Biceps Femoris Compensates for Semitendinosus After Anterior Cruciate Ligament Reconstruction With a Hamstring Autograft

A Muscle Functional Magnetic Resonance Imaging Study in Male Soccer Players

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Investigation performed at Ghent University, Ghent, Belgium

Background: Rates of reinjury, return to play (RTP) at the preinjury level, and hamstring strain injuries in male soccer players after anterior cruciate ligament reconstruction (ACLR) remain unsatisfactory, due to multifactorial causes. Recent insights on intramuscular hamstring coordination revealed the semitendinosus (ST) to be of crucial importance for hamstring functioning, especially during heavy eccentric hamstring loading. Scientific evidence on the consequences of ST tendon harvest for ACLR is scarce and inconsistent. This study intended to investigate the repercussions of ST harvest for ACLR on hamstring muscle function.

Hypothesis: Harvest of the ST tendon for ACLR was expected to have a significant influence on hamstring muscle activation patterns during eccentric exercises, evaluated at RTP in a population of male soccer athletes.

Study Design: Controlled laboratory study.

Methods: A total of 30 male soccer players with a history of ACLR who were cleared for RTP and 30 healthy controls were allocated to this study during the 2018-2019 soccer season. The influence of ACLR on hamstring muscle activation patterns was assessed by comparing the change in T2 relaxation times [$\Delta T2\ (\%) = \frac{\text{post} - \text{exercise} - \text{T2 pre} - \text{exercise}}{\text{T2 pre} - \text{exercise}}$] of the hamstring muscle tissue before and after an eccentric hamstring loading task between athletes with and without a recent history of ACLR through use of muscle functional magnetic resonance imaging, induced by an eccentric hamstring loading task between scans.

Results: Significantly higher exercise-related activity was observed in the biceps femoris (BF) of athletes after ACLR compared with uninjured control athletes (13.92% vs 8.48%; *P* = .003), whereas the ST had significantly lower activity (19.97% vs 25.32%; *P* = .049). Significant differences were also established in a within-group comparison of the operated versus the contralateral leg in the ACLR group (operated vs nonoperated leg: 14.54% vs 11.63% for BF [*P* = .000], 17.31% vs 22.37% for ST [*P* = .000], and 15.64% vs 13.54% for semimembranosus [SM] [*P* = .014]). Neither the muscle activity of SM and gracilis muscles nor total posterior thigh muscle activity (sum of exercise-related ΔT2 of the BF, ST, and SM muscles) presented any differences in individuals who had undergone ACLR with an ST tendon autograft compared with healthy controls.

Conclusion: These findings indicate that ACLR with a ST tendon autograft might notably influence the function of the hamstring muscles and, in particular, their hierarchic dimensions under fatiguing loading circumstances, with increases in relative BF activity contribution and decreases in relative ST activity after ACLR. This between-group difference in hamstring muscle activation pattern suggests that the BF partly compensates for deficient ST function in eccentric loading. These alterations might have implications for athletic performance and injury risk and should probably be considered in rehabilitation and hamstring injury prevention after ACLR with a ST tendon autograft.

Keywords: anterior cruciate ligament; knee; reconstruction; hamstrings; semitendinosus; mfMRI; soccer; rehabilitation

Anterior cruciate ligament (ACL) injuries are among the most common ligament injuries in sports medicine and, together with hamstring muscle strain injuries, make up the vast majority of injuries in soccer.[8,9,11] Both injuries may cause prolonged absence from sports and delayed

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return to play (RTP), and might even be career ending in some cases.[8,9,11,23,41] In contrast to hamstring injuries, however, ACL lesions are considered to be substantially more severe and disabling, necessitating surgical intervention and prolonged rehabilitation periods before safe RTP.[24,40]

In modern sports surgery, it is common practice to use autologous grafts for anterior cruciate ligament reconstruction (ACLR) because they are considered to provide the best biomechanical properties to regain knee stability and function.[15,21,36,39] The debate continues as to whether the hamstring (semitendinosus [ST]) tendon graft or the bone–patellar tendon–bone (BTB) graft is best suited for ACLR. To date, existing clinical trials and meta-analyses offer conflicting opinions regarding the most favorable graft. Therefore, graft choice remains dependent on the surgeon's preference.[15,21,36,39] A recent meta-analysis by Samuelsen et al[30] of 47,613 patients with ACLR showed that both graft types were viable options for primary ACLR, but hamstring tendon autografts had a higher failure rate compared with BTB graft (2.84% vs 2.80%; P = .01).

Both grafts are considered reliable due to their structural properties and assumed limited consequences for agonist muscle function.[15,21,39] Hamstring tendons are often preferred because of their ease of harvest, versatile length, lower donor-site morbidity, and the possibility of ACL augmentation and double-bundle reconstruction.[34,43] However, hamstring grafts have the disadvantage of generally greater residual laxity and persistent knee flexor weakness, whereas BTB grafts carry a greater risk of postoperative complications such as patellar fracture, quadriceps amyotrophy, flexion contracture, patellar ligament tendinopathy or tear, and anterior knee pain.[7,10,22]

Previous work has shown that the different entities of the hamstring muscles (ST, biceps femoris [BF], and semimembranosus [SM]) are activated in a specific sequence and with a diverging intensity of contraction in both functional (running and sprinting) conditions and isolated, strength training–related, muscle-loading conditions. More specifically, previous research revealed that the ST is most active toward the end of the front swing phase of the running cycle compared with the laterally oriented BF, which is activated to a higher extent in the stance and the front and back midswing phases.[5,32,33,34] The finding that the ST appears to have the highest muscle activity and is recruited more than the BF and the SM during strength exercises and locomotion indicates the essential role of the ST in performance and hamstring injury risk management in athletes. Soccer players with a history of hamstring injuries demonstrated an aberrant and less economic muscle activation pattern in which the BF partly compensated for the lack of activity of the ST, confirming the leading role of the ST in terminal eccentric muscle activity such as running and kicking.[33,34] The importance of sound hamstring functioning with regard to ACL injury prevention was stressed by Opar and Serpell[27] in a clinical commentary stating that there might be an important association between functional hamstring strength deficiencies and a greater risk of ACL rupture in athletes, although that statement was based mostly on a theoretical framework and requires additional research.

