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Title

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ABSTRACT

This study aimed to determine the spatial patterns of hamstring and gluteal muscle activation during high-speed overground running in limbs with and without a prior hamstring strain injury. Ten recreationally active males with a recent (<18 month) unilateral biceps femoris long head (BF_{LH}) strain injury underwent functional magnetic resonance imaging before and immediately after a repeat-sprint running protocol. Transverse relaxation (T2) time, an index of muscle activation, of the BF_{LH} and short head (BF_{SH}), semitendinosus (ST), semimembranosus (SM), gluteus maximus (G_{MAX}) and medius (G_{MED}) was assessed pre-post exercise. No significant between-limb differences in running-induced mean T2 changes were observed (p=0.949), however, decision tree induction revealed that previously injured limbs were characterised by highly variable intramuscular activation of the ST (standard deviation [SD]≥5.3). T2 times increased more for G_{MAX} than all other muscles (all p<0.001, *d*=0.5-2.5). Further, T2 changes were greater for ST than BF_{SH}, SM, G_{MED}, and BF_{LH} (all p≤0.001, *d*=0.5-2.9); and were greater for BF_{LH} than BF_{SH}, SM, and G_{MED} (all p<0.001, *d*=1.2-1.6). Athletes display heterogenous patterns of hip extensor activation when sprinting (G_{MAX}>ST>BF_{LH}>G_{MED}>SM>BF_{SH}) and may exhibit altered intramuscular but not intermuscular hamstring activation after returning to sport from BF_{LH} strain injury.

Key words: Imaging, Magnetic resonance; Muscle injuries; Physical therapy/Rehabilitation; Injury prevention

INTRODUCTION

Hamstring injuries are endemic in sports that involve high-speed overground running, representing the most common injury in track and field [1], Australian Rules football, [2] and soccer, [3] and the most prevalent non-contact injury in rugby union [4]. High rates of recurrence are arguably the most concerning aspect of these injuries, particularly given the tendency for re-injuries to result in more time-loss than the initial insult [5].

Hamstring strain injury (HSI) is commonly suffered when athletes run at maximal speeds [6] and ~80% of these injuries affect the long head of biceps femoris (BF_{LH}) [5]. Studies employing surface electromyography (sEMG) [7 8] suggest that the hamstrings are most active during the ostensibly injurious late-swing, where they actively lengthen to decelerate the forward swinging shank. However, while these studies have provided important insight into the temporal patterns (timing) of hamstring muscle use during high-speed running, the contribution of individual hamstring, and other hip extensor muscles (i.e., gluteals) is not well understood. Further, it remains unclear as to whether the spatial patterns of muscle activation (including both intermuscular and intramuscular activation) are altered following an HSI.

Fyfe and colleagues [9] propose that high rates of HSI recurrence might be partly explained by chronic neuromuscular inhibition of the previously injured muscle. In support of this, long-term deficits in voluntary activation have been observed in previously injured BF muscles during isokinetic testing [10-12] and during performance of the eccentric Nordic hamstring exercise [13]. Previously injured BF_{LH} muscles also display lower sEMG activity than uninjured contralateral BF_{LH} muscles [14], and ipsilateral gluteus maximus (G_{MAX}) and trunk muscles [15], during the late-swing phase of sprinting. Furthermore, higher levels of G_{MAX} sEMG in this portion of the gait cycle seem to be associated with a reduced risk of future HSI [16]. It is plausible that activation deficits that persist throughout rehabilitation and the return to training and competition, might mediate preferential eccentric knee flexor weakness [12] and reduced rates of

torque development [10], lasting BF_{LH} atrophy, [17] and a chronic shortening of BF_{LH} fascicles [18]. However, these spatial activation deficits have typically only been observed during single joint exercises [13] that do not readily replicate the high-velocity and multi-joint demands of high-speed overground running.

An improved understanding of the spatial patterns of hamstring and gluteal muscle activation during high-speed running, particularly in previously injured limbs, may be valuable in optimising rehabilitation programs and may have implications for understanding the mechanisms of running-induced HSI. Functional magnetic resonance imaging (fMRI) is a validated [19] and highly reliable [20] measure of skeletal muscle activation during exercise. The premise of using fMRI to assess muscle activation is based on signal intensity changes resulting from a transient increase in the transverse (T2) relaxation time of muscle water following exercise. These shifts increase proportionately to exercise intensity [21] and parallel electromyographical measures of muscle activity [19]. However, the unique ability of fMRI to non-invasively assess deep muscles at multiple sites within a single scan overcomes several spatial limitations associated with EMG [19] (i.e., cross-talk). As such, fMRI has become a popular tool for the assessment of muscle use during exercise with great potential to demonstrate aberrant activation patterns following injury [13 22].

