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In vivo ultrasonographic evaluation of patellar tendon stiffness after anterior cruciate ligament reconstruction with patellar tendon autograft

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Abstract. *Background:* Tendon mechanical properties have been used to evaluate the effects of therapies on the healing of animal tendons, but these measures have not been convenient to record *in vivo* in humans due to their invasive nature. The aims of this study were to assess the capability of an ultrasonography technique to track the change in stiffness of the healing human patellar tendon and to assess the correlation between stiffness parameters and clinical recovery measurements.

Method: Ten subjects undergoing anterior cruciate ligament reconstruction with a patellar tendon autograft were recruited for the study as well as 10 healthy control subjects. The surgical tendons' mechanical properties were evaluated at 2 and 6 months after surgery with a two-scan ultrasonography technique. A paired sign-rank test was performed to compare the change in biomechanical parameters and clinical recovery measurements across time between surgical and control groups.

Results: Tendon stiffness of the surgical group increased from ∼71% to ∼94% when compared to the stiffness of the contralateral tendons at the first visit; however, the difference was not significant ($P = 0.18$). Significant improvements were shown in all the clinical recovery outcomes. Poor correlation was found between the clinical recovery measurements and tendon stiffness.

Conclusions: No significant change in patellar tendon stiffness with healing was detected over the 2–6 month period after surgery, suggesting the tendon stiffness recovery in humans proceeds more slowly than other clinical measures.

Keywords: Tendon injury, healing, biomechanics, mechanical properties, ultrasound imaging

1. Introduction

Clinical tendon problems include acute lacerations, spontaneous ruptures, overuse injuries, and surgically induced injuries. These problems are common among

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athletes as well as the general population [1, 2]. These injuries are usually associated with significant pain, and limit the activity level of the injured limb. In addition, the healing process is often prolonged due to the limited blood supply and slow cell turnover of the tissues [3, 4]. Studies have suggested that the mechanical properties of tendon such as stiffness provide an indication of not only the functional capability of a tendon, but also the recovery level of the internal tissue material [5–7]. However, due to the invasiveness of traditional methods to evaluate the mechanical properties of tendon, these measures have not been convenient to record *in vivo*. In order to assess therapies for stimulating the healing of tendons in humans, clinical evaluation questionnaires, including pain, functional activity level assessments, and muscle strength testing have been commonly used. However, their relation with tendon healing and tendon stiffness is less clear.

Recent studies have described an ultrasound (USD) imaging technique for evaluating tendon mechanical properties *in vivo* in humans, and have used this technique to detect changes in tendon stiffness with strengthening programs or disuse [8–11]. The results of these studies suggest that this technique might be used to monitor the increase in tendon stiffness with healing after tendon injury to help determine effective therapies for accelerating tendon healing. The central third of the patellar tendon is a common autograft for anterior cruciate ligament reconstruction. The harvesting of the patellar tendon autograft for ACL reconstruction was used in this study as a controlled tendon injury model to investigate changes in tendon stiffness with healing. Previous animal model studies have shown using destructive mechanical testing that patellar tendon stiffness increases across the healing time after the patellar tendon autograft harvest [12–17].

The primary objective of this study was to evaluate the ability of a two-scan USD imaging technique to noninvasively track the recovery in patellar tendon stiffness with healing after ACL reconstruction in humans. It was hypothesized that patellar tendon stiffness would increase across the 2 to 6 month time frame after autograft harvesting and that the USD technique would be able to detect this increase. A secondary objective of this study was to determine if the tendon stiffness was correlated with other clinical outcome measures of recovery. It was hypothesized that the tendon stiffness may only be correlated with some of the clinical outcome measures. Therefore, we desired to determine which clinical outcome measures were useful in predicting the tendon recovery level if any.

2. Methods

2.1. Subjects

Ten subjects (6 male and 4 female) undergoing ACL reconstruction with a patellar tendon autograft were recruited for the study as well as 10 healthy control subjects (6 male and 4 female). Each healthy subject was matched with one surgical subject by sex, weight, activity level, age, and height. The average time from injury to surgery for the surgical subjects was 49.5 days (range: 20∼99). Inclusion criteria for the surgical group were 1) 18–40 years of age, 2) ACL reconstruction with autogenous patellar tendon graft within the past 2 months, and 3) no prior history of other knee pathology, knee surgery, or history of patella dislocation/ subluxation. Inclusion criteria for the control group were 1) 18–40 years of age, 2) no presence of lower extremity injury and pain that caused the individual to restrict physical activity for more than 2-weeks within the past 2-months, and 3) no prior history of knee surgery.

