In Vivo Evaluation of the Stiffness of the Patellar Tendon

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ABSTRACT

Hsin-yi Liu: *In Vivo* Evaluation of the Stiffness of the Patellar Tendon

(Under the direction of Paul Weinhold)

Tendon problems are common among athletes as well as the general population. The success of current repair and rehabilitation protocols remains controversial due to the subjective content of the clinical recovery measurements. Tendon mechanical properties have been considered the "gold standard" in evaluating the healing of tendon, but these measures have not been convenient to record *in vivo* due to the invasive nature of conventional mechanical testing. Recently, an *in vivo* ultrasonography technique has been successfully applied to detect the change in tendon mechanical properties over time. The overall objective of this study was to assess the capability of this technique to track the change of stiffness in the healing patellar tendon.

Initially, a single-scan ultrasound method was used to detect the change in the mechanical properties of patellar tendon in patients with patellofemoral pain syndrome. Our results showed the single-scan method was able detect the change in stiffnes due to this condition. However, the limitation of this method was that movement of the distal insertion of the tendon was unaccounted for. Therefore, we subsequently developed a two-scan method to monitor both insertion sites of the tendon and compared the between-day reliability of this method with the single-scan method. The results showed the deformation of the patellar tendon at the tibial insertion had a significant effect on the total tendon deformation and the two-scan method had

higher reliability across time. Therefore, in the last study, we proceeded with the two-scan method to evaluate the stiffness of the healing patellar tendon after ACL reconstruction with a patellar tendon autograft.

The result of this study did not show a significant increase in tendon stiffness properties over the 2 to 6 months after surgery, suggesting a longer healing time may be needed. However, this *in vivo* ultrasonographic technique was reliable in measuring the tendon stiffness properties across time in the controls and thus may be used to monitor the healing effect in a longer time frame. 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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TABLE OF CONTENTS

CHAPTER III

CHAPTER IV

CHAPTER IV

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Tendons are mechanically responsible for transmitting contractile forces to the skeleton to generate joint movement and enhance joint stability. For example, the patellar tendon is a major tendon that transmits the quadriceps force to the tibia to generate knee extension. Studies have indicated the maximal patellar tendon force can range from 2.5 to 6 times body-weight during daily activities (Kuster *et al.*, 1997; Collins, 1995). In order to transmit such large forces and produce joint motion, tendons must be stiff and strong. The material substance of a normal tendon consists of collagen, proteoglycan, glycoproteins, water and cells, with type I collagen accounting for about 70-80% of its dry weight. The main factors that increase the stiffness and strength of the tendon material are the collagen-I content, collagen alignment, and collagen cross-linking. Tendon stiffness and Young's modulus are the most common parameters to describe the biomechanical properties of a tendon under nondestructive loading conditions, and are often highly correlated with the tensile failure load and strength of the tissue (Muraoka *et al.*, 2005). Tendon stiffness is an extrinsic measurement of the tensile performance of the overall structure, while Young's modulus reflects the intrinsic material properties of a tendon.

Common clinical tendon problems include acute lacerations, spontaneous ruptures, overuse injuries, and surgically induced injuries associated with the harvesting of tendon grafts. Theses 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 generally limited blood supply and slow cell turn over of the tissues (Fenwick *et al.*, 2002; Kannus *et al.*, 1997). During tendon healing, collagen content, crosslinking, and alignment are altered causing increases in the stiffness of the tissue. Animal studies have demonstrated that disuse of a limb or injury to the tendon will alter the mechanical properties of tendons due to the reduction of the total area of collagen fibrils in the tendon cross-section and an increase in the numbers of thin and immature fibrils (Linder *et al.*, 1994; Yasuda and Hayashi, 1999). Other studies have also suggested that the mechanical properties of tendon provide an indication of not only the functional capability of a tendon, but also the recovery level of the internal tissue material (Atkinson *et al.*, 1998; Kasperczyk *et al.*, 1991; Kasperczyk *et al.*, 1993). However, due to the invasiveness of traditional methods to evaluate the mechanical properties of tendon, the function and recovery of human tendons after injuries remains unclear.

In 1995, the use of real-time ultrasonography to assess tendon stiffness was first published to obtain the mechanical properties of tibialis anterior tendon *in vivo* (Fukashiro *et al.*, 1995a). During isometric dorsal flexion at the ankle, they monitored the relative movement between the fascicle's insertion to its aponeurosis and a fixed skin marker. This method was then applied to evaluate the mechanical properties of different human tissues, including the gastrocnemius aponeurosis and tendon, distal myotendinous junction (MTJ) of the biceps brachii, and the patellar tendon during *in vivo* conditions. A force-deformation curve was plotted with the knowledge of the ultrasonic measurement of the deformation of the tendon under defined functional loads, and the stiffness of the tissue could be calculated from the slope of this curve (Hansen *et al.*, 2006; Loram *et al.*, 2006; Maganaris *et al.*, 2006b; Kubo *et al.*, 2004). Recent studies have also documented the ability of this

technique to detect the effect of complete disuse due to spinal cord injury (Gerrits *et al.*, 2005) and of strength training on tendon properties (Reeves *et al.*, 2003). At present, no study has been conducted to evaluate the potential of this real-time ultrasonography technique to evaluate changes in tendon stiffness with tendon healing, or to evaluate the effect of mild disuse after joint injury on tendon stiffness.

Significance of the Study

Although many investigators are trying to understand and evaluate repair processes after tendon injury, there remains much to be accomplished. To date, our understanding of tendon biology is quite descriptive, including various phases of repair and their time courses. Tendon biomechanical parameters appear to better quantify the state of healing of a tissue over time, but these measures have not been convenient to record *in vivo* due to the invasive nature of conventional mechanical testing methods available. However, with the development and refinement of the ultrasonic technique, assessment of the biomechanical properties of the repaired tissue may now be possible, thus allowing for better quantification of the state of healing of tendon tissue over time.

The overall objective of this study was to assess whether an ultrasonic technique can track changes in stiffness in the healing patellar tendon. As there are novel tissue engineering, biomaterials, cell therapies, and pain management therapies being considered to enhance the healing of the patellar tendon (Dressler *et al.*, 2005; Yasuda *et al.*, 2004), demonstration that the ultrasonic method can track the recovery of an injured tendon may provide a quantitative means for assessing the efficacy of these therapies.

A sequence of studies was carried out to accomplish the overall objective. Initially, a previously described ultrasonic method for assessing patellar tendon stiffness was applied to determine the ability of this technique to detect potentially subtle tendon stiffness changes associated with mild disuse after joint injury. Subsequently, the reliability of this established ultrasonic method and a refined method were evaluated in order to assess their suitability for use in a study to characterize patellar tendon stiffness changes across time. Finally, with a reliable method identified, the harvesting of the central third of the patellar tendon as an autograft for ACL reconstruction was utilized as a controlled model in humans for assessing the ability of the ultrasonic technique to monitor changes in tendon stiffness with healing. The specific objectives of these studies were the following:

Statement of Objectives:

1. To evaluate the capability of this USD technique in detecting the effect of patellofemoral pain syndrome on mechanical properties of the patellar tendon.

2. To compare the reliability of two ultrasound imaging methods in evaluating the patellar tendon stiffness *in vivo* across time.

3. To utilize ultrasonic measurements of patellar tendon stiffness to track the recovery of the patellar tendon after harvesting of the central third of the patellar tendon as an autograft for ACL reconstruction.

CHAPTER II

LITERATURE REVIEW

Tendon injuries are common during sports activities, and the healing process is often slow. Mechanical properties affect the levels of recovery after the injury and the functional activities. In this chapter, we first reviewed studies on the structural and mechanical properties, common injuries, and healing process of tendons. Then, the effect of tendon injury and healing on the mechanical properties of tendon is discussed. Finally, a newly-developed ultrasonography method used to evaluate tendon properties is introduced.

Structure and mechanical property of tendons

Tendons are composed of collagen molecules, fibrils, fiber bundles, and fascicles in a hierarchical fashion. These fiber bundles run parallel to its long axis, and provide tensile strength (Kastelic *et al.*, 1978). The fiber patterns and viscoelastic characteristics of tendon contribute to its unique mechanical behavior when subjected to dynamic mechanical forces *in vivo*. The structural properties of the tendon are an extrinsic measurement of the tensile performance of the overall structure. They depend on not only the size and shape of the tendon, but also on its unique material properties from its

mid-substance tissue to its insertion into bone. The structural properties can be obtained by loading a tendon to failure, and are represented as a resulting force-deformation curve. Three regions can be defined in the tendon force-deformation curve: the toe region, linear region, and failure region (Figure 2.1) (Butler and Awad, 1999). The tendon structural stiffness is defined as the slope in the linear region of the force-deformation curve, which is a measure of the structure's resistance to axial deformation. To account for inter-specimen dimensional differences, tendon forces are reduced to stress values (MPa) by normalization to the tendon cross-sectional area (CSA), and tendon deformations reduced to strain (%) by normalizing to their original lengths. The shape of the stress-strain curve is similar to that of the force-deformation curve, but it reflects the intrinsic material properties of the tendon. A typical tendon stress-strain curve has an initial toe region, where the tendon is strained up to 2%. In the linear region of the stress-strain curve where the tendon is lengthened less than 4%, collagen fibers lose their crimp pattern (Johnson *et al.*, 1994). The slope of this linear region is referred as the Young's modulus of the tendon. Young's modulus is also known as the modulus of elasticity, which is the product of the structural stiffness multiplied by the original length to CSA ratio of the specimen. Different approaches can be used to describe the material properties at different regions of the curve. a) Tangent approach: tangent modulus is defined as the slope of a line tangent to the force-deformation or stress-strain curve at a point of interest. Tangent modulus can have different values depending on the point at which it is determined. For example, tangent modulus is equal to Young's modulus when the point of interest falls within the linear region (Figure 2.2). b) Secant approach: secant modulus is the slope of a line drawn from the origin of the force-deformation or stress-strain diagram intersecting the curve at the point of interest (Figure 2.3).

