Title:

Hamstring muscle activation and morphology are significantly altered 1 to 6 years after anterior cruciate ligament reconstruction with semitendinosus graft

Word count: 3824

ABSTRACT

Purpose: Harvest of the semitendinosus (ST) tendon for anterior cruciate ligament reconstruction (ACLR) causes persistent hypotrophy of this muscle even after a return to sport, although it is unclear if hamstring activation patterns are altered during eccentric exercise. It was hypothesised that in comparison with contralateral control limbs, limbs with previous ACLR involving ST grafts would display i) deficits in ST activation during maximal eccentric exercise; ii) smaller ST muscle volumes and anatomical cross-sectional areas (ACSAs); and iii) lower eccentric knee flexor strength. **Methods:** Fourteen athletes who had successfully returned to sport after unilateral ACLR involving ST tendon graft were recruited. Median time since surgery was 49 months (range, 12-78 months). Participants underwent functional magnetic resonance imaging (MRI) of their thighs before and after the Nordic hamstring exercise (NHE) and percentage change in transverse (T2) relaxation time was used as an index of hamstring activation. Muscle volumes and ACSAs were determined from MRI and distal ST tendons were evaluated via ultrasound. Eccentric knee flexor strength was determined during the NHE. **Results:** Exercise-induced T2 change was lower for ST muscles in surgical than control limbs (95%CI = -3.8% to -16.0%). Both ST muscle volume (95%CI = -57.1cm^3 to -104.7cm^3) and ACSA (95%CI = -1.9cm^2 to -5.0cm^2) were markedly lower in surgical limbs. Semimembranosus (95%CI = 5.5cm^3 to 14.0cm^3) and biceps femoris short head (95%CI = 0.6cm^3 to 11.0cm^3) volumes were slightly higher in surgical limbs. No between-limb difference in eccentric knee flexor strength was observed (95%CI = 33N to -74N). **Conclusion:** ST activation is significantly lower in surgical than control limbs during eccentric knee flexor exercise 1 to 6 years after ACLR with ST graft. Lower levels of ST activation may partially explain this muscle's persistent hypotrophy post ACLR and have implications for the design of more effective rehabilitation programs.

Level of evidence: Level 4

Key Words: Imaging, Magnetic resonance; Physical therapy; rehabilitation; Injury prevention.

INTRODUCTION

Anterior cruciate ligament (ACL) ruptures are debilitating injuries that can lead to chronic deficits in medial hamstring volumes [13, 16, 23], knee flexor [13, 16, 23] and internal rotator strength [13], and knee stability [1], at least some of which may contribute to altered gait [1, 25] and early onset of knee osteoarthritis [4]. ACL reconstruction (ACLR) surgery is thought necessary to restore knee stability for sports participation [20] and it often involves autografts from the semitendinosus (ST), with or without the gracilis. However, despite the fact that ST tendons have been reported to eventually regenerate and make attachments to the tibia or other knee flexor muscle sheaths in a majority of cases [13, 16, 24], surgery typically results in long lasting ST muscle hypotrophy along with the aforementioned strength deficits.

Persistent deficits in hamstring muscle size and strength following ACLR with ST graft may be at least partly explained by chronic neuromuscular inhibition of the donor muscle. For example, medial hamstring surface electromyographic (sEMG) activity is diminished in limbs with previous ACLR during eccentric knee flexor exercise [3] and hopping [8]. However, limitations in spatial resolution of sEMG makes it impossible to determine whether only the ST muscle activity has changed. Functional magnetic resonance imaging (fMRI) offers a high resolution means of assessing spatial patterns of muscle use during exercise [10], which, as far as the authors are aware, has only once been employed to examine the hamstrings after ACLR involving ST grafts [24]. Takeda et al. [24] assessed hamstring muscle use after concentric exercise for the knee flexors 7-32 months after surgery and reported almost identical ST muscle activation between surgical and control limbs. However, neuromuscular inhibition of the hamstrings may be larger in supramaximal eccentric than concentric contractions [18] and fMRI has never been applied to eccentric exercise after ST grafts.

