

1 **TITLE**

2 Muscle activation patterns in the Nordic hamstring exercise: Impact of prior strain injury

3

4 **Authors**

5

6 Matthew N. Bourne^{1,2}, David A. Opar^{1,3}, Morgan D. Williams⁴, Aiman Al Najjar⁵, Anthony J.
7 Shield¹.

8

9

10 ¹Queensland University of Technology, Brisbane, Australia.

11 ²Queensland Academy of Sport, Centre of Excellence for Applied Sport Science Research, Brisbane,
12 Australia.

13 ³Australian Catholic University, Melbourne, Australia.

14 ⁴University of South Wales, Wales, United Kingdom.

15 ⁵Centre for Advanced Imaging, University of Queensland, Brisbane, Australia.

16

17 **Corresponding Author**

18 Dr Anthony Shield

19 School of Exercise and Nutrition Sciences and the Institute of Health and Biomedical Innovation,
20 Queensland University of Technology, Victoria Park Road, Kelvin Grove, 4059,
21 Brisbane, Queensland, Australia.

22 Email: aj.shield@qut.edu.au

23 Ph: +61 7 3138 5829

24 Fax: +61 7 3138 3980

25

26 **Running Title**

27 Hamstring activation in Nordic exercise.

28 **ABSTRACT**

29 This study aimed to determine: 1) the spatial patterns of hamstring activation during the
30 Nordic hamstring exercise (NHE); 2) whether previously injured hamstrings display
31 activation deficits during the NHE; and, 3) whether previously injured hamstrings exhibit
32 altered cross-sectional area. Ten healthy, recreationally active males with a history of
33 unilateral hamstring strain injury underwent functional magnetic resonance imaging (fMRI)
34 of their thighs before and after 6 sets of 10 repetitions of the NHE. Transverse (T2) relaxation
35 times of all hamstring muscles (biceps femoris long head, (BF_{lh}); biceps femoris short head
36 (BF_{sh}); semitendinosus (ST); semimembranosus (SM)), were measured at rest and
37 immediately after the NHE and cross-sectional area (CSA) was measured at rest. For the
38 uninjured limb, the ST's percentage increase in T2 with exercise was 16.8, 15.8 and 20.2%
39 greater than the increases exhibited by the BF_{lh}, BF_{sh} and SM, respectively ($p < 0.002$ for all).
40 Previously injured hamstring muscles ($n=10$) displayed significantly smaller increases in T2
41 post-exercise than the homonymous muscles in the uninjured contralateral limb (mean
42 difference -7.2%, $p=0.001$). No muscles displayed significant between limb differences in
43 CSA. During the NHE, the ST is preferentially activated and previously injured hamstring
44 muscles display chronic activation deficits compared to uninjured contralateral muscles.

45 **Key words:** Physical therapy, rehabilitation, inhibition

46

47

48 **INTRODUCTION**

49 **Paragraph number 1** Hamstring strains are the most prevalent of all injuries in sports that
50 involve high speed running (Woods et al., 2004; Drezner et al., 2005; Orchard et al., 2006;

51 Brooks et al., 2005; Brooks et al., 2006; Ekstrand et al., 2011) and 80% or more of these
52 insults involve the biceps femoris muscle (BF) (Verrall et al., 2003; Askling et al., 2007;
53 Koulouris et al., 2007; Silder et al., 2008). High rates of hamstring muscle strain injury (HSI)
54 recurrence (Heiser et al., 1984; Woods et al., 2004; Orchard et al., 2006; Brooks et al., 2006)
55 are also troublesome, particularly because re-injuries typically result in greater periods of
56 convalescence than first-time occurrences (Brooks et al., 2006; Ekstrand et al., 2011). These
57 observations highlight the need for improved HSI prevention and rehabilitation practices
58 while also suggesting that these exercise programs should specifically target (activate) the
59 BF.

60 **Paragraph number 2** The importance of eccentric conditioning in HSI prevention is
61 reasonably well recognised (Stanton & Purdham., 1989; Brockett et al., 2001; Askling et al.,
62 2013) and intuitively appealing in light of evidence that hamstring stresses are highest when
63 actively lengthening in the presumably injurious (Thelen et al., 2005; Schache et al., 2009),
64 terminal swing phase of sprinting (Schache et al., 2009; Chumanov et al., 2011). The Nordic
65 hamstring exercise (NHE), the most widely investigated of these eccentric movements, has
66 been reported to reduce first time (Arnason et al., 2008; Petersen et al., 2011; Van der Horst
67 et al., 2015) and recurrent (Petersen et al., 2011) HSIs in large scale interventions in soccer.
68 Furthermore, rugby union teams employing the NHE appear to have significantly lower HSI
69 rates than those that do not (Brooks et al., 2006). Despite the observed benefits of the NHE in
70 reducing injury risk, relatively little is known about the patterns of hamstring muscle
71 activation during this task. One study has reported a non-uniform pattern of hamstring
72 activation during the NHE in male soccer referees (Mendiguchia et al., 2013). However, there
73 is a need to extend these observations, particularly to athletes with a history of HSI, given the
74 prominent role of the NHE in prevention and rehabilitation programs.