The aim of the current study was to assess the repercussions of ST tendon harvesting in regard to hamstring muscle function in male soccer players who underwent ACLR; for this purpose, we evaluated exercise-induced metabolic hamstring muscle activity and intramuscular activation (using muscle functional magnetic resonance imaging [mfMRI]) in ACLR players' operated leg compared with the contralateral leg and compared with a matched healthy control group.[6,16,20,33,38]

METHODS

Participants

During the 2018-2019 soccer season, 60 male soccer players were recruited. All of them were competing at the regional and/or national level of the Belgian football competition. A total of 30 players had sustained a soccer-related injury of the ACL and had undergone ACLR with autologous ipsilateral ST tendon graft at the Departments of Orthopaedic Surgery of the University Hospital of Ghent or AZ Delta Roeselare. All patients underwent a classic ACLR (by T.T., T.L., N.A., or J.V.) with an autologous quadrupled ST tendon graft (in 4 patients, a 6-strand ST plus gracilis [GRA] graft was used). In 18 patients, a lateral extra-articular tenodesis was performed; in 3 patients, a partial lateral meniscectomy was needed; and in 5 patients, meniscal repair was performed (3 lateral, 2 medial). After surgery, rehabilitation was started using a standard evidence-based protocol.

Selection of participants for the control group was based on the features of the eligible candidates within the ACLR group. Clearance for RTP was given by the responsible orthopaedic surgeons (T.T., T.L., N.A., J.V.) and was based on the patient's clinical status, the evolution of physical therapy, and isokinetic strength profiles. For the latter, bilateral symmetry and antagonist ratios (H:Q) of the

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quadriceps (Q) and hamstring (H) muscles had to meet the <10% side-to-side difference and >0.60 H:Q ratio criteria.

Only athletes within the age limits of 18-35 years were included in order to minimize confounding effects of musculoskeletal maturity and age. A recent musculoskeletal injury (within the past 2 seasons) or a history of orthopaedic surgery was an absolute contraindication, and participants had to be completely ready to compete at the time of testing. Participants were instructed not to engage in intensive training or soccer competition 48 hours before testing to ensure a valid measure of the exercise-related T2 increase or signal intensity shift.

Participants of the ACLR group underwent physical therapy in a practice of their own preference, based on a standardized rehabilitation program. To rule out any potential biasing effect of different rehabilitation programs, we verified individual training content, volume, and frequency throughout the rehabilitation trajectory before study participation. Rehabilitation after ACLR consisted mostly of core and lower limb stability training, thigh muscle strength training, and stretching. Strength training was generally performed at the level of endurance, basic, and maximal strength, after which agility sessions were carried out while the athletes were allowed to start running again. None of the rehabilitation schemes essentially differed from this regimen. Differences in T2 shifts due to different rehabilitation protocols and outcomes were also ruled out by using normalized isokinetic strength test outcomes of the hamstring and quadriceps muscles as an inclusion criterion in this study sample. Patients were tested after RTP clearance based on the criteria mentioned in the methods. Testing was conducted at the time of RTP, which occurred at a mean ± SD of 10 ± 1.4 months after surgery.

MRI investigations were performed at the Ghent University Hospital and were systematically supervised by the same qualified researchers (J.S., T.T., H.V.). Participants were asked to fill out standardized questionnaires to document their clinical, functional, and athletic status, injury history, and level of recovery. Furthermore, the ACLR group was questioned using the International Knee Documentation Committee subjective knee form, the Lysholm and Tegner scores, and a visual analog scale score for pain. Before mfMRI investigation, participants were comprehensively informed with regard to the purpose and the content of the testing protocol. MRI safety checklists were verified to exclude possible contraindications, and informed consent forms were signed. This study was approved by the ethics committee of the Ghent University Hospital (EC UZG 2018/0841).

mfMRI Scanning Procedure With Eccentric Hamstring Exercise

mfMRI Scanning Sequence. The testing protocol consisted of 2 scanning sequences with a strenuous hamstring exercise between the scans. The difference in the transverse relaxation time of the separate hamstring muscle bellies before and after exercise (T2 increase or signal intensity shift) indicated the magnitude of underlying metabolic muscle activity.[1,38] The scanning procedure consisted of (1) a localizing sequence to adequately align the center of the scanning field of view (FOV) with the center of the upper leg (midpoint between the greater trochanter and lateral epicondyle of the distal femur), (2) an axial spin echo T1 sequence, and (3) 2 identical Carr Purcell Meiboom Gill (CPMG) T2 sequences, 1 before and 1 after exercise. The midthigh level was chosen as the FOV center because each of the hamstring muscle bellies presents a fairly high muscle-tendon (fascia) tissue ratio at this level, allowing one to predominantly take into account contractile (metabolically active) muscle tissue for muscle activity estimation. For the functional CPMG sequence, 5 transverse slices were acquired, on the basis of which T2 maps and associated isolated T2 relaxation values could be calculated using a customized MatLab Script (MatLab R2019b; MathWorks).

Figure 1. Localization of the midpoint between the greater trochanter and lateral epicondyle of the femur, where the middle of 5 transverse slices was positioned using the localizing sequence in the scanning protocol. A plastic tube filled with sodium chloride solution was applied for visualization of the respective field of view center and for optimal localization on the coronal magnetic resonance imaging scan obtained during the localizing sequence.

A 3.0-T MRI scanner (Siemens Trio Tim) was used for the mfMRI protocol. The participants were positioned supine on the scanning table, which was equipped with a spine coil, with both legs extended and their feet close to the magnet. A flexible body matrix was placed on the anterior thigh area and carefully aligned with the FOV center. Plastic tubes filled with sodium chloride solution were used for accurate localization and determination of the center of the FOV (the intended slice position) (Figure 1). Tape was used to indicate the center of image acquisition relative to the scanning table to ensure that the participant had the same position before and after exercise and to avoid the need for a second sagittal localizing sequence after exercise, in order to minimize the time span between the end of the exercise session and the second CPMG acquisition. Because the T2 shift half-life is only 7 minutes, running a second localizing sequence after exercise may have resulted in an underestimation of the exercise-induced metabolic changes within the hamstring muscles.[26,38]

TABLE 1
Spin Echo T1 and CPMG T2 Image Acquisition Parameters[a]

Scan Position and Acquisition Parameters	Spin Echo T1	CPMG T2
Number of slices	6	5
Slice thickness, mm	4	5
Interslice distance, mm	2	2
Field of view, mm	340	380
Middle slice location/center of field of view	Midthigh: midpoint greater trochanter to lateral epicondyle distance of the femur	Midthigh: midpoint greater trochanter to lateral epicondyle distance of the femur
Relaxation time, ms	550	1500
Echo time, ms	9	10.5-168
Number of echoes	1	16
Voxel size, mm	0.9 × 0.9 × 4.0	1.5 × 1.5 × 5.0

[a]CPMG, Carr Purcell Meiboom Gill.

