

1 **Title:**

2 Impact of exercise selection on hamstring muscle activation

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28 **Key Words**

29 Injury prevention, rehabilitation, physical therapy

30 **ABSTRACT**

31 **Objective:** To determine the extent to which different strength training exercises selectively
32 activate the commonly injured biceps femoris long head (BF_{LH}) muscle. **Methods:** This two-
33 part observational study recruited 24 recreationally active males. **Part 1** explored the
34 amplitudes and the ratios of lateral to medial hamstring (BF/MH) normalised
35 electromyography (nEMG) during the concentric and eccentric phases of 10 common
36 strength training exercises. **Part 2** used functional magnetic resonance imaging (fMRI) to
37 determine the spatial patterns of hamstring activation during two exercises which i) most
38 selectively, and ii) least selectively activated the BF in part 1. **Results:** Eccentrically, the
39 largest BF/MH nEMG ratio was observed in the 45° hip extension exercise and the lowest
40 was observed in the Nordic hamstring (NHE) and bent-knee bridge exercises. Concentrically,
41 the highest BF/MH nEMG ratio was observed during the lunge and 45° hip extension and the
42 lowest was observed for the leg curl and bent-knee bridge. fMRI revealed a greater BF_{LH} to
43 semitendinosus activation ratio in the 45° hip extension than the NHE (p<0.001). The T2
44 increase after hip extension for BF_{LH}, semitendinosus and semimembranosus muscles were
45 greater than that for BF_{SH} (p<0.001). During the NHE, the T2 increase was greater for the
46 semitendinosus than for the other hamstrings (p≤0.002). **Conclusion:** This investigation
47 highlights the non-uniformity of hamstring activation patterns in different tasks and suggests
48 that hip extension exercise more selectively activates the BF_{LH} while the NHE preferentially
49 recruits the semitendinosus. These findings have implications for strength training
50 interventions aimed at preventing hamstring injury.

51 **What are the new findings?**

- 52
- The hamstrings are activated non-uniformly during hip- and knee-based exercises
 - Hip extension exercise more evenly activates the three long heads of the hamstrings and the Nordic hamstring exercise preferentially recruits the semitendinosus (ST)

53 INTRODUCTION

54 **Paragraph 1** Hamstring strain injuries (HSIs) are commonly experienced by athletes
55 involved in running-based sports. They are the most prevalent injury in track and field,[1]
56 Australian Rules football,[2] and soccer[3] and up to 30% recur within 12 months.[4]
57 Upwards of 80% of HSIs involve the biceps femoris long head (BF_{LH}) muscle[5-7] and most
58 injuries are thought to occur during the late swing phase of high-speed running.[8] During
59 this phase of the gait cycle, the BF_{LH} reaches its peak length and develops maximal force
60 while undergoing a forceful eccentric contraction to decelerate the shank for foot strike,[9]
61 and it is thought that these conditions may at least partly explain its propensity for injury. It
62 has also been reported that prior BF_{LH} injury is associated with a degree of neuromuscular
63 inhibition[10 11] and prolonged atrophy[12], which suggests that current rehabilitation
64 practices do not adequately restore function to this muscle.

65 **Paragraph 2** It has been proposed that hamstring weakness is a risk factor for future strain
66 injury[6 13 14] and interventions aimed at increasing strength, particularly eccentric knee
67 flexor strength, have been effective in reducing HSI rates in several sports.[15-18] However,
68 despite an increased focus on hamstring strength in prophylactic programs,[19] exercise
69 selection is often implemented on the basis of clinical recommendations and assumptions
70 rather than empirical evidence.[20 21] There is currently a very small body of work on the
71 activation patterns of the hamstrings during commonly employed exercises. Studies using
72 functional magnetic resonance imaging (fMRI) have shown that activation differs within and
73 between hamstring muscles during different tasks.[11 22-24] For example, the
74 semitendinosus (ST) appears to be selectively activated during the Nordic hamstring exercise
75 (NHE)[11] and the eccentric prone leg curl,[24] while the semimembranosus (SM) is
76 preferentially recruited during the stiff leg deadlift.[23] Surface electromyography (sEMG)
77 has been used extensively in the analysis of hamstring exercises.[23-26] However, these

78 studies are sometimes contradictory and are often inconsistent with the results from fMRI.[11
79 22-25 27] The lack of complete agreement between fMRI and sEMG might reflect the
80 different physiological basis of each technique.[28] Surface EMG amplitude is sensitive to
81 the electrical activity generated by active motor units and is detected by electrodes overlying
82 the skin.[29] This provides valuable information on the neural strategies involved during
83 muscle activation with high temporal resolution, but is prone to cross talk[29] and cannot
84 discriminate between closely approximated segments of muscles[30] such as the medial
85 hamstrings (semimembranosus and semitendinosus). By contrast, fMRI is a relatively
86 new[31] technique which reflects the metabolic activity associated with exercise.[28] Muscle
87 activation is associated with a transient increase in the transverse (T2) relaxation time of
88 tissue water, which can be detected from signal intensity changes in fMR images. These T2
89 shifts, which, like sEMG, increase in proportion to exercise intensity,[31 32] can be mapped
90 in cross-sectional images of muscles and therefore provide significantly greater spatial clarity
91 than sEMG.[28 30]

