Remodeling of regenerated hamstring tendons: a magnetic resonance imaging study

Under review

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ABSTRACT

Background: Patients often report pain in the posterior thigh following harvest of the hamstring tendons. This is potentially caused by impaired regeneration of the tendons or altered morphological features of the regenerated structures. Therefore, this study aims to describe the regeneration and remodeling process of the hamstring tendons on magnetic resonance (MR) imaging.

Methods: Patients with anterior cruciate ligament (ACL) injury who underwent reconstruction using the hamstring tendons were included in the current study. MR imaging was preoperatively acquired and at 1- and 2-year follow-up after surgery. Hamstring tendon regeneration and sizes were evaluated at knee joint line level.

Results: 76 out of 93 patients had sagittal and transversal MR images available at 1- and 2-year follow-up. Two years after surgery, semitendinosus (ST) tendons regenerated in 65.8% and gracilis (G) tendons in 82.9%. At 2-years follow-up 10.5% of the patients showed an altered regeneration status compared to the first year after surgery. The sizes of native ST tendons (mean, interquartile range [IQR], 11.6 mm^2 [9.1-13.3]) and gracilis tendons (mean [IQR], 7.3 mm^2, [6.0-8.5]) significantly increased 2 years after surgery to 22.7 mm^2 (IQR 11.2-24.4, p=0.02) and 13.6 mm^2 (IQR 7.8-18.4, p=0.01) respectively. Additionally, musculotendinous junctions shifted proximally in 57.1% of the ST and in 78.6% of the G tendons.

Conclusions: The regeneration status of ST and G tendons changed in 10.5% of the patients over time, resulting in 65.8% and 82.9% respectively at 2-year follow-up. Regenerated tendons are hypertrophic and longer compared to their native ones.

Key words: Anterior Cruciate Ligament Reconstruction; Regenerative Medicine; Translational Research; Hamstring Tendons; Sports Medicine.

INTRODUCTION

Hamstring tendon autografts are widely used to anatomically reconstruct a variety of structures, such as the anterior cruciate ligament (ACL), the medial patellofemoral ligament, lateral ankle ligaments and the coracoclavicular ligament. More specifically, the semitendinosus (ST) tendon and/or gracilis (G) tendon are harvested for these reconstruction procedures. A particularly interesting feature of hamstring tendons is their potential to regenerate after harvest, which is observed in at least 70% of the patients[15]. This regeneration process of the hamstring tendons is less likely to occur with aging and in smokers[16]. Although hamstring tendon regeneration has been investigated extensively, knowledge about the tendon remodeling process is limited.

Hamstring tendon remodeling should be considered as a dynamic and continuous process that changes morphologic characteristics of the tendons over time, such as the cross-sectional area (CSA) and tendon lengths. In addition, impaired or delayed tendon remodeling might influence regeneration rates at different follow-up periods. However, the vast majority of the current literature assesses hamstring tendon regeneration dichotomously and only uses a single follow-up period[15]. Therefore, the process of tendon remodeling following tendon regeneration remains unclear.

Knowledge about hamstring tendon remodeling is of clinical importance for several reasons. First, impaired tendon remodeling and premature rupture of the regenerated structure might cause retraction of the muscle belly, resulting in clinical symptoms such as posterior thigh pain, weakness and cramping[7]. Second, many patients voice concerns about the resection of functional tendons and possibly accompanying functional deficits, potentially caused by impaired remodeling mechanisms. Although it has been suggested before that regenerated tendons could be used as grafts[14, 21], this might partially depend on the quality of the tendon remodeling process.

The current magnetic resonance (MR) study aims to describe the hamstring tendon remodeling process. In order to assess the remodeling process, the current study focuses on three specific outcomes that were repeated at 1-and 2-year follow-up. First of all, impaired or delayed remodeling potentially influences hamstring tendon regeneration rates. Therefore, hamstring tendon regeneration rates were measured at both 1- and 2-year follow-up to assess the quality of the remodeling process. Additionally, the remodeling process is likely to influence morphologic features of tendons, such as CSAs and tendon lengths.