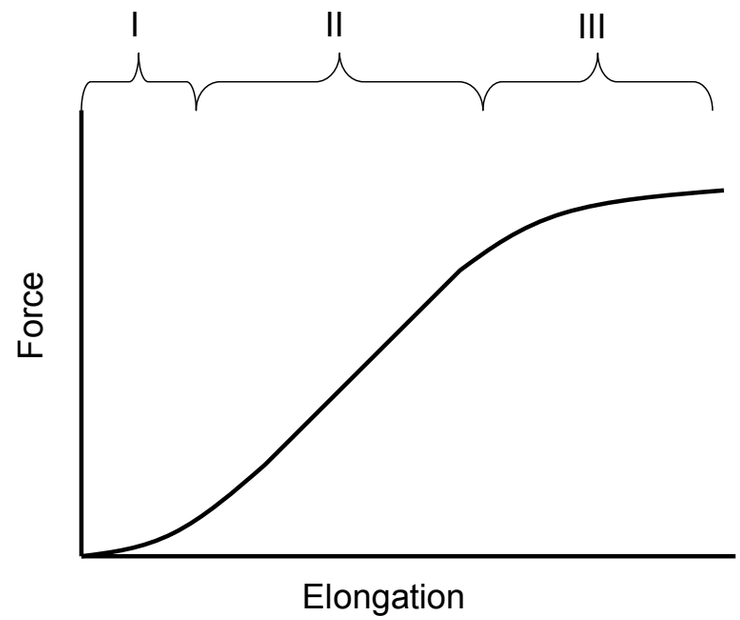

Figure 2.1 Typical force-elongation curve of a tendon pulled by a load exceeding the tendon elastic limit. I = toe region; II = linear region; and III = failure region.

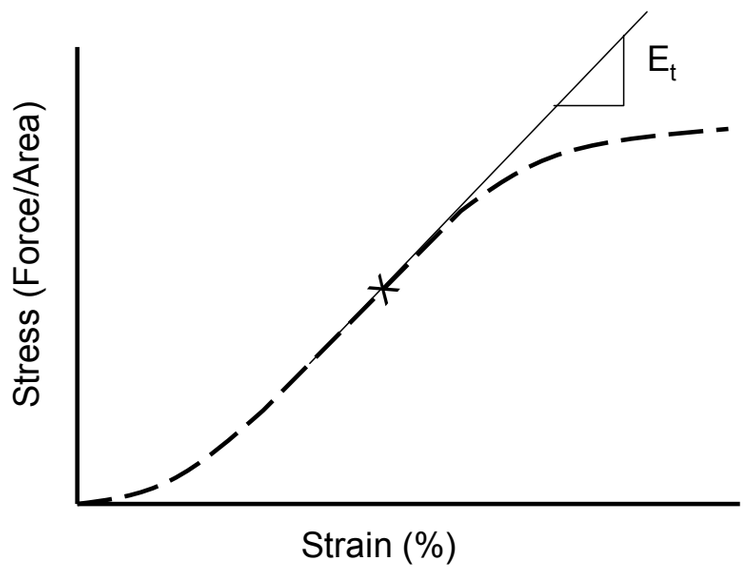

Figure 2.2 Tangent modulus (E_t) is defined as the slope of a line tangent to the stress-strain curve at a point of interest.

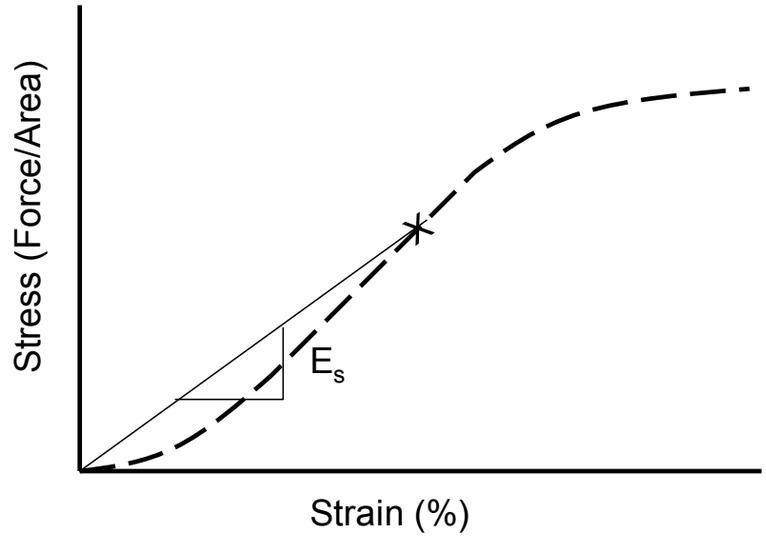

Figure 2.3 Secant modulus (E_s) is the slope of a line drawn from the origin of the stress-strain diagram and intersecting the curve at the point of interest.

Tendon injury and healing

Healthy tendons are brilliant white in color and have a fibroelastic texture. Within the extracellular matrix network, tenoblasts and tenocytes constitute about 90% to 95% of the cellular elements of tendon. The remaining 5% to 10% consists of chondrocytes at bony attachment and insertion sites, synovial cells of tendon sheath, and vascular cells. Tendons allow transmission of force generated by muscle to bone, resulting in joint movement. The oxygen consumption of tendons is 7.5 times lower than the skeletal muscle (Vailas *et al.*, 1978). The low metabolic rate and well-developed anaerobic energy-generation capacity are essential to carry loads and maintain tension for long periods. However, a low metabolic rate results in slow healing after injury (Williams, 1993). Tendon injuries usually produce considerable morbidity, and the associated disability may last for several months despite what is considered appropriate treatment (Almekinders and Almekinders, 1994). Tendon injuries can be acute or chronic, and are caused by intrinsic or extrinsic factors, either in isolation or in combination.

Chronic injury

Tendon overuse injuries, which are collectively referred to as tendinopathy, affect millions of people in occupational and athletic settings (Jarvinen *et al.*, 2001; Maffulli *et al.*, 2003). Clinical symptoms include pain and signs of inflammation, which impair the ability to function normally (Paavola *et al.*, 2002). Histological examination of tendinopathy shows a poor healing response, intratendinous degeneration, fiber disorientation and thinning, and hypercellularity due to the presence of fibroblasts (Sharma and Maffulli, 2005). A significant increase in tendon CSA and decrease in ultimate stress were also seen in

animal tendinopathy models (Wang, 2006). The traditional concepts of overuse injuries involve the repetitive, and excessive loading of the tendon and subsequent mechanical breakdown of the loaded tendon (Nakama *et al.*, 2005; Clement *et al.*, 1984; Korkia *et al.*, 1994). Therefore, training errors have been mentioned as an etiological factor in tendon overloading. The recently reported biomechanical studies suggest that the stress-shielded and transversely-compressed side of the tendon-bone junction (enthesis) has a distinct tendency to develop cartilage-like, or atrophic changes in response to the lack of tensile load (Almekinders *et al.*, 2002; Almekinders *et al.*, 2003). Over a long period, this process may develop a primary degenerative lesion in that area of the tendon. This may explain why the tendinopathy is not always activity-related, but sometimes more strongly correlated with age. In this manner, tendinopathy would be a result of stress-shielding rather than overuse. However, more research is needed to determine the significance of stress-shielding and compression in tendinopathy.

Acute injury

Spontaneous rupture (the sudden rupture of a tendon without preceding clinical symptoms) of Achilles tendon, digital tendon of biceps and patellar tendon are common and most frequently occurs in high-power sports events, such as high jump, basketball, and weight-lifting, at age of 15-30 years (Kannus and Natri, 1997). Although the incidence of the ruptures is difficult to determine accurately, it is generally agreed that the incidence has increased in the industrialized countries in recent years (Leppilahti *et al.*, 1996; Moller *et al.*, 1996). Studies have shown the presence of tendon degeneration at the site of the rupture, and suggest the degenerative changes may lead to reduced tensile strength, predisposing the tendon to rupture (Tallon *et al.*, 2001; Cetti *et al.*, 2003).

Surgical injury due to ACL reconstruction

Every year, more than 100,000 patients in the United States and 200,000~300,000 cases around the world undergo ACL reconstruction surgery (Brown and Carson, 1999). Currently recommended graft choices for ACL reconstruction include biologic autograft and allograft materials. Autograft choices include the bone-patellar tendon-bone (BPTB), quadrupled semitendinous-gracilis tendon (STG), or bone-quadriceps tendon autografts. Allograft options include Achilles, BPTB, and hamstring tendons. The BPTB autograft procedure has shown the most predictable long-term results, and in the 1990s this reconstruction was considered the "gold standard" procedure (Francis *et al.*, 2001).

After performing a diagnostic arthroscopic examination of the knee, the central third of the patellar tendon, a section approximately 10-11 mm in width and 25 mm in length, is harvested as the graft. This graft also includes small portions of the inferior pole of the patella and the tibial tuberosity referred to as bone plugs, hence the name "bone-patellar tendon-bone" (BPTB) graft. The BPTB autograft has been a popular graft for ACL replacement because of its high ultimate tensile load (approximately 1023 N), its stiffness (approximately 174 N/mm), and the possibility for rigid fixation, with its attached bony ends providing good mechanical strength of the graft (Kurosaka *et al.*, 1987; Steiner *et al.*, 1994). However, complications with the harvest of the BPTB graft include: rupture of the remaining patellar tendon (Delee and Craviotto, 1991; Marumoto *et al.*, 1996; Miller *et al.*, 1999), decreased strength of the quadriceps (Hashemi *et al.*, 2005; Kobayashi *et al.*, 2004), and patello-femoral problems (Tria *et al.*, 1994; Shino *et al.*, 1993) are often associated with the harvest of the BPTB graft.

Natural healing process

Tendons have a limited blood supply and a slow cellular turnover (Fenwick *et al.*, 2002; Kannus *et al.*, 1997). For this reason, healing is prolonged and often results in the formation of a dysfunctional scar and a tissue defect. Based on animal studies, tendon healing can be divided into three overlapping phases: the inflammatory, repairing, and remodeling phases. The initial inflammatory phase lasts about 24 hours after injury, followed by the repairing phase, which lasts from a few days to 6 weeks after injury. During the remodeling phase, the repaired tissue changes to fibrous tissue, which again changes to scar-like tendon tissue after 10 weeks. During the later remodeling phase, covalent binding between collagen fibers increases, which results in higher stiffness and tensile strength of the repaired tissue (Wang, 2006).

Biomechanics of natural healing

To investigate the biomechanical changes of the injured patellar tendon, Atkinson et al. removed the medial one-third of the patellar tendon in 18 female goats, and the mechanical properties of the tendons were measured at 0, 3, and 6 months after the operation. All the animals were allowed unrestricted mobilization on a farm. The mechanical properties of tendons were studied using the tensile testing method in which isolated tendon specimens are stretched by an external force, while both the specimen deformation and the applied force are recorded. Their results showed significant decreases in tensile stiffness and modulus of the operated tendons (right limbs) compared to the healthy tendons (left limbs) at all three time points, except the tensile modulus at time zero. In addition, the tendon stiffness increased 68% from 0 to 3 months ($p<0.05$), and another 26% from 3 to 6 months ($p<0.05$) after the operation (Atkinson *et al.*, 1998). A rapid early increase in tendon

strength and stiffness followed by slower improvements after surgery has also been observed in other studies (Butler *et al.*, 2004). Moreover, the tendons often did not achieve normal tendon failure force in the long-term (Jackson *et al.*, 1993; Bruns *et al.*, 2000).

