

1 **TITLE**

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

4
5 **Authors**

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32 **ABSTRACT**

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

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What are the new findings?

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- 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.

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- 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

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

67 There has been significant interest in exploring the patterns of muscle activity in hamstring
68 exercises,[10-15] however there is no research examining the architectural and morphological
69 adaptations of these muscles to different exercise interventions. The Nordic hamstring exercise
70 (NHE) has proven effective in increasing eccentric knee flexor strength[16] and reducing
71 hamstring injuries[17-19] in soccer, although there is disagreement in the literature as to which
72 hamstring muscles are most active during this exercise[10 14 15 20]. We have previously
73 reported that the NHE preferentially activates the semitendinosus (ST),[10 15] however, we
74 have also observed high levels of BF_{LH} activity in this exercise[15] which suggests that it may
75 still provide a powerful stimulus for adaptation within this most commonly injured muscle.[1-
76 4] Eccentric exercise has been proposed to increase muscle fascicle lengths via
77 sarcomerogenesis[21 22] and Timmins and colleagues[23] have recently observed such an
78 adaptation after eccentric knee flexor training on an isokinetic dynamometer while also noting
79 that concentric training caused fascicle shortening despite occurring at long muscle lengths.
80 Furthermore, we have recently reported that soccer players with shorter BF_{LH} fascicles
81 (<10.56cm) were at fourfold greater risk of hamstring strain injury than players with longer
82 fascicles.[23] Given the effectiveness of the predominantly eccentric NHE in hamstring injury
83 prevention and rehabilitation,[17-19] it is of interest to examine the impact of this and
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
86 activation of the two-joint hamstrings and greater BF_{LH} activity than the NHE[15]. HE
87 exercises are also performed at longer hamstring muscle lengths than the NHE and it has been
88 suggested that this may make them more effective in hamstring injury prevention than the
89 NHE.[24] However, HE and most other hamstring exercises are typically performed with both
90 eccentric and concentric phases and it remains to be seen how the combination of contraction
91 modes will affect fascicle length by comparison with an almost purely eccentric exercise like
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
94 observations of persistent atrophy in this muscle following injury.[25]

95 The primary purpose of this study was to evaluate changes in BF_{LH} architecture and hamstring
96 muscle volume and anatomical cross-sectional area (ACSA) following 10-week resistance
97 training programs consisting exclusively of NHE or HE training. We tested the hypotheses that
98 1) HE training would stimulate greater increases in BF_{LH} fascicle length than the NHE, on the
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
101 than the NHE; and 3) the NHE would result in more hypertrophy of the ST muscle than the HE
102 exercise.

103

104 **METHODS**

105 **Participants**

106 Thirty recreationally active males (age, 22.0 ± 3.6 years, height, 180.4 ± 7 cm, weight, $80.8 \pm$
107 11.1 kg) provided written informed consent to participate in this study. Participants
108 were free from soft tissue and orthopaedic injuries to the trunk, hips and lower limbs and had
109 no known history of hamstring strain, anterior cruciate ligament or other traumatic knee injury.
110 Before enrolment in the study, all participants completed a cardiovascular screening
111 questionnaire and a standard MRI questionnaire to ensure it was safe for them to enter the
112 magnetic field. This study was approved by the Queensland University of Technology Human
113 Research Ethics Committee and the University of Queensland Medical Research Ethics
114 Committee.

115 **Study design**

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

128 fascicles, the 7th to 9th longest fascicles and so forth until each group had 10 participants. The
129 NHE and HE groups completed a 10-week progressive strength training program consisting
130 exclusively of their allocated exercise (Table 1). The CON group were advised to continue
131 their regular physical activity levels but not to engage in any resistance training for the lower
132 body. At the beginning of every training session, participants in both training groups reported
133 their level of perceived soreness in the posterior thigh using a 1-10 numeric pain rating scale.
134 All CON participants were required to report to the laboratory at least once per week. For all
135 participants, BF_{LH} architecture was re-assessed 5 weeks into the intervention and within 5 days
136 of the final training session. MRI scans were acquired for all participants <7 days after the final
137 training session. Strength testing was conducted after all imaging had been completed.

