Effect of Time on MRI Appearance of Graft After ACL Reconstruction

A Comparison of Autologous Hamstring and Quadriceps Tendon Grafts

Joseph A. Panos,* MS, Brian M. Devitt,[†] MD, PhD, Julian A. Feller,^{†‡} MBBS(Hons), Haydn J. Klemm,[†] BFSc&Nutr(Hons), Timothy E. Hewett,[§] PhD, and Kate E. Webster,^{‡||} PhD

Investigation performed at OrthoSport Victoria, Epworth Healthcare, Melbourne, Victoria, Australia, and La Trobe University, Melbourne, Victoria, Australia

Background: After anterior cruciate ligament (ACL) reconstruction (ACLR), changes in the appearance of the ACL graft can be monitored using magnetic resonance imaging (MRI).

Purpose: The purpose of this study was to evaluate and compare the MRI signal intensity (SI) of hamstring and quadriceps tendon grafts during the first postoperative year after ACLR. As a secondary aim, the relationship of SI to clinical and anatomic measurements was analyzed.

Study Design: Cohort study; Level of evidence, 2.

Methods: A total of 78 patients who underwent ACLR with an autologous graft were reviewed; 55 received hamstring grafts and 23 received quadriceps tendon grafts. At 3 and 9 months postoperatively, 3-T MRI was performed using a dedicated knee coil, and the median SI of the intra-articular ACL graft was measured on sagittal-plane images. Postoperative lateral radiographs were analyzed to determine medial and lateral posterior tibial slope (PTS). Side-to-side difference in anterior knee laxity between injured and uninjured limbs was measured at 6 and 12 months postoperatively.

Results: The median SI of quadriceps grafts was significantly greater than hamstring grafts at 3 months after ACLR (P = .02). Between 3 and 9 months, the median SI of quadriceps grafts decreased (P < .001), while that of hamstring grafts did not significantly change (P = .55). The lateral PTS was significantly correlated with median SI measurements at 3 and 9 months such that greater lateral PTS values were associated with greater median SI. The side-to-side difference in anterior knee laxity decreased for the quadriceps group (P = .04) between 6 and 12 months but did not change for the hamstring group (P = .88).

Conclusion: The median SI of quadriceps grafts significantly decreased on MRI between 3 and 9 months after ACLR, while the median SI of hamstring grafts did not significantly change. The change in MRI appearance of the quadriceps grafts was paralleled by a reduction in anterior knee laxity between 6 and 12 months after surgery. In the absence of standardized imaging techniques and imaging analysis methods, the role of MRI in determining graft maturation, and the implications for progression through rehabilitation to return to sport, remain uncertain.

Keywords: anterior cruciate ligament reconstruction; ACL; graft; magnetic resonance imaging; MRI

Injuries to the anterior cruciate ligament (ACL) are a significant source of disability in young, active, and otherwise healthy individuals.⁹ Disruption of the ACL may compromise the stability of the knee and is often treated by surgical reconstruction, commonly using autologous tendon as a graft.²⁸ After surgery, the ACL graft undergoes a process of cytological rearrangement and adaptation to the

biological and mechanical environment of the joint.^{8,39} Previous studies have characterized the graft maturation process in animals from a histological and mechanical standpoint and have defined a process of 4 continuous and overlapping phases consisting of graft necrosis, revascularization, cellular proliferation, and structural remodeling.¹⁹ Investigations into the graft maturation process in humans have been limited to the histological characterization of samples acquired at various time points after ACL reconstruction (ACLR).^{1,10,20,23,35,37} However, these specimens have generally been acquired during so-called second-look

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arthroscopy—typically performed because of patient symptoms—and therefore may not be representative of the natural history of graft maturation during an uncomplicated recovery from ACLR.² As a result, many current research strategies employ noninvasive methods for the assessment of graft maturation.

Magnetic resonance imaging (MRI) is useful in the preoperative diagnosis of ACL injury and is increasingly implemented in the postoperative setting to evaluate graft healing and maturation.¹⁴ While the native ACL appears as a uniform, hypointense structure on MRI, the reconstructed ligament appears relatively hyperintense within the first year after ACLR surgery,³⁰ coinciding with the biological processes of cellular proliferation and extracellular matrix remodeling of the graft. The MRI signal of an ACL graft can be measured by a number of means, including signal-to-noise quotient (SNQ),^{16,26} median signal intensity (SI),⁴ and, recently, with more advanced quantitative methods that require specialized image acquisition protocols.^{7,42} Hofbauer et al¹⁶ used SNQ to evaluate the status of autologous hamstring grafts 6 months after ACLR and concluded that the increased signal intensity in the graft compared with a healthy native ACL indicated a lack of graft maturity. On the other hand, Li et al²⁴ evaluated the MRI SNQ of both ACL autografts and allografts at 3, 6, and 12 months after ACLR and did not find an association between MRI SI and clinical or functional outcomes during the first year, a period when decisions are being made about progression in terms of physical activity.

