Predictive factors of hamstring tendon regeneration and functional recovery after harvesting: a prospective follow-up study

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ABSTRACT

Background: Semitendinosus and gracilis tendons may regenerate after harvesting for ligament reconstruction procedures. However, predictive factors of tendon regeneration and the extent of functional recovery remain unclear.

Purpose: To identify predictive factors for hamstring tendon regeneration and to examine the morbidity of nonregenerated hamstring tendons.

Study design: Cohort study; Level of evidence, 3.

Methods: Of the 154 patients who were included in a prospective follow-up study, 79 underwent reconstruction of the anterior cruciate ligament entailing the hamstring tendons and met the following inclusion criteria: (1) anterior cruciate ligament rupture diagnosed by physical examination and magnetic resonance imaging (MRI), (2) MRI within 6 months after trauma, (3) age between 18 and 45 years, and (4) 2-year follow-up MRI data available. Hamstring tendon regeneration was assessed as complete if a tendon-like structure could be visualized at level of joint line or more cranially. Patient characteristics - such as age, gender, body mass index, alcohol/nicotine use, activity level (Tegner scores) and functional instability (1-legged hop test) – were evaluated preoperatively and at 2 years to determine predictive factors for tendon regeneration or examine functional recovery of hamstring tendon regeneration.

Results: At 2 years' follow-up, 67.1% of the patients showed regeneration of semitendinosus tendons, 81.0% of gracilis tendons and 59.5% of both tendons. The likelihood of semitendinosus regeneration significantly decreased with aging (odds ratio [OR], 0.92 change per year of age; 95% CI, 0.84-0.99; p=0.03) and smoking (OR, 0.20; 95% CI, 0.05-0.77; p=0.02). No predictive factor was found for gracilis tendon regeneration. Regeneration of the semitendinosus and gracilis tendons was negatively related with smoking (OR, 0.22; 95% CI, 0.06-0.79; p=0.02). Patients without regeneration showed similar postoperative visual analog scale scores during physical activity, similar Tegner scores, and a significant decrease of the upper leg circumference, as compared with their preoperative results. Regardless the regeneration status, 1-legged hop test results significantly increased at 2-year follow-up.

Conclusions: Hamstring tendon regeneration occurs less frequently in older patients and in smokers. However, absence of regenerated tendons does not seem to cause a loss of function.

Key Terms: hamstring tendon regeneration; predictive factors; functional outcome; recovery; anterior cruciate ligament reconstruction.

INTRODUCTION

Anterior cruciate ligament (ACL) rupture is a common sports-related injury of the knee. Estimations of annual incidences reach up to approximately 5 to 8 per 10,000 persons[26, 31]. Numerous graft choices exist for ACL reconstruction, such as hamstring tendons autografts and bone-patellar tendon-bone (BPTB) autografts. Because of donor site morbidity and patellar tendon ruptures with the use of BPTB autografts, hamstring tendon autografts are a commonly employed option[1, 15, 22, 42].

Cross et al. were the first to describe the potential of hamstring tendons to regenerate after harvesting procedures for ACL reconstructions[12]. In a previous study, semitendinosus and gracilis tendons regenerated in at least 70% of the patients after harvesting[37]. Currently, it is unknown why some tendons lack the capacity to regenerate[37]. Mechanical load and controlled mobilization are related to a beneficial effect on tendon recovery after injury[4, 41, 44]. On the contrary, smoking[23], aging[29, 33], and alcohol use[16] are related with tendon healing failure. The role of non-steroid anti-inflammatory drugs (NSAIDs) in healing processes remains unclear[14, 32]. However, the available literature does not describe predictive factors specifically for hamstring tendon regeneration, which could be considered an altogether different process from tendon healing.

A systematic review reported on the morbidity and function loss of nonregenerated hamstrings[37]. The exact mechanism of the absence of hamstring tendon regeneration is presently unclear. Several cases were described in which patients experienced a persistent sharp pain in the dorsal aspect of the thigh in the early stage after surgery, perhaps caused by rupturing of the regenerated structure[28]. Another explanation might be that the human body suspends its regenerating efforts in case of nonfunctional tissue, resulting in a removal of the newly formed but dysfunctional tissue. Although different studies investigated the clinical response to hamstring tendon regeneration, its consequences remain unclear because of conflicting evidence. A systematic review summarized studies that examined the effect of tendon regeneration on hamstring strength and function[37], reporting conflicting evidence regarding the relationship between regeneration status and deep knee flexion. Some studies cited a deep knee flexion deficit among patients without regeneration[11, 30], whereas other studies contradicted this finding[13, 19, 27]. In addition, there is no consensus about the clinical relevance of the number of regenerated hamstring tendons. Some studies suggested that the extent of deep knee flexion deficits is limited if both tendons regenerate[11]. Other studies did not find a relationship between the number of regenerated tendons and strength deficits[19].

Nevertheless, insight into determinants of hamstring tendon regeneration and its clinical consequences is relevant for several reasons. First of all, patients voice concerns about harvesting the tendons of functional muscles and the possible accompanying functional deficits. If predicting factors are identified, the chances of hamstring tendon regeneration

could be estimated more accurately. This may affect the choice of hamstring tendons as an autograft and provide insight in the clinical consequences of regenerated hamstring tendons. After all, knowledge of determinants for hamstring tendon regeneration may lead to life style modification before surgery and changes in rehabilitation programs after surgery.
The aim of the current study was to (1) identify predictive factors for hamstring tendon regeneration and (2) examine the effect of tendon regeneration on hamstring strength and function.