This study employed fMRI on recreational athletes with a recent history of unilateral BF_{LH} strain injury who had since undergone successful rehabilitation and returned to their pre-injury level of competition. The primary aim was to map the spatial patterns of hamstring and gluteal muscle activation during high-speed overground running in limbs with and without a history of injury. The secondary exploratory aim was to determine which combination of fMRI features best distinguished previously injured and uninjured contralateral limbs. We hypothesised that 1) the hamstring and gluteal muscles of uninjured limbs would be activated

non-uniformly during sprinting; and 2) previously injured BF_{LH} muscles would show reduced activation relative to homonymous muscles in the uninjured contralateral limb.

MATERIALS AND METHODS

This study employed a cross-sectional design in which all participants completed a single testing session. After providing written, informed consent, participants provided a detailed injury history to investigators with reference to imaging findings and clinical notations from the practitioner who diagnosed and treated their recent HSI. Subsequently, participants underwent an fMRI scan of their thighs before and immediately after a repeat-sprint running protocol. Participants were asked to rate their level of perceived pain in the posterior thigh before and after the run using a visual analogue scale (VAS).

Ten recreationally active male athletes (age, 25.5 ± 4.1 years; height, 182.3 ± 5.7 cm; mass, 81.8 ± 11.8 kg) currently competing in a running-based sport and who had suffered a time-loss unilateral strain injury to the BF_{LH} within the previous 18 months (median, 7; range, 3-18 months post-injury) were recruited (Table 1). A sample size of 10 was deemed sufficient to detect an effect size of 1.0 in T2 relaxation time between muscles and limbs, at a power of 0.80 and with $p<0.05$ [13 23]. All athletes had returned to their pre-injury levels of training and competition after completing a standard 4-12 week (median, 5.5; range, 2-12 weeks) progressive intensity rehabilitation program supervised by a physiotherapist or exercise physiologist. Participants completed an injury history questionnaire with reference to clinical notes provided by their treating practitioner and were free of orthopaedic abnormalities to the lower limbs, had no history of neurological or motor disorders and had no other soft tissue injuries to the lower limbs at the time of testing. Participants completed a cardiovascular risk factor questionnaire to ensure it was safe for them to perform intense exercise and a standardised MRI screening questionnaire provided by the imaging facility to make certain that

98 it was safe for them to enter the magnetic field. This study was approved by the XXXX Ethics
99 Committee.

100 **Insert Table 1 about here**

101 Participants completed three sets of six maximal intensity 40m sprints (with an additional 10m
102 acceleration and 15m deceleration distance) on a flat grass sports field adjacent to the imaging
103 facility. Participants were provided with 30s of rest between sprints and one-minute rest
104 between sets. Investigators verbally encouraged maximal effort throughout each interval.
105 Participants were returned to the scanner immediately following cessation of the running
106 protocol (<30s) and post-exercise T2-weighted imaging began within 3-4 mins (mean, 225s ±
107 31s), following localiser adjustments.

108 All fMRI scans were performed using a Phillips Ingenia (Koninklijke Phillips N.V) 3-Tesla
109 (3T) imaging system. Participants were positioned supine in the magnet bore with their knees
110 fully extended and hips in neutral. A 32-channel spinal coil was placed over the anterior thighs
111 and straps were positioned around both limbs to prevent any undesired movement. Consecutive
112 T2- transaxial MR images were taken of both limbs beginning at the level of the iliac crest and
113 finishing distal to the tibial plateau. T2-weighted images were used to assess the extent of
114 hamstring activation during exercise and were acquired pre- and immediately post-exercise
115 using a Car-Purcell-Meiboom-Gill (CPMG) spin-echo pulse sequence (T2 relaxation time =
116 2500ms; echo time = 8, 16, 24, 32, 40, 48ms; number of excitations = 1; slice thickness =
117 10mm; interslice gap = 10mm, field of view = 220 x 360mm) [13]. To minimise any
118 inhomogeneity in MR images caused by dielectric resonances at 3T, a B1 filter was applied to
119 all scans; this is a post-processing image filter that improves the image signal intensity profile
120 without affecting the image contrast. Participants were asked to avoid strength training and
121 strenuous exercise of the lower limbs for 72 hours prior to testing as muscle damage may

augment resting T2 values. Lastly, to reduce the effects of intramuscular fluid shifts before the pre-exercise scans, participants were seated for a minimum of 15 minutes before data acquisition [23].