Mean SI has also been used in prior clinical studies to evaluate ACL graft health and maturation.^{11,18,29,34,38} It has been found to be an independent predictor of graft and ligament failure properties in animal models.^{5,41} Biercevicz et al⁴ showed that in humans, ACL graft volume combined with median SI measured on standard MRI correlated with single-leg hop for distance performance at 3 and 5 years. Beyond 2 years postoperatively, the SI of autogenous grafts declines to the level of the native ACL,³⁰ which likely indicates functional adaptation of the graft to the mechanical and biological environment of the joint. Deviations from this pattern of graft SI on MRI throughout the postoperative period have been associated with graft impingement¹⁷ or graft failure.⁴⁰ Such conditions are identifiable on MRI as focal or diffuse elevations in SI about the graft. As such, previous studies in humans have associated lower SI with advancement of ACL graft maturity and healing.^{3,27,30,32} MRI-based SI measures of maturity have also identified differences between graft types at a single time point (6 months) of the maturation process.²⁷ To date, longitudinal imaging studies have focused on differences between autografts and allografts,^{6,26,30} and few studies have made direct comparisons between different graft types at multiple time points.

The primary aim of this study was to evaluate and compare the MRI appearance of 2 different autologous soft tissue grafts, hamstring tendon (HS) and quadriceps tendon (QT), at 2 time points—3 and 9 months—after ACLR. The secondary aim was to examine the relationship between MRI graft signal and clinical and anatomic factors.

METHODS

Patient Selection Criteria

Patients for this study were part of a larger prospective cohort study performed at a private knee clinic in Melbourne, Australia. The patients in this prospective cohort underwent primary ACLR from March 2016 to June 2018 performed by a single experienced knee surgeon (J.A.F.) using either an HS or a QT graft. Follow-up in these individuals included clinical and functional assessments at 6 and 12 months after ACLR. MRI evaluations were performed at 3 months to assess articular cartilage and bone tunnel position and at 9 months to assess graft integrity. Accordingly, all analyses in the present study utilized these clinical (6 and 12 months) and imaging (3 and 9 months) time points. To be included in the current study, patients were required to have had a knee MRI scan at 3 and 9 months and a clinical follow-up at 6 and 12 months, with an asymptomatic, mechanically stable knee at the 12-month follow-up. We excluded patients who were scheduled for additional surgery to address a concomitant posterior cruciate ligament injury or specific treatment of an associated fracture.

Graft Selection

Graft selection was made by the patient and, in the case of minors, his or her family, after a discussion of graft options with the treating surgeon. QT grafts were not used in skeletally immature patients due to the concern that the graft construct may contain the patellar periosteum, which could potentially pose a risk to the proximal tibial or distal femoral physes (depending on graft orientation). Other contraindications to QT graft choice were inadequate QT size based on body habitus or preoperative measurements of the native QT on MRI.

^{II}Address correspondence to Kate E. Webster, PhD, School of Allied Health, Human Services and Sport, La Trobe University, Victoria 3086, Australia (email: k.webster@latrobe.edu.au) (Twitter: @DrKateWebster).

^{*}Mayo Clinic, Rochester, Minnesota, USA.

[†]OrthoSport Victoria Research Unit, Epworth HealthCare, Melbourne, Victoria, Australia.

⁺School of Allied Health, Human Services and Sport, La Trobe University, Melbourne, Victoria, Australia.

[§]The Rocky Mountain Consortium for Sports Research, Edwards, Colorado, USA.

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Ethical approval for this study was obtained from Epworth Healthcare (study No. 57012).



Figure 1. Image analysis methods. (A) The SNQ was calculated using the following equation: (*Signal of ACL – Signal of PCL*)/ *Background Signal*. The SNQ was calculated for the proximal (circle 3), middle (circle 2), and distal zones (circle 1) of the ACL; circles 4 and 5 denote the PCL and background zones, respectively. (B) The median SI was generated from a manual segmentation of the border of the ACL on sagittal PD-weighted images (shaded green region). The (C) medial and (D) lateral PTS were measured on postoperative lateral radiographs (dashed red lines) as the angle formed between (E) the posterior tibial plateau and a line orthogonal to the tibial axis and tangential to the tibial plateau. ACL, anterior cruciate ligament; PD, proton density; PCL, posterior cruciate ligament; PTS, posterior tibial slope; SNQ, signal-to-noise quotient.