METHODS

Study Population

Between January 2009 and November 2010, patients were included in the Knee Osteoarthritis Anterior Cruciate Ligament Lesion (KNALL) study: a prospective multicenter cohort study with 2 years of follow-up. Patients were recruited from 3 hospitals in The Netherlands: Erasmus MC-University Medical Center Rotterdam, Medical Center Haaglanden (The Hague) and Reinier de Graaf Gasthuis (Delft). Inclusion criteria for the KNALL study were (1) ACL rupture diagnosed by physical examination and magnetic resonance imaging (MRI), (2) MRI made within 6 months after trauma, and (3) age between 18 and 45 years. Patients who did not speak Dutch, those with previous ACL injury or intra-articular knee trauma or surgery, those with disabling comorbidity and those with already osteoarthritic changes on radiographs (Kellgren and Lawrence grade > 0) at baseline were excluded. Patients were treated operatively or nonoperatively independent of the study, according to the decision of the treating physician in accordance with the Dutch ACL guideline[24]. In the current study, operatively treated patients were included when 2-year follow-up MRI, completed questionnaires, and data of physical examination at baseline and 2-year follow-up were available. Patients were excluded if the initial treatment was other than an ACL reconstruction entailing the hamstring tendons. Written informed consent was obtained from all included patients, and the institutions' medical ethics committees approved the study.

Measurements

Two-year follow-up MRI scans were acquired on a 1.5-T MRI scanner. The patient's legs were set in a neutral position through a dedicated knee coil. Details of MRI parameters are shown in Table 1.
Hamstring tendon regeneration was evaluated by an intensively trained researcher (M.S.) who was blinded for clinical information. Hamstring tendon regeneration was assessed at 2 years' follow-up for patients who underwent surgical ACL reconstruction with the hamstring tendons. Equivocal cases were discussed with a musculoskeletal

radiologist (E.O.) and a sports medicine-trained orthopaedic surgeon (D.M.), both with more than ten years of experience, and solved with consensus. Hamstring tendon regeneration was assessed at 2-year follow-up for patients who underwent surgical ACL reconstruction with the hamstring tendons. If regenerated tendons could be visualized at the level of the joint line or more cranially, regeneration was assessed as complete. If no neotendons could be visualized on any MRI scan on any level, this was considered no regeneration. Therefore, 4 subgroups of regeneration were distinguished: regeneration of the semitendinosus tendon and gracilis tendon, regeneration of only the semitendinosus tendon, or regeneration of only the gracilis tendon, and no regeneration of either tendon.

Table 1. Parameters of magnetic resonance imaging[a].

Pulse Sequence	Slice thickness, mm	TR/TE, ms
Sagittal and coronal proton density TSE sequence	3	2700/27
Coronal T2-weighted TSE sequence with fat saturation	3	5030/71
Axial proton density and T2-weighted TSE sequence	3	3500/25/74
Sagittal 3D water excitation double-echo steady state	1.5	21.35/7.97

[a]*TE, echo time; TR, repetition time; TSE, Turbo Spin Echo.*

Harvesting procedure

After an oblique skin incision just medial to the tibial tuberosity, the subcutaneous tissue was dissected to expose the sartorius fascia. A reversed L-shaped incision on this fascia was made to free the whole pes anserinus. The gracilis and semitendinosus tendons were divided from the conjoined tendon of the pes anserinus and whip stitched. Both tendons were harvested with a closed tendon stripper. The sartorius fascia was then sutured in its anatomic position. No drains were used.

Rehabilitation

Rehabilitation consisted of full weightbearing and use protective crutches use for 6 weeks. No immobilisation or brace was applied. Return to play was considered appropriate in concurrence with the advice of the physiotherapist, on average at 8-9 months after surgery. No specific functional or quantitative protocol, such as isokinetic testing, was obligatory.

Data collection

All included patients were requested to complete several questionnaires. One trained medical doctor (B.M.) who was blinded for the regeneration status, performed a standardized physical examination and history taking at baseline and 2 years' follow-up. To evaluate determinants for hamstring tendon regeneration and the clinical consequence of nonregenerated tendons, the following factors and outcome measurements were documented:

- Patient characteristics: sex, age and body mass index at baseline. The role of the patient's sex in the process of tendon regrowth remains unclear[37]. Aging seems to affect tendon regeneration negatively[29, 33]. No data about the correlation of body mass index and tendon regeneration were available. Therefore, patient's body mass index was determined and categorized into 1 or 3 groups: <25, 25-30, and > 30 kg/m^2.
- Mechanical load: mechanical load is associated with a beneficial effect on hamstring tendon regeneration[4, 41, 44]. Therefore, preinjury and 2-year follow-up Tegner scores were analyzed as a reflection of mechanical load.
- Hospital: some studies suggested an effect of surgical proceedings; therefore, the surgeon may be a factor that affects regeneration capacity[37].
- Toxins: smoking[23] and alcohol use[16] seem to negatively affect regeneration changes. The effect of NSAIDs on regeneration remains unclear[14, 32].
- Vascular status: diabetes mellitus (DM) complicates wound healing and has negative effects on tendon-healing processes in animal studies[3, 10]. Moreover, adequate blood supply has been shown to be an important factor for ligament healing[5].
- Clinical consequences
    - o All patients completed the following questionnaires regarding pain, sports activity and knee function: visual analogue scale for knee pain (rest and physical activity)[20], Tegner scale (pretrauma level)[20], Lysholm[6, 7, 21], and International Knee Documentation Committee (IKDC) questionnaire[18, 34, 40].
    - o One-legged hop test (OLHT) was performed, and the upper leg circumference of the affected knee was determined.