92 **Paragraph 3** An improved understanding of the patterns of hamstring muscle activation
93 during common strength training exercises may enable practitioners to make better informed
94 decisions regarding exercise selection in injury prevention and rehabilitation programs. These
95 data may also inform the design of training studies aimed at investigating the chronic
96 adaptations induced by different exercises. The purpose of this two-part study was to
97 determine the extent to which different exercises selectively activate the commonly injured
98 BF_{LH}. Part 1 used sEMG to determine the amplitude and ratio of lateral to medial hamstring
99 activation during 10 commonly employed exercises. Based on these findings, part 2
100 employed fMRI to map muscle activation during two exercises that appeared to a) most
101 selectively; and b) least selectively activate the BF according to sEMG. We hypothesised that
102 the patterns of hamstring muscle activation would be non-uniform between exercises and, on

103 the basis of previous work,[23] that more selective activation of the BF_{LH} would be observed
104 during hip-extension exercise.

105 **METHODS**

106 **Participants**

107 **Paragraph 4** Twenty-four recreationally active male athletes (age, 24.4 ± 3.3 years, height,
108 181.8 ± 6.1 cm, weight, 85.2 ± 13.4 kg) participated in this study. Eighteen athletes (age, 23.9
109 ± 3.1 , height, 180.6 ± 5.9 , weight, 86.0 ± 14.8) participated in part 1 and ten athletes (age,
110 24.6 ± 4.0 , height, 183.5 ± 7.0 , weight, 83.5 ± 8.7) participated in part 2. *A priori* sample size
111 estimates were based on 1) the capacity to detect a 10% difference in the ratio of BF to MH
112 (BF/MH) sEMG amplitude between exercises,[25] and 2) an effect size of 1.0 in the
113 percentage change in T2 relaxation time between muscles,[11] at a power of 0.80 and with
114 $p < 0.05$. Participants were free from soft tissue and orthopaedic injuries to the trunk, hips and
115 lower limbs at the time of testing and had no known history of cardiovascular, metabolic or
116 neurological disorders. Participants had no history of HSI in the previous 18 months and had
117 never suffered an anterior cruciate ligament injury. Prior to testing, all participants completed
118 a cardiovascular screening questionnaire to make sure it was safe for them to perform intense
119 exercise and those who were involved in part 2 also completed a standard MRI screening
120 questionnaire to ensure it was safe for them to enter the magnetic field. All participants
121 provided written, informed consent for this study, which was approved by the Queensland
122 University of Technology Human Research Ethics Committee and the University of
123 Queensland Medical Research Ethics Committee.

124 **Study Design**

125 **Paragraph 5** This cross-sectional study involved two parts. In the first we explored the
126 sEMG amplitudes and ratios of BF to medial hamstring (MH) sEMG activity during ten

127 commonly employed strength training exercises. Based on these findings, part 2 involved an
128 fMRI investigation of two exercises which appeared to a) most selectively, and b) least
129 selectively activate the BF muscle during eccentric contractions.

130 **PART 1**

131 **Paragraph 6** On a separate day prior to experimental testing, participants were familiarised
132 with the exercises used in this investigation. All were shown a demonstration of each exercise
133 (Figure 1) and performed several practice repetitions while receiving verbal feedback from
134 the investigators. Once the participant could complete the exercise with appropriate
135 technique, the loads were progressively increased until an approximate 12RM load was
136 determined (unless the exercise was already supramaximal, ie. NHE and glute-ham-raise). On
137 the day of testing, participants reported to the laboratory and were prepared for sEMG
138 measurement. The testing session began with two maximal voluntary isometric contractions
139 (MVICs) for the hamstrings. Subsequently, participants completed a single set of six
140 repetitions of each exercise, each with the predetermined 12RM load, in randomised order.
141 All data were sampled from a randomly selected limb (dominant or non-dominant), which
142 was the exercised limb during all unilateral movements and all testing sessions were
143 supervised by the same investigator (MNB) to ensure consistency of procedures.

144

145 **Exercise Protocol**

146 **Paragraph 7** The 10 exercises were chosen based on a review of the scientific literature[23
147 25 27]. They included the bilateral and unilateral stiff-leg deadlift, hip hinge, lunge, unilateral
148 bent and straight knee bridges, leg curl, 45° hip extension, glute-ham-raise and the NHE.
149 Unless the exercise was explosive (hip hinge) or supramaximal and eccentric-only (NHE and

150 glute-ham raise) participants completed both the concentric and eccentric phases of each
151 exercise using a 12-RM load at a constant pace (~2s up and ~2s down).

152

153 INSERT FIGURE 1

154

155 **Electromyography**

156 **Paragraph 8** Bipolar pre-gelled Ag/AgCl sEMG electrodes (10mm diameter, 15mm
157 interelectrode distance) (Ambu, BlueSensor N) were used to record electromyographical
158 activity from the BF and MH . The skin of the participants was shaved, lightly abraded and
159 cleaned with alcohol before electrodes were placed on the posterior thigh, midway between
160 the ischial tuberosity and tibial epicondyles. Electrodes were oriented parallel to the line
161 between these two landmarks, as per SENIAM guidelines,[33] and secured with tape to
162 minimise motion artefact. The reference electrode was placed on the ipsilateral head of the
163 fibula. Muscle bellies of the BF and MH were identified via palpation and correct placement
164 was confirmed by observing active external and internal rotation of the knee in 90° of
165 flexion.[10] During all exercise trials, hip and knee joint angles were measured
166 simultaneously with sEMG data using two digital goniometers. The hip sensor's axis of
167 rotation was aligned with the greater trochanter of the femur and the knee sensor was
168 positioned superficial to the lateral femoral epicondyle.