METHODS

Study population

The patients were recruited between January 2009 and November 2010 in a prospective multicenter follow-up study from three hospitals in The Netherlands: Erasmus MC – University Medical Center Rotterdam, Medical Center Haaglanden (The Hague) and Reinier de Graaf Gasthuis (Delft)[18]. In the current study, we included patients that participated in the KNALL (KNee osteoarthritis anterior cruciate Ligament Lesion) study and underwent a surgical ACL reconstruction entailing both the ST and G tendons. Inclusion criteria for this study were (1) ACL rupture diagnosed by physical examination and MRI, (2) MRI was preoperatively acquired within 6 months after trauma, (3) patients were between 18 and 45 years old. Patients who did not speak Dutch, those with previous ACL injury or intra-articular knee trauma or surgery, those with disabling co-morbidity and those with osteoarthritic changes on radiography (Kellgren and Lawrence grade > 0) were excluded. Written informed consent was obtained from all included patients and the institutions' Medical Ethics Committees approved the study (NL 21778.078.08, MEC-2008-068).

MRI measurements

MR examinations were performed before surgical reconstruction (baseline), at 1-and 2-year follow-up. At baseline, MR scans were acquired using three different MR scanners (Philips, Siemens or General Electric). The follow-up MR scans were acquired on the same MR scanner at 1.5 Tesla. Patients' knees were imaged in a neutral position using a dedicated knee coil. Included MR scans have the following MR pulse sequences: sagittal and coronal proton density weighted turbo spin echo (TSE) sequence (slice thickness 3 mm, TR/TE: 2700/27ms), coronal T2-weighted TSE sequence with fat saturation (slice thickness 3 mm, TR/TE: 5030/71 ms), axial proton density and T2-weighted TSE sequence (slice thickness 3 mm, TR/TE: 3500/25/74ms) and sagittal 3D water excitation double-echo steady state (slice thickness 1.5 mm, TR/TE 21.35/7.97ms).

Assessment of regenerated tendons on MRI

To assess presence of regenerated hamstring tendons, both axial and sagittal MR planes at 1- and 2-year follow-up had to be available. Based on previous findings, hamstring tendon regeneration in the current study was subdivided into three different categories: complete, incomplete or no regeneration[15]. If regenerated ST and G tendons could be visualized at the level of the joint line on axial and sagittal planes MR images, the regeneration was considered as complete. Tendon regeneration was considered as incomplete when a tendon-like structure was absent at the joint line level, but could be identified cranially

thereof. If no neotendon could be visualized on any MR image on any level, this was considered as no regeneration.

The cross-sectional area (CSA) of the hamstring tendons was assessed in patients with complete regeneration of both the ST and G tendons and with MR imaging available at baseline (native tendon), 1- and 2-year follow-up (regenerated tendons). CSAs were measured in the axial plane at the joint line level using a commercially available MR image analysis software (AW Server 2.0, GE Health care).

The location of the musculotendinous junction of the ST and G was determined on axial MR images as the most caudal image on which tissue with muscle signal intensity was visualized. This anatomic position was then co-localized on the sagittal plane and the distance between this location and the extension of the joint line was measured. Musculotendinous junctions could be determined in patients with complete regeneration of both hamstring tendons and with MR imaging visualizing the distal musculotendinous junction at all three time points.

All MR measurements were performed by a trained researcher (M.S.) under supervision of a musculoskeletal radiologist (E.O.) and a sports medicine trained orthopaedic surgeon (D.M.) with both more than ten years of experience. Baseline and follow-up MR scans were assessed concurrently and the order of MR measurements was known. Equivocal cases were discussed and solved by consensus.

Statistical analysis

All statistical analyses were performed with IBM SPSS Statistics for Windows (version 21.0, IBM Corp., Armonk, NY). To test for normality, a Shapiro-Wilk's test ($p>0.05$) and inspection of the histograms, normal Q-Q plots and box plots were performed. Interquartile range (IQR) was obtained for non-normally distributed variables. Furthermore, data was tested on skewedness and kurtosis. To determine the interobserver variability 20 randomly chosen scans were re-assessed by a blinded second observer (E.O.), and an inter- and intraclass correlation coefficient (two-way random effects model, absolute agreement) was calculated.

RESULTS

Study population

A flow chart of selection of eligible patients is shown in Figure 1. 93 patients met the inclusion criteria for the current study. Axial and sagittal MRI planes of 76 patients at both post-operative follow-ups were available for analysis. Baseline patient characteristics are presented in Table 1. Mean age at trauma was 25.8 years (SD 6.6) and 65.3% were men.