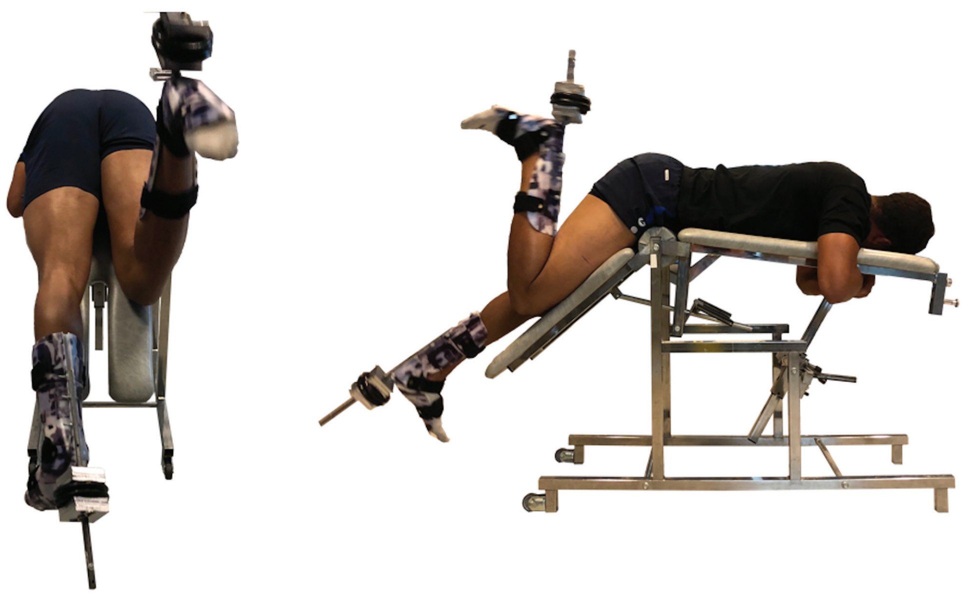

Figure 2. Prone leg-curl exercise with weights (5 kg) performed between the 2 Carr Purcell Meiboom Gill scanning sequences of the testing procedure.

The spin echo T1 sequence was added to the functional scanning protocol for more accurate identification and selection of the region of interest (ROI) in the post hoc analysis. The contrast of a T1 scan is substantially higher than the contrast within a T2 image, so the T1 image allowed more accurate discrimination of the different hamstring muscle bellies in the T2 map. The details of the entire scanning sequence are provided in Table 1. Concerning the reliability of this approach, we refer the reader to previous work that demonstrated intraclass correlation coefficients (ICCs) of 0.925, 0.724, and 0.737 for the BF, ST, and SM, respectively, for the baseline measurements. The ICCs for the postexercise measurements were 0.892, 0.801, and 0.856 for the BF, ST, and SM, respectively.[34]

Hamstring Exercise Integrated Within the Scanning Protocol. The participants performed a prone leg-curl exercise between the 2 CPMG sequences. Participants were positioned prone on a 60° inclined exercise table (inducing approximately 60° of flexion at hip joint level) and were instructed to flex and extend both knees alternately from 90° of knee flexion to full extension with a weight of 5 kg attached to each foot. Hip and knee joint deviations in the frontal (abduction-adduction) and transverse (internal-external rotation) planes were prohibited because this would influence the muscle activation patterns (Figure 2).

Participants were allowed to choose a self-selected, comfortable but constant movement pace until subjective exertion was reached (corresponding to a score of 20 on the Borg rating of perceived exertion scale and/or loss of motor control).[4] As stated in previous work, this exercise was chosen to mimic the biomechanics (and corresponding hamstring mechanics) of running (ie, hip flexion and knee extension that have to be controlled and decelerated against gravity) while providing a fairly high muscle loading to induce a sufficient metabolic activation response, as there is a linear relationship between exercise intensity and magnitude of T2 increase.[34,38]

When the point of maximal exertion was reached, participants were submitted to the second CPMG sequence within 1 minute. The number of adequate prone leg-curl repetitions was registered and included in the data analysis as an indication of the functional hamstring strength endurance and fatigue tolerance.

Data Processing

Acquired images were converted into T2 maps for calculation of the mean transverse relaxation times in the different ROIs using a custom-made MatLab script (T2Processor; MathWorks).

The T2 value was calculated via the formula Sn = S0 * exp (−TE/T2), where Sn represents the signal intensity, expressed in milliseconds, at a given echo time within the scanner's original signal intensity S0.

For the functional CPMG sequence, 5 slices were taken at the center of both thighs before and after exercise. In each of the 10 acquired images (10 slices), 8 ROIs were selected for relaxation time (T2) calculation, representing the long head of the biceps femoris (BF_{LH}), the ST, the SM, and the GRA muscles in both the right and left legs. Muscle bellies were systematically selected as ROIs, with strict inclusion of muscle fiber tissue and exclusion of fatty tissue, neurovascular structures, and connective or scar tissue (Figure 3). After selecting the ROIs and adjusting the threshold in the T2 map to ensure that only muscle tissue was included, we calculated the T2 relaxation time for each ROI in every slice. The final T2 relaxation time of each muscle before and after exercise was the mean T2 value from the 5 slices acquired at the midthigh. This procedure's intratester reliability was assessed and proven to be highly accurate.[1]

After selecting the ROI and calculating the T2 relaxation times before and after exercise, we derived this study's primary outcome parameter (ie, the amount of exercise-related metabolic muscle activity presented by each muscle belly in function of ACLR) based on the following:

1. Relative differences in baseline and postexercise activity: ΔT2
   $\Delta T2 = \frac{\text{T2 Postexercise} - \text{T2 Preexercise}}{\text{T2 Preexercise}}$
2. Proportion of relative metabolic muscle belly activity in reference to summated activity amount of the entire posterior thigh muscle unit: Proportional ΔT2
   Proportional $\Delta T2x = \frac{\Delta T2x}{\Delta T2\text{ BF} + \Delta T2\text{ ST} + \Delta T2\text{ SM} + \Delta T2\text{ Gra}}$

where x represents the muscle belly of which the proportional activity is to be calculated.