Surgical Technique and Rehabilitation

Arthroscopic ACLR was performed on all patients, with the femoral tunnel drilled via the anteromedial portal. For the HS graft, the semitendinosus and gracilis tendons were harvested and doubled over the loop of an EndoButton CL Ultra (Smith & Nephew). An Ethibond (Johnson & Johnson) whipstitch was used to secure the graft distally; tibial fixation was achieved with a metallic interference screw (Arthrex).

The QT graft was harvested without a bone block, to a width of 12 mm. The maximum length that could be harvested without entering the fibers of the rectus femoris muscle was obtained. QT graft thickness varied between patients, as the deep layer of the QT was kept intact during harvest. The smaller diameter end of the harvested QT graft was attached to an EndoButton (Smith & Nephew) via 2 Ethibond whipstitches. The graft was secured distally with an Ethibond whipstitch and fixed with a metallic interference screw. Measurements of the proximal and distal graft construct diameters were performed intraoperatively.

All patients received the same postoperative rehabilitation protocol. Weightbearing as tolerated was allowed in the immediate postoperative period, and no braces or splints were used. Early restoration of full knee extension and quadriceps strength were encouraged. The presence of pain and swelling was monitored; this dictated the progression through the rehabilitation program. Patients were allowed to ride a stationary bike as soon as they were comfortable (usually between 3 and 4 weeks) and were allowed to commence gymnasium exercises from approximately 5 weeks. Running was allowed once there was no knee effusion and after quadriceps strength was satisfactory (usually 12-16 weeks). Sport-specific drills were commenced from 4 months onward. Return to competition was permitted after at least 1 month of full and unrestricted training (usually after 9-12 months).

MRI Acquisition and Analysis

MRI scans were acquired at 3 and 9 months after surgery. All images were acquired with a 3-T MRI (Siemens) using a dedicated knee coil. Three-plane (sagittal, coronal, and axial) fast spin-echo proton density (PD)–weighted sequences were used, with standardized acquisition parameters as follows: repetition time, 2530 ms; echo time, 36 ms; matrix, 384 \times 384; slice thickness, 3 mm; and total field of view, 130 mm. For image analysis, the ACL graft was identified on sagittal images, manually outlined, and the median SI was calculated using InteleViewer software (Intelerad Medical Systems) (Figure 1). Median SI was used to mitigate the

| | Patient De | | | |
|------------------------|--|---|--|-------------------|
| | $\begin{array}{l} Total \\ (N=78) \end{array}$ | $\begin{array}{l} Ham string \\ (n=55) \end{array}$ | $\begin{array}{c} Quadriceps \\ (n=23) \end{array} \\$ | <i>P</i> Value |
| Age at surgery, y | | | | $.94^b$ |
| $Mean \pm SD$ | 22.1 ± 5.6 | 22.2 ± 5.5 | 22.0 ± 6.1 | |
| Median | 21.1 | 22.0 | 19.4 | |
| Range | 14.2 - 38.2 | 14.2 - 38.2 | 14.4 - 35.2 | |
| BMI, kg/m ² | | | | $.92^{b}$ |
| $Mean \pm SD$ | 24.6 ± 3.1 | 24.6 ± 3.0 | 24.5 ± 3.4 | |
| Median | 24.2 | 24.4 | 24.0 | |
| Range | 18.4 - 34.0 | 18.4 - 34.0 | 19.6 - 32.6 | |
| Sex, n (%) | | | | $.21^c$ |
| Female | 33(42.3) | 26(47.2) | 7(30.4) | |
| Male | 45(57.7) | 29(52.7) | 16 (69.6) | |

TABLE 1 Patient Demographics^a

^aBMI, body mass index; HS, hamstring; QT, quadriceps tendon. ^bUnpaired Student t test between HS and QT grafts.