75 **Paragraph number 3** Fyfe et al. (2013) have recently proposed that the high rates of HSI
76 recurrence might be partly explained by chronic neuromuscular **inhibition which results in a**
77 **reduced capacity to voluntarily activate** the BF muscle during eccentric but not concentric
78 knee flexor efforts (Opar et al., 2013a; Opar et al., 2013b). These contraction mode-specific
79 deficits in BF activation can persist despite rehabilitation and return to sport and may mediate
80 preferentially eccentric hamstring weakness (Jonhagen et al., 1994; Croisier et al., 2000;
81 Croisier et al., 2002), reduced rates of knee flexor torque development (Opar et al., 2013b)
82 and persistent BF long head (BF_{lh}) atrophy (Silder et al., 2008), all of which have been
83 observed months to years after HSI. It has been proposed that reduced activation of the BF
84 during active lengthening may diminish the stimuli that would otherwise promote adaptation
85 to the demands of running and strength exercises employed in rehabilitation and training
86 (Opar et al., 2012; Fyfe et al., 2013). However, the aforementioned activation deficits have
87 only been noted during eccentric isokinetic tasks and it remains to be seen whether they also
88 exist during the performance of exercises like the NHE.

89 **Paragraph number 4** Further insight into muscle activation patterns during the NHE in
90 uninjured and previously injured muscles will be critical in better understanding how this
91 exercise confers HSI-preventative benefits. Functional magnetic resonance imaging (fMRI)
92 allows for assessment of muscle size and this technique is also increasingly employed to
93 investigate muscle activation patterns during exercise (Akima et al., 1999; Mendiguchia et
94 al., 2013; Ono et al., 2011). fMRI enables the measurement of T2 relaxation times of imaged
95 skeletal muscles and these values increase in proportion with exercise intensity (Fleckenstein
96 et al., 1988) and in parallel with electromyographic measures of muscle activation (Adams et
97 al., 1992). Fortunately, the acute changes in T2 relaxation times last for 20-30 minutes after
98 intense physical activity (Patten et al., 2003) so post-exercise fMRI scans can reveal the
99 extent to which muscles have been activated even after exercise ceases. In addition, because

100 T2 relaxation times are mapped out across cross-sectional images of muscles, fMRI is able to
101 determine differences in activation within and between muscles and this excellent spatial
102 resolution overcomes several limitations of surface electromyography (sEMG) (Adams et al.,
103 1992).

104 **Paragraph number 5** The purpose of this study was to use fMRI to determine: 1) the spatial
105 patterns of hamstring activation during the NHE; 2) whether previously injured hamstrings
106 display activation deficits compared to homonymous muscles in the uninjured limb during
107 the NHE; and, 3) whether previously injured hamstrings exhibit reduced cross sectional areas
108 (CSAs) compared to homonymous muscles in the uninjured limb. We hypothesised that the
109 hamstrings of uninjured limbs would be activated non-uniformly during the NHE and that
110 previously injured hamstring muscles would display reduced activation and reduced CSA,
111 compared to homonymous muscles in the uninjured limb.

112 **METHODS**

113 **Experimental Design**

114 **Paragraph number 6** This study used a cross-sectional design in which all participants
115 visited the laboratory on two occasions. During the first, participants were familiarised with
116 the NHE and had baseline anthropometric measures taken. Experimental testing, completed
117 at least seven days later, involved the performance of a NHE session with pre- and post-
118 exercise fMRI scans to compare the extent of hamstring muscle activation during the NHE
119 and to assess hamstring muscle CSA between limbs.

120 **Participants**

121 **Paragraph number 7** Ten healthy and recreationally active males, aged 18-25 (age, $21.6 \pm$
122 1.9 years; height, 180.1 ± 7.4 cm; weight, 81.3 ± 6.5 kg) with a history of unilateral HSI

123 within the previous 24 months were recruited. A sample size of 10 was calculated to provide
124 sufficient statistical power (≥ 0.80) to avoid a type II error given a presumed effect size of 1.0
125 for the differences in exercise induced T2 relaxation time changes between muscles of the
126 same limb and between homonymous muscles in opposite limbs when $p < 0.05$. Since this
127 investigation was the first to explore between limb differences in T2 relaxation times
128 following a HSI, the effect size was estimated based on a previous fMRI study (Ono et al.,
129 2010) that reported an approximate change (mean \pm standard deviation) in T2 of $42 \pm 4\%$ in
130 ST, $7 \pm 1\%$ in SM and $11 \pm 6\%$ in BFH following eccentric knee flexor exercise using 120% of
131 the 1-repetition maximum load. Participants completed an injury history questionnaire with
132 reference to clinical notes provided by their physical therapist which detailed the location,
133 grade and rehabilitation period of their most recent HSI as well as the total number of HSIs
134 that they had sustained. Participants had all returned to full training and competition
135 schedules, were free of orthopaedic abnormalities of the lower limbs and had no history of
136 neurological or motor disorders. All completed a cardiovascular risk factor questionnaire
137 prior to testing. Additionally, all participants completed a standardised MRI screening
138 questionnaire provided by the imaging facility to ensure that it was safe for them to undergo
139 scanning. Participants were instructed to avoid strength training of the lower body and to
140 abstain from anti-inflammatory medications for the week preceding experimental testing.
141 This study was approved by the XXXX Ethics Committee and the XXXX Ethics Committee.

142 **Familiarisation Session**

143 **Paragraph number 8** A familiarisation session was conducted approximately 8 days (± 1
144 day) before experimental testing. Upon arrival at the laboratory, the participant's height and
145 mass were recorded before they received a demonstration and instructions on the
146 performance of the NHE. From the initial kneeling position with their ankles secured in
147 padded yokes, arms crossed on the chest and hips extended, participants were instructed to

148 lower their bodies as slowly as possible to a prone position (Figure 1). Participants performed
149 only the lowering (eccentric) portion of the exercise and after ‘catching their fall’, were
150 instructed to use their arms to push back into the starting position so as to minimise
151 concentric knee flexor activity. Verbal feedback was provided to correct any technique faults
152 while participants completed several practice repetitions (typically three sets of six
153 repetitions).