Based on animal models, mechanical properties of tendons decrease dramatically after injury or loss of activity, and increase gradually during healing/ recovery process. However, due to the invasiveness of the traditional tensile testing method, the mechanical properties of human tendons after injury have not been studied. On the other hand, novel technology, such as growth factors, gene therapy, and tissue engineering, have been considered to affect musculoskeletal tissue healing (Martinek *et al.*, 2000; Sharma and Maffulli, 2005). Monitoring tendon stiffness may provide a quantitative means for determining which treatments are most effective for promoting tissue healing. Therefore, a non-invasive method to evaluate the mechanical properties in tendons under *in vivo* environments is needed.

In vivo ultrasonography

Medical ultrasound waves have frequencies ranging from 2.5 to 40 MHz, which is many times higher than the upper limit for human hearing (14 to 20,000 Hz). High frequency ultrasound can provide information about tissue *in vivo* by producing a reflected sound image. Rapid image formation can reveal organ movement in real time. Ultrasound images are constructed by computing the time taken for an ultrasound beam to travel from a transducer, and return from a reflecting surface. The magnitude of the echo influences the brightness of a display, and is coded as gray-scale. Clinical ultrasound systems use computer methods for producing real-time images. Linear and curved array transducers are most commonly used

for diagnostic ultrasound. The linear array is commonly used for examining the abdomen and tendon. The linear array will produce a rectangular view, which provides good definition to both near and distant anatomy, and good image quality across a full image depth and field of view. Measurements of the cross-sectional area and volume of human skeletal muscles have been proved to be valid and reproducible while compared to MRI (Reeves *et al.*, 2004). A typical linear transducer would have a 3.5~7.5 MHz frequency range. A transducer with higher frequency will produce images with higher resolution.

In 1995, a real-time ultrasonography method was first introduced to study the elastic characteristics of human tendon *in vivo* (Fukashiro *et al.*, 1995a; Fukashiro *et al.*, 1995b). The *in vivo* method is based on real-time ultrasound scanning of a reference point along the tendon during voluntary isometric contraction of the in-series muscle. The limb is fixed to the load cell of a dynamometer to record the changes in joint moment during activation and subsequent relaxation in a maximal isometric contraction. The joint moments recorded correspond to muscle forces that can be calculated from the moment equilibrium equation. The muscle forces generated with activation pull the tendon proximally and cause a longitudinal deformation, which is measured by the recorded deformation of a reference landmark in the tendon. On relaxation following activation, the tendon recoils and the reference landmark shifts distally. The force-elongation plots obtained during loading-unloading can also be reduced to the respective stress-strain curve plots by normalization to the dimensions of the tendon, which can also be measured using ultrasonography.

Over the last 3 years, many investigators have utilized ultrasonography to study the viscoelastic characteristics in several tendons of the lower extremity or biceps brachii (Hansen *et al.*, 2006; Loram *et al.*, 2006; Reeves *et al.*, 2003; Maganaris *et al.*, 2006b; Maganaris *et al.*, 2006a; Kubo *et al.*, 2004; Kubo *et al.*, 2006). The myotendinous junction or the

bony insertion of a muscle is typically tracked using a 7.5 MHz, B-mode linear ultrasound probe. During the contraction experiments, video recordings of the sonograms are constructed (30Hz). The elongation of the tendon is then acquired by measuring the change in length between the myotendinous junction or bony insertion and the distal attachment of the tendon to bone from the sonogram over the contraction cycle. Most transducers in clinical use are not long enough to trace both ends of the tendon, thus special devices are typically utilized to fix one end of the tendon insertions (i.e. either proximal or distal). Most studies have also used a skin marker, made of an echoabsorptive material. This marker is used, to eliminate the errors in tendon deformations because of sliding between skin and probe. In this manner, the investigator is assured that the measured deformation is that of the tendon relative to the probe, and not vice versa. Test-retest reliability of measuring tendon stiffness using ultrasonography was reported 0.80 and 0.82 for right and left Achilles tendon during passive movement, indicating good reliability, and 0.88 for aponeurosis of vastus laterlais (VL) muscle during isometric maximal voluntary contraction (IMVC) knee extension task (Kubo *et al.*, 1999).

Studies on the patellar tendon

Previous studies which assessed patellar tendon stiffness of healthy subjects (age: 20~30 years) with the ultrasonic technique have reported values in the range from 1790-4334 N/mm (Hansen *et al.*, 2006; Kubo *et al.*, 2006). The large variation among studies could be associated with the different equipment utilized, testing procedures, fixation methods, and the variation in subjects with age, activity level, and anthropometric characteristics. This technique has also been shown to have the capability of detecting the effect of strength training on altering the mechanical properties of tendons (Kubo *et al.*,

2006).

Strengthening effect

Reeves et al. (2003) investigated the effect of strength training on the viscoelastic properties of the patellar tendon in elderly individuals (age: 74.3±3.5 years; N=9). Training was performed 3 times per week, and consisted of 2 series of 10 repetitions of knee extension and leg press at 80% of five-repetition maximum for 14 weeks. Measurements of tendon elongation during a ramp isometric knee extension were performed before and after training with *in vivo* ultrasonography. Their results showed tendon stiffness increased by 65%, and Young's modulus increased by 69% following training (Reeves *et al.*, 2003). In another study of the effect of isometric squat training on tendon stiffness, 8 younger adults (age: 20±1 years) were trained using a horizontal leg press training device. Isometric leg press resistance exercises were performed at 70% of maximal voluntary contraction for 15s x 10 repetitions per day. Their results also showed a significant increase in the stiffness of the tendon-aponeurosis complex in the vastus lateralis muscle and patella tendon by 16% after 12 weeks of training (4 days/week) (Kubo *et al.*, 2006). Their results were in agreement with previous *in vitro* studies indicating that strength training would increase the stiffness and Young's Modulus of tendons.

Disuse effect

There have only been a few studies to determine the effects of tendon disuse on tendon stiffness in humans. Therefore, our knowledge of these effects on the tendon is limited. Kubo et al. (2004) investigated the effects of 20 days' of

bed rest on the mechanical properties of knee extensors of 8 healthy males (age: 24±4 years). Subjects remained in a 6° head-down tilt bed rest at all times and did not assume any weight-bearing posture until all the tests were conducted. Their results showed tendon stiffness of vastus lateralis (VL) significantly decreased from 70.3 to 50.1 N/mm after bed rest. However, their measurements were determined from the deformation of the aponeurosis, which maybe more compliant than the free tendon (Magnusson *et al.*, 2003).

Summary

Tendon injuries occur frequently, and produce substantial disability. Due to the invasiveness of the traditional tensile testing method in studying tendon stiffness, no study has investigated how the mechanical properties change with the healing in human tendons. The *in vivo* ultrasonography technique has the potential of documenting the changes during the healing process. Currently, the effects of those novel management strategies, such as tissue engineering, gene therapy, and growth factors, on improving or shortening the healing process are still based on animal studies. Therefore, development of a protocol with the real-time ultrasound technique to track the change of mechanical properties of tendon during recovery will not only help us understand the time frame and nature of the healing process in humans, but will also provide a means to evaluate the efficacy of novel technologies in treating injured musculotendinous tissue.

CHAPTER III

Specific Aim 1: To evaluate the capability of this USD technique in detecting the effect of patellofemoral pain syndrome on mechanical properties of the patellar tendon.

Hypothesis: Patellofemoral pain will cause mild disuse of the affected limb, and thus cause a subsequent decline in tendon stiffness when compared to healthy control tendons. The USD imaging technique can detect the change.

The research design to address Specific Aim 1:

Introduction

Patellofemoral pain syndrome (PFPS) is the most commonly reported knee pain in the United States (Devereaux and Lachmann, 1984). It is defined as retropatellar or peripatellar pain resulting from physical and biochemical changes in the patellofemoral joint. This pain may limit activity level and decrease loading on the patellar tendon (Hahn and Foldspang, 1998). Previous animal studies suggest that immobilization of the limb alters the mechanical properties of tendons (Atkinson *et al.*, 1998; Kasperczyk *et al.*, 1991). An ultrasonic technique has been applied to evaluate mechanical properties of human

tendons *in vivo.* This technique has been used to evaluate the effect of complete disuse and strengthening exercise on tendon stiffness (Maganaris *et al.*, 2006b; Kubo *et al.*, 2004); however, no study has been conducted to evaluate the effect of PFPS on tendon properties *in vivo*.

The success of the rehabilitative treatments for PFPS remains controversial due to the subjective content of evaluation methods, such as pain scales and functional activity questionnaires (Witvrouw *et al.*, 2000; Lun *et al.*, 2005). An ultrasonic technique may be combined with the conventional methods to provide a more quantitative means for assessing the efficacy of these therapies. Therefore, the purpose of this study was to utilize the ultrasonic technique to assess the effect of PFPS on the mechanical properties of the patellar tendon.

Methods

Subjects:

Seven subjects with PFPS (age: 26±4 y/o, height: 176±10 cm, body mass: 79±21 kg; mean±SD) and seven age and weight-matched control subjects (age: 27±4 y/o, height: 177±9 cm, body mass: 79±22 kg; mean±SD) were recruited to participate after the study was approved by the institutional review board.

Data Collection:

Subjects were asked to perform five isometric maximal voluntary contractions (IMVC) of the knee extensors at 90º of flexion while secured on a strength testing system (Kincom125E Plus, TN). The force data were sampled with and reduced using a custom program within LabVIEW 6i (National Instruments, Austin, TX).

During the IMVC task a 7.5 MHz linear-array ultrasound (USD) transducer (Compact System Diasonics, Santa Clara, CA) was secured to the anterior surface of the knee and utilized to track the displacement of the inferior border of the patella. A silicon template was taped onto the skin over the patella-tendon area to prevent sliding of the USD probe. An echo-absorptive band was also attached to the skin over the scan region to monitor movement of the transducer relative to the skin. The image scans were recorded at 30 Hz with an image grabber PC card and commercial software (VCE-Pro, Imprex, Boca Raton, FL). The knee torque and ultrasound video data were synchronized with a triggering device and acquired on an IBM computer (Figure 3.1). In addition, surface electromyographic (EMG) electrodes were positioned in parallel over the area of greatest bulk of the hamstrings. Muscle activation amplitude, onset, and duration were assessed using a non-telemetered, surface EMG system (Delsys Bagnoli-8, Boston, MA) with a gain of 1000. The raw EMG data were collected by the Motion Monitor® software (Innovative Sports Training, Inc. Chicago, IL) and stored for analysis. Three static images of cross-sectional area (CSA) and original length of the patellar tendon were also recorded using the USD transducer.