The primary purpose of this investigation was to explore the extent and pattern of hamstring muscle activation during intense eccentric exercise in individuals with a previous unilateral ACLR involving ST autograft. Secondary goals were to examine hamstring muscle volumes and anatomical cross-sectional areas (ACSAs), ST muscle length and eccentric knee flexor strength. It was hypothesised that

in comparison with contralateral control limbs, limbs with a previous ACLR would display i) deficits in ST activation (according to T2 changes assessed via fMRI) during the eccentric Nordic hamstring exercise (NHE); ii) smaller ST muscle volumes, ACSAs and lengths; and iii) lower eccentric knee flexor strength.

MATERIALS AND METHODS

All participants provided written informed consent to participate in this study, which was approved by the Queensland University of Technology Human Research Ethics Committee (Approval Number: 1600000882). Fourteen recreationally active participants (5 men, mean age, 27.2 ± 4.0 years; mean height, 181.4 ± 3.2 cm; mean body weight, 80.4 ± 6.1 kg; and 9 women, mean age, 25.0 ± 5.3 years; mean height, 168.9 ± 5.3 cm; mean body weight, 65.3 ± 12.5 kg), with a history of unilateral ACLR were recruited for this study. The median time since surgery was 49 months (range, 12-78 months) at the time of testing. All had undergone rehabilitation under the supervision of a qualified physiotherapist and had returned to their pre-injury levels of training and competition. Inclusion criteria were: (i) age between 18 and 35 years, (ii) history of unilateral ACLR autograft from the ipsilateral semitendinosus, and (iii) ≥12 months post ACLR surgery. Exclusion criteria were (i) any contraindications to MRI, (ii) complex knee injuries with additional ligament surgery or meniscal injury, and (iii) any history of hamstring injury to the operated or non-operated contralateral limb. Prior to testing, all participants completed a cardiovascular screening questionnaire to ensure it was safe for them to exercise, and a standardised MRI questionnaire to ensure it was safe for them to enter the magnetic field.

Familiarization

Participants performed a familiarisation session of the NHE at least 5 days (range = 5-12 days) before experimental testing. Upon arrival at the laboratory, participants were provided with a demonstration of the NHE. Subsequently, participants performed several practice repetitions (typically two sets of five repetitions) whilst receiving verbal feedback from investigators.

Experimental procedures

Upon arriving at the imaging facility, participants were seated at rest for at least 15 minutes before data collection. Panoramic ultrasound images were then acquired for the hamstrings on both limbs. Finally, participants underwent an fMRI scan of their thighs before and immediately after performing the NHE.

Exercise protocol and eccentric strength testing

Participants performed the NHE on a NordBord (Vald Performance, Brisbane, Australia) as per previous studies [7, 15]. Participants completed five sets of 10 repetitions of the NHE with one-minute rests between sets. During the rest periods, participants lay prone to minimise activation of the knee flexors. Investigators provided strong verbal support throughout the exercise session to encourage maximal effort. All participants completed the 50 repetitions and were returned to the scanner immediately following the cessation of exercise (< 1 min). Post-exercise scans began within 189.7 ± 24s (mean ± SD). The NordBord measures forces at the ankles via load cells (sampling at 50Hz) that are attached to ankle hooks placed immediately superior to the lateral malleoli. Eccentric strength was determined for each limb from the peak force (N) produced during the first set (10 repetitions) of the exercise session.

Ultrasound Imaging

The distal ST tendons and adjacent muscle fascicles of both limbs were imaged via grey-scale ultrasound (US) images taken with an iU22 Philips scanner (Philips Healthcare, Eindhoven, Netherlands) equipped with a high resolution L12MHz linear transducer. All scanning was performed by a single sonographer with >20 years of musculoskeletal experience. The sonographer was not blinded to the ACLR limb. Participants lay in the prone position to allow the posterior thigh to be examined in the longitudinal and transverse planes. A standardised, pre-programmed general musculoskeletal setting was selected for the grey-scale US scanning protocol. Distal ST muscles and their tendons were compared for the absence or presence of grey scale abnormality (normal/abnormal). The sonographer made notes based on the following criteria; 1) integrity of distal semitendinosus tendon and appearance of adjacent muscle fascicles compared to those from semimembranosus and biceps

femoris long head (normal, partial loss of fibrillary pattern or echogenic complete loss of fibrillary pattern), 2) absence or presence of the surgical tendon scar (absent, thinned, normal reconstituted or hypertrophic), 3) observation of maturity of tendon scar (echogenic, mixed, hypoechoic or fluid), 4) colour doppler imaging indicative of vascularity of the post-surgical harvest site graded using the semi-quantitative method (none 0%, scant 1-24%, mild 25-49%, moderate 50-74% or severe 75-100%). All images and worksheets were recorded and stored with the picture archiving and communication system (PACs).