These outcome parameters are expressed in percentages and have repeatedly been used in previous research as well.[1,34]

Statistical Analysis

To compare the hamstring activation patterns in the ACL-reconstructed legs of the ACLR group with the patterns of

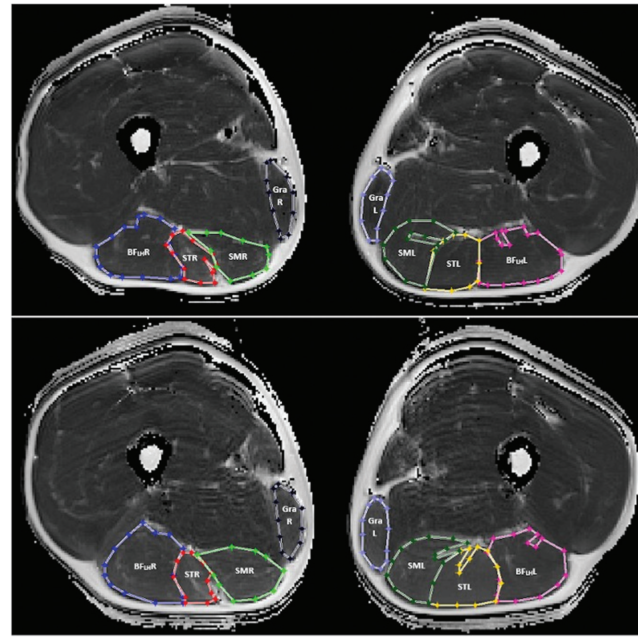

Figure 3. T2 map of the transverse Carr Purcell Meiboom Gill images before exercise (upper image) and after exercise (lower image) representing the process of selecting regions of interest within the T2 processor interface. BF_{LH}, long head of the biceps femoris; Gra, gracilis; L, left; R, right; SM, semimembranosus; ST, semitendinosus.

representative healthy legs of the control group without "leg dominance" as a confounder, the ratio of dominant-nondominant leg involvement in the ACLR group was verified.[1,11] This same distribution was applied for the control group by randomly including dominant or nondominant legs to achieve the same ratio in the respective groups. In the ACLR group, 62% of participants sustained an ACL rupture in the dominant knee. In the control group, the same percentage of dominant legs were included for analysis.

Normality of the data distribution for the different variables was evaluated using the Shapiro-Wilk test. To check for confounders, the similarity of anthropometric features as well as the level of competition and playing position in both groups was verified using multivariate analyses of variance and χ^2 hypothesis testing. Independent and paired Student t tests were used to identify differences in relative exercise-related T2 increases in each of the included muscles in reference to their baseline activity (ΔT2) between groups and between legs in the ACLR group, respectively. The same statistical approach was used to evaluate bilateral differences in the proportional muscle activity presented by each muscle belly in reference to the summated amount of muscle activity of the entire posterior thigh muscle unit (proportional ΔT2). To verify potential differences in functional load-bearing capacity of the hamstring muscles, between-group comparison of the mean (bodyweight normalized) number of repetitions was performed. SPSS Version 25 statistical software

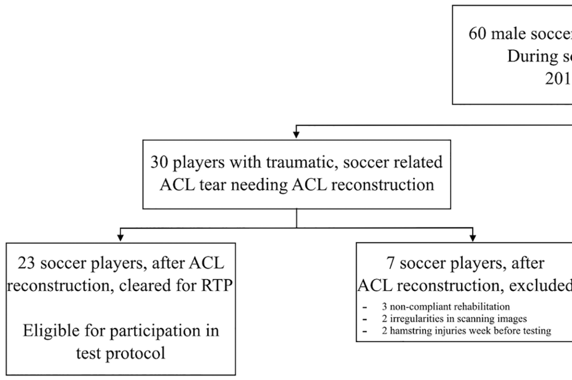

Figure 4. CONSORT (Consolidated Standards of Reporting Trials) flow diagram of the study. ACL, anterior cruciate ligament; RTP, return to play.

(IBM Corp) was used; the level of significance was set at α = .05.

RESULTS

Due to irregularities in scanning images and participant exclusion, the data of 52 of the 60 originally recruited participants were used for statistical analyses. The functional MRI data of 23 ACLR patients were compared with the data of 29 healthy controls with similar anthropometric and athletic profiles (Figure 4). Details on anthropometrics, demographic characteristics, level of football competition, and time between surgery and RTP clearance are given in Table 2.

Between-Group Analysis: ACLR Group Compared With Control Group

Hamstring muscle function was evaluated by comparing the exercise-related changes in T2 relaxation times of the BF_{LH}, ST, SM, and GRA, before and after a strenuous prone leg-curling exercise, in both the ACLR group and the healthy control group. The relative activity-related T2 shift (ΔT2) in the BF_{LH} appeared to be significantly higher in the ACLR group ($\Delta T2_{BFLH}$ ACLR = 13.92%) compared with the control group ($\Delta T2_{BFLH}$ control = 8.48%) (mean difference = 5.44% [95% CI, 2%-9%]; P = .003; Cohen d = 1), whereas the opposite was seen for the ST, which was significantly less activated in the ACLR group ($\Delta T2_{ST}$ ACLR = 19.97%) compared with the control group ($\Delta T2_{ST}$ control = 25.32%) (mean difference = 5.35% [95% CI, 0.3%-11%]; P = .049; Cohen d = 0.88). These between-group differences were not found for the SM and the GRA muscles or for the contralateral legs (Figure 5).

Next, assessing the proportional relative activity-related T2 shift (proportional ΔT2) in reference to the summated T2 shift of the entire posterior thigh muscle group (including the GRA), we observed significantly greater muscle activity in the BF_{LH} and significantly reduced activity in the ST muscles of the ACLR group compared with the healthy controls (proportional $\Delta T2_{BFLH}$ ACLR = 21.17%, proportional $\Delta T2_{BFLH}$ control = 12.65%, mean difference = 9.52% [95% CI, 3.79%-15.24%], P = .002; Cohen d = 1; proportional $\Delta T2_{ST}$ ACLR = 26.66%, proportional $\Delta T2_{ST}$ control = 37.38%, mean difference = 10.81% [95% CI, 5.44%-16.20%], P = .000, Cohen d = 1.4). The global posterior thigh muscle activity and the proportional activity patterns of the SM and the GRA were not significantly different between the ACLR and control groups or relative to the contralateral leg (Figure 6).