169 **Maximal voluntary contraction**

170 **Paragraph 9** Surface EMG activity was recorded during MVICs of the hamstrings using a
171 custom-made device which was fitted with two uniaxial load cells.[34] Participants lay prone
172 with their hips in 0° of flexion and knees fully extended (180°), with their ankles secured in

173 immoveable yokes and were asked to perform forceful knee flexion while investigators
174 provided strong verbal encouragement. After 1-2 warm-up contractions, participants
175 completed two 3-4s MVICs, with 30-sec of rest separating each attempt. The contraction that
176 elicited the highest average amplitude for the BF and MH was used to represent the maximal
177 EMG amplitude.

178 **Data analysis**

179 **Paragraph 10** All sEMG and joint angle data were sampled at 1 kHz through a 16-bit
180 PowerLab 26T AD unit (ADInstruments, New South Wales, Australia) (amplification =
181 1000; common mode rejection ratio = 110dB) and analysed using LabChart 8.0 (AD
182 Instruments, New South Wales, Australia). Raw sEMG data were filtered using a Bessel filter
183 (frequency bandwidth = 10-500Hz) and then full wave rectified. Joint angle data were used to
184 determine the concentric and eccentric phases of each repetition for each exercise. For each
185 phase, the filtered sEMG signal was normalised to values obtained during MVIC and these
186 normalised sEMG (nEMG) values were averaged across the six repetitions.

187

188 **Statistical analysis**

189 **Paragraph 11** Data were analysed using JMP version 10.02 (SAS Institute Inc, 2012).
190 Descriptive statistics were calculated for mean nEMG amplitudes of BF and MH for the
191 concentric and eccentric phases of each exercise and an activation ratio was determined by
192 dividing the average BF nEMG amplitude by the average MH nEMG amplitude (BF/MH);
193 ratios >1.0 indicated that the BF was more active than the MH muscles. For both the
194 concentric and eccentric phases, repeated measures linear mixed models fitted with the
195 restricted maximum likelihood method were used to determine differences between exercises.

196 For this analysis, *exercise* was the fixed factor and *participant identity* the random factor.
197 When a significant main effect was observed for *exercise*, post hoc t-tests with Bonferroni
198 corrections were used to identify the source and reported as mean differences with 95% CIs.
199 For these analyses, the Bonferroni adjusted p value was set at <0.002.

200

201 **PART 2**

202 **Paragraph 12** A cross-sectional design was used to map the spatial patterns of hamstring
203 muscle activation during the 45° hip extension and NHE. These exercises were chosen
204 because they a) most selectively (45° hip extension) and b) least selectively (NHE) activated
205 the BF muscle during eccentric contractions according to sEMG. Participants completed two
206 separate exercise sessions, separated by at least six days (14 ± 5 days), with each session
207 involving one of the aforementioned exercises. Functional MRI scans of both thighs were
208 acquired before and immediately after each exercise bout. All testing sessions were
209 supervised by the same investigator (MNB).

210 **Exercise Protocol**

211 **Paragraph 13** A depiction of the 45° hip extension and NHE can be found in Figure 1. All
212 exercise was completed using the same equipment as that used in part 1. Participants
213 completed five sets of 10 repetitions of each exercise with one-minute rest intervals between
214 sets. The higher volume of exercise (compared to part 1) was necessary because transient T2
215 changes reflect fluid shifts associated with glycolysis and have a higher detection threshold
216 than sEMG.[28] All subjects completed 50 repetitions successfully. During the rest periods,
217 participants remained in a seated position (for the hip extension exercise) or lay prone (NHE)
218 to minimise activation of the hamstrings. The 45° hip extension exercise was performed

219 unilaterally (with the limb chosen randomly) with a starting load corresponding to each
220 participant's approximate 12-RM (median = 10kg; range = 10 to 20kg). However if the
221 participant could no longer complete the exercise with the allocated load, the weight was
222 gradually reduced by increments of 5kg until it could be completed at the desired speed (2sec
223 up and 2 sec down), which was controlled by an electronic metronome. The NHE was
224 performed bilaterally with body weight only. Participants received verbal support from the
225 investigators throughout all exercise sessions to promote maximal effort. All participants
226 were returned to the scanner immediately following the cessation of exercise and post-
227 exercise scans began within 148.6 ± 24 sec (mean \pm SD).