MRI

All MRI scans were performed using a 3-Tesla imaging system (Phillips Ingenia, © Koninklijke Phillips N.V). Participants were positioned supine in the magnet bore with their knees fully extended, hips in neutral and straps secured around both limbs to prevent undesired movement. Scans of both lower limbs began at the level of the femoral head and finished immediately distal to the tibial plateau. Participants were positioned in the centre of the magnet bore with a 32-channel spinal coil placed over the anterior thighs. Prior to exercise, participants underwent two MRI scanning sequences of both upper limbs simultaneously to generate T2-weighted and mDixon axial images. T2-weighted imaging was repeated immediately after exercise. T2-weighted images were acquired using a Car-Purcel-Meiboom-Gill spin echo pulse sequence (Table 1) as per previous work [7, 15]. To ensure the signal intensity profile of T2-weighted images was not disturbed by abnormal fluid shifts, participants were instructed to avoid strength training of the lower limbs for 72 hours prior to data acquisition and were seated for 15 min [6, 17] before pre-exercise imaging. Axial mDixon images were taken using a T1-weighted 3-dimensional (3D) fast field echo (FFE) sequence (Table 1). The images were acquired in 4 stations (water only, fat only, in-phase and out-of-phase) with 180 slices per station. The FFE sequence provided smooth 3-D images allowing for improved visibility of the muscles' outer margins for manual segmentation.

Muscle activation

To determine the extent of hamstring muscle activation during the NHE, T2 relaxation times were measured in consecutive multi-echo T2-weighted images acquired before and after exercise (see figure 1). All images were transferred to a Windows computer in the digital imaging and communications in medicine (DICOM) file format. For all hamstring muscles, the T2 relaxation time was measured in five axial slices that corresponded 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%)) [6]. In the pre- and post- exercise scans, the signal intensity of each hamstring muscle in both limbs was measured using image analysis software (Sante Dicom Viewer and Editor, Cornell University). The signal intensity was measured in each slice using a 0.5-10cm^2 circular region of interest (ROI) [14], which was placed in a homogenous area of contractile tissue in the centre of each muscle belly (avoiding aponeurosis, fat, tendon, bone and blood vessels). 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 represented the mean value of all pixels within the ROI and was measured across six echo times (8, 16, 24, 32, 40 and 48ms). The T2 relaxation times where determined as per previous work [7, 15].

Muscle volume, anatomical cross-sectional area and muscle length

Muscle volume, anatomical cross-sectional area (ACSA) and muscle length for each of the hamstrings (biceps femoris long head (BF_{LH}), biceps femoris short head (BF_{SH}), ST and semimembranosus (SM)) were determined for both limbs from mDixon images using manual segmentation. Muscle boundaries were identified and traced on each image where the desired structure was present using image analysis software (Sante DICOM Viewer and Editor, Cornell University) (see figure 1). Volumes were determined for each muscle by multiplying the summed cross-sectional areas (CSAs) (from all slices containing the muscle of interest) by the slice thickness [21]. Maximum ACSA was determined by finding the 3.6mm slice with the greatest CSA and averaging this along with the two slices immediately cranial and caudal (5 slices). To determine muscle length, the total number of slices containing muscle tissue for each muscle of interest were summed and then multiplied by the slice thickness to represent