^cChi-square test between HS and QT grafts.

effects of outlier SI values on the region of interest, arising due to magnetic field inhomogeneities. The SNQ of the proximal, middle, and distal intra-articular portions of the graft was calculated as the difference between the graft signal and the signal of the posterior cruciate ligament divided by the background signal, measured approximately 2 cm anterior to the patellar tendon (Figure 1).⁴¹

To assess the intrarater reliability of the median SI and SNQ measurements, the 3-month images for all patients were measured 3 times, at 1-week intervals, by a single examiner (J.A.P.), and the intraclass correlation coefficient (ICC) was calculated. The ICC for median SI measurements was 0.961. The ICCs for proximal, middle, and distal graft SNQ values were 0.539, 0.763, and 0.692, respectively. Given the superior reliability of median SI over SNQ, only median SI data were reported.

Posterior Tibial Slope Measurement

Postoperative lateral radiographs were used to assess the posterior tibial slope (PTS) (Figure 1). Using ImageJ (National Institutes of Health), the anatomic axis of the tibia was defined as the line passing through the center of 2 circles, each bound by the cortices of the tibia and positioned in the metaphyseal-diaphyseal junction and proximal diaphysis of the tibia. A second line was placed perpendicular to the anatomic axis of the tibia, at the point where the proximal articular surface of the tibia intersected the anatomic axis. The third line was placed along the medial or lateral PTS. The PTS measurement was defined as the angle between the second line and the line placed along the medial or lateral PTS.³¹

Clinical Outcomes

Side-to-side difference (SSD) in anterior knee laxity between the injured and uninjured limb was measured with a KT-1000 arthrometer at an applied force of 134 N.





Figure 2. Median SI of QT and HS grafts at 3 and 9 months after ACLR. Median SI values for QT grafts were 192.6 ± 41.1 and 160.3 ± 42.2 at 3 and 9 months, respectively. For HS grafts, median SI values were 170.6 ± 50.9 and 167.2 ± 46.9 at 3 and 9 months, respectively. **Statistically significant difference (P < .05). ACLR, anterior cruciate ligament reconstruction; HS, hamstring tendon; QT, quadriceps tendon; SI, signal intensity.

The change in anterior knee laxity was calculated as the SSD at 12 months minus the SSD at 6 months. Knee extension deficit was measured using the method of Sachs et al.³⁶ With the patient in the prone position and both legs hanging off the examination table, the difference in heel height between the injured and uninjured limbs was converted to an angular measurement using a formula based on the height of the patient. Patients self-reported preinjury sporting level and the frequency of activity participation. Marx activity scores were collected 6 and 12 months postoperatively.

Statistical Analysis

All statistical analyses were performed in JMP Version 14 (SAS Institute Inc). Unpaired 2-sample Student t tests were used to compare the graft diameters between HS and QT grafts and the median SI values between HS and QT grafts at 3 and 9 months. Paired Student t tests were used to compare the change in median SI between 3 and 9 months and the change in extension deficit and anterior knee laxity for HS and QT grafts between 6 and 12 months. An a priori value of P < .05 was used for statistical significance.

RESULTS

A total of 78 patients (45 male, 33 female) were included in the study; 23 had a QT graft and 55 had a HS graft. The 2 graft type groups did not differ significantly in terms of patient age, body mass index, or sex ($P \ge .21$; Table 1).

| | $Total \ (N=78)$ | Hamstring $(n = 55)$ | $Quadriceps \; (n=23)$ | P Value ^b |
|-----------------------------------|------------------|----------------------|------------------------|----------------------|
| Graft diameter, mm | | | | |
| Proximal | 7.93 ± 0.68 | 7.83 ± 0.56 | 8.17 ± 0.86 | .09 |
| Distal | 8.65 ± 0.74 | 8.61 ± 0.72 | 8.76 ± 0.78 | .43 |
| Anterior knee laxity SSD, mm | | | | |
| 6 months postoperative | 1.67 ± 2.41 | 1.48 ± 2.00 | 2.10 ± 3.19 | .40 |
| 12 months postoperative | 1.42 ± 2.49 | 1.59 ± 2.36 | 1.04 ± 2.78 | .42 |
| P value ^{c} | .13 | .88 | .042 | |
| Extension deficit, deg | | | | |
| 6 months postoperative | 2.03 ± 3.12 | 2.46 ± 2.94 | 1.03 ± 3.36 | .09 |
| 12 months postoperative | 1.32 ± 3.01 | 1.35 ± 3.13 | 1.27 ± 2.78 | .91 |
| P value ^{c} | .07 | .048 | .62 | |
| PTS, deg | | | | |
| Medial PTS | 6.2 ± 2.0 | 6.3 ± 2.0 | 6.1 ± 2.0 | .73 |
| Lateral PTS | 8.5 ± 2.4 | 8.0 ± 2.2 | 9.0 ± 2.6 | .10 |
| Preinjury activity level | | | | |
| Nonsporting | 1 (1.3) | 1 (1.8) | 0 (0) | |
| Sports sometimes | 4 (5.1) | 4 (7.3) | 0 (0) | |
| Frequent sports participant | 35 (44.9) | 24 (43.6) | 11 (47.8) | |
| High-level competitive sports | 33 (42.3) | 23 (41.8) | 10 (43.5) | |
| Professional athlete | 5 (6.4) | 3 (5.5) | 2 (8.7) | |
| Preinjury frequency of activity | | | | |
| No response | 1 (1.3) | 1 (1.8) | 0 (0) | |
| 1-3 days per month | 1 (1.3) | 1 (1.8) | 0 (0) | |
| 1-3 days per week | 28 (35.9) | 23 (41.8) | 5 (21.7) | |
| 4-7 days per week | 48 (61.5) | 30 (54.5) | 18 (78.3) | |
| Marx activity score | | | | |
| 6 months postoperative | 7.6 ± 4.1 | 7.4 ± 4.1 | 8.3 ± 4.1 | .39 |
| 12 months postoperative | 10.9 ± 4.2 | 10.9 ± 3.8 | 10.7 ± 5.1 | .81 |
| P value ^{c} | <.001 | <.001 | .005 | |