154

155  Insert Figure 1 about here

156

157 **Experimental Session**

158 *Nordic hamstring exercise protocol*

159 **Paragraph number 9** Each participant completed 6 sets of 10 repetitions of the NHE with 1-
160 minute rest intervals between sets. During the 1min rest, the participant lay in the prone
161 position. Investigators verbally encouraged maximal effort throughout each repetition.
162 Participants were returned to the scanner immediately (<15s) following the exercise protocol
163 and post-exercise T2-weighted scans began within 90 ± 16 s (mean \pm SD) following localiser
164 adjustments.

165 *Functional magnetic resonance imaging*

166 **Paragraph number 10** All fMRI scans were performed using a Siemens 3-Tesla (3T)
167 TrioTim imaging system with a spinal coil. The participant was positioned supine in the
168 magnet bore with the knees fully extended and hips in neutral, while contiguous MR images
169 were taken of both limbs, beginning immediately superior to the iliac crest and finishing

170 immediately distal to the tibial plateau. Transaxial T2-weighted images were acquired before
171 and immediately after the NHE protocol using a CPMG spin-echo pulse sequence (transverse
172 relaxation time = 2000ms; echo time = 10, 20, 30, 40, 50 and 60ms; number of excitations =
173 1; slice thickness = 10mm; interslice gap = 10mm). All T2-weighted images were collected
174 using a 180 x 256 image matrix and a 400 x 281.3mm field of view. T1-weighted axial spin-
175 echo images were also obtained but only during the pre-exercise scan (transverse relaxation
176 time = 1180ms; echo time = 12ms; field of view = 400 x 281.3 mm; number of excitations =
177 1; slice thickness = 10mm; interslice gap = 10mm). The total acquisition time for pre-exercise
178 images was 15min 10s and for post-exercise images, 10min. Given the high field strength of
179 3T, a B1 filter was applied to minimise any inhomogeneity in MR images caused by
180 dielectric resonances (De Souza, 2011). Further, to minimise the effects of intramuscular
181 fluid shifts before the pre-exercise scans, the participant was seated for a minimum of 15
182 minutes before data acquisition.

183 **Data analysis**

184 **Paragraph number 11** All T1- and T2-weighted fMR images were transferred to a personal
185 computer in the DICOM file format and image analysis software (Sante Dicom Viewer and
186 Editor, Cornell University) was used for subsequent analysis. To evaluate the degree of
187 muscle activation during the NHE protocol, the T2 relaxation times of each hamstring muscle
188 were measured before and immediately after exercise for both the previously injured and
189 uninjured contralateral limb. To quantify T2 relaxation times, the signal intensity of each
190 hamstring muscle (BF_{lh}, BF_{sh}, SM and ST) was measured using a 5 mm² region of interest
191 (ROI) in three slices corresponding to 40%, 50% and 60% respectively, of the distance
192 between the inferior margin of the ischial tuberosity (0%) and the superior border of the tibial
193 plateau (100%) (Ono et al., 2010). For BF_{sh}, a single 5mm² ROI was selected at 50% of thigh
194 length because it was not always possible to identify this muscle in more cranial or caudal

195 slices. All ROIs were selected in the centre of the muscle belly with great care taken to avoid
196 scar and connective tissue, fatty deposits, aponeurosis, tendon, bone and blood vessels. The
197 signal intensity reflected the mean value of all pixels within the ROI and was determined for
198 each ROI across six echo times (10, 20, 30, 40, 50 and 60ms). The signal intensity at each
199 echo time was then graphed to a mono-exponential time curve using a least squares algorithm
200 [(SI= $M \times \exp(\text{echo time} / T2)$), where SI is the signal intensity at a specific echo time, and M
201 represents the pre-exercise fMRI signal intensity] to extrapolate the T2 relaxation times for
202 each ROI. The absolute T2 relaxation times at all three thigh levels (40%, 50% and 60%)
203 were averaged to provide a mean T2 value for each muscle (BF_{lh}, BF_{sh}, ST, SM) before and
204 after exercise. To assess muscle activation during the NHE protocol, the averaged post-
205 exercise T2 value for each muscle was expressed as a percentage change relative to the pre-
206 exercise value (Fleckenstein et al., 1988; Ono et al., 2011). Muscle cross-sectional area
207 obtained from pre-exercise T1-weighted images was analysed to determine differences in
208 hamstring muscle CSA in limbs with and without a history of HSI. The muscle boundaries of
209 BF_{lh}, SM and ST were identified and traced manually at slices 40%, 50% and 60% of the
210 distance between the inferior margin of the ischial tuberosity (0%) and superior border of the
211 tibial plateau (100%) (Ono et al., 2010) while BF_{sh} was only traced at 50% of thigh length
212 for reasons described previously. Muscle CSA was calculated as the total number of cm²
213 within each trace and was averaged across the three slices to provide a mean value for each
214 muscle. The averaged CSA of previously injured muscles was compared with homonymous
215 muscles in the uninjured contralateral limb to evaluate between-limb differences following an
216 HSI.

217

218 **Statistical Analysis**

219 **Paragraph number 12** To determine the spatial activation patterns in healthy (uninjured)
220 limbs, a repeated measures design linear mixed model fitted with the restricted maximum
221 likelihood (REML) method was used. Exercise-induced percentage changes in T2 relaxation
222 times were compared for each hamstring muscle in the 10 limbs without prior HSI. Muscle
223 (BF_{lh}, BF_{sh}, ST or SM) was the fixed factor with participant as a random factor. When a
224 significant main effect was detected, Bonferroni corrections were used for post-hoc testing
225 and reported as mean difference with 95% CIs.

