TITLE

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

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Running Title

Hamstring activation in Nordic exercise.

ABSTRACT

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

Key words: Physical therapy, rehabilitation, inhibition

INTRODUCTION

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

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

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

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

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

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

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

METHODS

Experimental Design

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

Participants

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

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

Familiarisation Session

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

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

Insert Figure 1 about here

Experimental Session

Nordic hamstring exercise protocol

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

Functional magnetic resonance imaging

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

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

Data analysis

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

slices. All ROIs were selected in the centre of the muscle belly with great care taken to avoid scar and connective tissue, fatty deposits, aponeurosis, tendon, bone and blood vessels. The signal intensity reflected the mean value of all pixels within the ROI and was determined for each ROI across six echo times (10, 20, 30, 40, 50 and 60ms). The signal intensity at each echo time was then graphed to a mono-exponential time curve using a least squares algorithm [(SI= $M \times \exp(\text{echo time} / T2)$, where SI is the signal intensity at a specific echo time, and M represents the pre-exercise fMRI signal intensity] to extrapolate the T2 relaxation times for each ROI. The absolute T2 relaxation times at all three thigh levels (40%, 50% and 60%) were averaged to provide a mean T2 value for each muscle (BFlh, BFsh, ST, SM) before and after exercise. To assess muscle activation during the NHE protocol, the averaged post-exercise T2 value for each muscle was expressed as a percentage change relative to the pre-exercise value (Fleckenstein et al., 1988; Ono et al., 2011). Muscle cross-sectional area obtained from pre-exercise T1-weighted images was analysed to determine differences in hamstring muscle CSA in limbs with and without a history of HSI. The muscle boundaries of BFlh, SM and ST were identified and traced manually at slices 40%, 50% and 60% of the distance between the inferior margin of the ischial tuberosity (0%) and superior border of the tibial plateau (100%) (Ono et al., 2010) while BFsh was only traced at 50% of thigh length for reasons described previously. Muscle CSA was calculated as the total number of cm^2 within each trace and was averaged across the three slices to provide a mean value for each muscle. The averaged CSA of previously injured muscles was compared with homonymous muscles in the uninjured contralateral limb to evaluate between-limb differences following an HSI.

Statistical Analysis

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

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

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

RESULTS

Participant injury histories

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

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

Table 1 approximately here

Spatial activation of the uninjured limb following the NHE

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

Figure 2 approximately here

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

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

Figure 3 approximately here

Between-limb comparisons of muscle CSA

Paragraph number 17 There were no statistically significant between-limb differences in CSA between the 10 homonymous muscles in the previously injured and uninjured limbs (mean difference = -0.29cm^2, CI = 1.21 to -1.80cm^2, p=0.670 (Figure 4).

Figure 4 approximately here

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

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

DISCUSSION

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

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

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

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

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

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

Paragraph number 23 Despite the presence of activation deficits, the current study found no evidence of atrophy in previously injured hamstring muscles. These findings differ from an earlier investigation that reported chronic atrophy of previously injured BFlh muscles and compensatory hypertrophy of the ipsilateral BFsh 5-23 months following an HSI in recreational athletes (Silder et al., 2008). However, subsequent work from the same group found no evidence of atrophy six months after completion of standardised hamstring rehabilitation (Sanfilippo et al., 2013) and this suggests that different rehabilitation and training practices might at least partially explain the disparate results. Methodological differences between the current study and that of previous work may also explain some of the discrepancies. The current investigation assessed hamstring muscle CSA at 40, 50 and 60% of thigh length, whereas previous investigations (Silder et al., 2008; Sanfilippo et al., 2013) assessed the volume of each hamstring muscle-tendon unit. Timmins and colleagues (2014) recently reported that ultrasound measures of biceps femoris muscle architecture revealed significantly shorter fascicles coupled with greater pennation angles and no significant differences in muscle thickness between previously injured muscles and uninjured 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

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

Conclusion

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

Perspective

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

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

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