Statistical analysis

All statistical analyses were performed with SPSS Statistics for Windows (v 21.0; IBM Corp). Descriptive statistics were used to describe baseline characteristics. Selection of variables was based on the available literature. To analyze predictive factors for hamstring tendon regeneration, the study population was subdivided into 3 groups based on the regeneration status. Multivariable binomial logistic regressions were used to calculate odds ratios (ORs) and 95% CIs for determinants of regeneration of hamstring tendon. Qualitative variables were coded in the following way: sex (man, 0; woman, 1), smoking (no, 0; yes, 1), alcohol use (no, 0; yes, 1), NSAID use (no, 0; yes, 1). Positive predictive values were calculated for the determinants that had a significant relationship in the multivariable model for hamstring tendon regeneration. Factors were tested for multicollinearity. To determine clinical recovery, outcomes of 4 questionnaires and physical examination were compared among the 4 regeneration subgroups (both tendons, semitendinosus tendon only, gracilis tendon only, none) and the nonoperatively treated group (control). Patients who were treated non-operatively were used as controls to examine clinical performance of native tendons after a ruptured ACL. Differences

between baseline and 2-year follow-up scores were statistically tested with paired t-tests. Significance was tested for p-value<0.05. To determine the interobserver variability, 20 randomly chosen scans were reassessed by a blinded second observer (E.O.), and an intraclass correlation coefficient (ICC; 2-way random effects model, absolute agreement) was calculated.

RESULTS

Study population

Of the 143 patients for whom MRI at 2-year follow-up was available, the baseline characteristics are presented in Table 2.

Table 2. Patient characteristics at baseline[a].

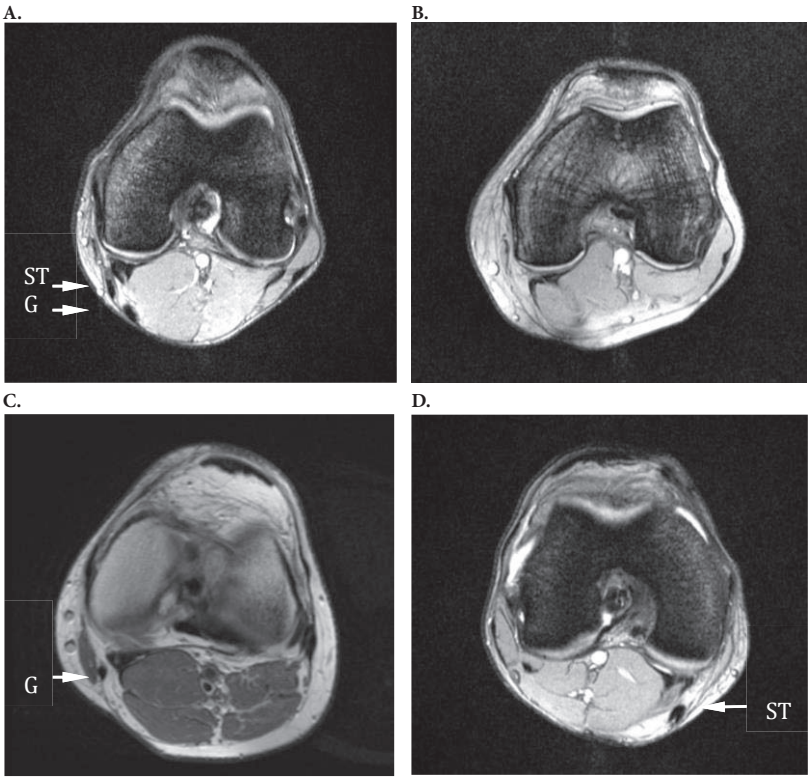
	Median (IQR) or No. (%)
Age, y	25.2 (21.4 – 32.6)
Male	94 (65.7)
Body mass index, kg/m^2	23.9 (22.0 – 26.2)
Injured side: right	76 (53.1)
Pretrauma Tegner score	9 (7 – 9)
Upper leg circumference of index knee, cm	46.7 (43.0 - 48.0)
One leg hop test of index leg, cm	55.0 (25.0 – 85.0)

[a]*IQR, interquartile range.*

During the 2-year follow-up period, 93 patients underwent an ACL reconstruction procedure. A surgical procedure entailing hamstring-tendon grafts was performed in 87 patients (93.5%), BPTB in 4 patients (4.3%), and a combination of hamstring tendon and allograft in 2 patients (2.2%). Postoperative MRI was available for 79 patients who underwent an ACL reconstruction with hamstring tendons. At 2 years' follow-up, semitendinosus and gracilis tendons regenerated in 53 (67.1%) and 64 (81.0%) patients, respectively. No tendon regeneration was reported for 9 (11.4%) patients. Figure 1 displays an overview of the regeneration subgroups. Figure 2 provides a flow chart of inclusion for eligible patients.

Predictive factors

Predictive factors were examined in cases of regeneration of the semitendinosus tendon (n=53), gracilis tendon (n=64), and both tendons (n=47). Regeneration of the semitendinosus tendon was significantly related with age (OR, 0.92 per change per year; 95% CI, 0.84-0.99; p=0.03) and smoking status (OR, 0.20; 95% CI, 0.05-0.77; p=0.02).



ST, semitendinosus; G, gracilis.

Figure 1: Representative magnetic resonance images at joint-line level after hamstrings harvesting.
A *Left knee with regeneration of the semitendinosus and gracilis tendon.*
B *Left knee without regeneration of the semitendinosus and gracilis tendon.*
C *Left knee with only regeneration of the gracilis tendon.*
D *Right knee with only regeneration of the semitendinosus tendon.*

Isolated gracilis tendon regeneration was not related with any of the analyzed predictive factors. Regeneration of both tendons was negatively related with patient's smoking status (OR, 0.22; 95% CI, 0.06-0.79; p=0.02). Table 3 represents an overview of the ORs. Because only 2 patients had diabetes mellitus and no patients were known to have abnormal cardiovascular status, we did not analyze those determinants for hamstring tendon regeneration outcome. Coefficients of determination varied from 26% (semitendinosus tendons) to 31% (semitendinosus and gracilis tendons). No multicollinearity was detected.