226

227 **Paragraph number 13** The between-limb analyses of muscle activation and CSA were
228 carried out on all participants. Paired t-tests were used to compare exercise-induced
229 percentage changes in T2 relaxation times and pre-exercise muscle CSA's of the 10
230 previously injured muscles (7 BF_{lh}, 2 ST, 1 SM) to the homonymous muscles in the
231 uninjured limbs. For these analyses, T2 relaxation times and CSA were reported as uninjured
232 limb versus injured limb mean differences both with 95% CIs. Bonferroni corrections were
233 again used for post-hoc testing and significance was set at $p < 0.05$.

234

235 Finally, given the possibility that changes in activation patterns and CSA after injury may be
236 muscle-specific, the between-limb analyses (injured v uninjured) were repeated using only
237 the seven participants who had injured their biceps femoris muscles.

238

239 **RESULTS**

240

241 **Participant injury histories**

242 **Paragraph number 14** All participants had a history of unilateral HSI within the previous
243 24 months, with an average time of 9.8 months (± 8.7 months) since the last insult. At the

244 time of injury, all participants had their HSI diagnosis confirmed with MRI (n=7) or
245 ultrasound (n=3). The details of all participants HSI histories can be found in Table 1.

246

247 *Table 1* approximately here

248

249

250

251 **Spatial activation of the uninjured limb following the NHE**

252 **Paragraph number 15** In the uninjured limbs, there was a significant main effect for muscle
253 with respect to exercise-induced T2 changes following the NHE protocol ($p < 0.001$). Post-hoc
254 tests revealed that the T2 changes induced by exercise within the ST were significantly larger
255 than those observed for the BF_{lh} (ST vs. BF_{lh} mean difference = 16.8%, 95% CI = 7.1 to
256 26.4%, $p = 0.001$), BF_{sh} (ST vs. BF_{sh} mean difference = 15.8%, CI = 6.1 to 25.4%, $p = 0.002$)
257 and SM (ST vs. SM mean difference = 20.2%, 95% CI = 10.6 to 29.9%, $p < 0.001$) (Figure 2).
258 All other between-muscle comparisons in the percentage change of T2 relaxation times were
259 small and non-significant (BF_{lh} vs. BF_{sh}, mean difference = 1.0%, 95% CI = -8.7 to 10.6%,
260 $p = 0.834$; BF_{lh} vs. SM, mean difference = 3.4%, 95% CI = -6.2 to 13.1%, $p = 0.467$; BF_{sh} vs.
261 SM, mean difference = 4.5%, 95% CI = -5.2 to 14.1%, $p = 0.351$).

262

263 *Figure 2* approximately here

264

265 **Between-limb comparisons of muscle activation in previously injured hamstring**
266 **muscles**

267 **Paragraph number 16** The 10 previously injured hamstring muscles displayed a
268 significantly lower percentage increase in T2 relaxation time (mean difference = -7.2%, 95%
269 CI = -3.8 to -10.7%, p=0.001) (Figure 3) after the NHE than the uninjured homonymous
270 muscles in the contralateral limbs.

271

272

273 Figure 3 approximately here

274 **Between-limb comparisons of muscle CSA**

275 **Paragraph number 17** There were no statistically significant between-limb differences in
276 CSA between the 10 homonymous muscles in the previously injured and uninjured limbs
277 (mean difference = -0.29cm², CI = 1.21 to -1.80cm², p=0.670 (Figure 4).

278

279 Figure 4 approximately here

280

281 When only BF1h injuries were considered (n=7), the previously injured BF1h's displayed a
282 significantly lower percentage increase in T2 relaxation time (mean difference = -7.9%, 95%
283 CI = -3.0 to -12.9%, p=0.008) after the NHE than the contralateral uninjured BF1h. However,
284 no additional significant between-limb differences were observed for the other muscles (BFsh
285 mean difference = -0.6%, 95% CI = -7.0 to 5.8, p=0.837; ST mean difference = 4.7%, 95%
286 CI = - 6.1 to 15.6, p=0.382; SM mean difference = 2.7%, 95% CI = -3.7 to 9.1, p=0.400).

287 Previously injured BFlh muscles did not display any significant deficits in CSA when
288 compared to uninjured contralateral BFlh muscles (mean difference = -0.26cm^2 , CI = -2.52 to
289 1.99cm^2 , $p=0.785$).

290

291 **DISCUSSION**

292 **Paragraph number 18** The results of this study suggest that in healthy, uninjured limbs, the
293 ST is activated significantly more than other hamstring muscles during the NHE.
294 Furthermore, previously injured hamstring muscles are activated less completely than the
295 homonymous uninjured muscles in the opposite limbs, although these activation deficits are
296 not associated with any significant differences in muscle CSA.

297 **Paragraph number 19** Selective recruitment of ST during the NHE is an interesting finding.
298 Maximum force-generating capacity of skeletal muscle is dependent on its physiological
299 CSA (Lieber et al., 2000), and as such, pennate muscles are generally stronger than fusiform
300 muscles. Nonetheless, the results of this study suggest that ST, which is long, thin and
301 fusiform (Woodley & Mercer., 2005), is more active during the NHE than BFlh and SM,
302 which are bulkier pennate muscles. These findings are consistent with a recent fMRI
303 investigation of the NHE (Mendiguchia et al., 2013) which reported a greater percentage
304 change in T2 for ST (14-20%) than for BFlh (6-7%) and non-significant changes in the SM.
305 In contrast to the current investigation, recent work employing sEMG in female athletes
306 reported no significant difference in the extent to which BFlh and ST muscles were activated
307 during the NHE (Zebis et al., 2013). However, sEMG is prone to cross-talk from
308 neighbouring muscles (Adams et al., 1992) and this may account to some extent for the
309 divergent results.