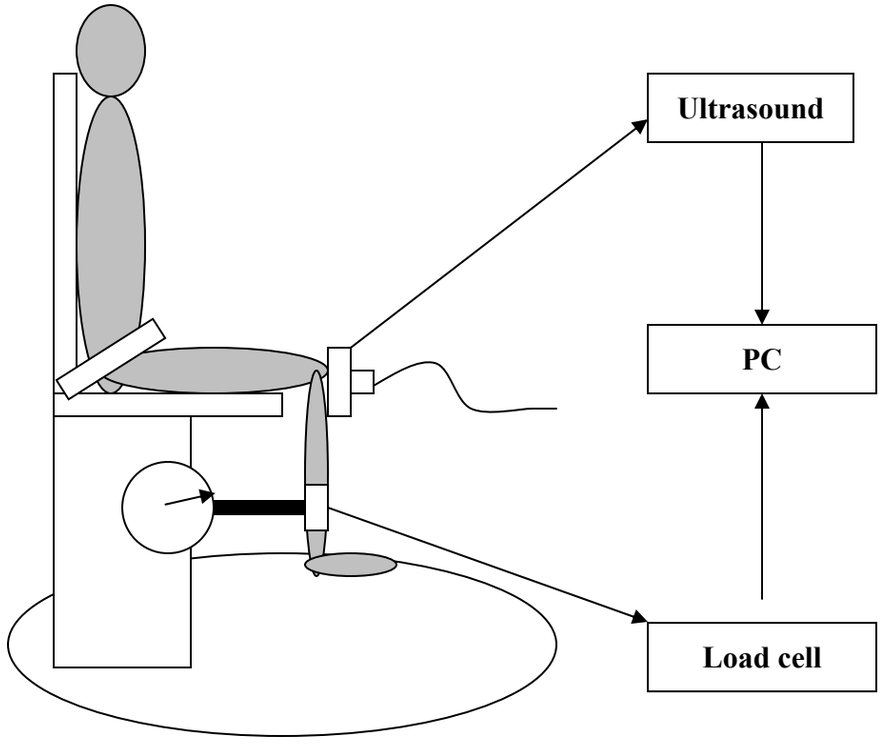

Figure 3.1 Experimental set-up: the USD transducer was placed at the inferior pole of the patella, and the load cell was attached to the ankle cuff that was fixed to the Kincom dynamometer system. The real-time USD images and loadcell signals were collected with an IBM laptop.

Data Reduction:

The raw EMG data were rectified by taking the Root-Mean-Square with a 20 ms sliding window using custom software (Datapak 2k2). Since the passive forces are very small when the muscle fibers are shorter than their optimal fiber lengths, the isometric linear EMG-Torque relationship was used to calculate the hamstrings torque during the knee extension task (Buchanan *et al.*, 2004). The force data from the loadcell during knee extension task were multiplied by the lower leg length to get the knee extensor torque. The measured knee extensor torque was then adjusted for the hamstrings torque, and the resulting net torque was converted to a linear force with knowledge of the patellar tendon moment arm (Figure 3.2).

$$\mathbf{M_{ptext} = M_{tot} + M_{hext}}$$

$$\mathbf{M_{hext} = (EMG_{hext} / EMG_{hmvc}) \times M_{hmvc}}$$

$$\mathbf{F_{pt} = M_{ptext} / D_{pt}} \qquad \textbf{(Eq. 1)}$$

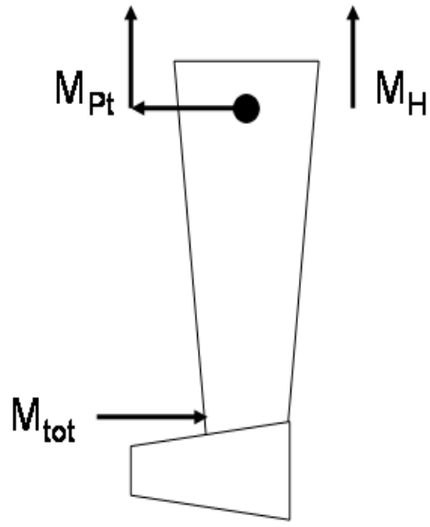

Figure 3.2 **M_{hext} is the torque produced by hamstrings during IMVC extension task; M_{hmvc} is the torque produced by hamstrings during IMVC flexion task; EMG_{hext} is the hamstrings EMG activity during IMVC extension task; EMG_{hmvc} is the RMS EMG activity during IMVC flexion task; M_{ptext} is the torque produced by the patellar tendon during IMVC extension task; M_{tot} is the resultant knee torque during IMVC extension task; F_{pt} is the force produced by patellar tendon; D_{pt} is the moment arm of patellar tendon.**

In our study, the length of moment arm of the patellar tendon was estimated from the thigh length and knee flexion angle of each subject using Equation 2.

$$\mathbf{D_{pt} / Thigh\ Length * 100\% = [0.201285 + 2 * (-0.000545) * \theta] * 180 / \pi} \qquad \textbf{(Eq. 2)}$$

while thigh length was defined as the distance between the most lateral part of the greater trochanter and the most distant part of the lateral femoral epicondyle. This equation was derived from the following equations (Bobbert *et al.*, 1986). .

$$\mathbf{\Delta L = A_0 + A_1\theta + A_2 (\theta)^2} \qquad \textbf{(Eq. 3)}$$

while ΔL is the distance between origin and insertion of a muscle relative to reference distance. By taking the first derivative at θ of this equation (Eq. 3), the following equation (Eq. 4) was obtained.

$$\mathbf{d = [A_1 + 2 A_2 \theta] * 180 / \pi} \qquad \textbf{(Eq. 4)}$$

where moment arm (d) is expressed as a percentage of segment length and θ is the angular deformation at knee joint expressed in degrees; A_1 and A_2 are constants calculated using equations for predicting ΔL (Eq. 3).

A_1 and A_2 used in our study was the average of the different muscle tendons in the quadriceps complexes measured in another study (Visser *et al.*, 1990). Length of muscle tendons complexes in the quadriceps complexes at different knee angles were measured using six human cadavers (3 males and 3 females). Then, the length-angle curves were fitted using

second degree polynomials and the coefficients of the best fitted polynomials relating length of muscle tendon complex to joint angle were determined.

The USD video of the patella-tendon movement was then extracted into a series of BMP images with spatial resolution of 640 x 480 pixels and 8-bit gray resolution. The images of each trial were analyzed with a pattern matching technique (IMAQ Vision; National Instruments, Austin, TX). Pattern matching was accomplished with a normalized cross correlation objective function.

$$c(i, j) = \frac{\sum_{x=0}^{L-1}\sum_{y=0}^{K-1}(w(x,y)-\overline{w})\left(f(x+i, y+j)-\overline{f}(i, j)\right)}{\left[\left(\sum_{x=0}^{L-1}\sum_{y=0}^{K-1}(w(x,y)-\overline{w})^2\right]^{\frac{1}{2}}\left[\sum_{x=0}^{L-1}\sum_{y=0}^{K-1}(f(x+i, y+j)-f(i, j))^2\right]^{\frac{1}{2}}}$$

(Eq. 5)

Where $\overline{w}$ is the average intensity value of the pixels in the template w and the variable $\overline{f}$ is the average of the value of f in the region coincident with the current location of w.

After selecting a template (size K x L) on the initial unloaded tendon grayscale image (size M x N), pattern matching performs a search of the template within each image. Pattern matching is the process of moving the template around the image area and computing the value of C in that area. This involves multiplying each pixel in the template by the image pixel that it overlaps, and then summing the results over all the pixels of the template. The maximal value of C indicates the position where the best match is. After searching for the template in the loaded tendon image, the center X and Y-coordinates

of the objects that matched the template were displayed as pixels from the local origin of the image. The deformation across each image was then calculated with the knowledge of the conversion factor 0.048 mm/pixel. The linear force data as a function of patellar tendon deformation were used to calculate the structural stiffness with a secant approach under maximal loading (Figure 3.3). Each tendon's maximal force level and the corresponding deformation were chosen as the points of interest; then the slope of a line drawn from the point of interest to the origin of the force-deformation curve was calculated.

The pixels within the CSA and the original length of the patellar tendon from the static images were measured with IMAQ Vision Builder and converted to metric units (mm^2 and mm, respectively) (Figure 3.4). The average tendon CSA and original length were used to calculate the tendon stress (force/CSA) and strain (deformation/original length). The same approach was used to calculate the secant modulus. These biomechanical parameters of the symptomatic knee were compared with the control group, using a paired-t test with a significance level of $p < 0.05$.