193 the total length of each respective muscle belly. All traces were performed by the same investigator
194 (DM) who was blinded to participant identity throughout all analyses.
195
196 **Insert Fig. 1 here**
197
198 **Statistical analysis**
199 Data were analysed using JMP Version 10.02 (SAS Institute, 2012). Hamstring muscle volume, ACSA,
200 length, pre- and post-exercise T2 values were reported as means ± SDs. Clinical interpretation of
201 ultrasound images was reported descriptively. A repeated measures linear mixed model fitted with the
202 restricted maximum likelihood (REML) method was used to compare transient exercise-induced
203 percentage changes in T2 relaxation times and resting values of muscle volume, ACSA and muscle
204 length for each hamstring muscle. For this analysis, muscle (BF_{LH}, BF_{SH}, ST, SM), limb
205 (surgical/control) and muscle by limb interaction were the fixed factors with participant identity (ID),
206 participant ID by muscle and participant ID by limb as the random factors. When a significant main
207 effect was detected post hoc Student's *t* tests with Bonferroni corrections were used to determine which
208 comparisons differed. Student's *t* tests were used for between-limb comparisons of muscle volumes and
209 ACSAs for the total lateral (BF_{LH} + BF_{SH}) and medial (ST + SM) hamstrings, the whole hamstrings and
210 eccentric knee flexor strength. Comparisons were reported as mean differences with 95% CIs and α was
211 set at $p < 0.05$. For all analyses, Cohen's *d* was reported as a measure for the effect size, with the levels
212 of effect being deemed small ($d = 0.20$), medium ($d = 0.50$) or large ($d = 0.80$).
213
214 As this is the first study to explore hamstring muscle activation during eccentric exercise in individuals
215 following ACLR, it was not possible to base sample size estimates on previously reported effect sizes.
216 However, previous studies exploring differences in strength and ST muscle volume have reported effect
217 sizes of 1.0 to 1.97 when comparing surgical to non-surgical limbs [13]. Therefore, conservative sample
218 size estimates were based on anticipated effect sizes of 0.7 and a sample size of 14 was deemed
219 sufficient to provide a statistical power of ≥ 0.8 when $p<0.05$.
220

RESULTS

Between limb comparisons

T2 relaxation time changes following eccentric exercise

A muscle by limb interaction was found ($p < 0.001$) for the percentage change in T2 relaxation time following the NHE. The average exercise-induced T2 change in surgical limb ST muscles was a third less (-9.9%; 95% CI = -3.8% to -16.0%; $p = 0.004$; $d = 0.93$) than controls. No significant differences in T2 changes were observed between the surgical and control limbs for SM (-2.2%; 95% CI = -10.0% to 6.1%; n.s; $d = 0.33$), BF_{LH} (-0.9%; 95% CI = -5.8% to 4.1%; n.s; $d = 0.24$) or BF_{SH} (0.6%; 95% CI = -2.4% to 3.5%; n.s; $d = 0.10$) (Fig. 2).

Insert Fig. 2 here

Hamstring muscle volumes

A muscle by limb interaction was detected for muscle volume ($p < 0.001$). The surgical limb ST volume was 45% lower (80.9cm^3; 95% CI = -57.1cm^3 to -104.7cm^3; $p < 0.001$; $d = 1.52$) than control limbs. Surgical SM volume was greater (9.7cm^3; 95% CI = 5.5cm^3 to 14.0cm^3; $p < 0.001$; $d = 0.20$) than control limbs. Between limb differences for both BF_{SH} (5.9cm^3; 95% CI = 0.6cm^3 to 11.0cm^3; $p = 0.032$; $d = 0.25$) and BF_{LH} (7.5cm^3; 95% CI = -1.4cm^3 to 16.0cm^3; n.s; $d = 0.17$) volumes were small and trivial (Fig. 3a). Medial hamstring muscle volume of the surgical limbs was 18% lower (-71.3cm^3; 95% CI = -48.9cm^3 to -93.6cm^3; $p < 0.001$; $d = 0.78$) than controls (Fig. 3a). Lateral hamstring volume did not differ significantly (13.4cm^3; 95% CI = -8.9cm^3 to 35.7cm^3; n.s; $d = 0.21$) between surgical and control limbs. Total hamstring muscle volume was 9% lower (-57.9cm^3; 95% CI = -38.0cm^3 to -77.6 cm^3; $p < 0.001$; $d = 0.39$) in surgical than control limbs.

Hamstring muscle ACSA

A main effect was observed for muscle ACSA between limbs ($p < 0.001$). ACSA of the ST was 28% lower (-3.5cm^2; 95% CI = -1.9cm^2 to -5.0cm^2; $p < 0.001$; $d = 0.89$) in surgical than control limbs, but ACSA of BF_{SH} was 9% larger (0.7cm^2; 95% CI = 0.2cm^2 to 1.2cm^2; $p = 0.008$; $d = 0.28$) in the surgical