No statistical significance was seen when we compared the hamstring muscle's strength endurance and fatigue tolerance by between-group comparison of the number of prone leg-curl repetitions, normalized to the participant's bodyweight: The mean number of repetitions per bodyweight was 1.78 ± 1.47 and 2.16 ± 1.51 for the ACLR and control groups, respectively (P = .379); the absolute number of curls was 154 ± 122 and 171 ± 117 for the ACLR and control groups, respectively (P = .630).

TABLE 2
Participant Characteristics[a]

	ACLR Group (n = 23)	Control Group (n = 29)
Age, y	24.57 ± 5.12	22.82 ± 2.04
Weight, kg	80.63 ± 8.88	74.86 ± 6.83
Height, cm	180.21 ± 5.92	180.21 ± 4.66
Body mass index	25.06 ± 3.70	22.30 ± 1.81
Level of competition, n		
National level	4	6
1st provincial	4	4
2nd provincial	3	6
3rd provincial	6	5
4th provincial	6	7
Playing position, n		
Attack	4	3
Midfield	12	9
Defense	7	16
Time to RTP after ACLR, mo	10 ± 1.4	

[a] Values are expressed as mean ± SD unless otherwise noted. ACLR, anterior cruciate ligament reconstruction; RTP, return to play.

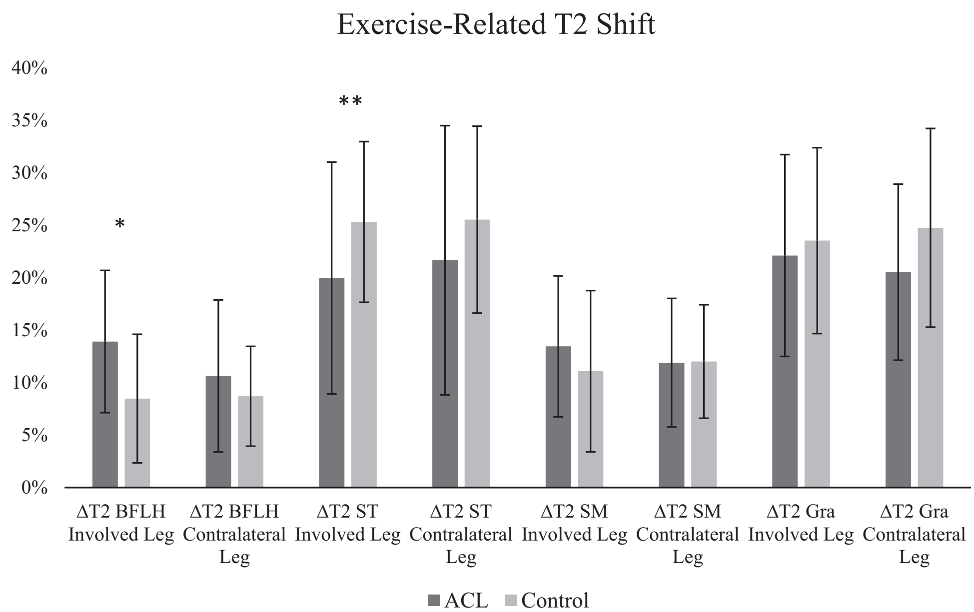

Figure 5. Relative activity-related T2 shifts (ΔT2) in each muscle belly of the control group and of both legs in the anterior cruciate ligament (ACL) reconstruction group. BF_{LH}, long head of the biceps femoris; Gra, gracilis; SM, semimembranosus; ST, semitendinosus; T2, T2 relaxation time constant of muscle water. *Significant between-group difference of 5.44% on average (P = .003). **Significant between-group difference of 5.35% on average (P = .049).

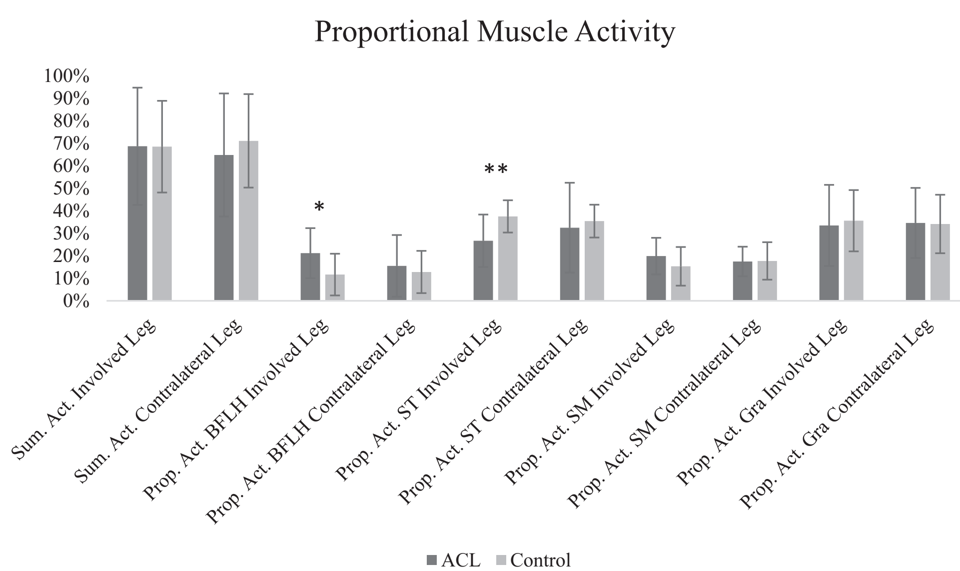

Figure 6. Proportional relative activity-related T2 shifts (proportional ΔT2) in each muscle belly of the control group and of both legs in the anterior cruciate ligament (ACL) reconstruction group, in reference to the summated muscle activity of the entire posterior thigh muscle portion. Act, activity; BF_{LH}, long head of the biceps femoris; Gra, gracilis; Prop, proportional; SM, semimembranosus; ST, semitendinosus; Sum, summated; T2, T2 relaxation time constant of muscle water. *Significant between-group difference of 9.52% on average (P = .002). **Significant between-group difference of 10.82% on average (P = .000).