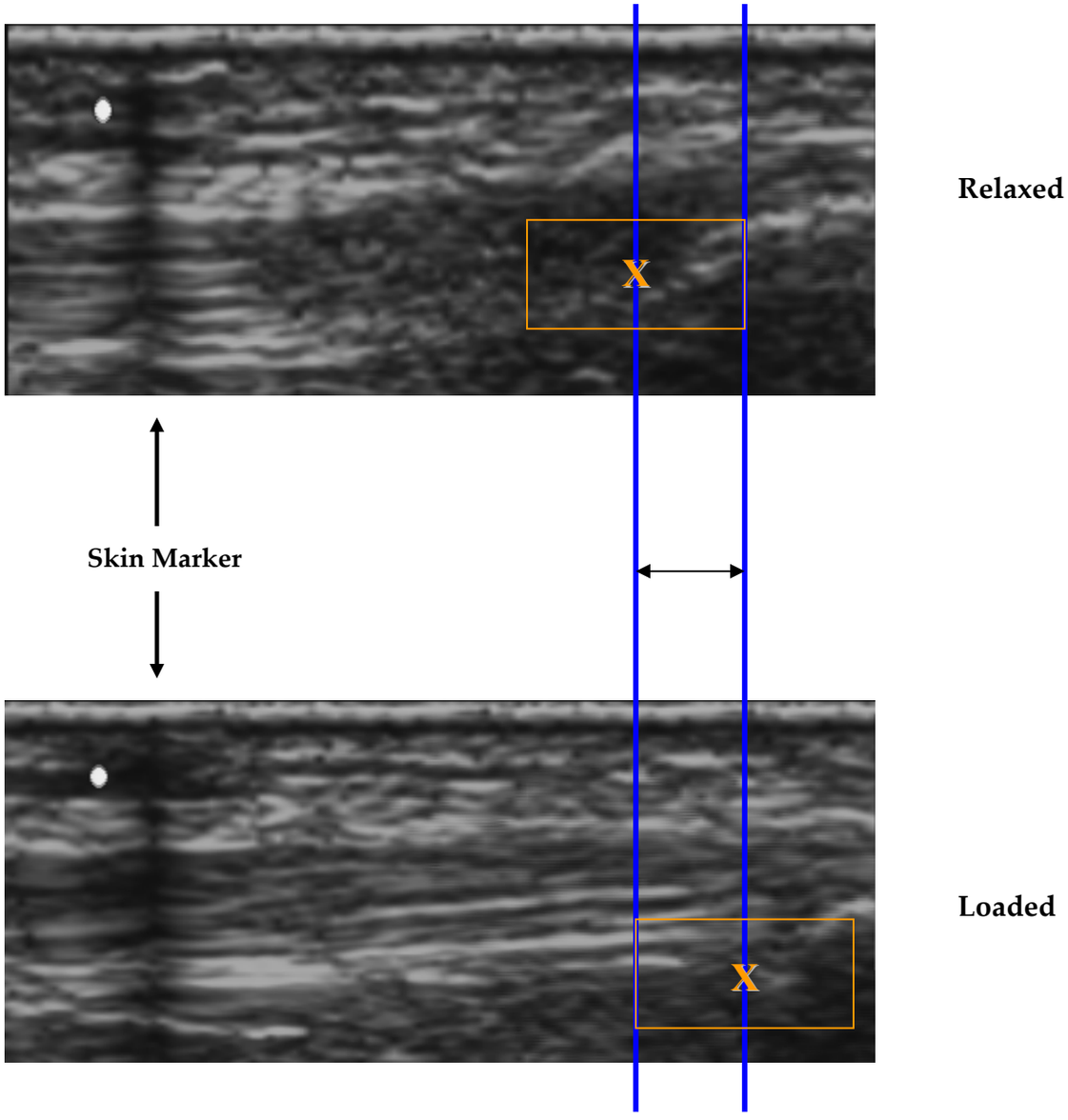

Figure 3.3 USD images of the deformation of the tendon at the inferior pole of the patella when the tendon is loaded.

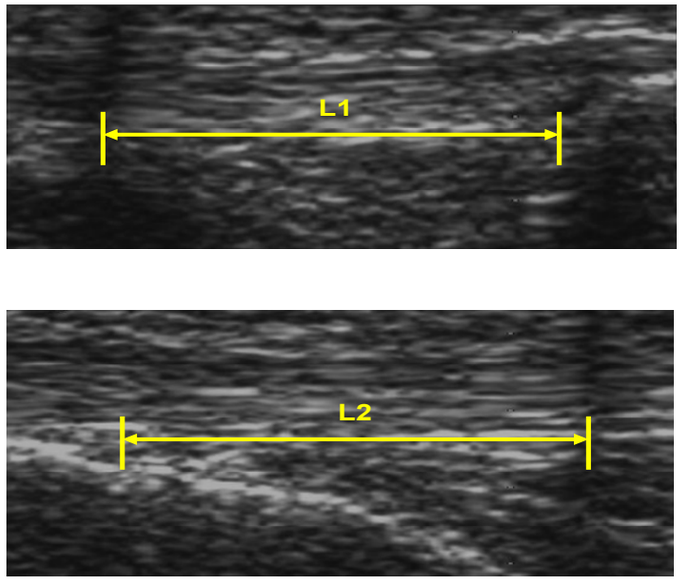

Figure 3.4 The length between the skin marker to the distal end of the patella (L1) and to the most proximal point of the tibial tubercle and patellar tendon junction (L2) were measured in pixels and then summed to get the total length of the tendon.

Results

The average length of symptoms for the PFPS subjects was 43±34 months. The resting length and CSA area of the patellar tendon were similar in the two groups ($P>0.05$). The maximal stress and strain levels during isometric knee extension were not significantly different between the two groups ($P>0.05$); however, tendon stiffness was decreased by ~30% in the PFPS subjects compared with the control subjects ($P<0.05$). Although tendon secant modulus was lower by 34% in the PFPS subjects compared with the control subjects, the difference was not significant statistically ($P>0.05$) (Table 3.1).

Table 3.1 Mean and SD of the main parameters of the PFPS and control subjects. Significance level (P) for a paired T-test comparing the groups is also shown.

Parameter	PFPS	Control	P
Tendon Length (mm)	46 ± 9	52 ± 15	P = 0.190
Tendon CSA (mm^2)	85 ± 13	92 ± 16	P = 0.140
Max. Tendon Force (N)	4906.48 ± 1513.15	6225.42 ± 1577.25	P = 0.005
Max. Tendon Deformation (mm)	2.70 ± 0.81	2.68 ± 0.96	P = 0.940
Tendon Strain (%)	6.55 ± 2.14	4.62 ± 1.79	P = 0.060
Tendon Stress (MPa)	54 ± 21	66 ± 19	P = 0.350
Stiffness (N/mm)	1735 ± 735	2477 ± 568	P = 0.030
Secant Modulus (GPa)	1.04 ± 0.59	1.57 ± 0.76	P = 0.090
* Mhext / Mptext (%)	4% ± 3%	8% ± 17%	

* Torque created by hamstrings muscle force presented as a percentage of torque created by patellar tendon force during IMVC extension task.

Discussion

In this study, we assumed the maximal deformation/strain and maximal force/stress happened at the same time point, and used the ratio of maximal force/stress divided by the maximal deformation/strain to calculate the stiffness/secant modulus. This approach provided us with an estimate of the mechanical response of the patellar tendon under the tension produced during IMVC of knee extensors. The average value of patellar tendon stiffness of our control group fell within the range reported from previous *in vivo* patellar tendon studies, which was from 1790-1904 N/mm to 3725-4334 N/mm (Hansen *et al.*, 2006; Kubo *et al.*, 2006).

The present results indicate that this newly developed ultrasonic technique is able to detect the changes in structural properties of tendon due to PFPS. The decrease in tendon stiffness in the PFPS group appears to be a combined effect of an apparent decrease in CSA and alteration of the material properties as neither of these changed significantly on their own and both can influence the stiffness. Our results coincide with similar changes in tendon properties reported in previous animal studies of disuse (Kasperczyk *et al.*, 1991; Atkinson *et al.*, 1998). Based on those studies, the reduction in mechanical properties of tendons could be due to the reduction of the total area of collagen fibrils in the tendon cross-section and the increase the numbers of thin and immature fibrils. As the effects of rehabilitative treatments on improving PFPS remain controversial due to the subjective content of evaluation methods, such as pain scale and functional activity questionnaires (Crossley *et al.*, 2004; Witvrouw *et al.*, 2000), this noninvasive ultrasonic technique may be incorporated with the conventional methods to provide a more quantitative means for assessing the efficacy of these therapies.

In this study, we observed some unexpected movements of the distal end of the patellar tendon that could be caused

by (a) imperfect external fixation on the joint, (b) compliance of anatomical structures mediating force transmission from skeleton to the hardware used (e.g., fat pads and ligaments), and (c) compliance of the hardware itself. By using a long ultrasound probe to monitor both ends of the patellar tendon at the same time, Hansen et al. suggested that the distally moved tibial tubercle corresponded to 45% of the overall tibia-patella deformation (Hansen *et al.*, 2006). However, without a long probe, other studies suggested using knee extension angle to estimate the deformation of tibial insertion, with the assumption that the femur was fixed during IMVC task. To test the validity of this assumption, we measured the translation and angular rotation of tibia with a Linear Variable Differential Transducer (LVDT) and an electrogoniometer during the IMVC task. The LVDT was mounted to a rigid stand and its piston contacted an L-shaped plate that was secured to the tibia. The electrogoniometer was attached across the knee. The results of these measurements indicated that the displacement of the tibial tubercle was not caused by a pure rotation of the tibia about the fixed knee axis, but the tibia-femur-patella composite appeared to rotate about the distal support. These findings lead us to suggest that the displacement at the tibial tubercle should not be estimated by knee rotational angle alone. Therefore, we decided to measure the deformation of the tibial insertion in addition to the patellar insertion in future studies.

Other limitations of this study also include: 1. the restriction to two-dimensional images; 2. with the secant method of calculating the tendon stiffness it is possible that observed differences in tendon stiffness between groups could be caused by differences in the extent of the toe region.

CHAPTER IV

Specific Aim 2: To compare the reliability of the conventional USD measurement method, which measures only the displacement of the patellar insertion of the tendon, to a two scan method that adjusts for tibial movement.

Hypothesis: The two scan method will display similar results for the tibial deformation as in previous studies with a long probe method. The two scan method will demonstrate increased reliability compared to the conventional method because deformation of the tendon at the tibial insertion will cause increased variability in evaluating the patellar tendon stiffness.

The research design address Specific Aim 2:

Introduction

Previous studies observed some unexpected movements at the distal end of the patellar tendon due to the compliance of the external fixation on the limb (Hansen *et al.*, 2006; Kubo *et al.*, 2000; Reeves *et al.*, 2003). By using a long ultrasound probe to monitor both ends of the patellar tendon at the same time, Hansen et al. suggested that the distal movement of the tibial tubercle accounted for 45% of the overall tibia-patella deformation (Hansen *et al.*, 2006). However,

without a long probe, other studies suggested using knee extension angle to estimate the deformation of tibial insertion, with the assumption that the femur was fixed during an IMVC task (Reeves *et al.*, 2003; Bojsen-Moller *et al.*, 2003). To test the validity of this assumption, we conducted pilot studies where we measured the translation and angular rotation of tibia with a linear deformation transducer and an electrogoniometer during the IMVC task. The findings from these studies lead us to believe that the deformation at tibia tubercle should not be estimated by knee rotational angle alone. Therefore, as an alternative approach, we investigated a two-scan method in which the deformation of the tibial insertion in addition to the patellar insertion was measured. The purpose of this study was to compare the stiffness values and reliability of the conventional USD measurement method, which measures only the displacement of the patellar insertion of the tendon, to a two-scan method that adjusts for tibial movement. We hypothesized that the two-scan method would show similar results for the tibial displacement as the previous study with a long probe. Furthermore, it was hypothesized that displacement of the tendon at the tibial insertion with the conventional method would decrease its reliability in evaluating patellar tendon stiffness.