For the significant determinants after multivariable analyses, see Table 4, which presents the positive predictive values for tendon regeneration.

Based on the multivariate binomial logistic regression analysis, an approximation of regeneration can be assessed for the semitendinosus tendon and both tendons. For the chance of semitendinosus tendon regeneration,

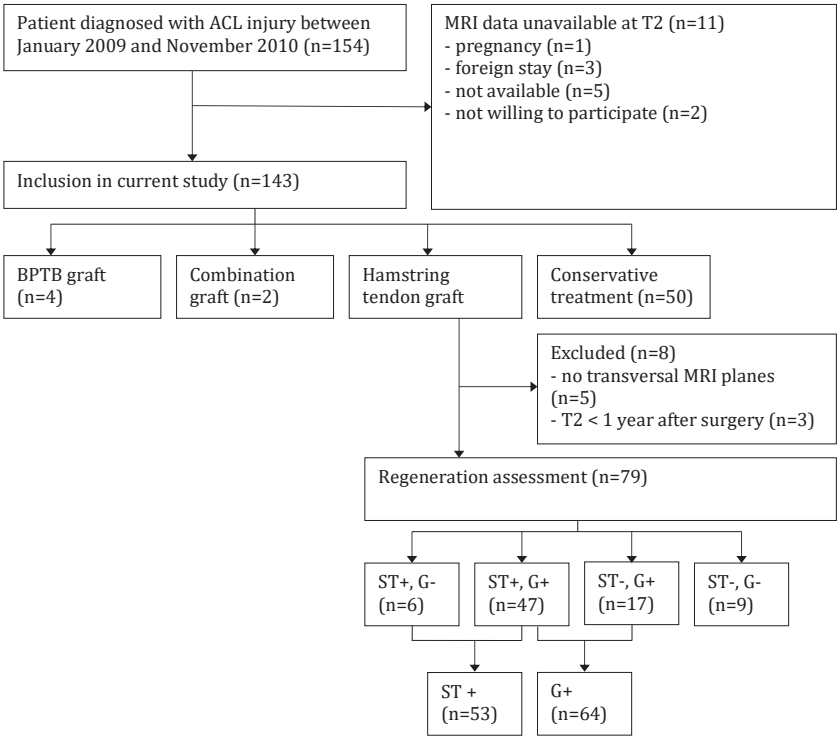


Figure 2. Flowchart.
ACL, anterior cruciate ligament; BPTB, bone-patellar tendon-bone; G, gracilis; MRI, magnetic resonance imaging; ST, semitendinosus. +, regeneration; -, no regeneration.

$$P\,(regeneration\ semitendinosus) = \frac{e^{2.245-(0.094\times age)+1.4(smoking)}}{1+e^{2.245-(0.094\times age)+1.4(smoking)}}.$$

For the chance of regenerating both tendons,

$$P\,(regeneration\ semitendinosus\ and\ gracilis) = \frac{e^{-0.619+1.363(smoking)}}{1+e^{-0.619+1.363(smoking)}}.$$

In both formulas, *e* represents the Euler number. If a patients smokes, the number 1 should be filled in the formula, whereas if the patients does not smoke, the number 0 should be filled in.

Clinical consequences

To analyze the clinical consequences of tendon regeneration, the study population was divided into 4 groups based on regeneration and 1 nonoperative group as control: semitendinosus tendon (n=53), gracilis tendon (n=64), both tendons (n=47), neither tendon (n=9), and control (n=50). When compared with preoperative scores, visual analog scale scores at physical activity significantly decreased for all groups at two years' follow-up (all p-values <0.001), except for the patients who showed no regeneration of

either tendon (p=0.14). Before trauma, Tegner scores were significantly higher for all groups versus 2-year follow-up, except for the patients with no regeneration of either tendon. Furthermore, the circumference of the upper leg decreased significantly from 47.1 cm to 45.5 cm (difference, 1.6; 95% CI of difference, 0.46-2.8; p=0.01) for patients with no regeneration of the semitendinosus and gracilis tendons, whereas patients with regeneration of at least one tendon did not show a similar decrease. One-legged hop test, Lysholm, and International Knee Documentation Committee scores significantly increased over time for all groups as compared with their preoperative scores. Table 5 presents an overview of the functional consequences and hamstring tendon regeneration.

Table 3. Multivariable analysis of possible predictive factors for hamstring tendon regeneration (n=79)[a].

	ST+ (n=53)		G+ (n=64)		ST+/G+ (n=47)	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Sex	1.7 (0.54 – 5.6)	0.35	1.4 (0.38 – 5.4)	0.60	0.69 (0.23 – 2.0)	0.50
Age	**0.92** (0.84 - 0.99)	**0.03**[b]	1.0 (0.92 – 1.1)	0.92	0.95 (0.88 – 1.0)	0.16
NSAID use	0.57 (0.15 – 2.2)	0.42	1.1 (0.23 – 5.1)	0.93	0.52 (0.15 – 1.9)	0.31
BMI[c], kg/m^2						
25-30	1.6 (0.43 – 6.3)	0.47	2.1 (0.46 – 9.3)	0.35	1.8 (0.52 – 6.1)	0.36
>30	0.27 (0.02 – 3.8)	0.33	1.0 (0.06 – 17)	0.99	0.27 (0.02 – 3.6)	0.32
Smoking	**0.20** (0.05 – 0.77)	**0.02**[b]	0.40 (0.10 – 1.6)	0.19	**0.22** (0.06 – 0.79)	**0.02**[b]
Alcohol	1.3 (0.36 – 4.9)	0.67	0.86 (0.21 – 3.5)	0.83	1.7 (0.50 – 5.7)	0.41
Surgeon[d]						
2	1.1 (0.17 – 7.1)	0.91	3.0 (0.31 – 30)	0.34	2.4 (0.41 – 14)	0.33
3	0.67 (0.08 – 5.4)	0.71	2.6 (0.21 – 33)	0.45	1.6 (0.22 – 11)	0.65
4	0.21 (0.01 – 6.2)	0.37	N/A		0.60 (0.02 – 15)	0.75
5	0.54 (0.11 – 2.6)	0.44	1.3 (0.24 – 6.7)	0.79	1.3 (0.31 – 5.5)	0.71
6	1.7 (0.20 – 15)	0.62	N/A		3.5 (0.45 – 27)	0.23