138 **Training intervention**

139 *Nordic hamstring exercise (NHE)*

140 An illustration of the NHE can be found in Figure 1a (see also video supplement). Participants
141 knelt on a padded board, with the ankles secured immediately superior to the lateral malleolus
142 by individual ankle braces which were attached to uniaxial load cells. The ankle braces and
143 load cells were secured to a pivot which allowed the force generated by the knee flexors to be
144 measured through the long axis of the load cells. From the initial kneeling position with their
145 ankles secured in yokes, arms on the chest and hips extended, participants lowered their bodies
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
148 knees to push back into the starting position so as to minimise concentric knee flexor activity.
149 When participants developed sufficient strength to completely stop the movement in the final
150 10-20° 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 of
152 supramaximal intensity. Participants were provided with 3min of rest between each set.

153 *Hip extension exercise (HE)*

154 Participants were positioned in a 45° hip extension machine (BodySolid, IL, USA) with their
155 trunk erect and hip joints extended and superior to the level of support pad (Figure 1b; see also
156 video supplement). The ankle of the exercised limb was ‘hooked’ under an ankle pad and the
157 unexercised limb was allowed to rest above its ankle restraint. Participants held one or more
158 circular weight plate(s) to the chest (centred to the xiphoid process) and were instructed to flex
159 their hip until they reached a point approximately 90° from the starting position. Once
160 participants had reached this position they were instructed to return to the starting position by
161 extending their hip, while keeping their trunk in a rigid neutral position throughout. Both limbs
162 were trained in alternating fashion; after completing a set on one limb participants rested for
163 30s before training the opposite limb, and then recovered for 3min before the next set. The load
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
166 completed with appropriate technique (Table 2).

167

168 INSERT FIGURE 1

169

170 **Hamstring training program**

171 Participants in both intervention groups completed a progressive intensity training program
172 consisting of 20 supervised exercise sessions (2 per week) over the 10 week period (Tables 1
173 & 2). Each session was followed by at least 48 hours of recovery and participants were

174 prohibited from engaging in any other resistance training for the lower body. The training
 175 program was based on the approximate loads, repetitions and sets employed in previous
 176 interventions using the NHE,[16-18] although the volume (number of repetitions) was reduced
 177 in the final two weeks to accommodate increases in exercise intensity. All sessions were
 178 conducted in the same laboratory, employed the same exercise equipment and were supervised
 179 by the same investigators (MNB and SJD) to ensure consistency of procedures.

180 **Table 1. Training program variables for both the Nordic hamstring and hip extension**
 181 **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**

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

10		desired repetitions and sets were achieved.
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187 **Strength assessments**

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

193 *Nordic eccentric strength test*

194 The assessment of eccentric knee flexor force using the NHE has been reported previously.[3
 195 4 23 26] Participants completed a single warm-up set of 5 submaximal repetitions followed, 1
 196 minute later, by a set of 3 maximal repetitions of the bilateral NHE. Eccentric strength was
 197 determined for each leg from the highest of 3 peak forces produced during the 3 repetitions of
 198 the NHE and was reported in absolute terms (N).

199 *Hip extension strength test*

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

205 **BF_{LH} architecture assessment**

206 BF_{LH} fascicle length was determined from ultrasound images taken along the longitudinal axis
207 of the muscle belly utilising a two-dimensional, B-mode ultrasound (frequency, 12Mhz; depth,
208 8cm; field of view, 14 x 47mm) (GE Healthcare Vivid-i, Wauwatosa, U.S.A). Participants were
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,
211 parallel to the presumed orientation of BF_{LH} fascicles. After the scanning site was determined,
212 the distance of the site from various anatomical landmarks were recorded to ensure its
213 reproducibility for future testing sessions. These landmarks included the ischial tuberosity,
214 head of the fibula and the posterior knee joint fold at the mid-point between BF and ST tendon.
215 On subsequent visits the scanning site was determined and marked on the skin and then
216 confirmed by replicated landmark distance measures. Images were obtained from both limbs
217 following at least five minutes of inactivity. To gather ultrasound images, the linear array
218 ultrasound probe, with a layer of conductive gel was placed on the skin over the scanning site,
219 aligned longitudinally and perpendicular to the posterior thigh. Care was taken to ensure
220 minimal pressure was placed on the skin by the probe as this may influence the accuracy of the
221 measures.[27] The orientation of the probe was manipulated slightly by the sonographer (RGT)
222 if the superficial and intermediate aponeuroses were not parallel.