All 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. T2 relaxation times of each muscle were measured before and after exercise to evaluate the degree of muscle activation during the repeat-sprint running protocol. For the BF_{LH} and short head (BF_{SH}), semitendinosus (ST), and semimembranosus (SM), the T2 relaxation times were measured in five axial slices corresponding to 30, 40, 50, 60 and 70% of thigh length [defined as the distance between the inferior margin of the ischial tuberosity (0%) and the superior border of the tibial plateau (100%)] (Figure 1A). For the G_{MAX} and gluteus medius (G_{MED}), T2 values were measured in five axial slices corresponding to 30, 40, 50, 60 and 70% of the distance between the most superior surface of the iliac crest (0%) and the gluteal tuberosity (100%) (Figure 1B). At each slice, the signal intensity of each muscle was measured in both the previously injured and uninjured limbs using a 0.6-16cm^2 region of interest (ROI) [22]. The size of each ROI varied due to the cross-sectional area and amount of homogeneous muscle tissue identifiable in each slice of interest. The signal intensity reflected the mean value of all pixels within the ROI and was determined for each ROI across all six echo times. Each ROI was selected in the centre of the muscle belly, at the same coordinates within each muscle for the pre- and post-exercise scans, with great care taken to avoid aponeurosis, tendon, bone and blood vessels. An ROI approach was deemed most appropriate as this method allowed investigators to avoid any areas of residual scar tissue associated with prior HSI [13 22]. T2 relaxation time was calculated by fitting signal intensity values at each echo time to a mono-exponential decay model using a least squares algorithm:

$$[(SI = M \times \exp(\text{echo time} / T2)\ ^{22\text{-}24}$$

where SI is the signal intensity at a specific echo time, and *M* represents the pre-exercise fMRI signal intensity. To assess muscle activation, the mean percentage change in T2 relative to the resting pre-exercise value [22-24] was calculated as:

[(mean post-exercise T2 / mean pre-exercise T2) x 100].

Insert Figure 1 about here

Before and immediately following the cessation of the repeat-sprint running protocol, participants were asked to rate their level of pain and discomfort in the posterior thigh (if any) on a VAS. Participants were instructed to choose a number between 0 (no pain) and 10 (unbearable pain).

All statistical analyses were performed using JMP version 10.02 (SAS Institute Inc). A repeated measures design linear mixed model fitted with the restricted maximum likelihood (REML) method was used to compare transient exercise-induced mean percentage changes in T2 relaxation times for each muscle in the previously injured and uninjured contralateral limbs. Muscle (BF_{LH}, BF_{SH}, ST, SM, G_{MAX}, and G_{MED}), limb (injured/uninjured) and muscle by limb interaction were the fixed factors with participant ID, participant ID by muscle and participant ID by limb as the random factors. When a significant main effect was detected for the mean percentage change in T2 relaxation time, post-hoc uncorrected t-tests were used to report the mean differences with 95% confidence intervals (95%CI). Where appropriate, Cohen's *d* effect sizes, classified as small ($d>0.2$), medium ($d>0.5$), and large ($d>0.8$), were also reported. To assess the potential impact of acute posterior thigh pain on between-limb differences in muscle activation, VAS scores obtained from participants before and after the repeat-sprint running protocol were reported descriptively as means ± SD. Additionally, coefficients of determination (r^2) were calculated from quadratic linear regression models to explore the extent

to which between-limb differences in running-induced T2 changes were explained by time since injury. For all comparisons, alpha was accepted as $p<0.05$.

Finally, an exploratory analysis was undertaken using decision tree induction to determine which combination of features best distinguished previously injured and uninjured contralateral limbs. The mean percentage change in T2 for each muscle and the SD (representing intramuscular variation) across muscle ROIs (30-70% of length) for each limb were entered into the model. Candidate variables were assessed using the G^2 statistic, which represents the likelihood ratio chi-square for the best split of the data. The model's ability to correctly classify previously injured and uninjured limbs was evaluated using the receiver operator characteristic (ROC) and area under the curve (AUC).