310

311 **Paragraph number 20** While the mechanism for selective recruitment of ST during the
312 NHE remains unclear, it is possible that differences between hamstring muscle moment arms
313 play a role. At the knee, ST has a larger sagittal plane moment arm than BF and SM (Thelen
314 et al., 2005) and it consequently possesses the greatest mechanical advantage which may
315 explain its preferential recruitment during movements at this joint. Indeed, preferential ST
316 recruitment has previously been observed during eccentric knee flexor exercise using a leg
317 curl machine (Ono et al., 2010) so this strategy appears to be characteristic of hamstring
318 recruitment associated with knee movements when the hip joint angle is fixed. These
319 observations suggest the possibility that the NHE, with its modest activation of BFlh in
320 comparison to ST, may not be the optimal exercise for the prevention of running related
321 strain injury. However, some large-scale intervention studies have shown that the NHE is
322 effective in reducing first time and recurrent HSIs (Arnason et al., 2008; Petersen et al., 2011;
323 Van der Horst et al., 2015). These benefits may be mediated via improvements in eccentric
324 knee flexor strength (Mjølshes et al., 2004) and/or a shift of the hamstring torque-joint angle
325 relationship to longer muscle lengths (Brockett et al., 2001). It is possible that even a
326 relatively mild training stimulus is sufficient to protect the BFlh from strain injury or that
327 activation of this muscle progressively increases with regular training as has been observed
328 for other muscle groups (Akima et al., 1999; Conley et al., 1997). Another possibility is that
329 NHE interventions do preferentially stimulate ST adaptations and that the BFlh is effectively
330 protected in running by an enhanced load bearing capacity of its agonist. Nevertheless, there
331 is evidence that BFlh is more selectively activated in the stiff leg deadlift exercise (Ono et al.,
332 2011) so further exploration of the injury prevention benefits of this and other hip-oriented
333 hamstring exercises is warranted.

334

335 **Paragraph number 21** Observations of reduced hamstring activation during the NHE after
336 strain injury are consistent with other findings. Opar et al. (2013a) recently reported
337 inhibition of previously injured BF muscles during eccentric knee flexor contractions using
338 surface electromyography and isokinetic dynamometry. However, by assessing hamstring
339 activation during the NHE, the present findings have more direct implications for
340 conventional rehabilitation practices. Importantly, these activation deficits persist despite
341 apparently successful rehabilitation and a return to pre-injury levels of training and match
342 play, which corroborates previous work (Opar et al., 2013a).

343 **Paragraph number 22** Neuromuscular inhibition, evident in the form of reduced strength
344 and voluntary activation of surrounding skeletal muscles has been shown to occur after a
345 range of musculoskeletal injuries including anterior cruciate ligament rupture (Urbach et al.,
346 2001) and ankle fractures (Stevens et al., 2006). Recently, it has been suggested that the acute
347 pain associated with a HSI may result in chronic neural inhibition that may compromise
348 hamstring rehabilitation (Fyfe et al., 2013). Short-lasting inhibition constitutes a well-
349 accepted protective strategy to minimise discomfort and preserve the injured structures from
350 further damage (Hodges et al., 2010; Opar et al., 2012). However, if inhibition is not
351 ameliorated during the rehabilitation process it may result in a ‘learned’ redistribution of
352 motor activity which would likely render the athlete weaker following a return to sport (Opar
353 et al., 2013a). Activation deficits that persist throughout rehabilitation might also be expected
354 to reduce the injured muscle’s loading, particularly during eccentric contractions and this
355 may compromise hypertrophy and sarcomerogenesis (Timmins et al., 2014; Brockett et al.,
356 2001), both of which are thought to be important in allowing muscles to adapt to the demands
357 of sprinting. Evidence of persistent inhibition, many months after conventional rehabilitation
358 and a full return to training and competition suggests that inadequate attention has been paid
359 to increasing voluntary activation of the previously injured muscle (Fyfe et al., 2013). Heavy

360 resistance training offers a practical and potent stimulus for improving voluntary activation of
361 skeletal muscle (Akima et al., 1999; Conley et al., 1997). However, in light of recent
362 evidence (Mendiguchia et al., 2013; Ono et al., 2010; Zebis et al., 2013) that different
363 exercises target different portions of the hamstring muscle group, it is possible that some
364 exercises employed in rehabilitation do not optimally target the injured muscle. An improved
365 understanding of the spatial patterns of hamstring muscle activation during different exercises
366 may help practitioners to better tailor rehabilitation programs to the site of injury and should
367 be a focus of future investigations.

368 **Paragraph number 23** Despite the presence of activation deficits, the current study found no
369 evidence of atrophy in previously injured hamstring muscles. These findings differ from an
370 earlier investigation that reported chronic atrophy of previously injured BF_{lh} muscles and
371 compensatory hypertrophy of the ipsilateral BF_{sh} 5-23 months following an HSI in
372 recreational athletes (Silder et al., 2008). However, subsequent work from the same group
373 found no evidence of atrophy six months after completion of standardised hamstring
374 rehabilitation (Sanfilippo et al., 2013) and this suggests that different rehabilitation and
375 training practices might at least partially explain the disparate results. Methodological
376 differences between the current study and that of previous work may also explain some of the
377 discrepancies. The current investigation assessed hamstring muscle CSA at 40, 50 and 60%
378 of thigh length, whereas previous investigations (Silder et al., 2008; Sanfilippo et al., 2013)
379 assessed the volume of each hamstring muscle-tendon unit. Timmins and colleagues (2014)
380 recently reported that ultrasound measures of biceps femoris muscle architecture revealed
381 significantly shorter fascicles coupled with greater pennation angles and no significant
382 differences in muscle thickness between previously injured muscles and uninjured
383 homonymous muscles in the opposite limb. This increase in pennation angle would tend to

384 counter any effects of muscle atrophy on measures of muscle thickness, so measures of cross-
385 section or thickness may not be as sensitive to atrophy as are measures of muscle volume.