2.2. Subject preparation

All subjects signed an informed consent form that was approved by the university's institutional review board. Each participant filled out a 10-cm visual analog pain scale (VAS) [25], the International Knee Documentation Subjective Knee Form (IKDC) [26], and the Activity Rating Scale (ARS) [27] at each visit. Electromyographic (EMG) electrodes were then positioned in parallel over the belly of the biceps femoris of the subjects' thighs, and the skin was cleaned with an alcohol pad to reduce the impedance. The ground electrode was positioned over the contralateral patella to the testing knee. EMG data were collected from both the surgical and contralateral limbs in the surgical subjects and only the injury-side-matched limb in the control subjects. The subjects were then asked to sit on an isokinetic strength testing system with their thighs stabilized on the seat and the knee fixed to 90° of flexion. The axis of rotation of the dynamometer was aligned with the lateral epicondyle of the femur. A restraint cuff connected to a load cell was placed at the distal end of the shank at a known distance from

Table 1
Demographic data of subjects

	Surgical	Control
Age	22.5 ± 3.74	24 ± 6.72
Height (m)	179.71 ± 7.86	179 ± 7.15
Body mass (Kg)	86.67 ± 9.07	88.12 ± 12.81
*Activity level	3.56 ± 0.62	3 ± 1.20

*Choose one from the following which describe the best about your activity level; 4-High competitive sports person; 3-Well-trained and frequent sporting; 2-Sporting sometimes; 1-Non-sporting.

the knee joint center. Each subject was instructed to perform isometric maximal voluntary knee extension contractions (IMVC) by slowly increasing the force to maximum over the first 3 seconds, and then to push against the ankle restraint cuff as hard as possible for another 3 seconds. The subject was asked to perform 6 trials of IMVC knee extension followed by 3 trials of IMVC knee flexion at the same position. Each subject was also asked to relax before each testing trial and rest as much as they needed between contractions. The demographic data of the subjects used in data reduction and analysis are summarized in Table 1.

2.3. Data collection

While the knee extension task was performed, an ultrasonic transducer (7.5 MHz linear array B-mode; Compact Systems Diasonics; Santa Clara, CA) was oriented longitudinally to the patellar tendon and alternately placed on either the patella to track the displacement of the inferior border of the patella, or the tibial tubercle to track the displacement of the distal insertion of the patellar tendon. Therefore, 3 USD images of the patellar tendon at both insertion sites were recorded during the 6 IMVC knee extension trials. Two silicone templates were secured to the skin at these locations to prevent sliding of the ultrasound transducer, and an echo-absorptive marker was used to monitor any sliding of the probe during testing. Because of the loss of bone and tendon from surgical harvesting procedure, the silicone templates were placed more laterally for the surgical tendons. The knee torque and the USD video data collection were initiated simultaneously with a triggering device. The biceps femoris muscle activation amplitude was assessed using a surface EMG system (Delsys Bagnoli-8, Boston, MA) with a gain of 10K and a hardwired analog bandpass filter of 20–450 Hz. Both load cell and EMG signals were sampled at 1020 Hz. The USD images were collected at 30 Hz, which is the highest frequency can be acquired with the image grabber PC card and commercial software (VCE-Pro, Imprex, Boca Raton, FL) used. Each USD image has a spatial resolution of 640 × 480 pixels with a sensitivity of 0.024 mm for displacement. The cross-sectional area (CSA) measured at the midpoint of both insertions and the original length of the patellar tendon were also recorded as static ultrasonic images. The pixels within the CSA (including the graft harvest region) and the original length of the patellar tendon from the images were then measured with image analysis software (IMAQ Vision Builder, National Instruments Inc. Austin, TX) and converted to metric units (mm^2 and mm, respectively).