228 **Functional muscle magnetic resonance imaging (fMRI)**

229 **Paragraph 14** All fMRI scans were performed using a 3-Tesla (Siemens TrioTim, Germany)
230 imaging system with a spinal coil. The participant was positioned supine in the magnet bore
231 with their knees fully extended and hips in neutral and straps were secured around both limbs
232 to prevent any undesired movement. Consecutive T2-weighted axial images were acquired of
233 both limbs beginning at the level of the iliac crest and finishing distal to the tibial plateau
234 using a 180 x 256 image matrix. Images were acquired before and immediately after exercise
235 using a Car-Purcel-Meiboom-Gill (CPMG) spin-echo pulse sequence and the following
236 parameters: transverse relaxation time (TR) = 2540ms; echo time (TE) = 8, 16, 24, 32, 40, 48
237 and 56ms; number of excitations = 1; slice thickness = 10mm; interslice gap = 10mm; field of
238 view = 400 x 281.3mm). The total acquisition time for each scan was 6min 24s. A localiser
239 adjustment (20s) was applied prior to the first sequence of each scan to standardise the field
240 of view and to align collected images between the pre- and post- exercise scans.[11] To
241 minimise any inhomogeneity in MR images caused by dielectric resonances at 3T, a (B1)
242 filter was applied to all scans; this is a post-processing image filter that improves the image
243 signal intensity profile without affecting the image contrast. In addition, to ensure that the

244 signal intensity profile of T2-weighted images was not disrupted by anomalous fluid shifts,
245 participants were instructed to avoid any exhaustive resistance training of the lower limbs in
246 the week preceding testing, and were seated for a minimum of 15 minutes[23] before pre-
247 exercise imaging.

248 **Paragraph 15** For each exercise session, the T2 relaxation times of each hamstring muscle
249 were measured in T2-weighted images acquired before and after exercise to evaluate the
250 degree of muscle activation during exercise. All fMRI scans were transferred to a Windows
251 computer in the digital imaging and communications in medicine (DICOM) file format. The
252 T2 relaxation times of each hamstring muscle (BF_{LH}, BF_{SH}, ST and SM) were measured in
253 five axial slices, corresponding to 30, 40, 50, 60 and 70% of thigh length; these values were
254 determined relative to the distance between the inferior margin of the ischial tuberosity (0%)
255 and the superior border of the tibial plateau (100%).[11 23] Image analysis software (Sante
256 Dicom Viewer and Editor, Cornell University) was used to measure the signal intensity of
257 each hamstring muscle in the exercised limb in both the pre- and post-exercise scans. The
258 signal intensity was measured manually in each slice using a circular region of interest
259 (ROI)[27] which was placed in a homogenous region of contractile tissue in each muscle
260 belly (avoiding fat, aponeurosis, tendon, bone and blood vessels). The size of each ROI
261 varied (0.2 to 5.6 cm²) based on the cross-sectional area and the amount of homogeneous
262 tissue available in each slice. The signal intensity reflected the mean value of all pixels within
263 the ROI and was measured across seven echo times (8, 16, 24, 32, 40, 48, 56ms). To calculate
264 the T2 relaxation time for each ROI, the signal intensity value at each echo time was fitted to
265 a mono-exponential decay model using a least squares algorithm:

$$266 \quad [(SI= M \times \exp(\text{echo time} / T2))][23]$$

267 where SI is the signal intensity at a specific echo time, and M represents the pre-exercise
268 fMRI signal intensity. To assess the extent to which each ROI was activated during exercise,
269 the mean percentage change in T2 was calculated as:

$$270 \quad [(mean \text{ post-exercise T2} / mean \text{ pre-exercise T2}) \times 100].$$

271 To provide a meaningful measure of whole-muscle activation, the percentage change in T2
272 relaxation time for each hamstring muscle was evaluated using ROIs from all five thigh
273 levels. Previous studies have demonstrated excellent reliability of T2 relaxation time
274 measures with intra-class correlation coefficients ranging from 0.87 to 0.94.[28 35]

275

276 **Statistical analysis**

277 **Paragraph 16** Absolute T2 values before and after each exercise session were reported
278 descriptively as mean \pm SD. Repeated measures linear mixed models fitted with the restricted
279 maximum likelihood (REML) method were used to determine the spatial activation patterns
280 of the hamstring muscles during the 45° hip extension and NHE. The percentage change in
281 T2 relaxation time was compared between each hamstring muscle (BF_{LH}, BF_{SH}, ST and SM)
282 for both exercises. For this analysis, *muscle* was the fixed factor and both *participant identity*
283 and *participant identity x muscle* the random factors. When a significant main effect was
284 detected for *muscle*, post hoc t tests with Bonferroni corrections were used to determine the
285 source; the adjusted alpha was set at $p < 0.008$. Given that the two examined exercises differed
286 in intensity and contraction mode(s), it was not appropriate to directly compare the magnitude
287 of the T2 shifts between exercises.[36] Instead, repeated measures linear mixed models fitted
288 with the REML method were used to determine differences in the ratio of BF to ST (BF_{LH}/ST
289 and BF_{SH}/ST) and SM to ST (SM/ST) percentage change in T2 relaxation time between
290 exercises. For these analyses *exercise* was the fixed factor and *participant identity* the random
291 factor. When a main effect was found for *exercise*, post hoc t tests were again used to

292 determine the source and reported as mean difference (and 95% CI). Alpha was set at $p < 0.05$
293 for these analyses.

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301 **RESULTS**

302 **Levels of hamstring muscle activation**

303 **Paragraph 17** Average BF muscle activity ranged from 21.4% (lunge) to 99.3% (unilateral
304 straight knee bridge) MVIC during the concentric phase and 10.7% (hip hinge) to 71.9%
305 (NHE) during the eccentric phase. Average MH muscle activity ranged from 18.1% (lunge)
306 to 120.7% (leg curl) during the concentric phase and 11.6% (hip hinge) to 101.8% (NHE)
307 during the eccentric phase.