386 **Paragraph number 24** Participants in this study had received their injuries in the 3 to 24
387 months prior to being tested so it might be argued that this group is not particularly
388 homogenous in terms of stage of recovery. However, when the activation deficits on the
389 injured limbs were plotted against time since injury, no relationship was observed ($R^2= 0.03$)
390 and all participants had resumed full training and competition schedules. Furthermore, there
391 are numerous reports in the literature suggesting that the deficits in eccentric hamstring
392 strength (Jonhagen et al., 1994; Croisier et al., 2002; Lee et al., 2009) and muscle volume
393 (Silder et al., 2008) persist long after strain injury. For example, Lee and colleagues (2009)
394 reported deficits in eccentric knee flexor performance in a group of athletes with an average
395 time since injury of 19 ± 12.5 months. Furthermore, Silder et al. (2008) provided evidence of
396 BFlh atrophy 5-23 months following injury. These observations are consistent with an
397 argument that some effects of hamstring strain are particularly persistent (Fyfe et al., 2013).

398 **Paragraph number 25** It should be acknowledged that some limitations are present in the
399 current study. Firstly, because of the retrospective design, we do not know whether activation
400 deficits in previously injured hamstring muscles are the cause or the result of prior HSI.
401 Furthermore, given the absence of a control group with no history of HSI in either limb, it is
402 not possible to know with certainty whether the participants in this study have normal
403 patterns of muscle activation in their uninjured legs. However, similar preferential
404 recruitment of ST has been reported during the NHE (Mendiguchia et al., 2013) and during
405 eccentric knee flexor exercise (Ono et al., 2010) so this pattern of activation is likely to be a
406 robust phenomenon. Finally, it is important to consider that T2 changes are multifactorial and
407 can be influenced by confounding factors such as the metabolic capacity and vascular
408 dynamics of the active tissue (Patten et al., 2003). Such factors have been proposed to

409 account for the high variability in exercise-induced T2 changes between individuals (Patten et
410 al., 2003). To minimise this effect we recruited a homogenous male population with limited
411 ranges in age and levels of physical activity.

412 **Conclusion**

413 **Paragraph number 26** The current study provides novel insight into the spatial activation
414 patterns of the hamstring muscles during the NHE and how these are altered by prior strain
415 injury. We have provided evidence that ST is selectively activated during the NHE and that
416 previously injured hamstring muscles are less active compared to uninjured homonymous
417 muscles in the contralateral limb. However, these activation deficits are not associated with
418 any significant between-limb differences in muscle CSA. The sub-optimal activation of the
419 BF_{lh} during the NHE may suggest the need to investigate the protective effects of alternative
420 hamstring exercises for the prevention of running related HSI. Furthermore, the observation
421 of persistent activation deficits in previously injured hamstring muscles suggests that
422 conventional rehabilitation practices are not addressing the mechanism(s) underpinning
423 neuromuscular inhibition following HSI (Fyfe et al., 2013). These findings provide evidence
424 for altered muscle use during eccentric hamstring exercise which should be a focus of future
425 investigations.

426 **Perspective**

427 This study demonstrated that during the performance of the NHE, the ST muscle is activated
428 significantly more than the BF and SM. This may have implications for the use of this
429 exercise in HSI prevention protocols given that the vast majority of HSIs involve the BF as
430 the primary site of injury (Verrall et al., 2003; Askling et al., 2007; Koulouris et al., 2007;
431 Silder et al., 2008). Furthermore, previously injured hamstring muscles were activated
432 significantly less than uninjured contralateral muscles during the NHE, in the absence of

433 diminished cross-sectional areas and despite apparently successful rehabilitation and a return
434 to full training and competition. From a practical point of view, these activation deficits may
435 compromise the rehabilitation process and would likely render the athlete weaker,
436 particularly during eccentric contractions, following a return to sport. Future work should
437 seek to clarify whether these activation deficits are a risk factor for hamstring strain re-injury.

438

439 **Acknowledgements**

440 We thank the Queensland Academy of Sport's Centre of Excellence for Applied Sport
441 Science Research, for funding this investigation. The authors also acknowledge the facilities,
442 and the scientific and technical assistance of the National Imaging Facility at the Centre for
443 Advanced Imaging, University of Queensland.

444

445

446 **References**

447

448 1. Adams GR, Duvoisin MR, Dudley GA. Magnetic resonance imaging and
449 electromyography as indexes of muscle function. *J Appl Physiol.* 1992;73(4):1578-1583.

450 [\[Abstract\]](#)

451 2. Akima H, Takahashi H, Kuno SY, Masuda K, Masuda T, Shimojo H, Anno I, Itai Y,
452 Katsuta S. Early phase adaptations of muscle use and strength to isokinetic training. *Med Sci*
453 *Sports Exerc.* 1999;31(4):588-594. [\[Medline\]](#)

454 3. Arnason A, Andersen TE, Holme I, Engebretsen L, Bahr R. Prevention of hamstring
455 strains in elite soccer: an intervention study. *Scand J Med Sci Sports.* 2008;18(1):40-48.

456 [\[Medline\]](#)

457 4. Askling CM, Tengvar M, Saartok T, Thorstensson A. Acute First-Time Hamstring Strains
458 During High-Speed Running A Longitudinal Study Including Clinical and Magnetic
459 Resonance Imaging Findings. *Am J Sports Med.* 2007;35(2):197-206. [\[Abstract/Full Text\]](#)

460 5. Askling CM, Tengvar M, Thorstensson A. Acute hamstring injuries in Swedish elite
461 football: a prospective randomised controlled clinical trial comparing two rehabilitation
462 protocols. *Br J Sports Med.* 2013;47(15):953-9. [\[Medline\]](#)

463 7. Brockett C, Morgan D, Proske U. Human hamstring muscles adapt to eccentric exercise by
464 changing optimum length. *Med Sci Sports Exerc.* 2001;33(5):783-790. [\[Medline\]](#)

465 8. Brooks JHM, Fuller CW, Kemp SPT, Reddin DB. Epidemiology of injuries in English
466 professional rugby union: part 1 match injuries. *Br J Sports Med.* 2005;39:757-766.