Patellar tendon stiffness was evaluated at 2 and 6 months after surgery in surgical subjects, while control subjects were evaluated simultaneously in time with their matched surgical subjects at 0 and 4 months after enrollment. Surgical subjects were evaluated for both knees (Surgical and Contralateral-tendon Group), while the control subjects were evaluated for only the knee that matched to the same side of the matched surgical knee (Control-tendon Group). The control and contralateral groups were included to determine if there is any external factor that may cause changes in tendon stiffness besides healing (i.e. changes in physical activity due to weather and/or athletic seasons). The contralateral group was also used to assess the relative recovery level in tendon stiffness (% of controalateral) of the surgical group.

Force, EMG, and USD image data were reduced and analyzed with custom programs within Labview (National Instruments Inc., Austin, TX). Force data were filtered with a second order, phase-corrected Butterworth low-pass filter with cutoff frequency at 5 Hz. EMG data were corrected for DC bias and filtered with a sixth order, phase-corrected Butterworth low-pass filter with cutoff frequency at 10 Hz to create a linear envelope. The hamstring force contribution was estimated from the EMG data multiplied by the ratio of peak force to peak EMG during the maximal knee flexion activation and then was added to the loadcell force data. Patellar tendon force at each time point during the IMVC extension task was calculated by the following equation (Eq. (1))

$$F_{pt} = (F_{tot} + F_{hext})^{*}MA_{leg}/D_{pt} \quad (1)$$

where F_{pt} was the patellar tendon force, F_{tot} was the resultant loadcell force, F_{hext} was the co-contraction

hamstring force applied at the loadcell, MA_{leg} was the moment arm of the lower leg from the knee joint center to the force application point at the load cell. D_{pt} was the patellar tendon moment arm estimated from femur length and knee flexion angle of each individual using (Eq. (2)).

$$D_{pt}/\text{Thigh Length}^{*}100\% = [0.201285 + 2^{*}(-0.000545)^{*}\ \theta]^{*}180/\ \pi \quad (2)$$

The D_{pt} of each tendon was multiplied by the ratio of the average D_{pt} across all subjects divided by an average D_{pt} reported from previous reports [23, 24]. In addition, the maximal knee extension torque without considering the co-contraction torque during isometric knee extension was also calculated. In order to compare the stiffness values at the same relative force level between groups, we followed four steps to compute the patellar tendon force-elongation data with our custom Labview programs. First, we found the peak tendon force of all the trials for each knee at a specific time point. The program then picked the ultrasonic scan number at the simultaneous point in time in which the patellar tendon force was at an increment of every 2% of the min-max force for each trial. The displacements calculated from the ultrasonic scans were also referenced to this same point in time. Second, we applied a pattern matching technique for automating the tracking of the displacement of the inferior border of the patella or proximal edge of the tibia tubercle in a series of USD images frame by frame. The matched location was determined by the highest score based on the normalized spatial cross correlation coefficient using a custom program developed within an image analysis programming environment (Vision, National Instruments, Austin, TX). Trials were excluded if sliding of the probe was detected by the movement of the anechoic marker or distal movement of the patella. We then calculated the displacements of the tracked entity across the entire extension task and found the displacements at the ultrasonic scan numbers corresponding to the 10% intervals of the min-max tendon force. Third, the displacement at the patellar insertion at each loading level was averaged across the 3 trials as well as the displacement at the tibial insertion. Displacements of the tibial and patellar insertion templates in the longitudinal and perpendicular directions relative to the scan surface were subtracted from each other and the resultant magnitude of total deformation was computed using the Pythagorean Theorem (Fig. 1).

The computed deformations were then plotted at every 10% of each individual's min-max load interval. With this procedure, we could analyze the stiffness of the paired surgical, contralateral and control tendons at the same relative loading level (i.e. 100% of the min-max tendon force). Finally, each force-deformation curve was fit to both a linear function and a nonlinear function ($y = ax^2 + bx + c$), where y was the tendon force and x was the deformation. Therefore, the instantaneous slope of the curve: $dy/dx = 2ax + b$, where the coefficients a, and b were obtained from the best fit of the data, was calculated as a measurement of the tendon's tangential stiffness at the min-max force level for each subject. Secant stiffness was also obtained by the slope of a line drawn from the 100% of the maximal tendon force level to the origin of the force-deformation curve. Normalized tendon stiffness was estimated by the product of stiffness and the ratio of original tendon length to CSA.