Methods

Subjects:

Thirteen healthy subjects (7 females and 6 males) with no history of knee pain or any lower extremity surgery (age: 26±7 y/o, height: 170±7 cm, body mass: 67±11 kg; mean±SD) volunteered to participate in this study.

Data Collection:

A similar experimental set-up and testing procedure as described in Aim 1 was conducted. Subjects were asked to perform six trials of IMVC of knee extension while the USD transducer was positioned at either the patellar or tibial insertion site for the first trial and switched alternately. The knee torque and USD video data were synchronized with a triggering device and acquired on two computers separately. Both USD image and knee torque were recorded at 30 Hz. No EMG data were collected because of the minimal effect of the hamstrings (less than 10%) on knee extension torque during the IMVC task from the previous study. The same procedure of data collection was performed for each subject on 2 separate days.

Data Analysis:

Patellar tendon force was calculated with the knowledge of knee torque and patellar tendon moment arm. The lowest peak tendon force was selected from all six trials of each subject so that the patellar tendon stiffness and secant modulus of each subject were evaluated at the same loading level. The corresponding USD images at the same loading status of each trial were then used to calculate the deformation data with pattern matching, which was described in Chapter 3. Three trials of tibial deformations and three trials of patellar deformations were averaged. Two methods were used to calculate the tendon stiffness and secant modulus. Method I only accounted the deformation at the patellar (proximal) insertion of the tendon, while Method II adjusted the Method I deformation data with the tibial movement by subtracting the average deformation of the tibial (distal) insertion from the average deformation of the patellar insertion.

Patellar Tendon Deformation = Patella Deformation – Tibial Tubercle Deformation

All the deformation data were given positive signs if they were in the proximal direction and negative signs in distal direction. The deformations caused by anterior-posterior displacements of the patella and tibia tubercle were ignored due to the minimal effect (< 10%) on total tendon deformation from this study. Biomechanical parameters were compared between the two days and between the two methods, using a repeated measures two-way analysis of variance (ANOVA) with a

significance level of $p < 0.05$. Between-day repeatability was also analyzed using Pearson's Correlation Coefficient (r) for both methods.

Results

In our study, the movement of the tibial tubercle during IMVC task was quite different between subjects. However, the tubercle moved proximally and then distally during quadriceps activation for most of the subjects (Figure 4.1). Both tendon stiffness and secant modulus were lower using Method II than that of Method I (Table 4.1 & 4.2). Significant differences in the results of tendon stiffness were found when using the different methods ($p=0.001$), but no difference was found between days ($p=0.98$). In addition, a significant difference in tendon secant modulus between methods was found ($p=0.002$), but no difference was found between days ($p=0.97$). Between-day r values for Method I for Method II were 0.64 and 0.84, respectively (Table 4.3).

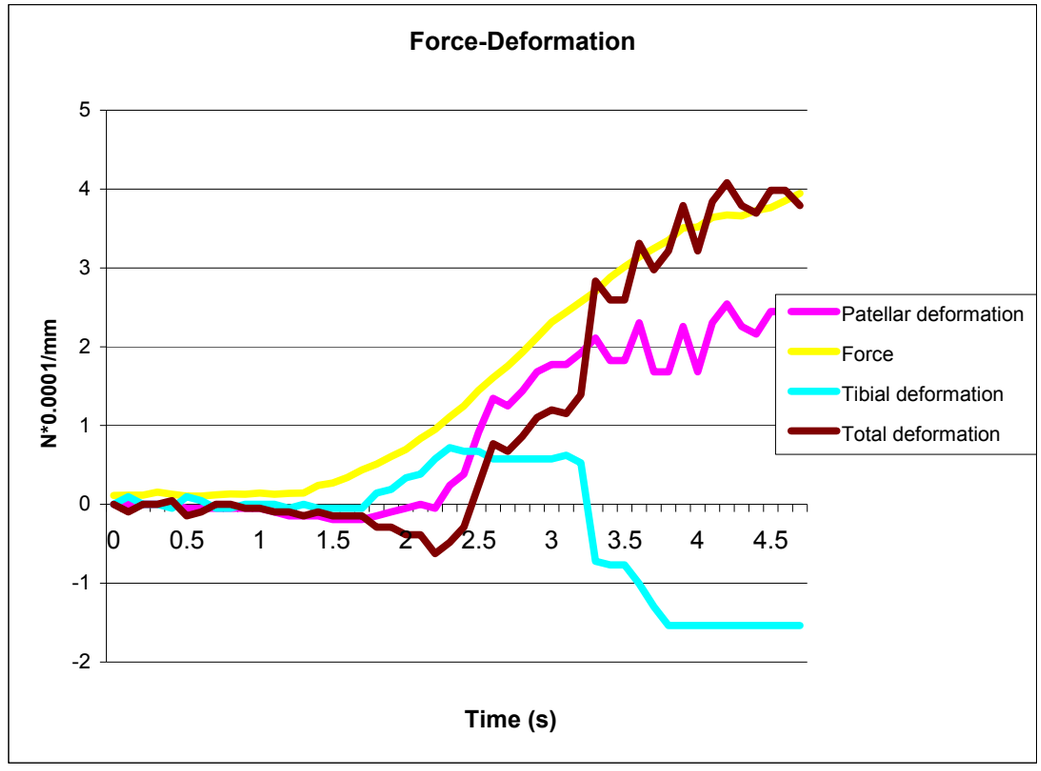

Figure 4.1 Tendon force and deformation across time during IMVC knee extension task.

Table 4.1 Mean and SD of tendon stiffness (N/mm) examined.

	Method I	Method II
Day1	2590.086 ± 1204.292	1653.484 ± 599.871
Day2	2649.713 ± 1488.737	1577.799 ± 550.010

Table 4.2 Mean and SD of tendon modulus (GPa) examined.

	Method I	Method II
Day1	1.231 ± 0.692	0.763 ± 0.279
Day2	1.263 ± 0.735	0.742 ± 0.263

Table 4.3 Pearson's Correlation Coefficient (r) of between-day tendon stiffness measurements examined.

	Method I	Method II
R	0.64	0.84

Discussion

The results of our study showed that Method II had higher between-day repeatability for evaluating the stiffness and modulus of the patellar tendon. Our study also showed the distal movement of the tibia tubercle accounted for 38% of the overall patella-tibia deformation, which revealed similar results to a previous study which used a long probe approach (Hansen *et al.*, 2006). The conventional USD method (Method I) tends to overestimate the stiffness of the tendon due to the fact that the distal tibial tubercle movement was not included in calculating the total tendon deformation. In addition, the superior translation of the tibial tubercle caused by the tibial extension with a fixed knee joint only happened at the very beginning of the entire IMVC. When the activation level of quadriceps muscle force increased, the femur was not fixed and appeared to move with the tibia, which maybe due to the compression of the soft interface cushions of the femur and tibia segments. This observation coincided with the results of the previous study with the LVDTs and electrogoniometer, suggesting that the tibial insertion should not be estimated using the knee rotational angle alone.

A limitation of this study was that the displacements at the patellar and tibial insertions were collected at different trials, thus the averaged data of the deformations at the two insertion sites were used with the averaged tendon force data to calculate the tendon stiffness values. We were not able to test the within-day reproducibility because only one stiffness value could be determined from the six trials of one subject.

CHAPTER V

Specific Aim 3: To track the recovery of patellar tendon stiffness properties after harvesting of the central third of the patellar tendon as an autograft for ACL reconstruction by the USD measurement method.

Specific Aim 4: To evaluate the correlation between other clinical evaluation tools for knee recovery, such as Visual Analog Scale (VAS), Activity Rating Scale (ARS), and International Knee Documentation Subjective Knee Form, (IKDC), and muscle strength with the tendon stiffness measurements.

Hypothesis for Aim 3: There will be a gradual increase in tendon stiffness after the surgery due to the restoring of weight-bearing to the limb and the healing of the tendon, and the USD measurement method will be able to detect this change in stiffness across time.

Hypothesis for Aim 4: The other recovery measurements will be poorly correlated with tendon stiffness.

The research design to address Specific Aim 3 and 4:

Introduction

Tendon injuries are common among athletes as well as the general population. 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 turn over of the tissues (Fenwick *et al.*, 2002; Kannus *et al.*, 1997). Studies have suggested that the mechanical properties of tendon provide an indication of not only the functional capability of a tendon, but also the recovery level of the internal tissue material (Atkinson *et al.*, 1998; Kasperczyk *et al.*, 1991; Kasperczyk *et al.*, 1993). 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 the recovery of tendons in human, 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 unknown. The results of our previous studies showed the *in vivo* USD imaging technique was able to not only detect the subtle change in tendon stiffness caused by limb mild disuse but was also reliable across time with the two scan method. Therefore, we felt confident to apply the two-scan technique to monitor the change in patellar tendon stiffness due to healing. The primary objective of this study was to evaluate the stiffness of the healing patellar tendon after the ACL reconstruction with patellar tendon autograft using the two-scan USD method. The secondary objective of this study was to determine if the tendon stiffness is correlated with other clinical outcome measures of recovery.

Methods

Subjects:

Ten subjects undergoing ACL reconstruction with a patellar tendon autograft were recruited for the study as well as 10 healthy control subjects. Each healthy subject was matched with one surgical subject by sex, weight, activity level, age, and height. Inclusion criteria for the surgical group were 1) age between 18 and 40 years, 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 Control group were 1) age between 18-40 years, 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.

Subject Preparation Procedure:

Upon arrival to the laboratory, subjects were asked to read and sign the informed consent agreement (IRB# 05-ORTHO-1005) outlining the procedures, protocols, and potential risks of the study. Subjects who agreed to participate in this study filled out a Visual Analog Scale (VAS), Activity Rating Scale (ARS), and International Knee Documentation Subjective Knee Form (IKDC) (Appendix I) at each visit. EMG electrodes were then positioned in parallel over the belly of the biceps femoris of the subjects' thighs, and the ground electrode was positioned at the contralateral patella to the testing

knee. All hair was removed from the area of electrode placement and the skin was scrubbed with an alcohol-soaked pad before the electrode placement. The electrodes were secured with adhesive tape, and a 10-mm distance was used between the electrodes' centers. The subjects were then asked to sit on the muscle strength system with their thighs stabilized on the seat and the knee flexed to 90°. The axis of rotation for 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 the knee joint center. Two silicone templates were placed on the patella and tibial tubercle as described in aim 2 to prevent sliding and an echo-absorptive marker was used to monitor the sliding of the probe during testing. Because of the loss of bone and tendon from the ACL reconstruction surgery, the silicone templates were placed more laterally for the surgical tendons. Each subject was instructed to perform isometric knee extension contractions by slowly increasing the force to maximum over the first 3 seconds, and then to maintain the maximal contraction 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.