[a]*BMI, body mass index; G, gracilis; N/A, not available; NSAID, nonsteroid anti-inflammatory drug; OR, odds ratio; ST, semitendinosus; +, regeneration.*
[b]*p-value<0.05.*
[c]*Reference: <25 kg/m^2.*
[d]*Reference: surgeon 1.*

Table 4 Positive predictive values of tendon regeneration[a].

	ST+			ST+/G+		
Smoking	Yes[b]	No	PPV	Yes[c]	No	PPV
Yes	9	11	0.45	7	13	0.35
No	44	15		40	19	

[a]*G, gracilis; PPV, positive predictive value; ST, semitendinosus.; +, regeneration.*
[b]*Prior chance: 67.1% (53 of 79).*
[c]*Prior chance: 59.5% (47 of 79).*

Table 5. Functional consequences and hamstring tendon regeneration[a].

		Mean (SD)		Difference	
		T0	T2	(95% CI)	p-value
VAS (at rest)[b]	ST+ (n=53)	1.1 (1.6)	0.47 (0.9)	0.65 (0.20 – 1.1)	0.005[c]
	G+ (n=64)	1.2 (1.8)	0.52 (1.0)	0.69 (0.22 - 1.2)	0.005[c]
	ST+/G+ (n=47)	1.1 (1.7)	0.52 (0.95)	0.61 (0.11 – 1.1)	0.017[c]
	ST-/G- (n=9)	0.83 (0.85)	0.34 (0.45)	0.49 (-0.26 – 1.2)	0.17
	control (n=50)	0.71 (1.2)	0.41 (0.75)	0.29 (-0.07 - 0.66)	0.11
VAS (during movement)	ST+	2.8 (2.5)	0.73 (0.99)	2.1 (1.4 - 2.8)	<0.001[c]
	G+	2.8 (2.6)	0.86 (1.2)	1.9 (1.2 – 2.6)	<0.001[c]
	ST+/G+	2.7 (2.5)	0.80 (1.0)	1.9 (1.1– 2.6)	<0.001[c]
	ST-/G-	2.5 (2.0)	1.1 (1.3)	1.3 (-0.52 - 3.2)	0.14
	control	2.3 (2.2)	1.0 (1.5)	1.3 (0.55 - 2.0)	<0.001[c]
Tegner	ST+	8.3 (1.4)[d]	7.1 (1.9)	1.2 (0.68 - 1.6)	<0.001[c]
	G+	8.3 (1.4)[d]	6.8 (1.9)	1.5 (1.0 – 1.9)	<0.001[c]
	ST+/G+	8.3 (1.4)[d]	7.1 (1.9)	1.1 (0.66 – 1.6)	<0.001[c]
	ST-/G-	7.8 (1.4)[d]	6.4 (1.5)	1.3 (-0.10 – 32.8)	0.65
	control	7.5 (1.6)[d]	5.5 (2.0)	2.0 (1.4 - 2.6)	<0.001[c]
Lysholm	ST+	77.2 (13.1)	93.0 (7.2)	15.8 (12.5 – 19.2)	<0.001[c]
	G+	75.4 (16.0)	92.8 (7.3)	17.4 (13.5 – 21.2)	<0.001[c]
	ST+/G+	76.9 (13.7)	92.8 (7.4)	15.9 (12.1 – 19.6)	<0.001[c]
	ST-/G-	64.6 (11.7)	87.8 (15.8)	23.2 (9.1 – 37.3)	0.005[c]
	control	74.6 (16.8)	91.6 (12.3)	17.0 (11.6 - 22.4)	<0.001[c]
IKDC	ST+	54.4 (14.9)	87.6 (10.4)	33.2 (28.9 – 37.5)	<0.001[c]
	G+	52.9 (16.3)	87.8 (11.2)	34.9 (30.4 – 39.3)	<0.001[c]
	ST+/G+	54.2 (14.9)	87.9 (10.3)	33.7 (29.0 – 38.4)	<0.001[c]
	ST-/G-	50.8 (11.3)	85.2 (16.0)	35.4 (18.7 – 50.0)	0.001[c]
	control	59.2 (19.0)	84.3 (14.7)	25.1 (10.2 – 30.9)	<0.001[c]
One-legged hop test (cm)	ST+	53.2 (38.7)	110.8 (29.4)	57.6 (46.8 – 68.3)	<0.001[c]
	G+	52.5 (38.9)	113.1 (30.9)	60.5 (50.7 -70.4)	<0.001[c]
	ST+/G+	54.1 (38.6)	111.2 (30.1)	57.1 (46.2 – 68.1)	<0.001[c]
	ST-/G-	37.4 (40.2)	94.6 (26.6)	57.1 (31.1 – 83.1)	0.001[c]
	control	57.9 (37.9)	101.3 (36.5)	43.3 (33.4 - 53.3)	<0.001[c]
Circumference upper leg (cm)	ST+	45.6 (4.8)	45.4 (3.7)	0.19 (-0.83 – 1.2)	0.71
	G+	47.3 (12.8)	45.7 (3.6)	1.6 (-1.5 – 4.8)	0.31
	ST+/G+	45.8 (5.0)	45.6 (3.8)	0.23 (-0.90 – 1.4)	0.68
	ST-/G-	47.1 (4.7)	45.5 (4.5)	1.6(0.46 – 2.8)	0.01[c]
	control	46.6 (5.5)	46.3 (4.4)	0.31 (-0.62 - 1.3)	0.50