Due to the small sample size in this study, our data were not normally distributed, and thus a paired sign-rank test was performed to compare the tendon length, CSA, maximal knee extension torque, stiffness, VAS, ARS, and IKDC scores across time within each group and the changes in scores among groups. Between-day correlation coefficients of the secant and tangential stiffness of the control group were calculated to examine the reproducibility of our measurements. A priori power analysis was performed to determine the required sample size. Under the assumption of the standard deviation = 30%, 10 subjects were required to obtain 80% statistical power to detect a 34% difference in tendon stiffness across time at $\alpha = 0.05$. Spearman's correlation was also performed to examine the relation between the commonly-used clinical evaluation tools for knee injury patients, including the VAS, ARS, IKDC, and the maximal knee extension torque across time, and the tangent stiffness across time. The knee extension torque was calculated without considering the hamstring contribution in order to simulate the results from customary isokinetic testing.

3. Results

The average scores of the VAS, ARS and IKDC Subjective Knee Evaluation collected from each group at the two visits are summarized in Table 2. The effect of healing time on the VAS, IKDC and ARS scores was significant in the surgical group ($P < 0.05$). The

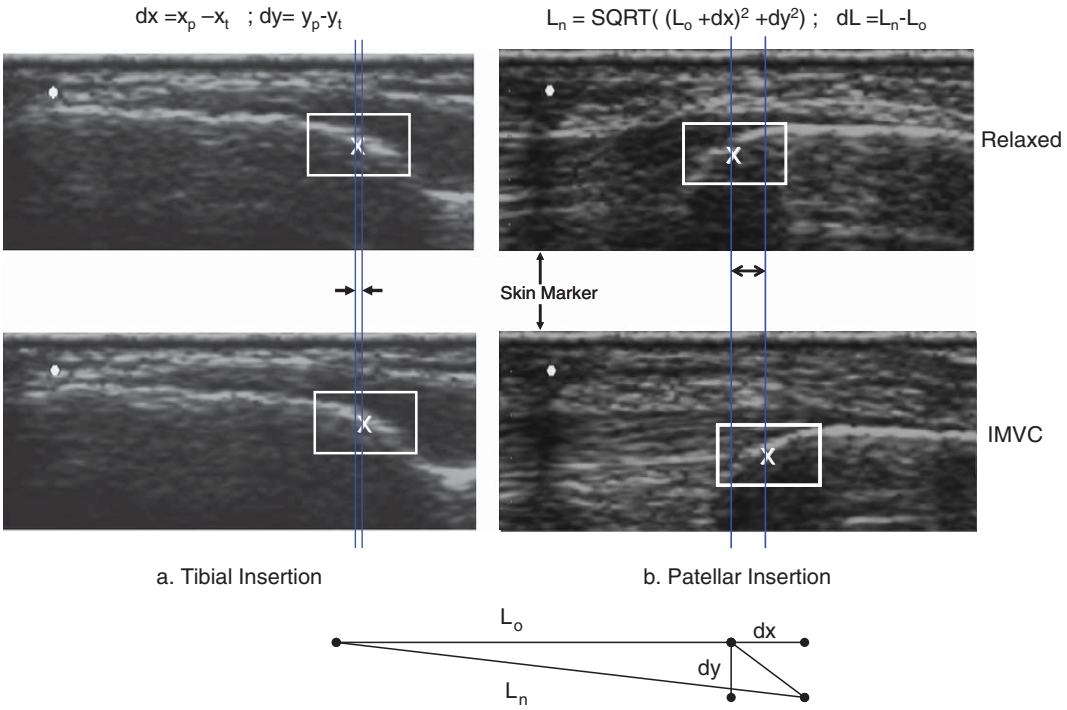

Fig. 1. Measurement of displacements at patellar and tibial patellar tendon insertion sites. The displacement of each insertion was calculated from the movement of a template located at each insertion. Displacements of the patella and tibial templates in the longitudinal and perpendicular directions relative to the scan surface were subtracted from each other and the resultant magnitude of total deformation was computed using the Pythagorean Theorem. (Xp: longitudinal displacement at patella; Xt: longitudinal displacement at tibia; Yp: perpendicular displacement at patella; Yt: perpendicular displacement at tibia; Lo: original length between centers of two templates; Ln: length between centers of two templates after displacement.)