223 Ultrasound images were analysed using MicroDicom software (Version 0.7.8, Bulgaria). For
224 each image, 6 points were digitised as described by Blazeovich and colleagues.[28] Following
225 the digitising process, muscle thickness was defined as the distance between the superficial and
226 intermediate aponeuroses of the BF_{LH}. A fascicle of interest was outlined and marked on the
227 image (Figure 2). Fascicle length was determined as the length of the outlined fascicle between
228 aponeuroses and was reported in absolute terms (cm). As the entire fascicles were not visible
229 in the probe's field of view, their lengths were estimated using the following equation:[28 29]

230
$$FL = \sin(AA + 90^\circ) \times MT / \sin(180^\circ - (AA + 180^\circ - PA)).$$

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

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

236

237 **INSERT FIGURE 2**

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

249 Muscle volumes and anatomical cross-sectional areas (ACSAs) of the BF_{LH} and short head
250 (BF_{SH}), semitendinosus (ST) and semimembranosus (SM) muscles were determined for both
251 limbs using manual segmentation. Muscle boundaries were identified and traced on each image
252 in which the desired structure was present using image analysis software (Sante Dicom Viewer

253 and Editor, Cornell University) (Figure 3). Volumes were determined for each muscle by
254 multiplying the summed CSAs (from all the slices containing the muscle of interest) by the
255 slice thickness.[25] ACSA was determined by locating the 10mm slice with the greatest CSA
256 and averaging this along with the two slices immediately cranial and caudal (five slices). All
257 traces (pre- and post-training) were completed by the same investigator (MNB) who was
258 blinded to participant identity and training group in all post-testing.

259

260

INSERT FIGURE 3

261 **Statistical analysis**

262 All statistical analyses were performed using SPSS version 22.0.0.1 (IBM Corporation,
263 Chicago, IL). Repeated measures split plot ANOVAs were used to determine training-induced
264 changes in BF_{LH} architecture, hamstring muscle volumes and ACSA, strength, and ratings of
265 perceived soreness, for each group. For the analysis of BF_{LH} fascicle length, the within-subject
266 variable was *time* (*baseline, mid-training, and post-training*) and the between-subject variable
267 was *group* (HE, NHE, CON). Because BF_{LH} architecture did not differ between limbs
268 (dominant vs non-dominant) at any time point ($p>0.05$), the left and right limbs were averaged
269 to provide a single value for each participant. To determine differences in the percentage
270 change in hamstring muscle volume and ACSA between groups, the within-subject variable
271 was *muscle* (BF_{LH}, BF_{SH}, ST, and SM) and the between-subject variable was *group* (HE, NHE,
272 CON). To explore changes in Nordic and 45° hip extension strength the within-subject variable
273 was *time* (*baseline and post-training*) and the between-subject variable was *group* (HE, NHE,
274 CON). Lastly, to determine if ratings of perceived soreness changed over time, or differed
275 between training groups, within-subject variable was *time* (weeks 1-10) and the between-
276 subject variable was *group* (HE, NHE, CON) For all analyses, when a significant main effect

277 was detected, post hoc independent t tests with Bonferroni corrections were used to determine
278 which comparisons differed. For all analyses, the mean differences were reported with their
279 95% confidence intervals (CIs), and where appropriate, Cohen's *d* was reported as a measure
280 of the effect size.

281 **Sample size**

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

286

287

288 **RESULTS**

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290 No significant differences were observed in age, height or body mass between the three groups
291 ($p > 0.05$) (Table 3). Compliance rates were excellent for both training groups (HE: 100%;
292 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*

300 A significant *group x time* interaction was observed for fascicle length during the training
301 period ($p < 0.001$) (Figure 4). No significant differences were observed between training groups
302 at either *baseline* ($d = 0.15$), *mid-* ($d = 0.49$) or *post-training* points ($d = 0.80$) (all $p > 0.05$).
303 However, the NHE group displayed significantly longer fascicles than the CON group at *mid-*
304 (mean difference = 1.50cm, 95% CI = 0.58 to 2.41cm, $d = 1.64$, $p = 0.001$) and *post-training*
305 (mean difference = 2.40cm, 95% CI = 1.28 to 3.53cm, $d = 2.19$, $p < 0.001$). Similarly, the HE
306 group exhibited significantly longer fascicles than the CON group at *mid-* (mean difference =
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*

310 Post hoc analyses revealed that BF_{LH} fascicle length increased significantly from *baseline* in
311 the NHE group at *mid-* (mean difference = 1.23cm, 95% CI = 0.84 to 1.63cm, $d = 1.39$, $p <$
312 0.001) and *post-training* (mean difference = 2.22cm, 95% CI = 1.74 to 2.69cm, $d = 2.17$, $p <$
313 0.001). The HE group also displayed significantly lengthened fascicles at *mid-* (mean
314 difference = 0.75cm, 95% CI = 0.35 to 1.15cm, $d = 1.12$, $p < 0.001$) and *post-training* (mean
315 difference = 1.33cm, 95% CI = -0.86 to 1.80cm, $d = 1.77$, $p < 0.001$). However, the CON group
316 remained unchanged relative to *baseline* values at all time points ($p > 0.05$, $d = 0.20 - 0.31$).