RESULTS

All participants completed the repeat running protocol and none reported any pain in the posterior thigh before or immediately after exercise.

Mean percentage change in T2 relaxation time

When comparing the running-induced mean percentage change in T2 relaxation times, we observed no differences between injured and uninjured limbs ($p=0.289$) and no muscle by limb interaction ($p=0.949$) (Table 2). However, the T2 responses between individual muscles were significantly different ($p<0.001$).

Insert Figure 2 about here

Insert Table 2 about here

Post hoc t tests revealed that the G_{MAX} was significantly more active than all other muscles (mean difference = 1.9% to 12.4%, d = 0.5-2.5, all $p\leq0.038$). In addition, ST was significantly

more active than BF_{SH}, SM, G_{MED}, and BF_{LH} (mean difference = 3.0% to 10.5%, *d* = 0.5-2.9, all $p \leq 0.001$). Finally, BF_{LH} displayed greater activity than BF_{SH}, SM, and G_{MED} (mean difference = 5.7%, to 7.5%, *d* = 1.2-1.6, all $p < 0.001$) (Table 3).

Insert Table 3 about here

Relationship between time since injury and between-limb difference in T2 relaxation time

A significant relationship was observed for the mean between-limb difference in running-induced percentage changes in T2 relaxation time (injured minus uninjured limb) and months since injury for the BF_{LH} (r^2=0.61, p=0.036) (Supplementary Figure 1). No significant relationships were observed for BF_{SH}, G_{MED}, ST, SM, or G_{MAX} (r^2=0.12 – 0.51, all p>0.05).

Decision tree induction

The SD for ST (G^2 = 5.3) and BF_{LH} (G^2 = 6.2) contributed to the final model. The model consisted of two splits: previously injured limbs were characterised by highly variable intramuscular ST activation (SD ≥ 5.3 [i.e., above the mean SD = 4.2]) and moderate to low variation in BF_{LH} intramuscular activation (SD < 4.6 [i.e., below the mean SD = 3.4]). Uninjured limbs were characterised by moderate and below intramuscular variation in ST activation (SD < 5.3) The receiver operator characteristic area under the curve (AUC) = 0.84; the model correctly classified all uninjured limbs, and 6 of the 10 previously injured limbs.

DISCUSSION

As far as we are aware, this study is the first to use fMRI to map the spatial patterns of hamstring and gluteal muscle activation during high-speed overground running. The results

suggest that athletes preferentially recruit the G_{MAX}, ST and BF_{LH} during sprinting, and that these intermuscular activation patterns do not appear to be impaired in limbs with a recent history of BF_{LH} strain injury. However, previously injured limbs are characterised by highly variable intramuscular activation of the ST, suggesting the possibility that this feature of coordination may be altered even after athletes have returned to their pre-injury level of training and competition.

It has been hypothesised that prior BF_{LH} strain injury may result in muscle-specific inhibition or reduced voluntary activation, at least in maximal eccentric contractions [9 10 12]. However, the present results suggest that there is no significant alteration in BF_{LH} use in repeated sprinting in participants with a unilateral history of strain injury in the previous 18 months to this muscle. This could be interpreted as evidence against injury-induced inhibition. Alternatively, it may be that limitations in the temporal resolution of fMRI renders the technique insensitive to the effects of prior HSI which may be specific to certain phases of the sprint gait cycle, [15] or limited to the performance of maximal voluntary eccentric contractions[10]. Functional MRI quantifies muscle activity via transient increases in the T2 relaxation time of tissue water, which can be measured from signal intensity changes in fMR images acquired before and after exercise. These T2 shifts are measured in cross-sectional MR images of muscles and therefore provide exceptional spatial resolution, however, they provide no information on the temporal patterns of muscle activity during exercise. Collegiate track athletes with a history of unilateral HSI have been reported to display significantly less BF_{LH} sEMG activity in their previously injured limb than the uninjured contralateral limb in the late- but not early-swing phase of sprinting [14]. Previously injured limbs also exhibit reduced BF sEMG activity relative to ipsilateral G_{MAX}, erector spinae, external oblique and contralateral rectus femoris muscles in the late-swing phase of sprinting [15]. Given the contraction-mode specific nature of the aforementioned activation deficits [10-12 15], and their tendency to be more pronounced at longer muscle-tendon

unit lengths [14 15], it is possible that more temporally robust measures of voluntary activation (e.g., fine wire EMG) are needed to accurately assess this parameter in running.