Within-Group Analysis: Injured Leg in Comparison With Contralateral Healthy Leg in the ACLR Group

With regard to the relative activity-related T2 shift, paired comparison of means demonstrated that both the BF_{LH} and the SM were significantly more active in the operated leg compared with the contralateral side ($\Delta T2_{BFLH}$ operated leg = 14.54%, $\Delta T2_{BFLH}$ contralateral leg = 11.63%, $\Delta T2_{SM}$ operated leg = 15.64%, $\Delta T2_{SM}$ contralateral leg = 13.54%; for BF_{LH}, mean difference = 2.9% [95% CI,

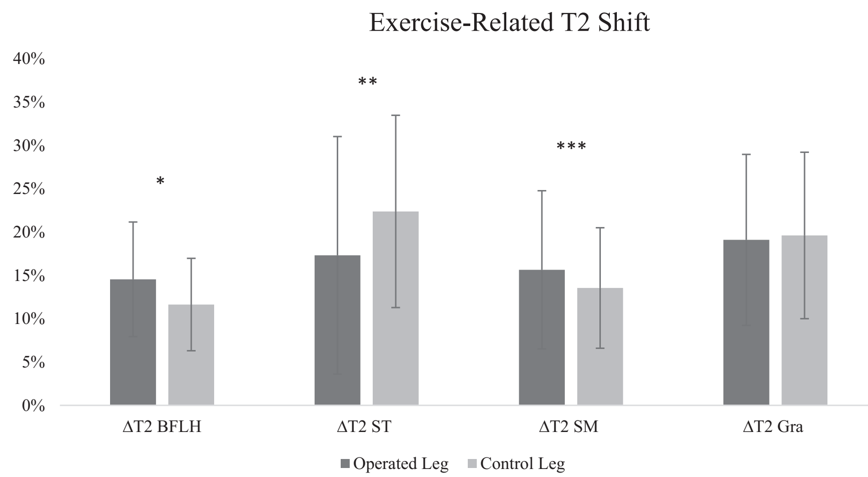

Figure 7. Relative activity-related T2 shifts (ΔT2) in each muscle belly of the operated leg compared with the contralateral leg in the ACLR group. BF_{LH}, long head of the biceps femoris; Gra, gracilis; SM, semimembranosus; ST, semitendinosus; T2, T2 relaxation time constant of muscle water. *Significant between-leg difference of 2.9% on average (P = .000). **Significant between-leg difference of 5.1% on average (P = .000). ***Significant between-leg difference of 2.1% on average (P = .014).

1.64%-4.18%], P = .000, Cohen d = 0.55; for SM, mean difference = 2.1% [95% CI, 0.43%-3.76%], P = .014, Cohen d = 0.30). The opposite was established for the ST, which was activated significantly less in the operated leg ($\Delta T2_{ST}$ operated leg = 17.31%) compared with the contralateral leg ($\Delta T2_{ST}$ contralateral leg = 22.37%) (mean difference = 5.1% [95% CI, 2.59%-7.54%]; P = .000; Cohen d = 0.46) (Figure 7).

With regard to the proportional relative activity-related T2 increase, statistical analysis revealed once again that both the BF_{LH} and the SM presented higher activity contributions in the operated leg in reference to the contralateral leg (proportional $\Delta T2_{BFLH}$ operated leg = 25.72%, proportional $\Delta T2_{BFLH}$ contralateral leg = 17.70%, proportional $\Delta T2_{SM}$ operated leg = 24.97%, proportional $\Delta T2_{SM}$ contralateral leg = 19.70%; for BF_{LH}, mean difference = 8.02% [95% CI, 2.59%-13.46%], P = .004, Cohen d = 1.20; for SM, mean difference = 5.28% [95% CI, 2.50%-8.06%], P = .000, Cohen d = 0.80). In contrast, the ST presented significantly less activity in the operated leg (proportional $\Delta T2_{ST}$ operated leg = 21.12%, proportional $\Delta T2_{ST}$ contralateral leg = 32.93%) (mean difference = 11.81% [95% CI, 7.25%-16.36%]; P = .000; Cohen d = 0.83) (Figure 8).

With regard to the gracilis muscle and global posterior thigh muscle activity after prone leg curls, no between-group or between-limb differences were found.

DISCUSSION

This study demonstrated significantly higher exercise-related activity of the BF_{LH} after ACLR with an ST tendon autograft when compared with the nonoperated side and with a healthy control group. In contrast, the ST muscle belly presented a significantly lower level of activity at the same postoperative evaluation time (10 ± 1.4 months of follow-up). Neither total posterior thigh muscle activity nor muscle activity of the SM and GRA muscles presented any significant association with ACLR. Because the SM demonstrated an increase in activity in the operated leg compared with the nonoperated side within the ACLR group and this difference was not found in comparing the injured group with the healthy controls, we suggest this finding is related to residual strength deficiencies of the hamstring muscles after surgery and probably not related to neuromuscular adaptations related to ST tendon harvest. Because the SM also has a very limited biomechanical influence on rotational torques imposed upon and controlled around the knee joint, this item is not addressed further in the discussion.[5,13,44]

Although the ST tendon autograft is one of the most popular grafts in ACLR, little attention has been paid to the consequences of harvesting this tendon. Contrary to the evidence linking quadriceps strength after ACLR and outcomes, only limited and contradictory knowledge is available regarding the changes in hamstring muscle function after ST tendon harvest.[3,12,17] Countering external tibial rotation and knee valgus moments, the ST is an established dynamic stabilizer of the knee.[1,16,26] The reduction in ST muscle activity with the corresponding increases in BF muscle activity might induce an essential rotational instability in the knee joint, as ST tendon harvest might even promote external rotation of the tibia due to higher lateral hamstring muscle recruitment while reducing muscle control of internal rotation. In the recent literature, there is increasing interest in this topic because ST tendon harvest might be an additional risk factor for development of early knee osteoarthritis, reinjury, and failure to return to preinjury level of sports.[1,16,26] Better understanding the consequences of autograft harvesting will enable a greater understanding of altered hamstring coordination and postoperative surgical performance and might improve postoperative rehabilitation and clinical