317 INSERT FIGURE 4

318

319 **Hamstring muscle volumes**

320 *Between-group comparisons*

321 A significant main effect was detected for the *muscle x group* interaction for hamstring muscle
322 volume changes ($p < 0.001$) (Figure 5). BF_{LH} volume increased significantly more in the HE
323 than the NHE (mean difference = 6.72%, 95% CI = 0.32 to 13.11%, $d = 1.03$, $p = 0.037$) and
324 CON groups (mean difference = 12.10%, 95% CI = 5.71 to 18.50%, $d = 2.24$, $p < 0.001$), and
325 a smaller nonsignificant difference was observed between the NHE and CON groups (mean
326 difference = 5.39%, 95% CI = -1.01 to 11.78%, $d = 1.13$, $p = 0.122$) (Figure 5). BF_{SH} volume
327 increased more in the HE (mean difference = 8.51%, 95% CI = 0.17 to 16.85%, $d = 1.49$, $p =$
328 0.044) and NHE groups (mean difference = 15.29%, 95% CI = 6.95 to 23.63%, $d = 2.09$, $p <$
329 0.001) than in the CON group. Both the NHE (mean difference = 21.21%, 95% CI = 11.55 to
330 30.88%, $d = 2.50$, $p < 0.001$) and HE (mean difference = 14.32%, 95% CI = 4.65 to 23.98%,
331 $d = 2.16$, $p = 0.002$) training groups exhibited a greater increase in ST volume than the CON
332 group. However, no significant difference in ST volume change was noted between NHE and

333 HE groups (mean difference = 6.90%, 95% CI = -2.77 to 16.56%, $d = 0.69$, $p = 0.239$). The
334 percentage change in volume for the SM was significantly greater for the HE group than for
335 CON (mean difference = 8.95%, 95% CI = 2.21 to 15.69%, $d = 1.57$, $p = 0.007$), while no
336 difference was observed between the NHE and CON group changes (mean difference = 3.38%,
337 95% CI = -3.36 to 10.12%, $d = 0.68$, $p = 0.636$) for this muscle.

338 *Within-group comparisons*

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

348

349 INSERT FIGURE 5

350

351 **Hamstring muscle anatomical cross-sectional area (ACSA)**

352 *Between-group comparisons*

353 A significant main effect was detected for the *muscle x group* interaction ($p < 0.001$) (Figure
354 6). The percentage change in BF_{LH} ACSA was greater in the HE training group than in the
355 NHE (mean difference = 5.24%, 95% CI = 0.061 to 10.41, $d = 0.98$, $p = 0.047$) and CON
356 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%
358 CI = -1.51 to 8.84%, $d = 1.07$, $p = 0.245$) (Figure 6). BF_{SH} ACSA increased significantly more
359 in the NHE than the CON group (mean difference = 13.26%, 95% CI = 4.98 to 21.54%, $d =$
360 1.97 , $p = 0.001$), while no difference was observed between changes exhibited by the HE and
361 CON groups for this muscle (mean difference = 5.69%, 95% CI = -2.59 to 0.70%, $d = 0.90$, p
362 = 0.273). The percentage change in ST ACSA was significantly greater in the NHE (mean
363 difference = 17.60%, 95% CI = 7.60 to 27.61%, $d = 2.17$, $p < 0.001$) and HE (mean difference
364 = 15.16%, 95% CI = 5.15 to 25.17%, $d = 1.95$, $p = 0.002$) groups than the CON group, however
365 no significant difference was noted between changes in the NHE and HE groups (mean
366 difference = 2.4%, 95% CI = -7.57 to 12.45%, $d = 0.24$, $p = 1.000$). The percentage increase
367 in SM ACSA was greater in the HE than the CON group (mean difference = 7.19%, 95% CI =
368 1.21 to 13.18%, $d = 1.34$, $p = 0.015$), but was not significantly greater in NHE than CON (mean
369 difference = 2.02%, 95% CI = -3.97 to 8.01%, $d = 0.49$, $p = 1.000$). No significant difference
370 in SM ACSA change was noted between the HE and NHE groups (main difference = 5.17%,
371 95% CI = -8.2 to 11.16%, $d = 0.85$, $p = 0.109$).