308

309 **Concentric biceps femoris to medial hamstring (BF:MH) activation ratio**

310 **Paragraph 18** The concentric BF/MH activation ratio for each exercise can be found in
311 Figure 2a. A significant main effect was detected between exercises ($p < 0.001$) with post hoc
312 t tests showing that the BF/MH ratio was greater during the lunge than the leg curl (mean
313 difference = 0.8, 95% CI = 0.5 to 1.1, $p < 0.001$) and bent-knee bridge (mean difference = 0.7,
314 95% CI = 0.4 to 1.1, $p < 0.001$). Similarly, the BF/MH ratio was greater in the 45° hip
315 extension exercise than the leg curl (mean difference = 0.6, 95% CI = 0.3 to 1.0, $p < 0.001$)
316 and bent-knee bridge (mean difference = 0.6, 95% CI = 0.2 to 0.9, $p = 0.001$).

317

318 **Eccentric biceps femoris to medial hamstring (BF:MH) activation ratio**

319 **Paragraph 19** The eccentric BF/MH activation ratio for each exercise can be found in Figure
320 2b. A significant main effect was observed for exercise ($p < 0.001$) with post hoc analyses
321 revealing that the BF/MH ratio was significantly greater in the 45° hip extension than the
322 NHE (mean difference = 0.7, 95% CI = 0.4 to 1.0, $p < 0.001$), bent-knee bridge (mean

323 difference = 0.7, 95% CI = 0.4 to 1.0, $p < 0.001$), leg curl (mean difference = 0.6, 95% CI =
324 0.3 to 0.9, $p < 0.001$) and the glute-ham raise (mean difference = 0.6, 95% CI = 0.3 to 0.9,
325 $p < 0.001$). No other between-exercise differences were observed once adjusted for multiple
326 comparisons ($p > 0.002$).

327

328

INSERT FIGURE 2

329

330 **Percentage change in T2 relaxation time following the 45° hip extension exercise**

331 **Paragraph 20** A significant main effect was observed for muscle ($p < 0.001$) with post hoc t
332 tests revealing that the exercise-induced T2 changes in the BF_{SH} were significantly lower than
333 those observed for the BF_{LH} (mean difference = 60.7%, 95% CI = 41.3 to 80.1%, $p < 0.001$),
334 ST (mean difference = 78.0%, 95% CI = 58.4 to 97.6%, $p < 0.001$) and SM muscles (mean
335 difference = 49.8%, 95% CI = 30.1 to 69.5%, $p < 0.001$) (Figure 3). The T2 change for ST was
336 significantly greater than SM (mean difference = 28.2%, 95% CI = 9.2 to 47.1%, $p = 0.005$)
337 however, no difference was observed between the BF_{LH} and SM ($p = 0.245$) or between the
338 BF_{LH} and ST muscles ($p = 0.067$). Absolute T2 values before and after the hip extension
339 exercise are reported in Table 1.

340

341

INSERT FIGURE 3

342

343 **Percentage change in T2 relaxation time following the Nordic hamstring exercise**

344 **Paragraph 21** A main effect was detected for muscle ($p < 0.001$). Post hoc analyses showed
345 that the T2 changes induced by exercise within the ST were significantly larger than those

346 observed for the BF_{LH} (mean difference = 29.8%, 95% CI = 20.5 to 39.2%, p<0.001), BF_{SH}
347 (mean difference = 16.2%, 95% CI = 6.4 to 26.0%, p=0.002) and SM (mean difference =
348 29.9%, 95% CI = 20.4 to 39.4%, p<0.001) muscles (Figure 4). In addition, the T2 increase
349 observed for BF_{SH} was significantly greater than for the BF_{LH} (mean difference = 13.7%,
350 95% CI = 3.9 to 23.4%, p=0.008) and SM (mean difference = 13.8, 95% CI = 3.8 to 23.7,
351 p=0.008) muscles. No difference was observed between the BF_{LH} and SM muscles (p=0.982).
352 The absolute T2 values before and after the NHE are reported in Table 1.

353

354

INSERT FIGURE 4

355

356 **Comparison of hamstring activation ratios between exercises**

357 **Paragraph 22** When comparing the BF_{LH}/ST ratio, a significant main effect was observed
358 for exercise (p<0.001) with post hoc analyses revealing a significantly greater ratio during
359 45° hip extension exercise than during the NHE (mean difference = 0.7, 95% CI = 0.6 to 0.9,
360 p<0.001) (Figure 5).

361

362

INSERT FIGURE 5

363

364 **Paragraph 23** A significant main effect was also detected for exercise when comparing the
365 BF_{SH}/ST ratio (p<0.001). Post hoc t tests demonstrated that this ratio was significantly greater
366 during the NHE than during the 45° hip extension exercise (mean difference = 0.42, 95% CI
367 = 0.24 to 0.62, p<0.001). When comparing the SM/ST ratio a significant main effect was

368 detected for exercise ($p < 0.001$) with post hoc t tests showing relatively higher ratios during
 369 the 45° hip extension than during the NHE (mean difference = 0.51, 95% CI = 0.39 to 0.64,
 370 $p < 0.001$).