than control limbs (Fig. 3b). No between-limb differences were observed for SM (0.4cm^2; 95% CI = -8.2cm^2 to 9.1cm^2; n.s; *d* = 0.15) or BF_{LH} ACSA (0.3cm^2; 95% CI = -46.8cm^2 to 47.3cm^2; n.s; *d* = 0.07). The combined ACSA for the surgical medial hamstrings was 11% lower (-3.1cm^2; 95% CI = -1.2cm^2 to -4.9cm^2; p = 0.001; *d* = 0.49) than the control limbs (Fig. 3b). For the lateral hamstrings, the combined ACSA was 5% greater in surgical than control limbs, although this difference was not statistically significant (1.0cm^2; 95% CI = -0.8cm^2 to 2.8cm^2; n.s; *d* = 0.17). The combined total of all hamstring muscle ACSAs was not different in surgical and control limbs (-2.1cm^2; 95% CI = -5.4cm^2 to 1.2cm^2; n.s; d = 0.17).

Hamstring muscle length

A main effect was observed for muscle length between limbs (p < 0.001). ST muscles of the surgical limb were 23% shorter (-7.2cm; 95% CI = -4.8cm to -9.5cm; p < 0.001; *d* = 1.99) than control limbs (Fig. 3c). No between-limb length differences were observed for the remaining homonymous hamstring muscle pairs (all p values n.s; all *d* values < 0.10).

Insert Fig. 3 here

Comparison of tendon and muscle morphology of semitendinosus between limbs

Of the 14 surgical ST tendons, seven showed partial and four showed a complete loss of fibrillary pattern while three appeared normal under ultrasound. All ST tendons from control limbs appeared normal (Supplementary file 1a). Distal ST muscle fascicles were abnormal only in the surgical limbs. Ultrasound of the tendon harvest site showed variable degrees of scarring, (Supplementary file 1b) while ten surgical tendons exhibited no vascularity in the region of the scar, three displayed ‘scant’ and one displayed ‘mild’ vascularity.

Eccentric knee flexor strength

Eccentric knee flexor strength, as determined from the highest forces generated in the first set of the NHE, were small and trivial (-21N; 95% CI = 33N to -74N; n.s; *d* = 0.26) between surgical (289 ± 87N)

277 and control limbs (310 ± 71N) (Fig. 4). Three participant's strength tests were not recorded due to
278 equipment failure during testing.
279
280 **Insert Fig. 4 here**
281
282 **DISCUSSION**
283 The most important finding of this study was that one to six years after surgical intervention, the graft
284 donor ST is activated significantly less than the homonymous muscle in the control limb during the
285 NHE, an exercise known to place high demands on this muscle [7]. Deficits in ST muscle size and
286 length and ultrasound evidence consistent with chronic ST tendon unloading were also apparent in
287 surgical limbs. BF_{SH} volume and ACSA and SM volume were slightly higher in surgical than control
288 limbs and there were only minor deficits in total hamstrings volume (9%) while the total hamstrings
289 ACSA was not significantly different. These modest differences in total muscle size may explain the
290 statistically insignificant between-limb difference eccentric knee flexor strength, despite large deficits
291 in ST ACSA (~28%). To the authors' knowledge, this is the first fMRI study to explore hamstring
292 muscle activation during eccentric exercise in recipients of ACLR involving ST grafts.
293
294 One previous study used fMRI to evaluate hamstring activation after ACLR [24] and it showed no
295 difference in exercise-induced T2 changes in ST muscles of surgical and contralateral limbs after
296 concentric isokinetic knee flexion exercise. It is possible that the greater demands imposed by the
297 supramaximal eccentric exercise in this study revealed muscle activation deficits while submaximal
298 concentric exercise as employed by Takeda and colleagues [24] could not.
299
300 Deficits in ST volume and ACSA after ACLR involving ST grafts have previously been reported [13,
301 16, 23]. In contrast to this study, Konrath et al. [13] reported that BF_{LH} muscles were and BF_{SH} muscles
302 were not larger in surgical than control limbs. BF_{SH} muscles in the surgical limbs in the current study
303 were larger than those in control limbs, while there was no significant between-limb difference in BF_{LH}
304 size. It is possible that the larger BF muscles in surgical limbs have experienced compensatory

hypertrophy after ST tendon grafts, although this is obviously impossible to prove in retrospective studies like these. Differences in relative hamstring muscle volumes between studies [13, 16, 23] may reflect variable rehabilitation strategies or subsequent training of the participants in each study. Alternatively, the diversity of relative hamstring volumes may reflect differences that pre-dated surgery. Like Konrath et al. [13], this study showed that SM volume but not ACSA was larger in surgical than control limbs and that the summed volumes and ACSAs of the medial hamstrings were in deficit in surgical limbs. These observations have implications for internal knee rotation strength, which has been reported to be in deficit long after ACLR with ST grafts [13].