Although we did not observe any significant intermuscular differences in mean running-induced T2 changes between limbs, decision tree induction revealed that previously injured limbs exhibited highly variable intramuscular ST activation and low to moderate variability in BF_{LH} activation. By comparison, uninjured limbs were characterised by low variation in ST activation. As far as we are aware, this is the first study to explore intramuscular hamstring activation following hamstring injury, and so the mechanisms underpinning the observed effect remains unclear. Furthermore, given the small sample and the absence of validation data, the reader should interpret these findings with caution. However, recent work has demonstrated that the ST is an important agonist to the BF_{LH}. Schuermans et al. [25] observed, with fMRI, that previously injured hamstrings exhibit less ST and relatively more BF activity than uninjured hamstrings following ~255s of exhaustive leg curl exercise. A prospective follow-up study [26] of this cohort demonstrated that this reduced reliance upon the ST was associated with an increased susceptibility to primary HSI in the following 18 months. Subsequently injured players also reached task failure in the leg curl test significantly earlier than those who remained injury free. These observations are at least partly supported by more recent sEMG findings, which demonstrated that a disproportionate reliance upon any of the hamstring muscles was related to poor endurance when 20% of maximal knee flexor force was held until task failure [27]. It might therefore be reasonable to suggest that altered intramuscular coordination of the hamstrings contributes to hamstring fatigue [25-27] and this may increase injury risk [26] via its influence on 'load sharing' between the hamstring muscles. However, future studies are needed to confirm this hypothesis.

Non-uniform patterns of hamstring and gluteal activity during high-speed overground running are a novel finding. According to the present study, the magnitude of muscle activity in

sprinting appears to be hierarchical, whereby G_{MAX} > ST > BF_{LH} > G_{MED} > SM > BF_{SH}. These data suggest the possibility that the G_{MAX}, ST and BF_{LH} contribute proportionately more than other hip and knee spanning muscles to high-speed running performance. In support of this, elite sprint athletes have been reported to display 31%, 54% and 26% larger G_{MAX}, ST and BF muscles, respectively, than sedentary young adults (relative to body size) [28]. In comparison, G_{MED} and SM muscles were only 6% and 20% larger, respectively [28]. Although we cannot infer anything about long-term training adaptations from the present study, recent evidence suggests that the transient exercise-induced T2 shifts observed after a single bout of hamstring exercise [23] parallel the hypertrophic adaptations experienced after 10 weeks of training [29]. No other studies have used fMRI to characterise the spatial patterns of hamstring and gluteal muscle use during overground sprinting, however, Sloniger et al. [31] employed this technique to assess lower limb muscle use during exhaustive treadmill running in a group of recreationally active females. This study reported that the gluteals were the most heavily activated muscle group, closely followed by the BF, ST and SM, which were all activated to a similar extent; however, the musculoskeletal demands of submaximal treadmill running are considerably different than overground sprinting so comparison to the present study should be made with caution.

It should be acknowledged that this study has some limitations. Firstly, the high cost of fMRI limited our sample size (n=10) and as a consequence we were not adequately powered to detect small to moderate effects. Further, the retrospectivity of our observations makes it impossible to determine if the altered intramuscular activation patterns in previously injured limbs were the cause or result of HSI. Given the absence of a control group without a history of HSI in either limb, it is difficult to determine whether participants had normal patterns of muscle activation in their uninjured limbs. However, T2 relaxation time changes in the uninjured limbs of previously injured athletes has been shown to match, very closely, the T2 changes of hamstring muscles from athletes with no history of injury during the Nordic hamstring exercise

[13 22 23]. We were also unable to measure hip and knee kinematics during the running protocol which could possibly contribute to altered patterns of muscle use. Lastly, it is important to consider that the T2 response to an exercise stimulus is highly dynamic and can be influenced by intrinsic factors such as the metabolic capacity and vascular dynamics of the active tissue [19]. These effects were minimised by recruiting a homogenous male population within a limited age range and with a similar training status, that had all suffered BF_{LH} strains in the prior 18 months. Nevertheless, 61% of the variance in between-limb difference in T2 change for the BF_{LH} was explained by time since injury (Supplementary Figure 1), so future investigations might consider recruiting participants within a narrower time window.