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 alternately placed on either the patella to track the deformation of the inferior border of the patella, or the tibial tubercle to track the deformation of distal insertion of the patellar tendon. The knee torque and the USD video data collection were initiated simultaneously with a triggering device, and acquired on two computers. The

load cell signal and its known distance relative to the knee joint center allowed us to monitor the knee extension torque in real time. The biceps femoris muscle activation amplitude was assessed using a non-telemetered, surface EMG system (Delsys Bagnoli-8, Boston, MA) with a gain of 10K. An analog bandpass filter (20-450 Hz) was hardwired into the EMG system. Both load cell and EMG signals were sampled at 1020 Hz. The USD images were collected at 30 Hz. The cross-sectional area and the original length of the patellar tendon were also measured from ultrasonic images. Surgical subjects had their patellar tendon stiffness evaluated at 2 and 6 months after surgery 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 while the control subjects were evaluated for only the knee that matched to the same side of the matched surgical knee. The control and contralateral groups were used to see if there is any external factor that may cause the change of tendon stiffness besides healing. The contralateral group was also used to assess the relative recovery level in tendon stiffness (% of intact) of the surgical group.

Data Analysis:

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 filter with cutoff frequency at 5 Hz. EMG data were adjusted to the offset by subtracting the average value from the entire time series and filtered with a sixth order, phase-corrected Butterworth filter with cutoff frequency at 10 Hz to calculate the linear envelope.

The hamstring contribution was estimated with the EMG data multiplied by the ratio of peak force to 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.

$$\mathbf{F_{pt} = (F_{tot} + F_{hext}) * MA_{leg} / D_{pt}} \qquad \textbf{(Eq. 6)}$$

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 and D_{pt} was the the patellar tendon moment arm estimated from femur length and knee flexion angle of each individual using Eq.2. The patellar moment was then scaled to the average values computed from previous reports. (Baltzopoulos, 1995; Krevolin *et al.*, 2004; Kellis and Baltzopoulos, 1999; Wretenberg *et al.*, 1996). Thus, at 75°, 80°, 85°, and 90°, D_{pt} was 42.1, 42.8, 44.8, and 44.7 mm, respectively. With our custom Labview programs, we followed mainly four steps to compute the patellar tendon force-elongation data. First, we found the peak tendon force of all the trials for each subject, and then picked the minimal tendon force (min-max force) among the trials for each paired surgical and control subject. The program then picked the ultrasonic scan number at 0, 10%, 20%, 30%, 40%, 50%, 60%, 80%, 90% and 100% of the min-max force for each trial. In order to select a better defined initial loading state for the start of the computed load-deformation response, zero load was defined as the point in time when the load cell of the dynamometer increased to 25N of load. The deformations calculated from the ultrasonic scans were also referenced to this point in time. Second, we applied the pattern matching technique for automating the tracking 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 Vision program. The matched template of the previous frame became the new template for the next frame. The trials were excluded if the sliding of the probe was detected by the movement of the anechoic marker or distal movement of the patella. We then calculated the tendon deformation of the tracked entity across the entire extension task and found the deformations at the ultrasonic scan numbers corresponding to the 10% intervals of the min-max tendon force. Third, another program automatically picked the corresponding deformation from both the patellar and tibial trials at the same load level. The deformation at the patellar insertion at each loading level was averaged across the 3 trials as well as the deformation at the tibial insertion. The deformation at the tibial insertion at the same loading level was adjusted to the deformation at the patellar insertion by subtracting the averaged tibial deformation from the average patellar deformation at each load level. The deformation in the longitudinal (X) and perpendicular (Y) directions relative to the scan surface was summed to get the total deformation (H) using the Pythagorean Theorem (Eq. 7).

$$H = \sqrt{X^2 + Y^2} \qquad \textbf{(Eq. 7)}$$

The computed deformations were then plotted at every 10% load interval. With this procedure, we could analyze the stiffness of the paired surgical, contralateral and control tendons at the same loading level. Finally, each force-deformation curve was fitted to both a linear function (y = mx, where y was the tendon force and x was the deformation; m was the linear stiffness) and a nonlinear function. The force-deformation data was fit to an exponential function ($y = a (e^{bx}-1)$, where y was the tendon force and x was the deformation) (Stromberg and Wiederhielm, 1969; Fung, 1967) using a nonlinear curve fitting

program based on the Marquardt-Levenberg Algorithm (Fung *et al.*, 1979; Marquardt, 1963). The initial guesses of a and b were determined by initially fitting each curve manually and then a range of a and b was used successively to approximate the data. The best fit coefficients a and b were determined using the least mean squared error. By taking the first derivative of the nonlinear function y at x, the tangential stiffness (dy/dx, also the instantaneous slope of force-deformation curve) was calculated as a function of tendon force (Eq. 8).

$$\mathbf{y = a\,(e^{bx} - 1)}$$

$$\mathbf{dy/dx = a\,b\,e^{bx}}$$

$$\mathbf{y = a\,e^{bx} - a}$$

$$\mathbf{a\,e^{bx} = y + a}$$

$$\mathbf{dy/dx = b\,(y + a)} \qquad \textbf{(Eq. 8)}$$

Therefore, the function: dy/dx= b*F + a*b with coefficients a and b that were obtained from the nonlinear fit of the force-deformation data were then used to calculate the tangent stiffness at specific tendon force levels. The tangent stiffness was obtained at two force levels: the same force level for all the matched subjects and the same force level for all the subjects. 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 the stiffness and original tendon length divided by the CSA.

Due to the small sample size in this study, a paired sign-rank test was performed to compare the tendon length, CSA, and maximal knee extension torque across time for all the 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 paired sign-rank test was also performed to examine the group effect on the change in VAS, ARS, IKDC, CSA, tangent stiffness across time at both force levels, and normalized tangent stiffness, with a significance level of $p<0.05$. A priori power analysis was performed to determine the required sample size. Under the assumption of the standard deviation = 30%, 10 pairs of subjects were required to obtain 80% statistical power to detect a 34% difference 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 with the tendon CSA, 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.

Assumptions of the USD measurement method:

1. Sliding of USD probe relative to skin can be detected or eliminated with the use of the skin marker and silicon template.
2. Skin movement with the patella and tibia is assumed negligible during quadriceps isometric activation.

Results

A total of 10 surgical subjects (6 male and 4 female) and 10 control subjects (6 male and 4 female) were screened and recruited for the study. The average time from injury to surgery for the surgical subjects was 49.5 days (range: 20~99). Two surgical and two control subjects were not able to come for the follow-up testing session and thus were excluded from the data analysis. The average age, height, and body mass of the surgical subjects used in data reduction analysis were 22.5±3.74 y/o, 179.71±7.86 cm and 86.67±9.07 kg, respectively. The average age, height, and body mass of the control+ subjects used in data reduction analysis were 24±6.72 y/o, 179±7.15 cm and 88.12±12.81 kg, respectively (Table 5.1).

The average scores of the VAS, ARS and IKDC Subjective Knee Evaluation collected from each groups at the two visits were summarized in Table 5.2. Day effects on the VAS, IKDC and ARS scores were significant in the surgical group ($P<0.05$). The VAS scores decreased significantly at the second visit, while the IKDC and ARS scores improved significantly at the second visit. No between-day difference was shown in any of these scores for our control group (Table 5.3).

Table 5.1 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- Are you a high competitive sports person?

3- Are you well-trained and frequent sporting?

2- Sporting sometimes

1- Non-sporting

Table 5.2 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

*Appendix I: VAS is scored by calculating the ratio of the length from the vertical mark to the point indicated no pain at all and the total length of the horizontal line, and then transforming the score to a scale that ranges from 0 to 10. ARS is scored by summing the scores of each activity. The activity level ranges from less than one time in a month (0 point) to 4 or more times in a week (4 points). The IKDC Subjective Knee Evaluation Form is scored by summing the scores for the individual items and then transforming the score to a scale that ranges from 0 to 100.

Table 5.3 A paired sign-rank test was performed to examine the day effect on IKDC, VAS, and ARS.

	IKDC	VAS	ARS
Surgical	P = 0.008	P = 0.016	P = 0.016
Control	P = 1.000	P = 1.000	P = 1.000

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 (Table 5.4 & 5.5 & 5.6). The maximal knee extension torque without considering the co-contraction torque during isometric knee extension was also analyzed. The maximal extension torque in the surgical and contralateral groups showed a significant increase at the second visit ($P<0.05$), and no difference was shown in the control subjects (Table 5.4 & 5.5 & 5.6).

The force-elongation plots of the control and contralateral tendons demonstrated similar curves with larger toe-regions when compared with the surgical tendons, and thus the secant and linear methods may underestimate the tendon stiffness of the linear region (Figure 5.1; Table 5.4 & 5.5 & 5.6) for the control and contralateral tendons. Therefore, in order to more effectively assess the stiffness in the linear region we decided to use the tangent stiffness at two load levels to compare between groups. The higher load level was the lowest peak tendon force among the matched subjects, so that we could compare between paired subjects. The lower load level (642.17 N) is the lowest peak tendon force among all the subjects so that we can compare between subjects.

Table 5.4 Mean and SD of the main parameters of the surgical tendon of the surgical subjects. Significance level (P) for a paired sign-rank test comparing parameters across time is also shown.

Parameter	2 month	6 month	P
Tendon Length (mm)	44.95 ± 6.34	44.27 ± 4.17	P = 0.195
Tendon CSA (mm^2)	118.72 ± 15.12	100.17 ± 26.39	P = 0.039
Max. Knee Ext. Torque (Nm)	547.44 ± 227.72	1088.49 ± 402.79	P = 0.008
Secant Stiffness (N/mm)	1359.13 ± 310.85	984.52 ± 781.70	P = 0.078
Linear Stiffness (N/mm)	981.63 ± 417.89	946.40 ± 766.05	P = 0.313

Table 5.5 Mean and SD of the main parameters of the contralateral tendon of the surgical subjects. Significance level (P) for a paired sign-rank test comparing parameters across time is also shown .