The persistent deficit in medial hamstring muscle mass after ACLR with ST graft is a concern given the role of these muscles in countering external tibial rotation torques and knee valgus moments [9], both of which may be risk factors for ACL injury [2, 20]. Given the devastating effects of ST grafts, it may be beneficial to develop rehabilitation strategies that target the SM, the only other internal rotator of the knee that also acts as a hip extensor. Bourne et al. [5] reported that 10 weeks of hip extension strength training resulted in significant SM hypertrophy while training with the NHE (in which overload is largely limited to the knee) did not. So hip-extension exercises may be effective in compensating for the medial hamstrings size deficits that this study and others have reported [13]. In uninjured athletes, the ST hypertrophies significantly in response to both hip extensor and knee flexor strength training, with a trend towards greater responses after the knee-oriented exercise [5]. However, it is doubtful that similar benefits occur after ST grafts, because the persistent deficits in ST muscle size shown here and by others [13, 16] are evident 1 to 6 years after surgery despite the completion of standard rehabilitation programs and successful return to sport. The present findings of relatively low levels of post-surgical ST activation in the demanding NHE also suggest that this muscle receives limited stimulus for adaptation, even during a supramaximal exercise known to preferentially target this muscle [5, 7]. It should also be considered that ST tendon regeneration after ACLR may take approximately 18 months [19] and may not occur at all in 10 to 50% of patients [13, 16, 23]. Rehabilitation during this time and for individuals with no tendon regeneration would presumably not load the ST significantly. Future studies may examine the effectiveness of hip-extension exercises in promoting SM hypertrophy,

improving knee internal rotation strength and altering dynamic lower limb function during running gait after ACLR with ST grafts.

Contrary to this study's hypothesis, there were no significant differences in eccentric knee flexor strength between surgical and control limbs, although there was considerable between-subject variability. The literature regarding knee flexor strength after ACLR is mixed, with most studies reporting persistent deficits [16, 26] and others showing none [22]. The study by Timmins et al. [26] is the most similar to the current study because it also assessed eccentric forces during the NHE. By contrast, they observed a ~14% strength deficit in surgical limbs, with an effect size approximately twice as big as the one reported here (d = 0.51 v 0.26). Future work should investigate the impact of different ACLR graft techniques (hamstring vs bone-patellar-tendon-bone grafts) on knee flexor muscle use after rehabilitation and successful return to sport [11, 12].

The limitations of this study include its lack of internal knee rotation strength measurements and the large range in times since surgery; the latter of which could conceivably influence compensatory muscle hypertrophy in the postoperative limb. Variability in participant rehabilitation and sports participation before and after the injury and surgery is also likely to have impacted these findings. Finally, while there was no control group (without a history of ACLR) in this study, the activation patterns of the control limbs are very similar to those previously observed in uninjured limbs [6, 7, 15].

CONCLUSION

In conclusion, this is the first fMRI study to show ST activation is significantly reduced during eccentric exercise 1 to 6 years after ACLR with ST graft. Diminished ST activation may partially explain this muscle's persistent hypotrophy and have implications for the design of more effective rehabilitation programs.

ACKNOWLEDGEMENTS

The authors acknowledge the facilities, and the scientific and technical assistance of the staff at the Imaging at Olympic Park Centre, Melbourne.

CONTRIBUTORS

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

TRANSPARENCY DECLARATION

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

COPYRIGHT DECLARATION

DATA SHARING

Consent was not obtained for data sharing but the presented data are anonymised and risk of identification is low.

FUNDING

This study was funded by a grant from the Institute of Health and Biomedical Innovation at the Queensland University of Technology.

COMPETING INTERESTS

AS is listed as a co-inventor on a patent filed for the knee-flexor testing device employed in this study (PCT/AU2012/001041.2012) as well as being a minority shareholder in Vald Performance Pty Ltd, the company responsible for comercialisng the device. All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that (1) the Institute of Health and Biomedical Innovation, Queensland University of Technology funded this study; (2) DM, MW, RT and MB have no relationships with companies that might have an interest in the submitted work in the previous 3 years; (3) their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and (4) DM, MW, RT and MB have no non-financial interests that may be relevant to the submitted work.