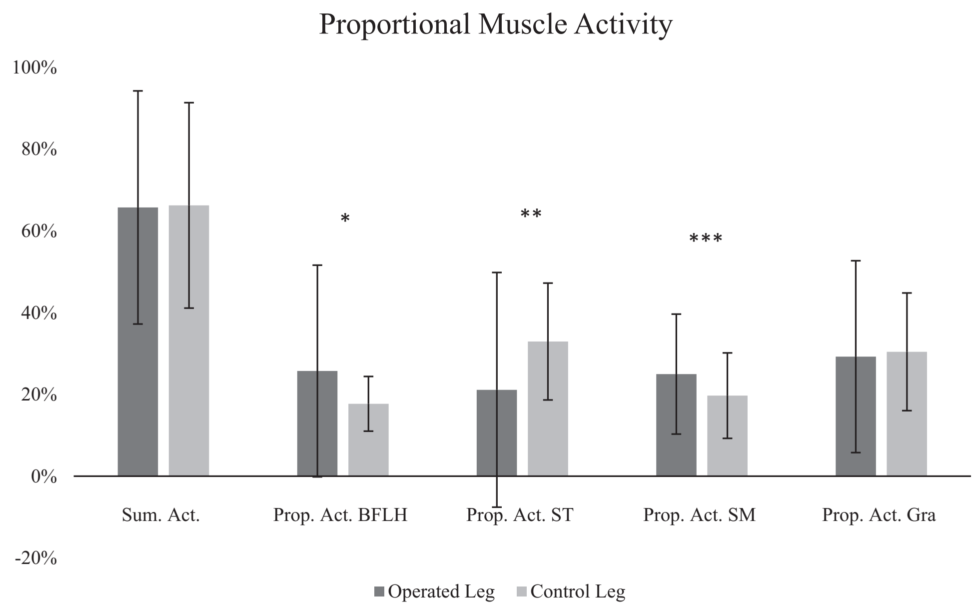

Figure 8. Proportional relative activity-related T2 shifts (proportional ΔT2) in each muscle belly of the control group and of both legs in the ACLR group, in reference to the summated muscle activity of the entire posterior thigh muscle portion. Act, activity; BF_{LH}, long head of the biceps femoris; Gra, gracilis; Prop, proportional; SM, semimembranosus; ST, semitendinosus; Sum, summated; T2, T2 relaxation time constant of muscle water. *Significant between-leg difference of 8.02% on average (*P* = .004). **Significant between-leg difference of 11.81% on average (*P* = .000). ***Significant between-leg difference of 5.28% on average (*P* = .000).

outcomes. Not only would ACLR rehabilitation and injury prevention benefit from these improved insights, but also in terms of hamstring injury prevention and rehabilitation. In terms of hamstring injury prevention and rehabilitation, future research investigating the repercussions of ST tendon harvest on hamstring muscle function is definitely needed. Opar and Serpell[27] stated that a history of hamstring injury and persisting deficits in eccentric hamstring strength might induce excessive biomechanical loads on the ACL in athletes, potentially making them more susceptible to ACL injury. Although this statement is hypothetical, lacking supporting evidence from prospective studies, it is in agreement with the work of Schuermans et al,[34] who established a causal association between decreases in ST and increases in BF activity during prone leg curls and the occurrence of hamstring injury in male soccer players. The finding that patients with either hamstring injuries or ACLR with an ST graft both present aberrant hamstring activation patterns indicates a related origin.

The present study demonstrated that global posterior thigh muscle activation was similar in the ACLR and control groups as well as in both legs of the ACLR group. This finding is in accordance with the similarity in average number of prone leg-curl repetitions performed in the ACLR and the control groups, confirming no significant residual presence of functional deficiencies after surgical intervention and, as such, adequate completion of rehabilitation. Furthermore, this finding also supports our hypothesis that ST harvest has significant repercussions on hamstring muscle function after ACLR; the BF_{LH}, being the ST's most important agonist, will inevitably compensate for ST dysfunction tendon after harvest for ACLR in male soccer players. This is exactly what the present study findings confirmed: a significant relative increase in the activity-related T2 shift in the BF_{LH} in the ACLR group compared with the control group and the contralateral side. Inversely, the ST was activated significantly less in the ACLR group compared with both the control group and the contralateral side within the ACLR group. Similar results were established for the proportional relative activity-related T2 shift (relative activity-related T2 shift in reference to the summated T2 shift of the entire posterior thigh muscle group, including the GRA), with highly significant differences for both the BF_{LH} and ST with, respectively, higher and lower activity after ACLR in comparison with both the control group and the nonoperated leg. Notably, no between-group differences were found for the SM and the GRA. These findings are similar to the results of a recent study by Messer et al,[20] also based on mfMRI, which showed significantly less (up to one-third) activity of the ST in the operated leg compared with the nonoperated leg during the Nordic hamstring exercise at 1 to 6 years after ACLR, with no overall differences in eccentric knee flexor strength. Contrary to our findings, however, Messer et al did not observe significant differences in T2 changes for the BF_{LH} as a consequence of reconstruction. This is a questionable conclusion, as they observed no overall differences in strength but also no significant alterations in BF_{SH} or SM activity. A possible explanation for these contrasting results might be the essential difference in exercise protocol used to induce the activity-related T2 shift in their participants. Messer et al used an isolated eccentric Nordic hamstring exercise, whereas in the present study we used a more dynamic prone leg-curl task that activated the hamstring muscles both concentrically and eccentrically. Other reasons for these different study outcomes might include the Messer study's rather small sample size, remarkably wide range of time since surgery, and lack of a control group. In 2006, Takeda et al[38] used mfMRI to evaluate hamstring muscle function after tendon harvest for ACLR, but they did not find substantial differences in exercise-induced T2 changes in ST muscles relative to the nonoperated leg after a concentric isokinetic knee flexion protocol. When assessing hamstring muscle strength and morphology after ACLR, Nomura et al[26] did not see compensatory BF, SM, or GRA hypertrophy, whereas Maeda et al[19] and Segawa et al[35] described increased contributions from the lateral biceps femoris to accommodate for strength loss in the medial hamstrings.