This study provides novel insight into the spatial patterns of hip extensor muscle use during high-speed overground running in limbs with and without a history of HSI. Our data suggest that limbs with a prior BF_{LH} strain injury display similar spatial patterns of hamstring and gluteal activation, but more variable intramuscular activation of the ST, than uninjured contralateral limbs. We also provide evidence to suggest that the G_{MAX}, ST and BF_{LH} are preferentially activated during sprinting. Future work should seek to determine if greater variation in intramuscular coordination contributes to an elevated risk of HSI or re-injury.

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447 **Table 1.** Participant characteristics and injury history details.

ID	AGE (YRS)	HEIGHT (CM)	MASS (KG)	INJURED LIMB	GRADE (1-3)	MONTHS SINCE INJURY	REHAB DURATION (WKS)
1	31	190	94	Dom	2	18	4
2	28	176	90	Dom	1	7	3
3	22	187	86	Dom	2	17	12
4	24	178	76	Dom	2	16	5
5	24	185	81	Non-dom	1	10	2
6	23	172	53	Non-dom	2	3	4
7	23	181	78	Non-dom	2	4	7
8	24	181	80	Dom	3	7	9
9	34	186	88	Non-dom	2	7	6
10	22	187	92	Dom	3	5	8

448 ID, participant identity; BF_{LH}, biceps femoris long head; Dom, dominant limb; Non-dom, non-
449 dominant limb; Rehab, Rehabilitation.

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Table 2. Mean (± SD) running-induced percentage changes in T2 relaxation time for each muscle in previously injured and uninjured contralateral limbs. BF_{LH}, biceps femoris long head; BF_{SH}, biceps femoris short head; ST, semitendinosus; SM, semimembranosus; G_{MAX}, gluteus maximus; G_{MED}, gluteus medius.

MUSCLE

	Previously Injured	*Uninjured*	*Mean difference (95%CI)*
BF_{LH}	11.93 (5.65)	11.21 (5.97)	0.71 (-1.8, 3.3)
BF_{SH}	4.02 (1.63)	4.02 (1.75)	0.00 (-2.6, 2.6)
ST	15.12 (6.41)	13.92 (4.59)	1.21 (-1.3, 3.8)
SM	5.49 (2.43)	4.48 (2.53)	1.01 (-1.5, 3.6)
G_{MAX}	16.24 (5.91)	16.63 (6.86)	-0.40 (-2.2, 2.9)
G_{MED}	6.28 (3.51)	5.44 (3.28)	0.84 (-1.7, 3.4)

Table 3. Mean difference and 95% confidence interval (95%CI) between muscles for the running-induced percentage changes in T2 relaxation time. BF_{LH}, biceps femoris long head; BF_{SH}, biceps femoris short head; ST, semitendinosus; SM, semimembranosus; G_{MAX}, gluteus maximus; G_{MED}, gluteus medius. * represents a significant difference between muscles ($P<0.05$).