ETHICAL CLEARANCE

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

REFERENCES

1. Abourezk MN, Ithurburn MP, McNally MP, Thoma LM, Briggs MS, Hewett TE, et al. (2017) Hamstring Strength Asymmetry at 3 Years After Anterior Cruciate Ligament Reconstruction Alters Knee Mechanics During Gait and Jogging. Am J Sports Med 45:97-105
2. Alentorn-Geli E, Myer GD, Silvers HJ, Samitier G, Romero D, Lazaro-Haro C, et al. (2009) Prevention of non-contact anterior cruciate ligament injuries in soccer players. Part 1: Mechanisms of injury and underlying risk factors. Knee Surg Sports Traumatol Arthrosc 17:705-729
3. Arnason SM, Birnir B, Guethmundsson TE, Guethnason G, Briem K (2014) Medial hamstring muscle activation patterns are affected 1-6 years after ACL reconstruction using hamstring autograft. Knee Surg Sports Traumatol Arthrosc 22:1024-1029
4. Beynnon BD, Johnson RJ, Abate JA, Fleming BC, Nichols CE (2005) Treatment of anterior cruciate ligament injuries, part I. Am J Sports Med 33:1579-1602
5. Bourne MN, Duhig SJ, Timmins RG, Williams MD, Opar DA, Al Najjar A, et al. (2017) Impact of the Nordic hamstring and hip extension exercises on hamstring architecture and morphology: implications for injury prevention. Br J Sports Med 51:469-477
6. Bourne MN, Opar DA, Williams MD, Al Najjar A, Shield AJ (2016) Muscle activation patterns in the Nordic hamstring exercise: Impact of prior strain injury. Scand J Med Sci Sports 26:666-674
7. Bourne MN, Williams MD, Opar DA, Al Najjar A, Kerr GK, Shield AJ (2017) Impact of exercise selection on hamstring muscle activation. Br J Sports Med 51:1021-1028
8. Briem K, Ragnarsdottir AM, Arnason SI, Sveinsson T (2016) Altered medial versus lateral hamstring muscle activity during hop testing in female athletes 1-6 years after anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc 24:12-17
9. Buchanan TS, Kim AW, Lloyd DG (1996) Selective muscle activation following rapid varus/valgus perturbations at the knee. Med Sci Sports Exerc 28:870-876

10. Cagnie B, Elliott JM, O'Leary S, D'Hooge R, Dickx N, Danneels LA (2011) Muscle functional MRI as an imaging tool to evaluate muscle activity. J Orthop Sports Phys Ther 41:896-903
11. Heijne A, Hagstromer M, Werner S (2015) A two- and five-year follow-up of clinical outcome after ACL reconstruction using BPTB or hamstring tendon grafts: a prospective intervention outcome study. Knee Surg Sports Traumatol Arthrosc 23:799-807
12. Heijne A, Werner S (2010) A 2-year follow-up of rehabilitation after ACL reconstruction using patellar tendon or hamstring tendon grafts: a prospective randomised outcome study. Knee Surg Sports Traumatol Arthrosc 18:805-813
13. Konrath JM, Vertullo CJ, Kennedy BA, Bush HS, Barrett RS, Lloyd DG (2016) Morphologic Characteristics and Strength of the Hamstring Muscles Remain Altered at 2 Years After Use of a Hamstring Tendon Graft in Anterior Cruciate Ligament Reconstruction. Am J Sports Med 44:2589-2598
14. Mendiguchia J, Arcos AL, Garrues MA, Myer GD, Yanci J, Idoate F (2013) The Use of Mri to Evaluate Posterior Thigh Muscle Activity and Damage during Nordic Hamstring Exercise. J Strength Cond Res 27:3426-3435
15. Messer DJ, Bourne MN, Williams MD, Al Najjar A, Shield AJ (2018) Hamstring Muscle Use in Women During Hip Extension and the Nordic Hamstring Exercise: A Functional Magnetic Resonance Imaging Study. J Orthop Sports Phys Ther 48:607-612
16. Nomura Y, Kuramochi R, Fukubayashi T (2015) Evaluation of hamstring muscle strength and morphology after anterior cruciate ligament reconstruction. Scand J Med Sci Sports 25:301-307
17. Ono T, Higashihara A, Fukubayashi T (2011) Hamstring functions during hip-extension exercise assessed with electromyography and magnetic resonance imaging. Res Sports Med 19:42-52
18. Opar DA, Williams MD, Timmins RG, Dear NM, Shield AJ (2013) Knee flexor strength and bicep femoris electromyographical activity is lower in previously strained hamstrings. J Electromyogr Kinesiol 23:696-703