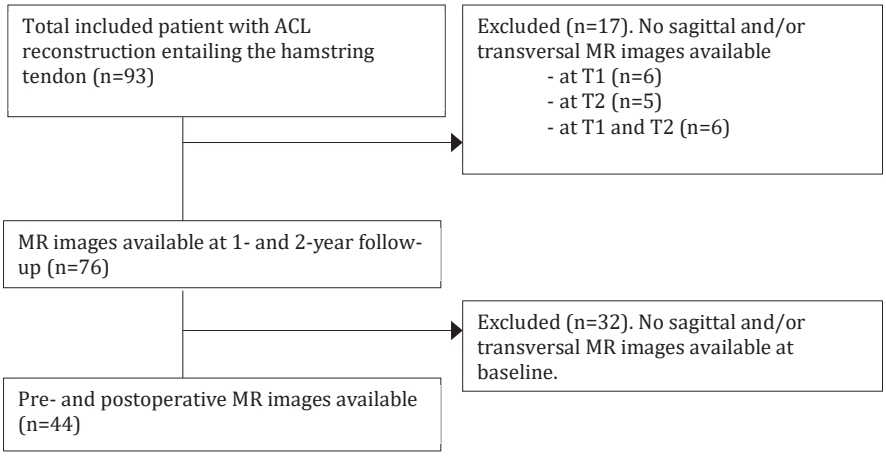

Figure 1. Overview of included patients.
[a]*ACL, Anterior Cruciate Ligament; MR, Magnetic Resonance; T1, 1-year follow-up; T2, 2-year follow-up.*

Table 1. Patient characteristics[a].

	n=76
Age at trauma, y	25.8 ± 6.6
Sex (male) – n (%)	49 (65.3)
Body mass index, kg/m^2	24.2 ± 3.3
Time from surgery to MRI at 1-year follow-up, m	9.5 ± 2.7
Time from surgery to MRI at 2-year follow-up, m	21.1 ± 4.2

[a]*Data are presented as mean ± SD unless otherwise specified.*

Regeneration rates

At 1-year follow-up after harvesting of the hamstring tendons, 43.3% of the patients showed complete regeneration of both tendons. On the contrary, 10.5% of the patients showed no regeneration at all. Complete regeneration of the ST tendon was visualized in 53.9% of the patients, whereas incomplete regeneration was found in 17.1% of the patients. No signs of ST regeneration were found in 28.9% of the patients. The G tendon regenerated in 59.2% of the patients and showed incomplete regeneration in 25.0% of the patients. In 10 patients, complete regeneration of one tendon was accompanied with no regeneration of the other tendon. Regeneration rates 9.5 months after harvest are displayed in Table 2A.

At 2-year follow-up, both tendons regenerated in 42.1% and 11.8% of all patients had no tendon regeneration (Table 2B). Complete regeneration of the ST tendon took place in 53.9% of the patients two years after surgery and incomplete regeneration was found in 11.8%. At 2-year follow-up, the G tendon regenerated completely in 59.2% of the patients and did not regenerate in 17.1% of the patients.

Interestingly, the regeneration status changed in 8 patients (10.5%) over time. The regeneration status of the semitendinosus tendon deteriorated in 50% (4 out of 8), whereas the gracilis tendon deteriorated in only 12.5% (1 out of 8) of the patients. The regeneration status of the semitendinosus and gracilis improved in 37.5% (3 out of 8) and 25% (2 out of 8) respectively at 2-year follow-up. This implies that 6.7% (5 out 76) of the patients improved their regenerated structure over time (Table 3).

At 1-year follow-up, the regeneration starting point was in all 33 cases of incomplete tendon regeneration found at the distal muscle sites. This was confirmed in all 28 cases of incomplete regeneration at 2-year follow-up. No concurrent regeneration sites could be identified.

Table 2. Regeneration rates.

A. Regeneration at 1-year follow-up.

		Semitendinosus			
		Complete	Incomplete	No	
Gracilis	Complete	33 (43.4)	5 (6.6)	7 (9.2)	45 (59.2)
	Incomplete	5 (6.6)	7 (9.2)	7 (9.2)	19 (25.0)
	No	3 (3.9)	1 (1.3)	8 (10.5)	12 (15.8)
		41 (53.9)	13 (17.1)	22 (28.9)	76 (100.0)

n (%).

B. Regeneration 2-year follow-up.

		Semitendinosus			
		Complete	Incomplete	No	
Gracilis	Complete	32 (42.1)	3 (3.9)	10 (13.2)	45 (59.2)
	Incomplete	6 (7.9)	5 (6.6)	7(9.2)	18 (23.7)
	No	3 (3.9)	1 (1.3)	9 (11.8)	13 (17.1)
		41 (53.9)	9 (11.8)	26 (34.2)	76 (100.0)

n (%).

Table 3. Patients with altered regeneration status[a].