| TABLE 2 |
|---|
| Clinical and Radiographic Outcomes ^a |

^{*a*}Data are reported as mean \pm SD or n (%). Bolded *P* values indicate statistically significant difference between groups compared (P < .05). PTS, posterior tibial slope; SSD, side-to-side difference.

^bUnpaired Student t test between hamstring and quadriceps grafts.

^cPaired Student t test between 6- and 12-month points.

The median SI for QT grafts was significantly greater than for HS grafts at 3 months (P = .02) but not at 9 months (P = .74). Between 3 and 9 months, the median SI of QT grafts significantly decreased (P < .001), while the median SI for HS grafts did not change significantly (P = .55). At 9 months, the median SI did not significantly differ between HS and QT grafts (P = .52) (Figure 2).

There was no difference in anterior knee laxity between the QT and HS groups at both 6 and 12 months, but there was a significant decrease in anterior knee laxity from 6 to 12 months in the QT group (Table 2). There was no difference between the 2 groups in terms of extension deficit at either 6 or 12 months (Table 2). Overall, there was no correlation between median SI at 3 or 9 months and anterior knee laxity at either 6 months (median SI at 3 months: r = 0.08; P = .51; median SI at 9 months: r = 0.12;P = .28) or 12 months (median SI at 3 months: r = 0.06; P = .61; median SI at 9 months: r = 0.09; P = .46). However, in the QT group, the significant decrease in median SI between 3 and 9 months was paralleled by a decrease in anterior knee laxity between 6 and 12 months (Table 2). Median SI was not correlated with extension deficit at any time under study (data not shown).

Medial and lateral PTS values did not significantly differ between the HS and QT groups ($P \ge .10$) (Table 2). Lateral PTS measurements were positively but weakly correlated with median SI value at both 3 months (r = 0.29; P = .01) and 9 months (r = 0.25; P = .03) (Figure 3). The medial PTS was not correlated with median SI values at 3 or 9 months ($r \le 0.10$; $P \ge .52$).

DISCUSSION

In this study, we found that on standard MRI, the median SI was greater in QT grafts compared with HS grafts at 3 months but there was no difference between the 2 graft types at 9 months after ACLR. Interestingly, the changes in graft median SI were paralleled by a small but significant decrease in anterior knee laxity in the QT graft group between 6 and 12 months, which may indicate a clinical relevance of the observed decrease in median SI. However, it is unclear whether these MRI changes represent QT graft maturation or some other phenomenon.

Previous studies have characterized the MRI appearance of HS graft after ACLR; several have reported constant



Figure 3. Correlations between median SI and lateral PTS. The lateral PTS was correlated with median SI at both (A) 3 months and (B) 9 months, such that a larger lateral PTS was associated with greater median SI, which indicated a less mature graft. (C) Lateral PTS was not associated with the percentage change in median SI between 3 and 9 months. HS, hamstring tendon; QT, quadriceps tendon; PTS, posterior tibial slope; SI, signal intensity.