19. Papandrea P, Vulpiani MC, Ferretti A, Conteduca F (2000) Regeneration of the Semitendinosus Tendon Harvested for Anterior Cruciate Ligament Reconstruction Evaluation Using Ultrasonography. Am J Sports Med 28:556-561

20. Paterno MV, Schmitt LC, Ford KR, Rauh MJ, Myer GD, Huang B, et al. (2010) Biomechanical measures during landing and postural stability predict second anterior cruciate ligament injury after anterior cruciate ligament reconstruction and return to sport. Am J Sports Med 38:1968-1978

21. Silder A, Heiderscheit BC, Thelen DG, Enright T, Tuite MJ (2008) MR observations of long-term musculotendon remodeling following a hamstring strain injury. Skeletal Radiol 37:1101-1109

22. Simonian PT, Harrison SD, Cooley VJ, Escabedo EM, Deneka DA, Larson RV (1997) Assessment of morbidity of semitendinosus and gracilis tendon harvest for ACL reconstruction. Am J Knee Surg 10:54-59

23. Snow BJ, Wilcox JJ, Burks RT, Greis PE (2012) Evaluation of muscle size and fatty infiltration with MRI nine to eleven years following hamstring harvest for ACL reconstruction. J Bone Joint Surg Am 94:1274-1282

24. Takeda Y, Kashiwaguchi S, Matsuura T, Higashida T, Minato A (2006) Hamstring muscle function after tendon harvest for anterior cruciate ligament reconstruction: evaluation with T2 relaxation time of magnetic resonance imaging. Am J Sports Med 34:281-288

25. Tashman S, Collon D, Anderson K, Kolowich P, Anderst W (2004) Abnormal rotational knee motion during running after anterior cruciate ligament reconstruction. Am J Sports Med 32:975-983

26. Timmins RG, Bourne MN, Shield AJ, Williams MD, Lorenzen C, Opar DA (2016) Biceps Femoris Architecture and Strength in Athletes with a Previous Anterior Cruciate Ligament Reconstruction. Med Sci Sports Exerc 48:337-345

Fig. 1 (a) Tracings of hamstring muscles in a mDixon image and a T2-weighted image (b) before and; (c) immediately after 50 repetitions of the Nordic Hamstring Exercise. BF_{LH}, biceps femoris long head; BF_{SH}, biceps femoris short head; ST, semitendinosus; SM, semimembranosus. For all images, the *right* side of the image corresponds to the participant's *left* side as per radiology convention.

Fig. 2 Percentage change in fMRI T2 relaxation times of each hamstring muscle following the Nordic hamstring exercise. Values are displayed as the mean percentage change compared to values at rest. * Indicates significant difference between limbs ($p = 0.004$). Data are presented as mean values (± SD). BF_{LH}, biceps femoris long head; BF_{SH}, biceps femoris short head; ST, semitendinosus; SM, semimembranosus.

Fig. 3 (a) Mean volumes, (b) anatomical cross-sectional areas (ACSAs) and (c) lengths of hamstring muscles in surgical and control limbs. Values were measured at rest. Data are presented as mean values (± SD). For between limb muscle comparisons, * indicates ($p < 0.001$), ** indicates ($p = 0.001$) and *# signifies ($p < 0.05$). BF_{LH}, biceps femoris long head; BF_{SH}, biceps femoris short head; ST, semitendinosus; SM, semimembranosus; Hams, hamstrings; Medial Hams, medial hamstrings; Lateral Hams, lateral hamstrings.

Fig. 4 Peak eccentric knee flexor force measured at the ankles during the Nordic hamstring exercise. Bars depict the average peak knee flexor forces, while the dots represent each participant's responses. Strength is reported in absolute terms (N).