1 **Title:**

2 Impact of exercise selection on hamstring muscle activation

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28 **Key Words**

29 Injury prevention, rehabilitation, physical therapy

ABSTRACT

Objective: To determine the extent to which different strength training exercises selectively activate the commonly injured biceps femoris long head (BF_{LH}) muscle. **Methods:** This two-part observational study recruited 24 recreationally active males. **Part 1** explored the amplitudes and the ratios of lateral to medial hamstring (BF/MH) normalised electromyography (nEMG) during the concentric and eccentric phases of 10 common strength training exercises. **Part 2** used functional magnetic resonance imaging (fMRI) to determine the spatial patterns of hamstring activation during two exercises which i) most selectively, and ii) least selectively activated the BF in part 1. **Results:** Eccentrically, the largest BF/MH nEMG ratio was observed in the 45° hip extension exercise and the lowest was observed in the Nordic hamstring (NHE) and bent-knee bridge exercises. Concentrically, the highest BF/MH nEMG ratio was observed during the lunge and 45° hip extension and the lowest was observed for the leg curl and bent-knee bridge. fMRI revealed a greater BF_{LH} to semitendinosus activation ratio in the 45° hip extension than the NHE ($p<0.001$). The T2 increase after hip extension for BF_{LH}, semitendinosus and semimembranosus muscles were greater than that for BF_{SH} ($p<0.001$). During the NHE, the T2 increase was greater for the semitendinosus than for the other hamstrings ($p\leq0.002$). **Conclusion:** This investigation highlights the non-uniformity of hamstring activation patterns in different tasks and suggests that hip extension exercise more selectively activates the BF_{LH} while the NHE preferentially recruits the semitendinosus. These findings have implications for strength training interventions aimed at preventing hamstring injury.

What are the new findings?

- 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)

INTRODUCTION

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

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

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

PART 1

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

Exercise Protocol

Paragraph 7 The 10 exercises were chosen based on a review of the scientific literature[23 25 27]. They included the bilateral and unilateral stiff-leg deadlift, hip hinge, lunge, unilateral bent and straight knee bridges, leg curl, 45° hip extension, glute-ham-raise and the NHE. 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

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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.

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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 ± 24sec (mean $\pm$ SD).

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

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

Paragraph 15 For each exercise session, the T2 relaxation times of each hamstring muscle were measured in T2-weighted images acquired before and after exercise to evaluate the degree of muscle activation during exercise. All fMRI scans were transferred to a Windows computer in the digital imaging and communications in medicine (DICOM) file format. The T2 relaxation times of each hamstring muscle (BF_{LH}, BF_{SH}, ST and SM) were measured in five axial slices, corresponding to 30, 40, 50, 60 and 70% of thigh length; these values were determined relative to the distance between the inferior margin of the ischial tuberosity (0%) and the superior border of the tibial plateau (100%).[11 23] Image analysis software (Sante Dicom Viewer and Editor, Cornell University) was used to measure the signal intensity of each hamstring muscle in the exercised limb in both the pre- and post-exercise scans. The signal intensity was measured manually in each slice using a circular region of interest (ROI)[27] which was placed in a homogenous region of contractile tissue in each muscle belly (avoiding fat, aponeurosis, tendon, bone and blood vessels). The size of each ROI varied (0.2 to 5.6 cm^2) based on the cross-sectional area and the amount of homogeneous tissue available in each slice. The signal intensity reflected the mean value of all pixels within the ROI and was measured across seven echo times (8, 16, 24, 32, 40, 48, 56ms). To calculate the T2 relaxation time for each ROI, the signal intensity value at each echo time was fitted to a mono-exponential decay model using a least squares algorithm:

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

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 [(mean post-exercise T2 / mean pre-exercise T2) x 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]

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Statistical analysis

277 **Paragraph 16** Absolute T2 values before and after each exercise session were reported

278 descriptively as mean ± 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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RESULTS

Levels of hamstring muscle activation

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

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

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

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

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

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

INSERT FIGURE 2

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

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

INSERT FIGURE 3

Percentage change in T2 relaxation time following the Nordic hamstring exercise

Paragraph 21 A main effect was detected for muscle (p<0.001). Post hoc analyses showed 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

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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

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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.

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DISCUSSION

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

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

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

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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.

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

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

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

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

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

21

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 45^0 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.

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?

- Hamstring injury prevention and rehabilitation exercises can potentially be targeted to the site of injury.
- Hip extension exercise may be more useful than the NHE for selectively activating the commonly injured BF_{LH}.

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CONTRIBUTORS

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

TRANSPARENCY DECLARATION

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

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576 *party material where-ever it may be located; and, vi) licence any third party to do any or all of the*

577 *above.*

DATA SHARING

579 Consent was not obtained for data sharing but the presented data are anonymised and risk of

580 identification is low.

COMPETING INTERESTS

585 None declared. All authors have completed the Unified Competing Interest form

586 at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare

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591 MW, DO, GK, AA and TS have no non-financial interests that may be relevant to the submitted work.

592

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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