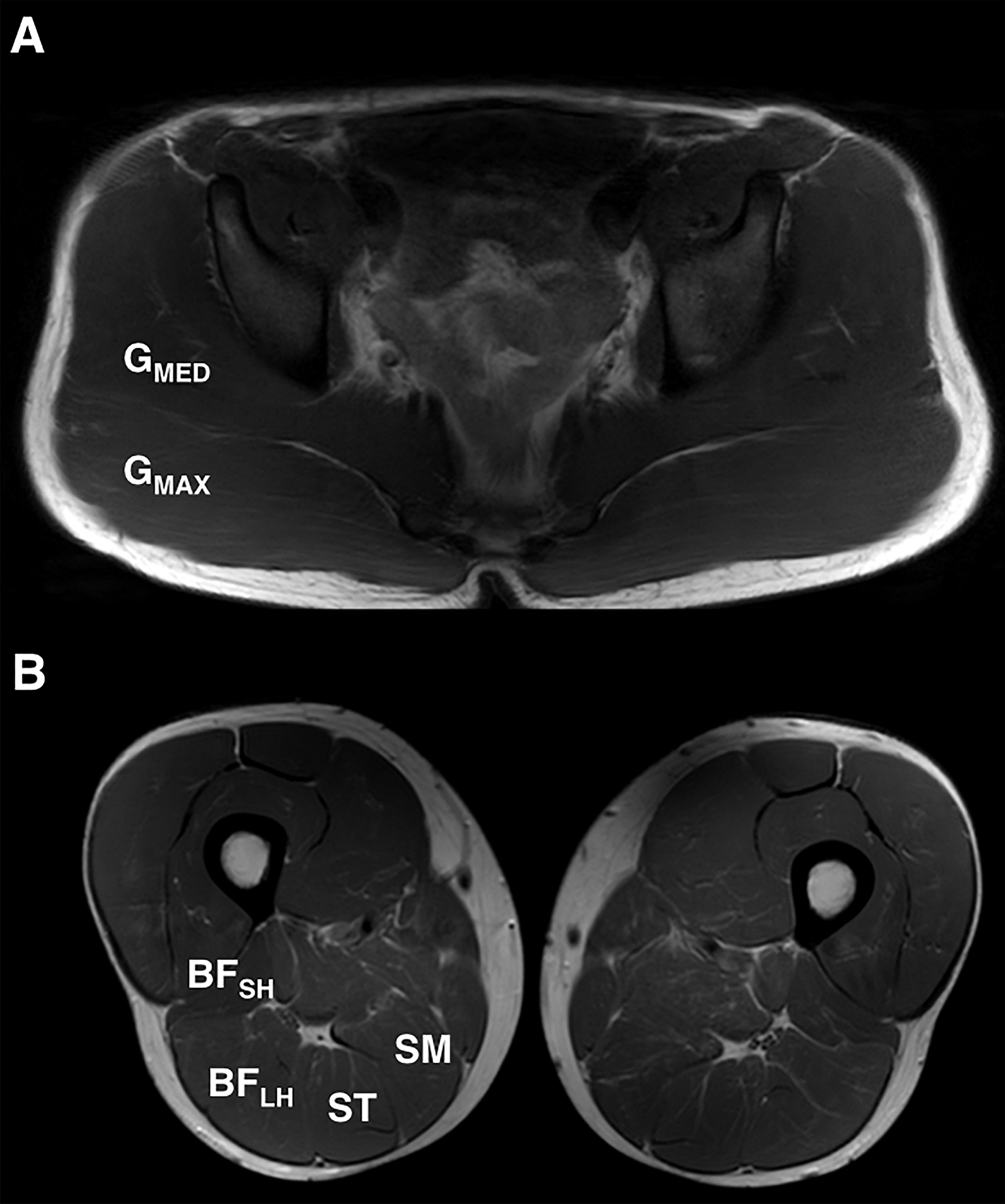
MUSCLES		MEAN DIFFERENCE (%)	95%CI	P
G_{MAX}	BF_{SH}	12.4	10.6, 14.2	<0.001*
G_{MAX}	SM	11.4	9.6, 13.2	<0.001*
G_{MAX}	G_{MED}	10.6	8.8, 12.4	<0.001*
ST	BF_{SH}	10.5	8.7, 12.3	<0.001*
ST	SM	9.5	7.7, 11.3	<0.001*
ST	G_{MED}	8.7	6.9, 10.5	<0.001*
BF_{LH}	BF_{SH}	7.5	5.7, 9.4	<0.001*
BF_{LH}	SM	6.6	4.8, 8.4	<0.001*
BF_{LH}	G_{MED}	5.7	3.9, 7.5	<0.001*
G_{MAX}	BF_{LH}	4.9	3.0, 6.7	<0.001*
ST	BF_{LH}	3	1.1, 4.8	0.001*
G_{MAX}	ST	1.9	0.1, 3.7	0.038*
G_{MED}	BF_{SH}	1.8	0.0, 3.6	0.046*
SM	BF_{SH}	1	-0.8, 2.8	0.293
G_{MED}	SM	0.9	-0.9, 2.7	0.349