Patient number	1-year follow-up		2-year follow-up		Change in regeneration status	
	ST	G	ST	G	ST	G
1	+	+	+	±	=	↓
2	+	+	-	+	↓	=
3	+	+	-	-	↓	↓
4	+	±	-	+	↓	↑
5	±	+	-	+	↓	=
6	±	+	+	+	↑	=
7	±	±	+	±	↑	=
8	±	±	+	+	↑	↑

[a]*ST, semitendinosus; G, gracilis; +, complete regeneration; ±, incomplete regeneration; -, no regeneration, ↑ improvement of regeneration status; ↓ deterioration of regeneration status; =, no change in regeneration status.*

Cross-sectional areas

Of the 30 patients with complete regeneration of both tendons, a total of 15 patients had MR imaging available at baseline, 1- and 2-year follow-up. Representative images are displayed in Figure 2A-C.

The mean CSA of the native ST tendons was 11.6 mm^2 (IQR 9.1-13.3), whereas 9.5 months after tendon harvesting the mean CSA of regenerated ST tendons was increased to 25.1 mm^2 (IQR 15.0-27.0) (p=0.04). Compared to the native tendon, the mean CSA of the ST tendons increased to 22.7 mm^2 (IQR 11.2-24.4, p=0.02) at 2-year follow-up (Figure 3A).

The average CSA of native G tendons was 7.3 mm^2 (IQR 6.0-8.5). This CSA increased to an average of 17.5 mm^2 (IQR 11.2-21.5, p<0.01) 9.5 months after surgery and to 13.6 mm^2 (IQR 7.8-18.4, p=0.01) at 2-year follow-up (Figure 3B).

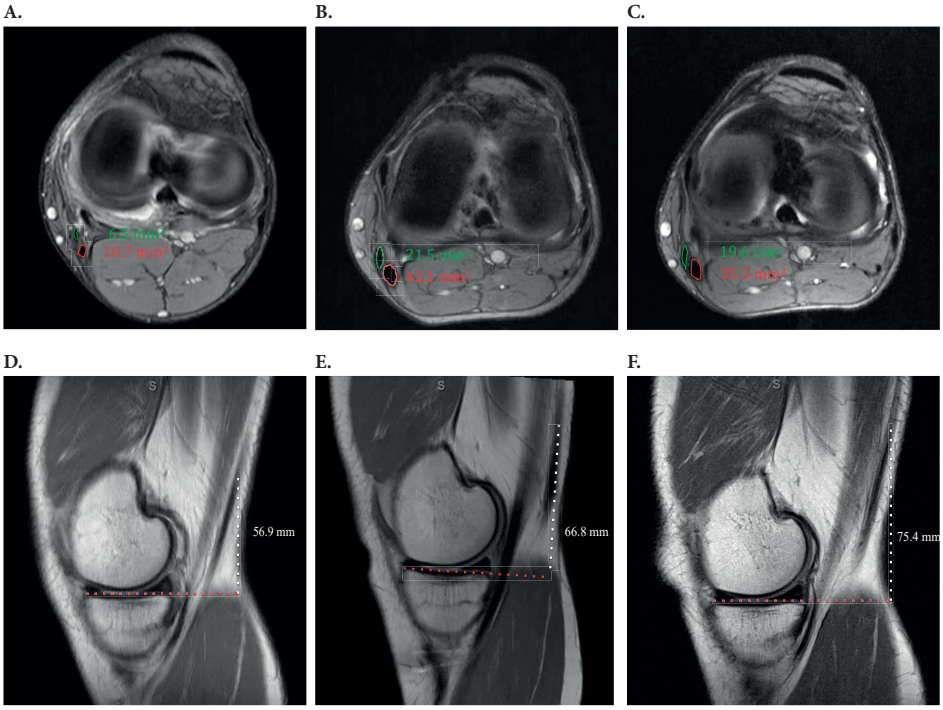

Figure 2. Representative MR images of same patient.

***A-C**: Images of the cross-sectional area of the left knee before surgery (A), at one year follow-up (B), and 2-year follow-up (C). Red color indicates semitendinosus tendon, green color indicates gracilis tendon.*

***D-F**: images of the musculotendinous junction of the semitendinosus tendon in a single patient A) before harvesting, B) at 1-year follow-up, C) at 2-year follow-up. Red dotted line indicates extension of the joint line, white dotted line indicates distance between musculotendinous junction and extension of joint line.*

Tendon lengths

Of the 30 patients with complete regeneration of both tendons, musculotendinous junctions of the ST and G could be visualized at all follow-up measurements in 8 and 14 patients, respectively. A shift in the location of musculotendinous junction of the ST could be determined in 8 patients: the junction was found to be located more proximally at both post-operative time points compared to that of the native ST tendons. Similarly, of 14 patients in whom the musculotendinous junction of the G tendon could be visualized pre-operatively, a proximal shift occurred in 11 patients at both post-operative time-points. Representative images are displayed in Figure 2D-F.