 $SNQ^{6,24}$ and SI^{18} values during the first postoperative year. Other groups have compared the effect of remnant preservation on MRI appearance of HS graft.^{13,22} In these studies, control patients undergoing ACLR with standard debridement demonstrated constant SNQ beyond 2 months in 1 report,²² and an increase in SNQ at 6 months, decreasing by 12 months to the early postoperative level, in the other.¹³ Hakozaki et al¹⁵ similarly described an increase in SI at 6 months, followed by a decrease at 12 months to baseline values. While our study did not include imaging at the 6-month point, if HS graft SI values do peak at this time, our results suggest that their return to early postoperative levels could occur as soon as 9 months.

Conversely, there are limited studies exploring the MRI signal of QT grafts and the changes over time. Ma et al²⁷ conducted an MRI-based comparison of HS and QT grafts at 6 months and showed a lower SNQ for QT grafts compared with HS grafts. The results of the current study appear to conflict with these findings; the median SI of QT grafts was greater than that of HS grafts at 3 months but was not different at 9 months. In the current study, SI measurements were used rather than SNQ, due to enhanced intrarater reliability. It is worth noting that Ma et al also reported excellent inter- and intrarater reliability for the SI of the region of interest, but nonetheless used SNQ values, despite the absence of reliability measures for SNQ in their paper. The single period assessed by Ma et al,

which lies between the 2 periods assessed in the present study, could explain the variation in findings; however, it remains unclear why the direction of the differences between the 2 graft types would be dissimilar.

In the current cohort, a modest correlation was observed between PTS and the median SI at both 3 and 9 months, such that a steeper PTS was related to increased median SI values. This is in keeping with the findings of Kiapour et al,²¹ who reported an association between lateral but not medial PTS values, such that a steeper lateral PTS was correlated with increased graft signal on MRI. On the other hand, Li et al²⁵ found no association between medial or lateral PTS and graft appearance on MRI in a mixed cohort of autografts and allografts. In a biomechanical study, Giffin et al¹² examined the effects of increasing tibial slope on knee kinematics and determined that a steeper PTS was associated with greater anterior tibial translation in normal activities of daily living, thus stressing the ACL, which is the primary restraint to anterior translation of the tibia relative to the femur. This may explain the association between increased PTS and higher median SI observed in the current study.

Whether there is value in serial postoperative imaging to inform clinical decisions such as return to sport after ACLR is unclear. In a study by Petersen and Zantop,³³ only 4% of surveyed surgeons cite MRI appearance as a criterion on which to base return-to-sport decisions. However, the relationship between MRI-based measurements of maturity and graft mechanical properties^{5,41} may point to a role of MRI to guide progression through the rehabilitation process, as opposed to making a binary decision about return to sport at the end of rehabilitation protocols. The prognostic capacity of MRI-based maturity assessments in terms of graft ruptures is yet to be investigated. Intuitively, greater graft maturity may be associated with a lower reinjury rate, but at present this is speculative. If this proves to be the case, there would be significant time and costs associated with successive imaging studies, and the interval at which such assessments are most appropriate must be determined. A further limitation of the potential clinical use of MRI as a prognostic tool during the postoperative period after ACLR is the heterogeneity in image acquisition parameters, analysis methodologies, and the variable use of contrast agents.

There are limitations to this study. The median SI and SNQ methods employed are influenced by image acquisition parameters as well as variations in slice selection and definition of the region of interest. To mitigate this shortcoming, we conducted a reliability analysis, which demonstrated superior performance of the median SI over the SNQ method. In addition, the points at which the MRIs were performed were 3 and 9 months postoperatively, whereas the measurements of anterior knee laxity were made at 6 and 12 months. Although the time between the 2 types of assessment was the same (6) months), whether this 3-month offset influenced the results is unknown. Furthermore, baseline measurements of graft SI were not performed and the SI of native QT relative to HS is unknown; how these attributes affect SI values at later points is unclear. Finally, the factors that influence the appearance of the ACL graft remain poorly defined, and it is therefore speculative to relate appearances to graft maturation.

CONCLUSION

The median SI of QT grafts significantly decreased on MRI between 3 and 9 months after ACLR, while the median SI of HS grafts did not significantly change. The change in MRI appearance of the QT grafts was paralleled by a reduction in anterior knee laxity between 6 and 12 months after surgery. While the lateral PTS was correlated with the median SI at discrete points throughout this study, it did not correlate with the change in the median SI of either QT or HS grafts. In the absence of standardized imaging techniques and imaging analysis methods, the role of MRI in determining graft maturation, and the implications for progression through rehabilitation to return to sport, remain uncertain.

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