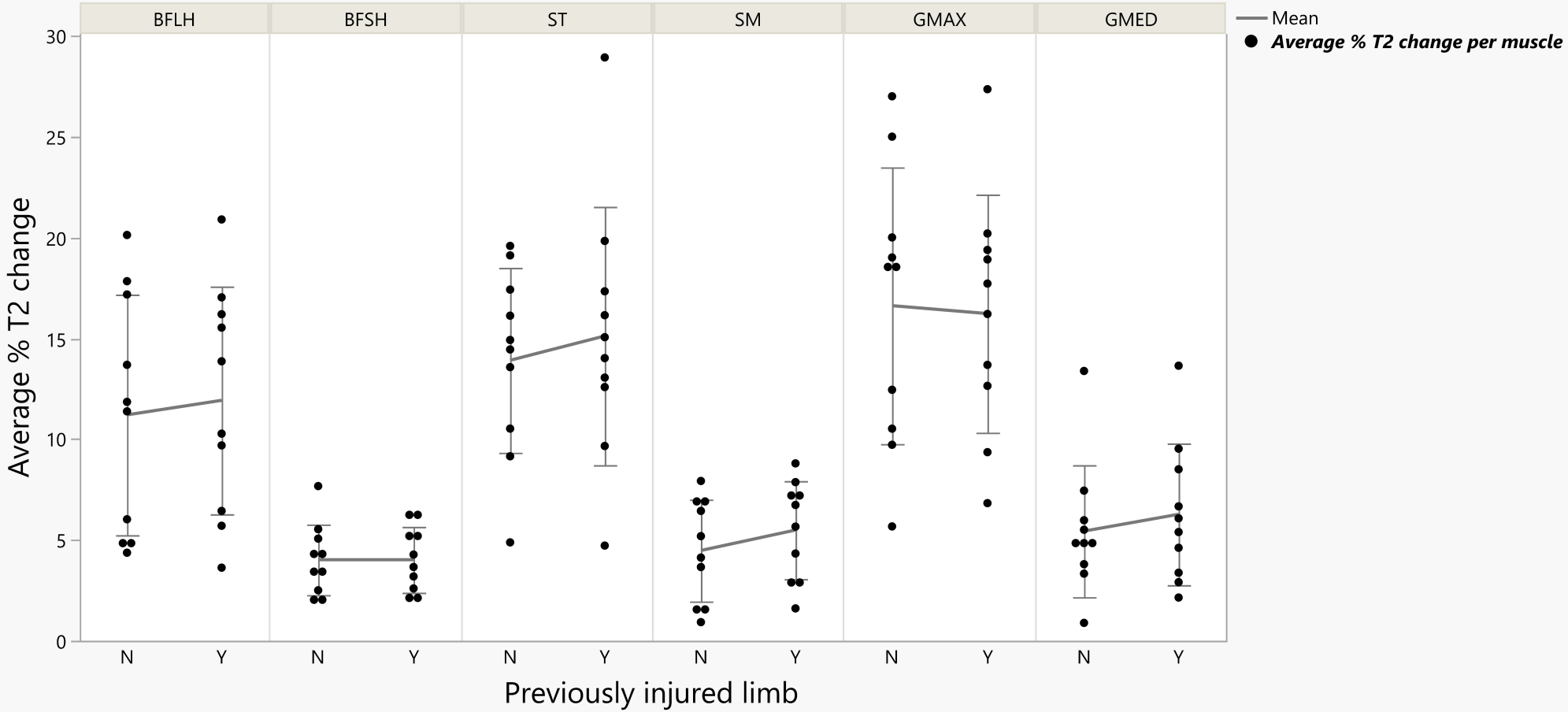
FIGURE CAPTIONS

Figure 1. Typical T2-weighted images (transverse relaxation time = 1180ms; echo time = 12ms; slice thickness = 10mm), depicting the regions of interest for **(A)** the gluteus maximus (G_{MAX}) and gluteus medius (G_{MED}); and **(B)** the biceps femoris long head (BF_{LH}), biceps femoris short head (BF_{SH}), semitendinosus (ST), and semimembranosus (SM). For both A & B, the *right* side of the image corresponds to the participant's *left* side as per radiology convention.

Figure 2. Running-induced percentage changes in T2 relaxation time for the biceps femoris long head (BF_{LH}) and short head (BF_{SH}), semitendinosus (ST), semimembranosus (SM), gluteus maximus (G_{MAX}), and gluteus medius (G_{MED}) for all previously injured (Y) and uninjured contralateral (N) limbs. Values are expressed as mean percentage change compared to values at rest. Error bars depict standard deviation.

Supplementary Figure 1. Quadratic linear regression models displaying the coefficient of determination (r^2) between time since injury and the magnitude of between-limb differences in running-induced mean percentage changes in T2 for the biceps femoris long head (BF_{LH}) and short head (BF_{SH}), semitendinosus (ST), semimembranosus (SM), gluteus maximus (G_{MAX}), and gluteus medius (G_{MED}).


A
G_{MED}
G_{MAX}
B
BF_{SH}
SM
BF_{LH}
ST


BFLH
BFSH
ST
SM
GMAX
GMED
Mean
Average % T2 change per muscle
Average % T2 change
30
25
20
15
10
5
0
N
Y
Previously injured limb