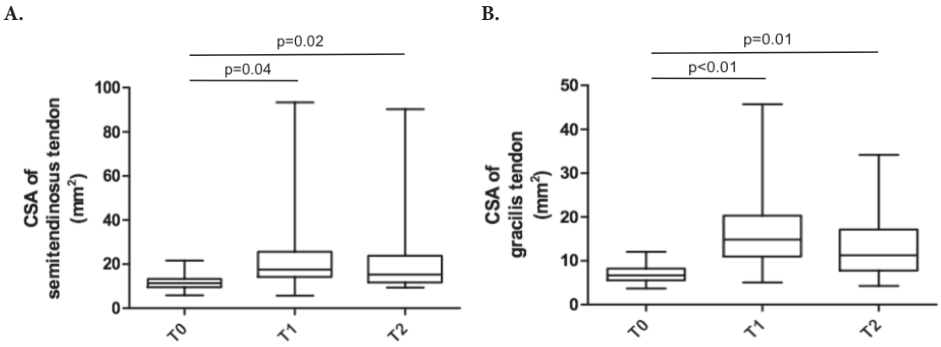

Figure 3. Remodeling of cross-sectional areas.

Boxplots displaying the absolute values of CSA (in mm²) at different time points of the (A) semitendinosus and (B) gracilis tendon.

Mean with second and third quartile. Wickets representing the lowest and highest value.

[a] T_0, baseline; T_1, 1-year follow-up; T_2, 2-year follow-up; ns, not significant.

Inter- and intraclass correlation coefficients

The two observers agreed on presence of complete regeneration and absence of regeneration in every case. Assessment of incomplete regeneration was concordant in 95% of the cases.

The interclass correlation coefficient of CSA at 1-year follow-up in the regenerated ST tendons ranged from 0.97 to 0.99 and for regenerated G tendons from 0.92 to 0.96.

The intraclass correlation coefficient of CSA at 2-year follow-up in the regenerated ST tendons ranged from 0.97 to 0.99 and for regenerated G tendons from 0.93 to 0.97.

DISCUSSION

Due to the prospective follow-up, we were able to increase knowledge about how hamstring tendons remodel. At a 2-year follow-up period ST tendons regenerated in 65.8% and

G tendons regenerated in 82.9%. Interestingly, we reported that the initial regeneration status alters in 10.5% of the patients. Additionally, we reported that regenerated hamstring tendons are hypertrophic and longer compared to their native ones.

With regeneration rates of 65.8% for the ST and 82.9% for the G tendons at a 2-year follow-up period, our findings are in line with previous work[15]. Compared to the ST tendon, the G tendon was more capable of at least partial regeneration. Surprisingly, 5 tendons that showed signs of regeneration one year after surgery, did not have signs of regeneration at the 2-year follow-up time point. We hypothesize that disappearance of the initial visualized structure at 1-year follow-up may be caused by the rupturing of the regenerated structure[10]. Another explanation for this phenomenon is that the human body may have a certain "time point of no success" and that after this time point, the body suspends its own regenerating efforts in the case of non-functionality leading to removal of the newly formed, but dysfunctional tissue. On the other hand, five patients with initially incomplete regenerated tendons, showed complete regeneration at 2-year follow-up.

Over time, various theories have been postulated aiming to explain the capacity and direction of hamstring tendons to regenerate after harvest. Analogous to repair of nerve lesions along an intact neural sheath, several authors considered the anatomic space between the fascial planes of the medial thigh as pathway for regenerating tendons[4, 12, 19]. Based on this, it has been previously hypothesized that tendons regenerate in a proximal to distal fashion along the fascial plane[7]. With presence of the partially regenerated tendons on the distal muscle ends, this study might indirectly support this hypothesis. As more fascial layers cover the ST tendon, regeneration rates of ST tendons could be expected to be higher than those of the G tendon[17]. However, in the current study we found that regeneration rates of the G are increased compared to regeneration rates of the ST. These findings are in line with previous studies[13, 20]. Furthermore, the anatomic space between medial layer I and II is not tubular in shape[2]. Taken this into account, we conclude that this pathway cannot result in a similar shape of the regenerated tendon compared to the native tendons.