467 [\[Abstract/Full Text\]](#)

468 9. Brooks JHM, Fuller CW, Kemp SPT, Reddin DB. Incidence, risk, and prevention of
469 hamstring muscle Injuries in professional rugby union. *Am J Sports Med.* 2006;34(8):1297-

470 1306. [\[Abstract/Full Text\]](#)

- 471 10. Chumanov ES, Heiderscheit BC, Thelen DG. Hamstring musculotendon dynamics during
472 stance and swing phases of high-speed running. *Med Sci Sports Exerc.* 2011;43(3):525-532.
473 [\[Medline\]](#)
- 474 11. Conley MS, Stone MH, Nimmons M, Dudley GA. Resistance training and human
475 cervical muscle recruitment plasticity. *J Appl Physiol.* 1997;83(6):2105-2111. [\[Abstract/Full](#)
476 [Text\]](#)
- 477 12. Croisier JL, Crielaard JM. Hamstring muscle tear with recurrent complaints: an isokinetic
478 profile. *Isokin Exer Sci.* 2000;8:175-180.
- 479 13. Croisier JL, Forthomme B, Namurois MH, Vanderthommen M, Crielaard JM. Hamstring
480 muscle strain recurrence and strength performance disorders. *Am J Sports Med.*
481 2002;30(2):199-203. [\[Abstract/Full Text\]](#)
- 482 14. De Souza P, Vignaud A, Fleury S, Carlier G. Fast monitoring of T₁, T₂ and relative
483 proton density (M₀) changes in skeletal muscles using an IR-TrueFISP sequence. *J Magn*
484 *Reson Imaging.* 2011;33:921-930.
- 485 15. Drezner J, Ulager J, Sennet M. Hamstring muscle injuries in track and field athletes: A 3-
486 year study at the Penn Relay Carnival [abstract]. *Clin J Sport Med.* 2005;15(5):386.
- 487 16. Ekstrand J, Hagglund M, Walden M. Injury incidence and injury patterns in professional
488 football: the UEFA injury study. *Br J Sports Med.* 2011;45(7):553-558. [\[Abstract/Full Text\]](#)
- 489 17. Fleckenstein JL, Canby RC, Parkey RW, Peshock RM. Acute effects of exercise on MR
490 imaging of skeletal muscle in normal volunteers. *AJR Am J Roentgenol.* 1988;151(2):231-
491 237. [\[Medline\]](#)
- 492 18. Fyfe JJ, Opar DA, Williams MD, Shield AJ. The role of neuromuscular inhibition in
493 hamstring strain injury recurrence. *J Electromyogr Kinesiol.* 2013;23(3):523-530. [\[Medline\]](#)

- 494 19. Heiser TM, Weber J, Sullivan G, Clare P, Jacobs RR. Prophylaxis and management of
495 hamstring muscle injuries in intercollegiate football players. *Am J Sports Med.*
496 1984;12(5):368-370. [\[Abstract\]](#)
- 497 20. Hodges PW, Tucker K. Moving differently in pain: a new theory to explain the adaptation
498 to pain. *Pain.* 2010;152(3):90-98. [\[Medline\]](#)
- 499 21. Jonhagen S, Nemeth G, Eriksson E. Hamstring injuries in sprinters. The role of
500 concentric and eccentric hamstring muscle strength and flexibility. *Am J Sports Med.*
501 1994;22:262. [\[Abstract\]](#)
- 502 22. Koulouris G, Connell DA, Brukner P, Schneider-Kolsky M. Magnetic resonance imaging
503 parameters for assessing risk of recurrent hamstring injuries in elite athletes. *Am J Sports*
504 *Med.* 2007;35(9):1500-1506. [\[Abstract/Full Text\]](#)
- 505 23. Lee MJ, Reid SL, Elliott BC, et al. Running biomechanics and lower limb strength
506 associated with prior hamstring injury. *Med Sci Sports Exerc.* 2009;41(10):1942-51.
- 507 24. Lieber RL, Friden J. Functional and clinical significance of skeletal muscle architecture.
508 *Muscle Nerve.* 2000;23(11):1647-1666. [\[Medline\]](#)
- 509 25. Mendiguchia J, Arcos AL, Garrues MA, Myer GD, Yanci J, Idoate F. The use of MRI to
510 evaluate posterior thigh muscle activity and damage during the Nordic hamstring exercise. *J*
511 *Stength Cond Res.* 2013;27(12):3426-3435. [\[Medline\]](#)
- 512 26. Mjøl̄snes R, Arnason A, Osthagen T, Raastad T, Bahr R. A 10-week randomized trial
513 comparing eccentric vs. concentric hamstring strength training in well-trained soccer players.
514 *Scand J Med Sci Sports.* 2004;14(5):311-317. [\[Medline\]](#)
- 515 27. Ono T, Higashihara A, Fukubayashi T. Hamstring functions during hip-extension
516 exercise assessed with electromyography and magnetic resonance imaging. *Res Sports Med.*
517 2011;19(1):42-52. [\[Medline\]](#)