[a]*G, gracilis; IKDC, International Knee Documentation Committee; ST, semitendinosus; T0, preoperative; T2, 2-year follow-up; VAS, visual analog scale; + regeneration; -, no regeneration.*
[b]*Sample sizes apply to each grouping.*
[c]*$p<0.05$.*
[d]*Pretrauma Tegner.*

Inter- and intracorrelation coefficients

The 2 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 cross-secitonal areas in the regenerated semitendinosus tendons ranged from 0.97 to 0.99 and for regenerated gracilis tendons from 0.92 to 0.96.
The intraclass correlation coefficient of cross-sectional areas in regenerated semitendinosus tendons ranged from 0.97 to 0.99 and for regenerated gracilis tendons from 0.93 to 0.97.

DISCUSSION

Hamstring tendon regeneration occurs in at least 70% of the patients[37]. The results of this prospective observational follow-up study show that hamstring tendon regeneration occurs significantly less frequent in patients who smoke. Furthermore, semitendinosus tendons are less likely to regenerate in older patients. If none of the harvested tendons regenerated, patients did not report improved physical activity and a significant decrease of their upper leg circumference was observed.
In the current study, semitendinosus tendons regenerated more often than gracilis tendons. This finding is in line with previous studies[37-39]. To explain the difference in regenerative capacity, we developed the following hypothesis: that hamstring tendon regeneration occurs behind the deep layer of the thigh fascia. Regarding this fascia, the gracilis tissue plane is covered and protected to a lesser extent than to the semitendinosus tendon. This anatomic difference may explain inferior gracilis tendon regeneration rates versus those of the semitendinosus tendons.
Although previous literature described several determinants for tendon healing, potential predictive factors for hamstring tendon regeneration have not been investigated; therefore, this study is the first to evaluate potential predictive factors for hamstring tendon regeneration based on known factors for tendon healing. For regeneration of the semitendinosus tendon, we identified age and smoking as predictive factors. Age-related changes in tendons include loss of cellularity, loss of vascularity, and fatty infiltration[17]. The latter two are mainly thought to be responsible for less regenerative capacity in tendons. The exact mechanism of smoking on hamstring tendon regeneration remains unclear. It could be that nicotine, as a known major vasoconstrictor, affects tendons' regeneration chances by decreasing the blood supply to former harvest sites[25]. However, nicotine use could also be a marker for unhealthy lifestyles. Nonetheless, based on these results, it remains hard to predict an individual's capacity for hamstring tendon regeneration after harvest procedure. As with common orthopaedic conditions, we suggest a model of intrinsic and extrinsic factors that produce an indication of susceptibility for regenerating

processes. However, identifying the cause and genetic linkage of orthopaedic phenotypes has proven to be complex and requires further investigation. Therefore, the current study points out that regeneration of the semitendinosus is related to patient's age and smoking habits, but it may be that genetic factors also contribute to one's regeneration capacity.

This study is the first to examine functional consequences of hamstring tendon regeneration in 5 subgroups: regeneration of 1 tendon only, regeneration of both tendons, no regeneration of either tendon, and a nonoperatively treated group (control). Although the primary function of the hamstring muscles is to flex the knee or to decelerate its extension, the hamstring muscles control anterior translation of the tibia, sharing the stress with the ACL. However, we found that all patients experience better knee stability at 2 years' follow-up, regardless of regeneration status of the hamstring tendons. Second, several previous studies used the 1-legged hop test for distance to examine strength and confidence in the tested leg[34, 35]. In the current study, all groups showed a significant increase in the 1-legged hop test results, suggesting that the number of regenerated tendons does not affect clinical performance. An increase of 1-legged hop test results has been reported[2]; however, this study did not differentiate between patients with and without regenerated tendons. In addition, Choi et al. reported no statistically significant difference between the number of regenerated tendons and 1-legged hop test results[11]. This is in line with the findings of the current study.

Furthermore, we found that the circumference of the operated upper leg is significantly decreased among patients without regeneration versus patients showing regeneration of one or more hamstring tendons. A previous study reported that the majority of the upper leg atrophy involves the semitendinosus and gracilis muscles[36], although this could not be confirmed with measurements in the current study.

A previous study of 45 patients investigated the relationship among tendon regeneration, flexor strength, and functional tests at a minimum follow-up of 2 years, reporting that individual tendon regeneration was associated with fewer knee flexion deficits at 70° and with improved performances on the carioca test[11]. Taken together, the results may suggest that lack of regeneration results in knee flexion deficits because of muscle atrophy of the harvested tendons.

The current study confirms previous studies' findings of a significant decrease of upper leg circumference in the case of no tendon regeneration, which suggests muscle atrophy[8, 43]. These studies showed a compensatory hypertrophy of the biceps femoris. However, this could not adequately compensate the loss of muscle volume measured in the harvested medial hamstrings[9, 36]. Unfortunately, most of these studies compared clinical outcomes postoperatively regardless of an individual's regeneration status. In addition, only relatively short-term follow-up studies are available. So, despite some strong indications to the clinical relevance of hamstring tendon regeneration, it remains to be seen if different

degrees of muscle atrophy and tendon regeneration will have any clinically relevant effect on patients at longer-term follow-ups.