A second theory is that after harvest some peritendinous tissue and tendon sheath is left at the most distal end of the ST and G tendon[5, 11]. Fibroblast precursor cells in this tissue then migrate towards the haematoma that is formed in the void space after harvest. The precursor cell then start to proliferate and start to produce collagen. In this hypothesis, the haematoma acts as a scaffold for tendon regeneration[5, 11].

In the current study, we also investigated the CSAs and musculotendinous junction shift in regenerated tendons and these findings were compared with native tendons. We found significantly increased CSAs of the regenerated tendons at both post-operative time points compared to the native tendons. This is in contradiction with Choi et al. who reported no statistical significant difference in CSA in regenerated hamstring tendons

compared with the native tendons[3]. However, the average follow-up period of their study was 3 years. This fits with the observed trend in the current study that showed an initial increase in CSA of the regenerated tendons and gradual decrease over time. Another important finding is that the range of CSAs of the regenerated tendons is increased compared to the range of the native tendons. The phenomenon of tendon hypertrophy after tendon lesion has been described before[1]. The range of increased CSAs is wide, as some regenerated tendons are hypertrophic, whereas others hardly are. This suggests that the extent of hypertrophy depends on patient characteristics. Our finding that the musculotendinous junction of both tendons shifted proximally is in line with previous studies[3, 9]. This finding is of clinical relevance as it has been previously suggested that the extent of muscle retraction might correlate with symptoms, such as cramping, weakness and pain of the posterior thigh[6-8]. Although smaller retractions might be relatively common in asymptomatic patients, patients with higher retractions might report the previous mentioned symptoms. In addition, this study reports that both hamstring tendons can regenerate independently from each other. The extent of retraction might be less if one of both tendons regenerates. Also, the fact that all kind of variations in regeneration are possible might affect clinical outcome.

The primary strength of our study is the large number of included patients. This is, to our knowledge, the first study that has investigated hamstring tendon regeneration in a prospective MRI study in 76 patients. An additional strength of our study is that hamstring tendon regeneration could be assessed at 1- and 2-year follow-up, which has never been performed before. Besides, this is the first study that did not describe regeneration as a dichotomous process, but differentiated between complete, incomplete and no regeneration.

Our study has some limitations. Some patients already underwent an MRI scan before assessment of eligibility for the study, resulting in the use of different MRI scanners. Secondly, a relatively low number of patients could be included for the analysis of the CSA and the musculotendinous junction shift. Although one might suggest that the multicenter aspect of the study and subtle surgical differences affect regeneration rates, this argument has been invalidated by our previous study[16].

Although 65.8% of the ST tendons and 82.9% of the G tendons regenerate, it remains unclear why tendons in some patients only regenerate partially or do not regenerate at all. Therefore, future studies should focus on identifying determinants and molecular mechanisms underlying regeneration processes. Furthermore, the current literature is unclear about the clinical consequences of absence of regeneration[15] and therefore possible symptoms reported by patients without regeneration should be investigated.

In conclusion, the results of this prospective multicenter MR imaging study indicate that the ST tendons regenerate in 65.8% and the G tendons in 82.9% of the patients. There was a change in extent of regeneration in 10.5% of the patients over times, in which

both improvement and deterioration were seen. Additionally, regenerated tendons are hypertrophic and longer compared to their native ones. Future research should focus understanding the cellular and molecular mechanisms of the tendon regeneration and remodeling process.

Perspective

This study provides insight in the dynamics of tendon regeneration processes, in terms of regeneration rates, morphological characteristics and possible molecular pathways of regeneration. An important finding is that an initial regenerated structure may fail over time[10]. Although regenerated tendons might be used for re-reconstruction purposes, the failure of regenerated tendons questions its quality and therefore the use of regenerated hamstring tendons in re-reconstruction procedures[21]. Another interesting observation is that regenerated hamstrings tendons are hypertrophic compared to native tendons, as has been described before for the patellar tendon[1]. The reason for this remains unclear. However, one may hypothesize that this is a protection mechanism of the human body regarding previous injuries. On the other hand, it may be postulated that the quality of the regenerated tendons may be inferior to native tendons and one needs the hypertrophic tendons to resist similar strengths as before. Also, this study contributes to the direction of future translational research in the field of tendon repair processes. As regeneration starts proximally in any case, fibroblast precursor cells in the muscles may migrate towards the hematoma that is formed in the void space after harvest[5, 11].

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