- 518 28. Ono T, Okuwaki T, Fukubayashi T. Differences in activation patterns of knee flexor
519 muscles during concentric and eccentric exercises. *Res Sports Med.* 2010;18(3):188-198.
520 [\[Medline\]](#)
- 521 29. Opar DA, Williams MD, Shield AJ. Hamstring strain injuries: factors that lead to injury
522 and re-injury. *Sports Med.* 2012;42(3):209-226. [\[Medline\]](#)
- 523 30. Opar DA, Williams MD, Timmins RG, Dear NM, Shield AJ. Knee flexor strength and
524 bicep femoris electromyographical activity is lower in previously strained hamstrings. *J*
525 *Electromyogr Kinesiol.* 2013a;23(3):696-703. [\[Medline\]](#)
- 526 31. Opar DA, Williams MD, Timmins RG, Dear NM, Shield AJ. Rate of torque and
527 electromyographic development during anticipated eccentric contraction is lower in
528 previously strained hamstrings. *Am J Sports Med.* 2013b;41(1):116-125. [\[Abstract/Full Text\]](#)
- 529 32. Orchard J, Seward H. Injury Report 2009: Australian Football League. *Sport Health.*
530 2010;28(2):10-19.
- 531 33. Orchard JW, James T, Portus MR. Injuries to elite male cricketers in Australia over a 10-
532 year period. *J Sci Med Sport.* 2006;9:459-467. [\[Medline\]](#)
- 533 34. Patten C, Meyer RA, Fleckenstein MD. T2 Mapping of Muscle. *Seminars in*
534 *Musculoskeletal Radiology.* 2003;7(4):297-305.
- 535 35. Petersen J, Thorborg K, Nielsen MB, Budtz-Jørgensen E, Hölmich P. Preventive Effect of
536 Eccentric Training on Acute Hamstring Injuries in Men's Soccer: A Cluster-Randomized
537 Controlled Trial. *Am J Sports Med.* 2011;39(11):2296-2303. [\[Abstract/Full Text\]](#)
- 538 36. Sanfilippo JL, Silder A, Sherry MA, Tuite MJ, Heiderscheid BC. Hamstring strength and
539 morphology progression after return to sport from injury. *Med Sci Sports Exerc.*
540 2013;45(3):448-454. [\[Medline\]](#)
- 541 37. Schache AG, Dorn TW, Blanch PD, Brown NA, Pandy MG. Mechanics of the human
542 hamstring muscles during sprinting. *Med Sci Sports Exerc.* 2012;44(4):647-658. [\[Medline\]](#)

543 38. Schache AG, Wrigley TV, Baker R, Pandy MG. Biomechanical response to hamstring
544 muscle strain injury. *Gait Posture*. 2009;29(2):332-338. [\[Medline\]](#)

545 39. Silder A, Heiderscheit B, Thelen D, Enright T, Tuite M. MR observations of long-term
546 musculotendon remodeling following a hamstring strain injury. *Skeletal Radiol*.
547 2008;37:1101-1109. [\[Medline\]](#)

548 40. Stanton P, Purdham C. Hamstring injuries in sprinting - the role of eccentric exercise. *J*
549 *Orthop Sports Phys Ther*. 1989;10(9):343-349. [\[Medline\]](#)

550 [41. Stevens JE, Pathare NC, Tillman SM, Scarborough MT, Gibbs CP, Shah P, Jayaraman A,
551 Walter GA, Vandeborne K.. Relative contributions of muscle activation and muscle size to
552 plantar flexor torque during rehabilitation after immobilization. *J Orthop Res*.
553 2006;24\(8\):1729-36.](#)

554 42. Thelen DG, Chumanov ES, Hoerth DM, Best TM, Swanson SC, Li L, Young M,
555 Heiderscheit BC. Hamstring muscle kinematics during treadmill sprinting. *Med Sci Sports*
556 *Exerc*. 2005;37(1):108-114. [\[Medline\]](#)

557 43. Timmins RG, Shield AJ, Williams MD, Lorenzen C, Opar DA. (2014). Biceps femoris
558 long head architecture: a reliability and retrospective injury study. *Med Sci Sports Exerc*,
559 accepted.

560 44. Urbach D, Nebelung W, Becker R, Awiszus F. Effects of reconstruction of the anterior
561 cruciate ligament on voluntary activation of quadriceps femoris a prospective twitch
562 interpolation study. *J Bone Joint Surg Br*. 2001;83(8):1104-10.

563 45. Van der Horst N, Wouter Smits D, Petersen J, Goedhart E, Backx F. The preventive
564 effect of the Nordic Hamstring Exercise on hamstring injuries in amateur soccer players: a
565 randomized controlled trial. *Br J Sports Med*. 2015;[Epub ahead of print] [\[Medline\]](#)

566 46. Verrall GM, Slavotinek JP, Barnes PG, Fon GT. Diagnostic and prognostic value of
567 clinical findings in 83 athletes with posterior thigh injury: comparison of clinical findings

568 with magnetic resonance imaging documentation of hamstring muscle strain. Am J Sports
569 Med. 2003;31(6):969-973. [\[Abstract/Full Text\]](#)

570 47. Woodley SJ, Mercer SR. Hamstring muscles: architecture and innervation. Cells Tissues
571 Organs. 2005;179(3):125-141. [\[Medline\]](#)

572 46. Woods C, Hawkins RD, Maltby S, Hulse M, Thomas A, Hodson A. The Football
573 Association Medical Research Programme: an audit of injuries in professional football-
574 analysis of hamstring injuries. Br J Sports Med. 2004;38(1):36-41. [\[Abstract/Full Text\]](#)

575 48. Zebis MK, Skotte J, Andersen CH, Mortensen P, Petersen HH, Viskaer TC, Jensen TL,
576 Bencke J, Andersen LL. Kettlebell swing targets semitendinosus and supine leg curl targets
577 biceps femoris: an EMG study with rehabilitation implications. Br J Sports Med.
578 2013;47(18):1192-8.