The strengths of the current study are its prospective design, availability of baseline and follow-up MRI, extensive physical examination, and questionnaires at baseline and follow-ups. Because of these strengths, we were able to identify predictive factors and clinical consequences of hamstring tendon regeneration for different subgroups.

This study also has some limitations. The parameters used to evaluate the clinical consequences of regeneration may be debatable, as they may be not specific for hamstring tendons. However, there is currently no test to evaluate the function of the semitendinosus and gracilis muscles. Although there are no functional consequences, determining muscle function with Biodex measurements may be useful. Another limitation of our study is that patients showing regeneration of both t tendons were included in analysis for semitendinosus and gracilis regeneration separately; therefore, those 3 groups have a certain overlap.

In conclusion, the current study reported that semitendinosus and gracilis tendons regenerated in 67.1% and 81.0% of patients, respectively. Furthermore, it points out that regeneration of the semitendinosus tendon is related with an individual's age and smoking habits. Likewise, regeneration of both hamstring tendons is negatively related to smoking habits. However, absence of regenerated tendons does not seem to cause a loss of function.

REFERENCES

1. Ahlden M, Samuelsson K, Sernert N, Forssblad M, Karlsson J, Kartus J. The Swedish National Anterior Cruciate Ligament Register: a report on baseline variables and outcomes of surgery for almost 18,000 patients. *Am J Sports Med.* 2012;40(10):2230-2235.
2. Ahlen M, Liden M, Bovaller A, Sernert N, Kartus J. Bilateral magnetic resonance imaging and functional assessment of the semitendinosus and gracilis tendons a minimum of 6 years after ipsilateral harvest for anterior cruciate ligament reconstruction. *Am J Sports Med.* 2012;40(8):1735-1741.
3. Bedi A, Fox AJ, Harris PE, et al. Diabetes mellitus impairs tendon-bone healing after rotator cuff repair. *J Shoulder Elbow Surg.* 2010;19(7):978-988.
4. Berendsen AD, Smit TH, Walboomers XF, Everts V, Jansen JA, Bronckers AL. Three-dimensional loading model for periodontal ligament regeneration in vitro. *Tissue Eng Part C Methods.* 2009;15(4):561-570.
5. Bray RC, Leonard CA, Salo PT. Vascular physiology and long-term healing of partial ligament tears. *J Orthop Res.* 2002;20(5):984-989.
6. Briggs KK, Kocher MS, Rodkey WG, Steadman JR. Reliability, validity, and responsiveness of the Lysholm knee score and Tegner activity scale for patients with meniscal injury of the knee. *J Bone Joint Surg Am.* 2006;88(4):698-705.
7. Briggs KK, Lysholm J, Tegner Y, Rodkey WG, Kocher MS, Steadman JR. The reliability, validity, and responsiveness of the Lysholm score and Tegner activity scale for anterior cruciate ligament injuries of the knee: 25 years later. *Am J Sports Med.* 2009;37(5):890-897.
8. Burks RT, Crim J, Fink BP, Boylan DN, Greis PE. The effects of semitendinosus and gracilis harvest in anterior cruciate ligament reconstruction. *Arthroscopy.* 2005;21(10):1177-1185.
9. Carofino B, Fulkerson J. Medial hamstring tendon regeneration following harvest for anterior cruciate ligament reconstruction: fact, myth, and clinical implication. *Arthroscopy.* 2005;21(10):1257-1265.
10. Chbinou N, Frenette J. Insulin-dependent diabetes impairs the inflammatory response and delays angiogenesis following Achilles tendon injury. *Am J Physiol Regul Integr Comp Physiol.* 2004;286(5):R952-957.
11. Choi JY, Ha JK, Kim YW, Shim JC, Yang SJ, Kim JG. Relationships among tendon regeneration on MRI, flexor strength, and functional performance after anterior cruciate ligament reconstruction with hamstring autograft. *Am J Sports Med.* 2012;40(1):152-162.
12. Cross MJ, Roger G, Kujawa P, Anderson IF. Regeneration of the semitendinosus and gracilis tendons following their transection for repair of the anterior cruciate ligament. *Am J Sports Med.* 1992;20(2):221-223.
13. Eriksson K, Hamberg P, Jansson E, Larsson H, Shalabi A, Wredmark T. Semitendinosus muscle in anterior cruciate ligament surgery: Morphology and function. *Arthroscopy.* 2001;17(8):808-817.
14. Ferry ST, Dahners LE, Afshari HM, Weinhold PS. The effects of common anti-inflammatory drugs on the healing rat patellar tendon. *Am J Sports Med.* 2007;35(8):1326-1333.
15. Granan LP, Forssblad M, Lind M, Engebretsen L. The Scandinavian ACL registries 2004-2007: baseline epidemiology. *Acta Orthop.* 2009;80(5):563-567.
16. Hapa O, Cakici H, Gideroglu K, Ozturan K, Kukner A, Bugdayci G. The effect of ethanol intake on tendon healing: a histological and biomechanical study in a rat model. *Arch Orthop Trauma Surg.* 2009;129(12):1721-1726.

17. Hashimoto T, Nobuhara K, Hamada T. Pathologic evidence of degeneration as a primary cause of rotator cuff tear. *Clin Orthop Relat Res.* 2003(415):111-120.

18. Irrgang JJ, Anderson AF, Boland AL, et al. Development and validation of the international knee documentation committee subjective knee form. *Am J Sports Med.* 2001;29(5):600-613.

19. Janssen RP, van der Velden MJ, Pasmans HL, Sala HA. Regeneration of hamstring tendons after anterior cruciate ligament reconstruction. *Knee Surg Sports Traumatol Arthrosc.* 2013;21(4):898-905.