372 *Within-group comparisons*

373 After HE training, the change in ACSA observed for the ST was significantly greater than the
374 BF_{LH} (mean difference = 6.46, 95% CI = 0.84 to 12.10%, $d = 0.78$, $p = 0.017$), BF_{SH} (mean
375 difference = 9.98%, 95% CI = 4.25 to 15.71%, $d = 1.09$, $p < 0.001$) and SM (mean difference
376 = 6.73%, 95% CI = 1.54 to 11.92%, $d = 0.78$, $p = 0.006$). No other significant pairwise
377 between-muscle differences in ACSA change were noted after HE training (all $p > 0.05$). After
378 NHE training, the change in ACSA was greater for BF_{SH} than BF_{LH} (mean difference = 9.30%,
379 95% CI = 3.47 to 15.12%, $d = 1.34$, $p = 0.001$) and SM (mean difference = 9.50%, 95% CI =
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%, $d = 1.75$, $p < 0.001$).

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INSERT FIGURE 6

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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, $d = 2.36$, $p < 0.001$) 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 *post-training*
395 compared to *baseline* while the CON group did not change (mean difference = 8.91N, 95% CI
396 = -42.51 to 24.69N, $d = 0.14$, $p = 0.590$). No groups differed at *baseline* ($p > 0.461$), however,
397 at *post-training* 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 *post-training* (mean difference = 29.16N, 95% CI = -54.34
401 to 112.66N, $d = 0.41$, $p = 0.999$).

402

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INSERT FIGURE 7

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405 *Hip extension strength test*

406 A significant *group x time* interaction effect was also observed for 3-RM strength as assessed
407 during the 45° HE strength test ($p < 0.001$) (Figure 8). Post hoc analyses demonstrated that the
408 HE (mean difference = 41.00kg, 95% CI = 35.97 to 46.03kg, $d = 4.59$, $p < 0.001$) and NHE
409 groups (mean difference = 26.00kg, 95% CI = 20.97 to 31.03kg, $d = 2.36$, $p < 0.001$) improved
410 significantly from *baseline* whereas the CON group did not change (mean difference = 3.50kg,
411 95% CI = -1.53 to 8.53kg, $d = 0.33$, $p = 0.165$). No groups differed significantly at *baseline* (p
412 > 0.091) however at *post-training*, both the HE (mean difference = 43.50kg, 95% CI = 30.93
413 to 56.07kg, $d = 4.21$, $p < 0.001$) and NHE groups (mean difference = 32.0kg, 95% CI = 19.43
414 to 44.57kg, $d = 2.66$, $p < 0.001$) were significantly stronger than CON. *Post-training*, no
415 significant difference was observed between training groups (mean difference = 11.50kg, 95%
416 CI = -1.07 to 24.07kg, $d = 1.09$, $p = 0.082$).

417

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INSERT FIGURE 8

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421 **Perceived soreness**

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

426

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INSERT FIGURE 9

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431

432 **DISCUSSION**

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

442 Fascicle lengthening is one possible mechanism by which the NHE[17-19] and other eccentric
443 or long length hamstring exercises[22] protect muscles from injury. We have recently shown,
444 prospectively, that professional soccer players with fascicles $<10.56\text{cm}$ were ~ 4 times more
445 likely to suffer a hamstring strain than athletes with longer fascicles and that the probability of
446 injury was reduced by $\sim 74\%$ for every 0.5cm increase in fascicle length.[23] In the current
447 study, participants increased their fascicle lengths from $\sim 10.6\text{cm}$ prior to training, to 12.8 and
448 12.0cm in the NHE and HE groups, respectively, which would likely result in large reductions
449 in hamstring injury risk.

450 Despite its success in reducing hamstring strain injuries, the adoption of the NHE in elite
451 European soccer has been reported to be poor with only $\sim 11\%$ of Norwegian premier league
452 and UEFA teams deemed to have adequately implemented the NHE programs that have proven
453 effective in randomised controlled trials[17-19]. Some conditioning coaches and
454 researchers[24] believe that the exercise does not challenge the hamstrings at sufficient lengths
455 to optimise injury prevention benefits. However, this study shows, for the first time, that the
456 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
458 than the HE, although the current study lacked the statistical power to distinguish between the
459 two. Together with observations that long length concentric hamstring training can shorten
460 muscle fascicles,[33] the current findings are consistent with the possibility that the
461 combination of concentric and eccentric contractions somewhat dampens the elongation of
462 BF_{LH} fascicles. The advantage of the NHE may be its almost purely eccentric or eccentrically-
463 biased nature. Further work is needed to clarify whether eccentrically-biased or purely
464 eccentric HE exercise may yield greater improvements in BF_{LH} fascicle length than the
465 combined concentric and eccentric contraction modes used in this investigation.