Table 2
Mean and SD of the Visual Analog Scale (VAS), Activity Rating Scale (ARS) and the International Knee Documentation Committee (IKDC) Subjective Knee Evaluation scores collected from subjects at both testing sessions

	Surgical			Control		
	*VAS	*ARS	*IKDC	VAS	ARS	IKDC
2 month	3.93 ± 2.4	0.13 ± 0.35	26.69 ± 22.46	0.15 ± 0.42	10.25 ± 5.68	75.14 ± 10.06
6 month	2.26 ± 1.42	8.38 ± 5.42	47.25 ± 28.42	0.87 ± 1.07	8.13 ± 5.72	74.57 ± 9.53

* Significant difference across time $P < 0.05$.

VAS pain scores decreased significantly at the second visit, while the IKDC and ARS scores improved significantly at the second visit. No difference across time was shown in any of these scores for our control group.

The resting length of control and contralateral tendons at the first visit were 44.17 ± 4.02 mm, and 43.52 ± 6.20 mm, respectively. The CSA of surgical, control, and contralateral tendons at the first visit were 118.72 ± 15.12 mm^2, 91.05 ± 8.32 mm^2, and 104.38 ± 11.43 mm^2. The CSA of surgical, control, and contralateral tendons at the second visit were 100.17 ± 26.39 mm^2, 91.24 ± 5.31 mm^2, and 104.89 ± 10.13 mm^2. The resting length and CSA area of the patellar tendon showed no difference across time for both the control and contralateral tendon groups ($P > 0.05$); however, the CSA of the surgical tendon was decreased by ∼15% at the second visit. The maximal extension torque in the surgical (71.89 ± 29.84 Nm) and contralateral (144.56 ± 64.57 Nm) groups showed a significant increase at the second visit (surgical: 138.79 ± 47.80 Nm; contralateral: 201.14 ± 49.59 Nm) ($P < 0.05$), and no difference was

shown in the control subjects (171.14 ± 69.28 Nm). The average percentages of hamstring contribution on the maximal extension torque were 17% for the control group and 28% for the surgical group.

The force-elongation plots of the control and contralateral tendons demonstrated similar curves, with each having larger toe-regions when compared with the surgical tendons. As such, the secant and linear curve fitting methods may underestimate the tendon stiffness of the linear region for the control and contralateral tendons (Fig. 2). Therefore, in order to more effectively assess the stiffness in the linear region we used the tangent stiffness values obtained using the nonlinear curve fitting approach to compare between groups.

During the second testing session, the tendon stiffness of the surgical group at the min-max load level increased from ~71% to ~94% when compared to the stiffness of the contralateral tendons at the first visit; however, the difference was not significant ($P = 0.18$) (Fig. 3). The normalized tendon stiffness increased from ~61% to ~96% when compared to the stiffness of the contralateral tendons at the first visit; however, this difference did not reach statistical significance ($P = 0.25$) (Fig. 4). The structural and normalized tendon stiffness at the min-max load level also showed no significant difference across time for the control and contralateral tendon groups ($P > 0.05$) (Tables 3 and

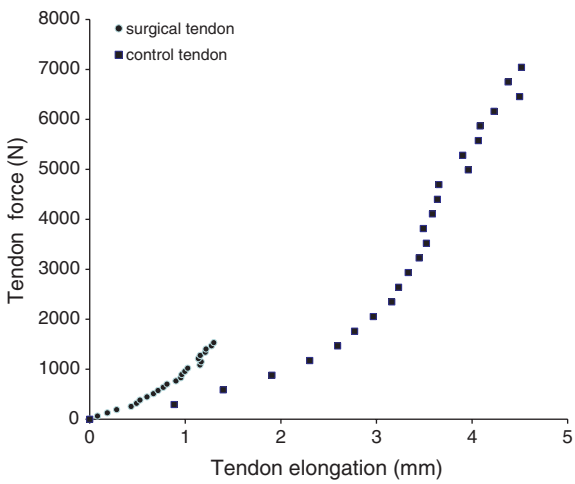

Fig. 2. Force-deformation curve of surgical and control tendons at 2 months after surgery. The curve of control tendon demonstrated an extended toe-region. The average deformation at every 2% force level, and the averaged absolute force levels of all the 8 subjects were used to plot the curve. The force-elongation plots of the control and contralateral tendons demonstrated similar curves, with each having larger toe-regions when compared with the surgical tendons. As such, the secant and linear curve fitting methods may underestimate the tendon stiffness of the linear region for the control and contralateral tendons. The plot of the contralateral tendon demonstrated a very similar curve as the control tendons and thus is not shown here.