371

372 **Table 1.** T2 relaxation time values measured before (T2 Pre) and immediately after (T2 Post)
 373 the 45° hip extension and Nordic hamstring exercise (NHE) sessions.

	45° hip extension		NHE	
	T2 Pre (ms)	T2 Post (ms)	T2 Pre (ms)	T2 Post (ms)
BF_{LH}	35.61 (±8.13)	62.56 (±17.34)	35.84 (±8.83)	39.76 (±11.13)
BF_{SH}	31.03 (±3.85)	36.17 (±4.65)	32.36 (±6.05)	40.57 (±9.64)
ST	38.68 (±11.89)	74.18 (±20.06)	37.31 (±10.74)	53.04 (±19.97)
SM	44.26 (±13.23)	71.09 (17.48)	41.39 (±10.87)	45.4 (±11.67)

374

375 Data are presented as mean values (±SD). BF_{LH}, biceps femoris long head; BF_{SH}, biceps
 376 femoris short head; ST, semitendinosus; SM, semimembranosus.

377

378

379 **DISCUSSION**

380 **Paragraph 24** The primary aim of this study was to determine movements that most
381 selectively activate the commonly injured BF_{LH}. The results support the hypothesis that
382 hamstring activation patterns differ markedly between exercises and provide evidence to
383 suggest that hip extension exercise more selectively targets the BF_{LH} than the NHE.

384 **Paragraph 25** The NHE has been shown, in a number of studies,[15 16 18] to be very
385 effective at reducing HSIs in soccer players as long as compliance is adequate.[37] However,
386 we[11] and others[22] have previously reported that the NHE preferentially activates the ST
387 and this might be interpreted as evidence that the exercise is sub-optimal to protect against
388 running-related strain injury. It is entirely possible that the NHE confers injury-preventive
389 benefits via an improved load-bearing capacity of its agonist,[38] however, in this study, we
390 have provided EMG evidence which shows, despite the relatively selective activation of the
391 ST, that the lateral hamstrings were still strongly activated during the NHE. Indeed, BF
392 nEMG was higher during the NHE than during the eccentric phase of any other exercise and
393 the evidence for this exercise's protective effects[15 16 18] suggests that eccentric actions
394 alone in a training program are sufficient to make the hamstrings more resistant to strain
395 injury. High levels of BF nEMG during the NHE are consistent with previous
396 investigations[25] and are the result of the supramaximal intensity of the exercise, which
397 potentially explains why high levels of BF nEMG were also observed in the eccentric glute-
398 ham-raise. High levels of BF nEMG in concentric actions were observed in several other
399 exercises including the straight-knee bridge, leg curl and the 45° hip extension which
400 corroborates previous observations.[25] However, the importance of hamstring activation
401 patterns during concentric actions remains unclear from the perspective of injury prevention.

402 **Paragraph 26** While high levels of nEMG are an important stimulus for improving strength
403 and voluntary activation,[39] exercise selectivity may still have important implications for

404 rehabilitation. For example, inhibition of previously injured BF muscles during eccentric
405 actions has been reported many months after rehabilitation,[10 11 40] and it has been
406 proposed[41] that these deficits might partly explain observations of persistent eccentric knee
407 flexor weakness,[10] BF_{LH} atrophy[12] and a chronic shortening of BF_{LH} fascicles.[42] These
408 data[10-12 40 42] are consistent with the possibility that conventional rehabilitation strategies
409 may not adequately target the commonly injured BF_{LH}. Previous studies have shown that the
410 ratio of lateral to medial hamstring (BF/MH) sEMG varies with foot rotation[43] and differs
411 between exercises.[25] In the current study, the eccentric phase of the 45° hip extension
412 exercise exhibited the greatest BF/MH nEMG ratio (1.5 ± 0.1) while the NHE (0.8 ± 0.1) and
413 bent-knee bridge exercises (0.8 ± 0.1) displayed the lowest ratios. These observations were
414 confirmed in the subsequent fMRI analysis whereby the ratio of BF_{LH} to ST in the 45° hip
415 extension exercise (0.96 ± 0.09) was markedly higher than that observed for the NHE ($0.23 \pm$
416 0.08). It is also noteworthy that the eccentric phase of other hip-oriented exercises (straight-
417 knee bridge, unilateral and bilateral stiff-leg deadlift and hip hinge) displayed BF/MH nEMG
418 ratios >1.0 . In contrast, the eccentric phase of exercises that involved significant movement at
419 the knee (leg curl, glute-ham-raise, bent-knee bridge and NHE) had higher levels of medial
420 hamstring nEMG (BF/MH ratio <1.0). These data suggest that hamstring activation strategies
421 are partly dependent on the joints involved in each movement. During concentric
422 contractions, the most selective BF activation was observed in the lunge exercise which
423 corroborates a previous fMRI investigation.[27] However, it is important to consider that the
424 lunge also exhibited the lowest BF nEMG amplitude ($21.4 \pm 7.4\%$) of any exercise which
425 likely renders it an inadequate stimulus for improving strength or stimulating hypertrophy in
426 this muscle.[39] Interestingly, the exercise that least selectively activated the BF during
427 concentric contractions was the leg curl, which mimics the joint positions and hamstring
428 muscle-tendon lengths experienced in the NHE.