20. Jensen MP, Miller L, Fisher LD. Assessment of pain during medical procedures: a comparison of three scales. *Clin J Pain.* 1998;14(4):343-349.

21. Kocher MS, Steadman JR, Briggs KK, Sterett WI, Hawkins RJ. Reliability, validity, and responsiveness of the Lysholm knee scale for various chondral disorders of the knee. *J Bone Joint Surg Am.* 2004;86-a(6):1139-1145.

22. Magnussen RA, Granan LP, Dunn WR, et al. Cross-cultural comparison of patients undergoing ACL reconstruction in the United States and Norway. *Knee Surg Sports Traumatol Arthrosc.* 2010;18(1):98-105.

23. Mallon WJ, Misamore G, Snead DS, Denton P. The impact of preoperative smoking habits on the results of rotator cuff repair. *J Shoulder Elbow Surg.* 2004;13(2):129-132.

24. Meuffels DE, Poldervaart MT, Diercks RL, et al. Guideline on anterior cruciate ligament injury. *Acta Orthop.* 2012;83(4):379-386.

25. Mosely LH, Finseth F. Cigarette smoking: impairment of digital blood flow and wound healing in the hand. *Hand.* 1977;9(2):97-101.

26. Moses B, Orchard J, Orchard J. Systematic review: Annual incidence of ACL injury and surgery in various populations. *Res Sports Med.* 2012;20(3-4):157-179.

27. Nakamae A, Deie M, Yasumoto M, et al. Three-dimensional computed tomography imaging evidence of regeneration of the semitendinosus tendon harvested for anterior cruciate ligament reconstruction: a comparison with hamstring muscle strength. *J Comput Assist Tomogr.* 2005;29(2):241-245.

28. Nakamae A, Ochi M, Deie M, Adachi N. Unsuccessful regeneration of the semitendinosus tendon harvested for anterior cruciate ligament reconstruction: report of two cases. *Orthop Traumatol Surg Res.* 2012;98(8):932-935.

29. Nho SJ, Yadav H, Shindle MK, Macgillivray JD. Rotator cuff degeneration: etiology and pathogenesis. *Am J Sports Med.* 2008;36(5):987-993.

30. Nishino A, Sanada A, Kanehisa H, Fukubayashi T. Knee-flexion torque and morphology of the semitendinosus after ACL reconstruction. *Med Sci Sports Exerc.* 2006;38(11):1895-1900.

31. Nordenvall R, Bahmanyar S, Adami J, Stenros C, Wredmark T, Fellander-Tsai L. A population-based nationwide study of cruciate ligament injury in Sweden, 2001-2009: incidence, treatment, and sex differences. *Am J Sports Med.* 2012;40(8):1808-1813.

32. O'Connor JP, Lysz T. Celecoxib, NSAIDs and the skeleton. *Drugs Today (Barc).* 2008;44(9):693-709.

33. Provenzano PP, Hayashi K, Kunz DN, Markel MD, Vanderby R, Jr. Healing of subfailure ligament injury: comparison between immature and mature ligaments in a rat model. *J Orthop Res.* 2002;20(5):975-983.

34. Sachs RA, Daniel DM, Stone ML, Garfein RF. Patellofemoral problems after anterior cruciate ligament reconstruction. *Am J Sports Med.* 1989;17(6):760-765.

35. Seto JL, Orofino AS, Morrissey MC, Medeiros JM, Mason WJ. Assessment of quadriceps/hamstring strength, knee ligament stability, functional and sports activity levels five years after anterior cruciate ligament reconstruction. *Am J Sports Med.* 1988;16(2):170-180.

36. Snow BJ, Wilcox JJ, Burks RT, Greis PE. Evaluation of muscle size and fatty infiltration with MRI nine to eleven years following hamstring harvest for ACL reconstruction. *J Bone Joint Surg Am.* 2012;94(14):1274-1282.

37. Suijkerbuijk MA, Reijman M, Lodewijks SJ, Punt J, Meuffels DE. Hamstring Tendon Regeneration After Harvesting: A Systematic Review. *Am J Sports Med.* 2014.

38. Tadokoro K, Matsui N, Yagi M, Kuroda R, Kurosaka M, Yoshiya S. Evaluation of hamstring strength and tendon regrowth after harvesting for anterior cruciate ligament reconstruction. *Am J Sports Med.* 2004;32(7):1644-1650.

39. Takeda Y, Kashiwaguchi S, Matsuura T, Higashida T, Minato A. Hamstring muscle function after tendon harvest for anterior cruciate ligament reconstruction: evaluation with T2 relaxation time of magnetic resonance imaging. *Am J Sports Med.* 2006;34(2):281-288.

40. van Meer BL, Meuffels DE, Vissers MM, et al. Knee injury and Osteoarthritis Outcome Score or International Knee Documentation Committee Subjective Knee Form: which questionnaire is most useful to monitor patients with an anterior cruciate ligament rupture in the short term? *Arthroscopy.* 2013;29(4):701-715.

41. Wang JH. Mechanobiology of tendon. *J Biomech.* 2006;39(9):1563-1582.

42. West RV, Harner CD. Graft selection in anterior cruciate ligament reconstruction. *J Am Acad Orthop Surg.* 2005;13(3):197-207.

43. Williams GN, Snyder-Mackler L, Barrance PJ, Axe MJ, Buchanan TS. Muscle and tendon morphology after reconstruction of the anterior cruciate ligament with autologous semitendinosus-gracilis graft. *J Bone Joint Surg Am.* 2004;86-a(9):1936-1946.

44. Woo SL, Gelberman RH, Cobb NG, Amiel D, Lothringer K, Akeson WH. The importance of controlled passive mobilization on flexor tendon healing. A biomechanical study. *Acta Orthop Scand.* 1981;52(6):615-622.