The present study revealed that although the overall posterior thigh activity presented by the ACLR group at 10 ± 1.4 months postoperatively was comparable with the total hamstring activity in healthy controls, important shifts in relative activity contributions of the BF_{LH} and ST were seen. Our previous work[33,34] stressed the importance of hamstring muscle interplay and intermuscular coordination in prevention of hamstring injuries, with particular emphasis on sufficient recruitment of the ST in knee-oriented, dynamic hamstring muscle loading. Given those findings and the findings of the current study, ST tendon harvest for ACLR should not be assumed to be harmless, and surgeons should take into account functional consequences of this surgical approach in the athletic patient population. One should consider the available scientific evidence regarding the effects of hamstring tendon harvest for ACLR, as these might increase the risk of reinjury and/or hamstring strain injury and could prevent athletes from safely returning to their preinjury level of performance and competition. Indeed, a recent systematic review and meta-analysis of risk factors for recurrent hamstring strain injuries in sports revealed that athletes with a history of ACL injury have a 70% increased risk of hamstring strain injuries.[11] In the investigators' search, the mechanisms responsible for the increased risk after ACL injury remained unclear. Furthermore, it was mentioned that susceptibility to hamstring strain injuries after an ACLR may be associated with ongoing hamstring muscle deficits due to the graft used. In previous work, we have demonstrated that the ST, as a result of a sophisticated, complex neuromuscular coordination within the hamstring muscle complex, plays a prominent role in eccentric hamstring loading and is most activated during the prone leg-curl exercise (compared with the BF and SM muscles).[33,34] It has been demonstrated that the ST is recruited more than both the BF_{LH} and the SM in strength exercises and locomotion and has the highest level of activity during the terminal swing phase, whereas the BF_{LH} is predominantly active from the middle to late swing phase.[14,31] Gathering the available evidence in terms of hamstring mechanics and function/activation properties in strength training and running conditions, it can be concluded that the ST plays a prominent role in producing and controlling the torques around the hip and knee joints under high loading conditions, which will inevitably result in overloading of the neighboring hamstring muscles in case of aberrant recruitment patterns, such as muscle strain.[33,34]

Previous work has shown that morphological alterations occur in the hamstring unit after ST tendon harvest, with a decrease in muscle volume and a proximal shift of the muscle-tendon junction.[2,37] Furthermore, research has shown symmetrical peak knee flexion torque with subsequent hamstring muscle hypertrophy and even ST tendon regrowth to compensate for harvest in 92% of cases.[37] Suydam et al[37] demonstrated a bilateral recovery of biomechanical properties associated with time after surgery, with more symmetrical viscoelastic properties in the second postoperative year. Other studies described compensatory BF_{LH} and SM hypertrophy after ACLR, although study results have been conflicting.[37]

Hamstring muscle strength symmetry after ACLR has been investigated in several studies, yielding inconsistent results. Some studies reported no difference in peak flexion strength between the operated and nonoperated legs up to 2 years after ACLR, whereas other studies identified deficits in internal rotation, reduced knee varus angles during gait and jogging, and decreased knee flexion strength in the operated limb when compared with the nonoperated side.[1,25,28,29,35] In 2017, Abourezk et al[1] showed that hamstring strength asymmetry is common at 3 years after ACLR with a hamstring tendon autograft and significantly affects involved knee mechanics during gait and jogging. Isokinetic strength profiling of the hamstrings, used as a discharge test (to date, still the most important item surgeons use to determine RTP clearance), appears to be a weak indicator for successful RTP.[18,42] This is consistent with our results, as all players were cleared based on an isokinetic test, but the plausibly normalized bilateral strength profile determined by means of isokinetic dynamometry clearly failed to identify the established alterations in hamstring coordination.

This study has some limitations. First, participants in this multicenter study underwent surgery by different surgeons. However, all surgeons were trained by a single senior surgeon (J.V.), and all used a standardized technique for hamstring tendon harvest. A second limitation is the relatively limited sample size, which could impose a type II error. The risk for error is limited, however, because of the power analysis (based on the effect sizes established in previous studies) performed a priori as well as the statistical approach used throughout the data analysis, which allowed us to sufficiently control for both type I and type II errors. Third, this study included only male soccer players. We deliberately chose to solely include male athletes to avoid a sex-confounding factor. Given that muscle coordination patterns might be sex specific and ACL injuries are very common in female athletes as well, future research should assess the effects of hamstring graft harvest for ACLR in a female population. Fourth, the prone leg-curl exercise was used with free weights to impose adequate exercise intensity and to simulate hamstring activation characteristics in running and kicking while providing sufficient eccentric loads. However, hamstring loading in the prone leg-curl exercise is very different from hamstring loading in high-speed running, so the muscle recruitment pattern could differ substantially. This indoor exercise was chosen over a sprinting or kicking protocol because of the importance of exercise intensity and the short half-life of the activity-related signal intensity increase. A fifth limitation that should be taken into account in interpreting the present findings is that the study entailed an exercise performed to the athlete's subjective exertion, which might have given rise to interindividual differences in the actual amount of effort performed during the prone leg curls, as each individual has his own particular load-bearing capacity. However, because we did not find differences in the number of prone leg curls performed in comparing both groups, we consider this limitation to be of minor importance. Sixth, this study focused on the long head of the biceps, because the short head is a monoarticular muscle and is therefore almost never the subject of a strain injury. Furthermore, adding a second center of FOV would have increased the scanning time significantly.

Hence, the present findings might contribute to the valid implementation of more focused, muscle belly–specific, hamstring training in ACLR rehabilitation, in RTP screening protocols, and in primary and secondary injury prevention.[5] Because duration of rehabilitation and RTP clearance are shifting from a time line–based approach toward more criterion-based prerequisites, this study may provide surgeons and physical therapists with improved insights for hamstring muscle conditioning in rehabilitation and prevention programs to improve long-term functional outcomes after ACLR. Whether the effect of these interventions might cause a further decrease of ACL injury incidence, decrease in reinjury rate, and higher rates of return to previous level of performance is a subject for further research.

CONCLUSION

These findings demonstrate an association between the increased amount of BF metabolic muscle activity during hamstring exercises and ST tendon harvest for ACLR, where the ST appeared to be activated significantly less. This reconstruction-associated shift in intramuscular coordination suggests that the BF partly takes over ST function in eccentric loading. These differences might have implications for athletic performance and the risk of injury or reinjury and should be considered in rehabilitation and hamstring injury prevention after ACLR.

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