429 **Paragraph 27** The mechanism for higher levels of BF_{LH} activity during hip extension-
430 oriented movements remains unclear, however, it is possible that hamstring muscle moment
431 arms play a role. For example, the BF_{LH} exhibits a larger moment arm at the hip than at the
432 knee[44] and therefore possesses a greater mechanical advantage at this joint. As a result, the
433 BF_{LH} undergoes significantly more shortening during hip extension than knee flexion.[44] By
434 contrast, the ST displays a larger sagittal plane moment arm at the knee than both BF_{LH} and
435 SM,[44] which may explain its preferential recruitment during movements at this joint, such
436 as the NHE and leg curl exercises. It is also noteworthy that the ST is a fusiform muscle with
437 long fibre lengths and many sarcomeres in series, which potentially makes it well-suited to
438 forceful eccentric contractions[45] such as those experienced in the NHE. Further work is
439 needed to clarify the mechanisms underpinning these unique strategies of hamstring
440 activation during hip and knee movements.

441 **Paragraph 28** The current findings are different to some others that have investigated
442 hamstring activation patterns. For example, Zebis and colleagues[25] recently reported that
443 both the NHE and the prone isokinetic leg curl were performed with very similar levels of ST
444 and BF_{LH} nEMG. However, in the current investigation, the NHE and leg curl exercises
445 resulted in more selective activation of the medial hamstrings and, in the case of the NHE, the
446 fMRI results also suggest selective use of the ST muscle. Differences between these studies
447 may conceivably be related to participant sex (females[25] versus males in the current study),
448 electrode placement, and the fact that this earlier work did not differentiate between the
449 concentric and eccentric phases of each exercise. However, it is also important to consider
450 that sEMG does not have the spatial resolution of fMRI and cannot reliably distinguish
451 between neighbouring muscles,[30] such as the long and short heads of BF or the ST and SM,
452 which appear to display distinct activation magnitudes.[11 23 24] These data highlight the

453 limitations of relying exclusively on sEMG to infer strategies of hamstring muscle activation
454 during exercise and suggest the need for more spatially robust methods in future work.

455 **Paragraph 29** In interpreting the results of this study, it is important to consider that sEMG
456 and fMRI techniques measure different aspects of muscle activity. The absence of T2
457 relaxation time changes in people with McCardle's disease[46] suggests that fMRI is
458 sensitive to glycolysis and it is thought that the osmotic fluid shifts which persist after
459 exercise and give rise to T2 changes are a consequence of the accumulation of glycolytic
460 metabolites.[36] Fortunately, the proportion of Type II glycolytic fibres does not appear to
461 vary across the hamstring muscles[47] so this is unlikely to be a confounding factor in this
462 study. However, exercise induced changes in T2 will be influenced by contraction mode
463 because concentric work is characterised by higher nEMG amplitudes[29] and is markedly
464 less efficient than eccentric work against the same loads.[48] As a consequence, the
465 differences in T2 relaxation time changes after the 45° hip extension exercise which involved
466 concentric and eccentric actions and the almost entirely eccentric NHE do not reflect only the
467 levels of voluntary muscle activation. Instead, fMRI can offer insights into the relative
468 metabolic activity and reliance upon different hamstring muscles in each exercise. According
469 to fMRI, the NHE involves preferential ST use with modest use of the other hamstrings,
470 while the 45° hip extension exercise appears to heavily recruit both the BF_{LH} and ST muscles.
471 These observations are largely consistent with the sEMG component of this study, which also
472 suggested higher activation of the medial than lateral hamstrings in the NHE and more even
473 activation of the medial and lateral hamstrings in the 45° hip extension.

474 **Paragraph 30** Characterising the activation patterns of the hamstrings during different tasks
475 is an important first step in identifying exercises worthy of further investigation, however,
476 electrical or metabolic activity of muscles should not be the only factors considered in
477 exercise selection. Indeed, despite the BF_{LH} being more active in hip extension, there is

478 currently no evidence to suggest that training with this exercise actually leads to a reduction
479 in the risk of HSI. Further work is required to understand how the hamstrings adapt to this
480 and other exercises and adaptation is influenced by a range of factors, such as contraction
481 mode[49] and range of motion,[45] which were not a part of the current investigation. For
482 example, there is little reason to believe that concentric or concentrically-biased exercise is
483 effective in HSI prevention or rehabilitation programs.[17] Indeed, there is evidence that
484 concentric training shortens BF_{LH} fascicles[49] and shifts knee flexor torque-joint angle
485 relationships towards shorter muscle lengths[50] and neither of these adaptations are
486 considered beneficial for HSI prevention.[7 51] Because eccentric and concentric training
487 programs appear to have opposing effects on fascicle lengths,[49] it is possible that exercises
488 combining contraction modes may have minimal or at least blunted effects on muscle
489 architecture. Future studies are needed to assess the impact of certain exercises on known or
490 proposed risk factors for HSI such as eccentric strength[6] and fascicle lengths,[7] and only
491 then will there be sufficient evidence to justify use of those exercises in intervention studies
492 aimed at reducing the risk of injury. Based on the current findings, for example, it seems
493 logical to compare the effects of training programs including the NHE and the 45⁰ hip
494 extension exercises on the abovementioned variables.