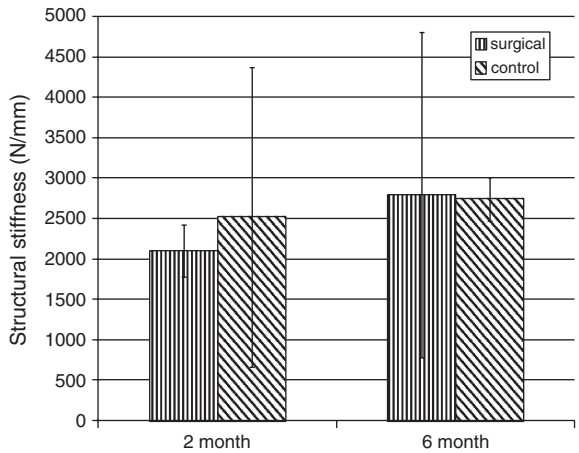

Fig. 3. Structural stiffness of the two groups during the two visits. The stiffness of the surgical tendon was approximately 71% of the contralateral tendon at the first session and about 94% at the second session. Each group did not change significantly ($P > 0.05$) across the two visits. The contralateral group was not included in the paired sign-rank test comparison.

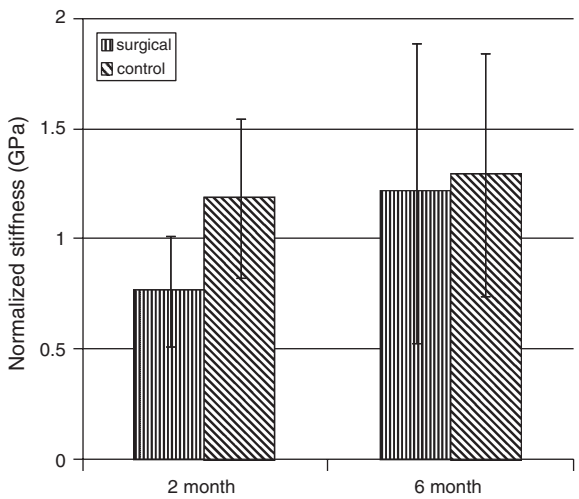

Fig. 4. Normalized stiffness of the two groups during the two visits. The stiffness of the surgical tendon was about 61% of the contralateral tendons at the first session and about 96% at the second session. Each group did not change significantly ($P > 0.05$) across the two visits. The contralateral group was not included in the paired sign-rank test comparison.

Table 3
Mean and SD of the structural stiffness (N/mm)

Group	2 month	6 month	*P*
Surgical	1483.63 ± 264.46	1725.20 ± 1036.33	*P* = 0.18
Control	2005.34 ± 741.32	2076.01 ± 1249.47	*P* = 0.68
Contralateral	2337.98 ± 1818.88	2820.64 ± 1714.32	*P* = 0.87

Significance level (*P*) for a paired - *t* test comparing values across time.

Table 4
Mean and SD of the normalized tendon stiffness (GPa)

Group	2 month	6 month	*P*
Surgical	0.57 ± 0.25	0.86 ± 0.68	*P* = 0.17
Control	0.94 ± 0.36	0.97 ± 0.55	*P* = 0.88
Contralateral	0.98 ± 0.70	1.19 ± 0.73	*P* = 0.59

Significance level (*P*) for a paired - *t* test comparing values across time.

Table 5
Spearman correlation was performed to examine the correlation between IKDC, VAS, ARS scores, maximal knee extension torque, and tendon stiffness

	IKDC	VAS	ARS	Torque	Stiffness
IKDC	1.00	−0.85*	0.83*	0.62*	0.23
VAS	−0.85*	1.00	−0.61*	−0.50*	−0.20
ARS	0.83*	−0.61*	1.00	0.75*	0.36
Torque	0.62*	−0.50*	0.75*	1.00	0.51*
Stiffness	0.23	−0.20	0.36	0.51*	1.00

*$P < 0.05$.

