF_{LH} fascicle length and, contrary to our 435 hypothesis, that the longer muscle lengths encountered in the HE exercise do not result in 436 greater lengthening of fascicles than are observed after NHE training. As hypothesised, HE 437 training appears to elicit more hypertrophy in the BF_{LH} than does the NHE; while contrary to 438 439 our hypothesis, the NHE was not significantly more effective at increasing ST volume or cross 440 sectional area than the HE. Both exercises resulted in significant strength increases which were similarly evident in the NHE and HE strength tests. 441

Fascicle lengthening is one possible mechanism by which the NHE[17-19] and other eccentric 442 or long length hamstring exercises[22] protect muscles from injury. We have recently shown, 443 prospectively, that professional soccer players with fascicles <10.56cm were ~4 times more 444 445 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 446 study, participants increased their fascicle lengths from ~10.6cm prior to training, to 12.8 and 447 12.0cm in the NHE and HE groups, respectively, which would likely result in large reductions 448 in hamstring injury risk. 449

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 457 increasing BF_{LH} 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 458 two. Together with observations that long length concentric hamstring training can shorten 459 460 muscle fascicles, [33] the current findings are consistent with the possibility that the combination of concentric and eccentric contractions somewhat dampens the elongation of 461 BF_{LH} fascicles. The advantage of the NHE may be its almost purely eccentric or eccentrically-462 biased nature. Further work is needed to clarify whether eccentrically-biased or purely 463 eccentric HE exercise may yield greater improvements in BF_{LH} fascicle length than the 464 465 combined concentric and eccentric contraction modes used in this investigation.

466 Observations of increased fascicle length following eccentric hamstring exercise are largely consistent with existing literature. For example, Potier and colleagues[32] reported a 34% 467 increase in BF_{LH} fascicle length following eight weeks of eccentric leg curl exercise, while 468 469 Timmins and colleagues[33] reported a 16% increase in BF_{LH} fascicle length after six weeks of eccentric training on an isokinetic dynamometer.[33] These adaptations most likely result 470 471 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 472 increase in serial sarcomeres accounts for both a rightward shift in a muscle's force-length 473 474 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 475 aponeurotic stiffness and further research is needed to clarify the precise mechanism(s) 476 477 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_{SH} growth and the latter resulting in significantly more 482 hypertrophy of the BF_{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 483 exercises that almost exactly fit this pattern, [15] so it appears that the acute changes observed 484 485 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 486 hamstring strain injury, so the exact significance of these findings is unknown. Indeed, we have 487 488 previously reported that BF_{LH} muscle thickness measured via ultrasound is not a risk factor for hamstring injury in elite soccer.[23] Nevertheless, BF_{LH} muscle atrophy has been noted as long 489 490 as 5-23 months after injury in recreational athletes, [25] so unilateral HE exercises may prove more beneficial than the NHE at redressing this deficit in rehabilitation. Interestingly, reduced 491 492 muscle volumes of the ST have been observed 12-72 months after anterior cruciate ligament 493 injury[36] and the results of the current investigation suggest that the NHE may be valuable in rehabilitation of this injury. 494

Hamstring strengthening is an important component of injury prevention strategies. [24 37 38] 495 496 Indeed, several large scale interventions employing the NHE have shown ~65% reductions in 497 hamstring strain injury rates in soccer [17-19] and recent prospective findings in elite Australian football[3] and soccer[23] suggest that eccentric strength improvements like those 498 499 reported here and previously[16] are at least partly responsible for these protective benefits. For example, elite athletes in these sports who generated less than 279N (Australian football) 500 and 337N (soccer) of knee flexor force at the ankles during the NHE strength test were ~4 times 501 more likely to sustain hamstring injuries than stronger counterparts.[3 23] In this study, our 502 recreational level athletes were able to generate, on average, 460N and 431N after 10 weeks of 503 NHE and HE training, respectively, making them substantially stronger than these elite 504 505 Australian football[3] and soccer players.[23] Significant improvements in 3-RM HE strength were also observed for both training groups, which suggests that hamstring strengthening, at 506

507 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, 508 the observed effects of HE training on BF_{LH} fascicle lengths and eccentric knee flexor strength 509 510 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 511 HE training is effective in reducing hamstring strain injuries, however, access to exercise 512 equipment (ie., a 45° HE machine) may be a limiting factor in designing such studies. It is also 513 noteworthy that strength improvements can be achieved with very modest levels of hamstring 514 muscle soreness when training is appropriately structured and progressively overloaded. These 515 observations are in agreement with Mjolsnes and colleagues[16] who have previously reported 516 517 very limited muscle soreness with a gradual increase in NHE volume.

The authors acknowledge that there are some limitations associated with the current study. 518 Firstly, muscle architecture was only assessed in the BF_{LH} and it may not be appropriate to 519 generalise these findings to other knee flexors, given that each hamstring muscle displays 520 unique architectural characteristics.[40] Further, the assessment of fascicle length using two-521 dimensional ultrasound requires some degree of estimation, because the entire length of the 522 BF_{LH} fascicles are not visible in ultrasound images. While the estimation equation used in this 523 study has been validated against cadaveric samples, [29] there is still the potential for error, and 524 future studies employing extended field of view ultrasound methods may be needed to 525 526 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. 527 However, our participants were, on average, as strong as elite Australian football players[3] 528 and stronger than professional soccer players[23] at the start of the study. Furthermore, our 529 cohort displayed average fascicle lengths before training that were within one standard 530

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 533 architectural and morphological adaptations within the hamstring muscle group. We have 534 provided evidence to suggest that both HE and NHE training are effective in lengthening BF_{LH} 535 fascicles and that the greater excursion involved in the HE does not result in greater increases 536 in fascicle length. However, HE training appears to be more effective for promoting 537 hypertrophy in the commonly injured BF_{LH} than the NHE, which preferentially develops the 538 ST and BF_{SH} muscles. HE and NHE had very similar effects on ST volume and cross-sectional 539 540 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 541 HE-oriented exercises in hamstring strain injury prevention protocols. Future prospective 542 543 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 544 NHE training is more effective than the NHE alone. 545

546

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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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554 CONTRIBUTORS

555 MB was the principle investigator and was involved with study design, recruitment, analysis and

556 manuscript write up. SD, RT were involved in data collection. MW, DO, GK and TS were involved

557 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.

560 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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575 DATA SHARING

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

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581 COMPETING INTERESTS

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

590 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.

594 **Figure legends**

Figure 1. (a) The Nordic hamstring exercise (NHE) and (b) the 45^o 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) (cm²) 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.

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