495 **Limitations**

496 **Paragraph 31** Given the high cost of fMRI, it was not possible to include all participants in
497 both parts of the experiment. Therefore, comparing the results of part 1 and 2 should be done
498 with caution. Furthermore, all of our participants were recreationally active so it remains to
499 be seen if these findings can be applied to more highly trained athletes. We have previously
500 shown that recreationally active young men with a history of unilateral hamstring strain
501 exhibited less T2 change in previously injured muscles than in their uninjured homologous
502 muscles from the contralateral limbs after performing the NHE.[22] More work will be

503 needed to establish whether the patterns of selective muscle activation observed in the current
504 study are also evident in athletes with a history of strain injury.[10 11 40] Lastly, it should be
505 acknowledged that the T2 response to an exercise stimulus is highly dynamic and can be
506 influenced by a range of factors such as the metabolic capacity and vascular dynamics of the
507 active tissue.[28 36] We attempted to minimise this by recruiting only male participants with
508 a similar age and training status.

509 **Conclusion**

510 **Paragraph 32** The current study suggests that the patterns of hamstring muscle activation are
511 heterogeneous across a range of different strength exercises. We have provided sEMG
512 evidence to suggest that, during eccentric contractions, hip extension exercise more
513 selectively activates the lateral hamstrings while knee flexion-oriented exercises
514 preferentially recruit the medial hamstrings. However, despite being the least selective
515 activator of the BF, the NHE still elicited higher levels of BF nEMG during eccentric actions
516 than any other exercise which may help to explain how it confers HSI-preventive benefits.[15
517 16 18] The results of the fMRI investigation largely confirm our initial sEMG observations,
518 showing that, relative to the ST, the BF_{LH} was ~4 times more active in hip extension than the
519 NHE. However, they also show that the BF_{LH}, BF_{SH}, ST and SM display distinct patterns of
520 muscle use during different tasks. Collectively, the results of this study highlight the
521 limitations of relying on a single method to infer strategies of muscle activation and suggest
522 that the hip extension exercise may be useful for improving strength and voluntary activation
523 of the commonly injured BF_{LH}. Future work is needed to determine the effect of this and
524 other exercises on hamstring architecture and morphology before we can justify their
525 inclusion in interventions aimed at reducing the risk of HSI.

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How might it impact upon clinical practice in the future?

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- Hamstring injury prevention and rehabilitation exercises can potentially be targeted to the site of injury.

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- Hip extension exercise may be more useful than the NHE for selectively activating the commonly injured BF_{LH}.

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557 **CONTRIBUTORS**

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

563 **TRANSPARENCY DECLARATION**

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

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577 *above.*

578 **DATA SHARING**

579 Consent was not obtained for data sharing but the presented data are anonymised and risk of
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585 None declared. All authors have completed the Unified Competing Interest form
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589 that might have an interest in the submitted work in the previous 3 years; (3) their spouses, partners,
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592

593 **ETHICAL CLEARANCE**

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

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745 **Figure 1.** The 10 examined exercises. (a) bilateral stiff-leg deadlift, (b) hip hinge, (c)
746 unilateral stiff-leg deadlift, (d) lunge, (e) unilateral bent knee bridge, (f) unilateral straight
747 knee bridge, (g) leg curl, (h) 45° hip extension, (i) glute-ham-raise, (j) Nordic hamstring
748 exercise (NHE).

749 **Figure 2.** Biceps femoris (BF) to medial hamstring (MH) normalised EMG (nEMG)
750 relationship for the (a) concentric and (b) eccentric phases of each exercise. (SDL) Bilateral
751 stiff-leg deadlift, (HH) hip hinge, (USDL) unilateral stiff-leg deadlift, (L) lunge, (bKb)
752 unilateral bent knee bridge, (SKB) unilateral straight knee bridge, (LC) leg curl, (HE) 45° hip
753 extension, (GHR) glute-ham-raise, (NHE) Nordic hamstring exercise. Exercises to the left of
754 and above the 45° line exhibited higher levels of BF than MH nEMG and exercises to the
755 right and below the line displayed higher levels of MH than BF nEMG.

756 **Figure 3.** Percentage change in fMRI T2 relaxation times of each hamstring muscle
757 following the 45° hip extension exercise. Values are expressed as mean percentage change
758 compared to values at rest. ** indicates significantly different from ST, BF_{LH} and SM
759 (p<0.001). * indicates significantly different from ST (p=0.005). Error bars depict standard
760 error. BF_{LH}, biceps femoris long head; BF_{SH}, biceps femoris short head; ST, semitendinosus;
761 SM, semimembranosus.

762 **Figure 4.** Percentage change in fMRI T2 relaxation times of each hamstring muscle
763 following the Nordic hamstring exercise. Values are expressed as mean percentage change
764 compared to values at rest. ** indicates significantly different from BF_{LH}, BF_{SH} and SM
765 (p≤0.002). * indicates significantly different from BF_{LH} and SM (p=0.008) Error bars depict
766 standard error. BF_{LH}, biceps femoris long head; BF_{SH}, biceps femoris short head; ST,
767 semitendinosus; SM, semimembranosus.

768 **Figure 5.** Ratio of biceps femoris long head (BF_{LH}) to semitendinosus (ST) (BF_{LH}/ST)
769 percentage change in fMRI T2 relaxation times following the 45° hip extension and the
770 Nordic hamstring exercise (NHE). * indicates a significant difference between exercises
771 ($p < 0.001$). Error bars depict standard error.

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