4). Spearman's correlation was performed to examine the correlation between the common clinical evaluation tools (i.e. VAS, ARS, IKDC scores, and maximal knee extension torque) and tendon stiffness. Our results showed strong correlations between VAS and IKDC ($r = -0.85$); IKDC and ARS ($r = 0.83$); notable to strong correlations between maximal knee torque and VAS, IKDC, and ARS, respectively, ($r = -0.50$–0.75); and notable correlation between tendon stiffness and maximal knee torque ($r = 0.51$). However, no significant correlations were found between tendon stiffness and IKDC, VAS, or ARS (Table 5).

4. Discussion

Tendon disorders are a major problem in both sports and occupational activities. However, little is known about how the mechanical properties of tendon change across time during human tendon healing and to what extent clinical outcome measures of recovery are predictive of the tendon mechanical properties. A better understanding of these aspects of tendon healing may improve our ability to evaluate the effectiveness of new and existing therapies. This study, to our knowledge, was the first to investigate the *in vivo* healing effect on tendon mechanical properties with a non-invasive real-time USD imaging technique. A pattern matching imaging technique was used to measure the movement of features on sonographic images. The average tangent stiffness of the control tendons was 2005.34 N/mm, which was within the range (1790–4334 N/mm) reported from previous patellar tendon stiffness studies [18, 19]. The average tangent stiffness of the control group did not show any difference between visits, suggesting there was no seasonal or other external factors that may influence the tendon stiffness during this time period. Previous studies on the time table of tendon healing have been based mainly on animal results, which have suggested up to a 68% increase in patellar tendon stiffness over the first three months after harvesting a patellar tendon autograft [5, 13, 15, 17]. Based on these animal studies, we expected to see a significant increase in tendon stiffness in our human subjects. However, our results showed no significant increase in either the structural tendon stiffness or the material properties (i.e. normalized stiffness) from 2 months to 6 months after surgery. These results suggest: 1) the recovery of human patellar tendon stiffness with healing is slower than what has been observed in animal studies, and slower than that observed for the clinical recovery measures utilized in this study; 2) the surgical subjects compliance with the rehabilitation and exercise program, which were not recorded in this study, may have confounding effect on the tendon properties and a large variation between surgical tendons; 3) the subjects who maintained a relative sedentary life style after the surgery may have prevented increases in the tendon stiffness, and thus the effect of rehabilitation program on tendon healing should be further investigated.

In the contralateral tendon group, both structural and normalized stiffness showed a pattern for increasing after 4 months though not statistically significant. This trend for increasing stiffness may have resulted from an increase in weight bearing and activity level in the contralateral limb. Reeves et al. investigated the effect of training on the mechanical properties of the human patellar tendon. Their results showed a 65% increase in patellar tendon stiffness and 69% in Young's Modulus after 14 weeks of progressive isotonic resistance training [19].

Additional findings also suggest the healing response to patellar tendon autograft harvest may differ

between humans and animals. Previous studies on goat and rabbit animal models of the healing patellar tendon after autograft harvest suggested the ratio of surgical limb/contralateral limb patellar tendon CSA immediately following surgery was 54%, while at 3 months and 12 months the ratio was 237% and 184% [12, 14, 15, 17]. In another study on rabbit patellar tendons after removal of the central one-third, the microscopic findings of the regenerated tissue suggested that a large increase in the tissue volume was mainly composed of scar-like connective tissue [16]. In contrast, the cross-sectional areas based on USD images of our surgical tendons did not increase from 2 to 6 months after surgery. A more carefully performed surgical procedure on humans to minimize injuries to the surrounding tissue, and unrestricted activity of animals after surgery compared to heavy activity restrictions in human patients may have caused less scar tissue formation as compared to the animal studies. However, in 2009, Reeves et al. also reported the CSA of human patellar tendons was 21% larger between 1 year to 10 years after graft harvest for ACL surgery in comparison to the contralateral tendon. In addition, the Reeves et al. study reported that the elastic modulus of the patellar tendon subjected to graft harvest was significantly decreased compared to the contralateral tendon [22]. In contrast, in the current study the elastic modulus of the surgical tendon at 6 month was found to approach the value of the control tendon. A possible explanation for these differing results is that because the tendon was still healing (2 to 6 months after surgery) in the current study that we may have been unable to assess the true outer-border of the tendons, causing us to underestimate the CSA, which could also cause us to overestimate the normalized tendon stiffness at 6 month after surgery.