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

478 To the authors' knowledge, this is the first study to explore the morphological adaptations of
479 the hamstrings to different strengthening exercises. These data suggest that the NHE and HE
480 exercises induce heterogeneous patterns of hamstring muscle hypertrophy, with the former
481 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
483 have previously noted transient T2 relaxation time changes after 50 repetitions of each of these
484 exercises that almost exactly fit this pattern,[15] so it appears that the acute changes observed
485 via functional MRI match quite well with the hypertrophic effects observed after 10 weeks of
486 training. However, neither muscle volume nor ACSA have been identified as risk factors for
487 hamstring strain injury, so the exact significance of these findings is unknown. Indeed, we have
488 previously reported that BF_{LH} muscle thickness measured via ultrasound is not a risk factor for
489 hamstring injury in elite soccer.[23] Nevertheless, BF_{LH} muscle atrophy has been noted as long
490 as 5-23 months after injury in recreational athletes,[25] so unilateral HE exercises may prove
491 more beneficial than the NHE at redressing this deficit in rehabilitation. Interestingly, reduced
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
494 rehabilitation of this injury.

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

507 least in recreationally trained athletes, is not highly specific to the chosen exercise. While the
508 benefits of high levels of HE strength remain unclear from the perspective of injury prevention,
509 the observed effects of HE training on BF_{LH} fascicle lengths and eccentric knee flexor strength
510 suggest the potential for this exercise to reduce injury risk. Future intervention studies
511 analogous to those employing the NHE previously,[17-19 39] are needed to clarify whether
512 HE training is effective in reducing hamstring strain injuries, however, access to exercise
513 equipment (ie., a 45° HE machine) may be a limiting factor in designing such studies. It is also
514 noteworthy that strength improvements can be achieved with very modest levels of hamstring
515 muscle soreness when training is appropriately structured and progressively overloaded. These
516 observations are in agreement with Mjolsnes and colleagues[16] who have previously reported
517 very limited muscle soreness with a gradual increase in NHE volume.

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

531 deviation of the values reported in elite soccer players previously,[23] so it is unlikely that they
532 were unrepresentative of higher-level athletes, in these parameters at least.

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

546

547

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.
558 All authors had full access to all of the data (including statistical reports and tables) in the study and
559 can take responsibility for the integrity of the data and the accuracy of the data analysis.

560 **TRANSPARENCY DECLARATION**

561 The lead author* (MB) affirms that this manuscript is an honest, accurate, and transparent account of
562 the study being reported; that no important aspects of the study have been omitted; and that any
563 discrepancies from the study as planned (and, if relevant, registered) have been explained. * = The
564 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 www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare
584 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
589 the submitted work.

590 **ETHICAL CLEARANCE**

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

594 **Figure legends**

595

596 **Figure 1.** (a) The Nordic hamstring exercise (NHE) and (b) the 45⁰ hip extension (HE)
597 exercise, progressive from left to right.

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

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

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

614 **Figure 5.** Percentage change in volume (cm³) for each hamstring muscle after the intervention.
615 Values are expressed as a mean percentage change compared to the values at *baseline* with
616 error bars representing standard error (SE). For all pairwise comparisons between groups, *
617 indicates p<0.05 and ** signifies that p<0.001. BF_{LH}, biceps femoris long head; BF_{SH}, biceps
618 femoris short head; ST, semitendinosus; SM, semimembranosus.

619 **Figure 6.** Percentage change in anatomical cross sectional area (ACSA) (cm²) for each
620 hamstring muscle after the intervention. Values are expressed as a mean percentage change
621 compared to the values at *baseline* with error bars representing standard error (SE). For all
622 pairwise comparisons between groups, * indicates p<0.05 and ** signifies that p<0.001. BF_{LH},
623 biceps femoris long head; BF_{SH}, biceps femoris short head; ST, semitendinosus; SM,
624 semimembranosus.

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

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

635 **Figure 9.** Mean (\pm standard error) weekly soreness measured using a numeric pain rating scale
636 (1-10) at the beginning of each training session.

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