Due to the difference in toe-region of the force-deformation curves between surgical and control tendons, errors may have been introduced if we assessed time and group differences using secant stiffness. Therefore, the nonlinear curve fitting approach to estimate tendon stiffness at the same relative tendon force level (100% min-max) was used to compare between time points and groups. Another approach was also applied to compare the tendon stiffness of the paired surgical subjects (both contralateral and surgical tendons) and control subjects at the same absolute force level [29]. In order to normalize to the weakest subject and time point, we did not include the data from the higher force region with this approach. Using this approach the difference in stiffness across the healing time in the surgical group was smaller, and again was not statistically significant.

IKDC, VAS and ARS scores and maximal knee extension torque are commonly used by clinicians to evaluate recovery of knee injuries. These questionnaires are based on the patients' subjective evaluation of their knee function, such as symptoms, and activity levels. Significant improvements on all the scores of these questionnaires for the surgical groups were noted at the second visit. Strong correlation between the knee torque production and the VAS was also shown in another study [28]. This correlation suggested that the muscle strength of the knee injured patients improved as symptoms dissipated. The knee functional activity score was poorly correlated with tendon stiffness, suggesting that the knee functional activity level is not indicative of tendon healing. As such, basing clinical decisions regarding return to physical activity and athletic participation on functional activity level and symptomatology may put the healing tendon at risk of re-injury and other complications. The higher correlation ($r = 0.51$) between the maximal knee extension torque and tangent stiffness than we expected may be due to the curvilinear nature of the force-deformation response (Fig. 2). The correlation dropped to 0.11 when we compared the change of the tangent stiffness and the change in maximal knee torque across time. Although Muraoka et al. showed a significant correlation between muscle strength and mechanical properties of healthy tendons, this relation may be different for injured tendons [20]. A confounding factor in examining the correlation between the clinical recovery measures and the tendon stiffness is that the surgical subjects have both a patellar tendon and ACL injury and thus the clinical recovery measures might be influenced by the ACL injury while the tendon stiffness might not be influenced by the ACL injury causing a poorer correlation between these measures.

There are several limitations of this study. First, misalignment of the USD scan with the line of action of the tendon is a possible source of error. Second, because of the larger variation than we expected in mechanical properties of the injured tendons from 2 to 6 months after surgery, we likely did not have sufficient power to detect a significant change in the tendon stiffness due to healing. With the larger standard error (44%) we observed from this study when compared to our assumption of 30%, 15 subjects would be needed in order to obtain 80% statistical power to detect a 34%

difference for a future study. Third, due to the lack of a central region of the surgical tendons, the measurements of tendon elongation were based on the relative movement of the patellar and tibia insertions at the lateral patellar tendon for the surgical tendons and central part for the control and contralateral tendons. Error may be introduced if the tendon experiences differential strain across its width (i.e. lateral to central). However, at 30° to 110° of knee flexion, previous studies have suggested the difference in lengthening attributable to the image location is negligible [21]. Fourth, using EMG to predict force levels at low levels of maximal voluntary contraction may cause errors in estimating the patellar tendon force, and thus stiffness measurements. Fifth, we did not measure the patellar tendon stiffness immediately after the reconstruction operation due to concerns of damaging the ACL graft and other soft tissue of the knee joint while performing the MVC trials. Therefore, the increase in tendon stiffness between pre-surgery to 2-month after surgery was unknown. However, the increase during this time should be minimal due to the restricted weight bearing activity of the injured limb before and immediately after surgery. Lastly, the removal of some trials due to sliding of the USD probe was another limitation for this study.

5. Conclusions

The results of this study did not show a significant increase in tendon stiffness properties over the 2 to 6 months after surgery, suggesting a longer time frame may be needed to evaluate the increase in tendon stiffness with healing. However, this in vivo USD technique displayed sufficient reliability in measuring tendon stiffness in the control tendons and should be able to monitor the healing effect on tendon properties for a longer time frame. Therefore, future studies may include: 1) A longer-term study over at least 12 months after injury with more than two time points to help chart the trend of the healing process for the human tendon; 2) A longer ultrasonic probe allowing both insertions to be viewed may be helpful to minimize the chance of losing trials because of slippage of the probe.

In addition, our results also showed that some commonly-used clinical recovery measurements were poorly correlated with the tendon stiffness, and thus should be interpreted cautiously in evaluating tendon healing.

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