Parameter	2 month	6 month	P
Tendon Length (mm)	43.54 ± 6.20	42.12 ± 5.77	P = 0.289
Tendon CSA (mm^2)	104.38 ± 11.43	104.89 ± 10.13	P = 0.727
Max. Knee Ext. Torque (Nm)	1117.36 ± 635.12	1369.12 ± 707.87	P = 0.070
Secant Stiffness (N/mm)	1400.23 ± 575.24	1519.89 ± 645.38	P = 1.000
Linear Stiffness (N/mm)	1227.74 ± 524.08	1400.53 ± 532.48	P = 0.820

Table 5.6 Mean and SD of the main parameters of the tendon of the control subjects. Significance level (P) for a paired sign-rank test comparing parameters across time is also shown.

Parameter	2 month	6 month	P
Tendon Length (mm)	44.17 ± 4.02	42.42 ± 3.63	P = 0.180
Tendon CSA (mm^2)	91.05 ± 8.32	91.24 ± 5.31	P = 0.523
Max. Knee Ext. Torque (Nm)	1633.76 ± 846.53	1357.00 ± 430.93	P = 0.234
Secant Stiffness (N/mm)	1187.57 ± 572.08	1044.29 ± 419.25	P = 0.070
Linear Stiffness (N/mm)	1139.37 ± 493.82	933.78 ± 383.35	P = 0.070

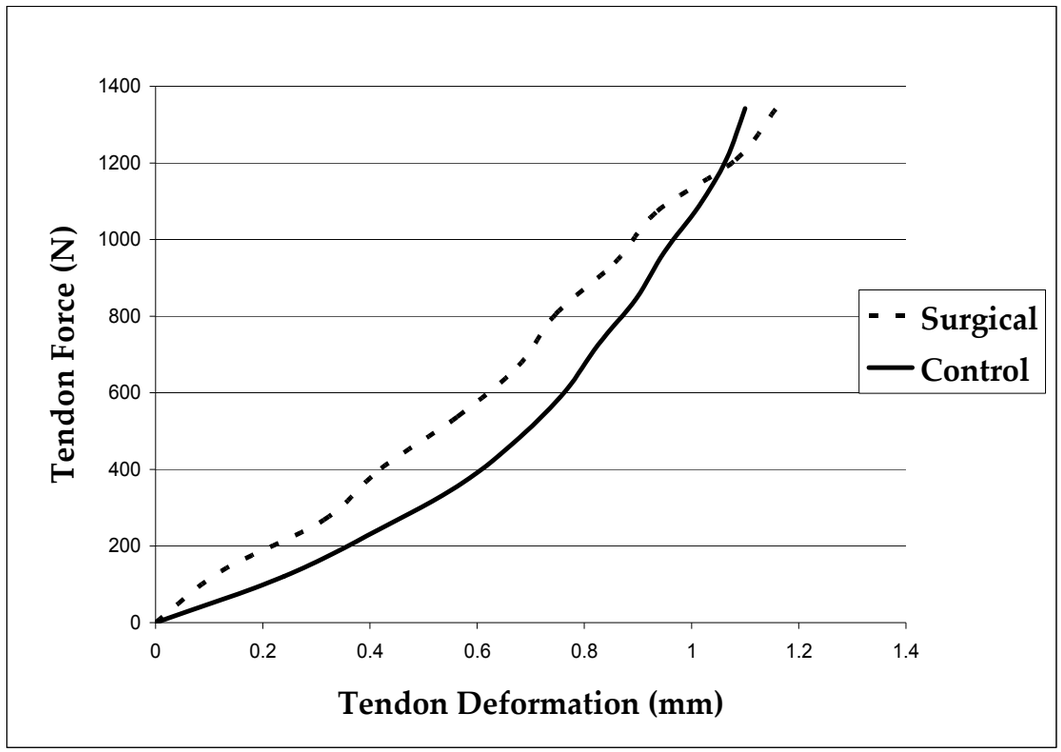

Figure 5.1 Force-deformation curve of surgical and control tendons: the curve of control tendon demonstrated an extended toe-region. The average deformation at every 10% force level, and the averaged absolute force levels were used to plot the curve. In order to normalize to the weakest subject for the paired comparison, the force level may not have exceeded the toe-region for the control tendons. Therefore, the secant and linear approaches may underestimate the stiffness in the linear region in 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.

During the second testing session, the tangent stiffness of the surgical group at the higher load level increased from ~63% to ~74% when compared to the stiffness of the contralateral tendons at the first visit; however, the difference was not significant (P=0.74) (Table 5.7 & Figure 5.2). The normalized tendon stiffness increased from ~58% to ~88% when compared to the stiffness of the contralateral tendons at the first visit; however, this difference did not reach statistical significance (P=0.25) (Table 5.8; Figure 5.4). The tangent and normalized tendon stiffness at the higher load level also showed no significant difference across time for the control and contralateral tendon groups (P>0.05).

During the second testing session, the tangent stiffness of the surgical group at the lower load level (642.17 N) increased from ~80% to ~86% when compared to the stiffness of the contralateral tendons at the first visit; however, the difference was not significant (P=0.73). The tangent stiffness at lower load level also showed no difference across time for the control and contralateral tendon groups (P>0.05) (Table 5.9 & Figure 5.3).

Table 5.7 Mean and SD of the tangent stiffness (N/mm) at higher load level. Significance level (P) for a paired sign-rank test comparing parameters across time is also shown.

Group	2 month	6 month	P
Surgical	1483.63 ± 264.46	1725.20 ± 1036.33	P = 0.74
Control	2005.34 ± 741.32	2076.01 ± 1249.47	P = 0.24
Contralateral	2337.98 ± 1818.88	2820.64 ± 1714.32	P = 0.73

Table 5.8 Mean and SD of the normalized tendon stiffness (GPa). Significance level (P) for a paired sign-rank test comparing parameters across time is also shown.

Group	2 month	6 month	P
Surgical	0.57 ± 0.25	0.86 ± 0.68	P = 0.25
Control	0.94 ± 0.36	0.97 ± 0.55	P = 0.14
Contralateral	0.98 ± 0.70	1.19 ± 0.73	P = 0.73

Table 5.9 Mean and SD of the tangent stiffness (N/mm) at lower load level. Significance level (P) for a paired sign-rank test comparing parameters across time is also shown.

Group	2 month	6 month	P
Surgical	1231.56 ± 233.13	1316.90 ± 827.10	P = 0.73
Control	1466.79 ± 657.75	1276.39 ± 335.74	P = 0.73
Contralateral	1534.82 ± 597.41	2436.85 ± 1740.74	P = 0.10

Table 5.10 A paired sign-rank test was performed to compare the change across time between surgical and control groups for the CSA, maximal knee extension torque, tangent stiffness and normalized tangent stiffness.

Parameter	Surgical (6mo.-2mo.)	Control (6mo.-2mo.)	P
Tendon CSA (mm^2)	-18.54 ± 18.03	-3.21 ± 10.71	P = 0.29
Max. Knee Ext. Torque (Nm)	541.05 ± 224.43	-326.085 ± 585.07	P = 0.07
Tangent Stiffness (N/mm)	115.72 ± 765.54	663.62 ± 1255.63	P = 0.73
Normalized Stiffness (N/mm)	0.25 ± 0.49	0.32 ± 0.50	P = 1.00

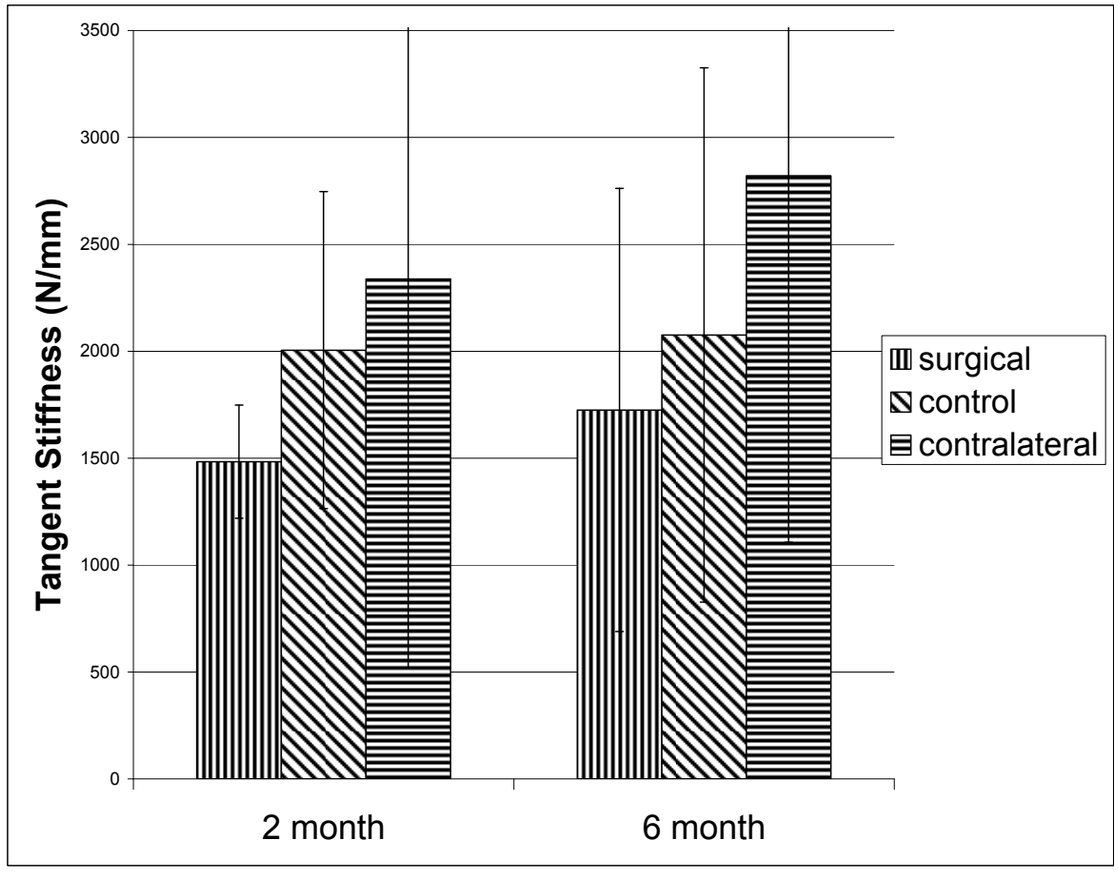

Figure 5.2 Tangent stiffness at the higher Load level of the three groups during the two visits: the stiffness of the surgical tendon was about 63% of the contralateral tendons at the first session and about 74% at the second session. The contralateral group was not included in the paired sign-rank test comparison.

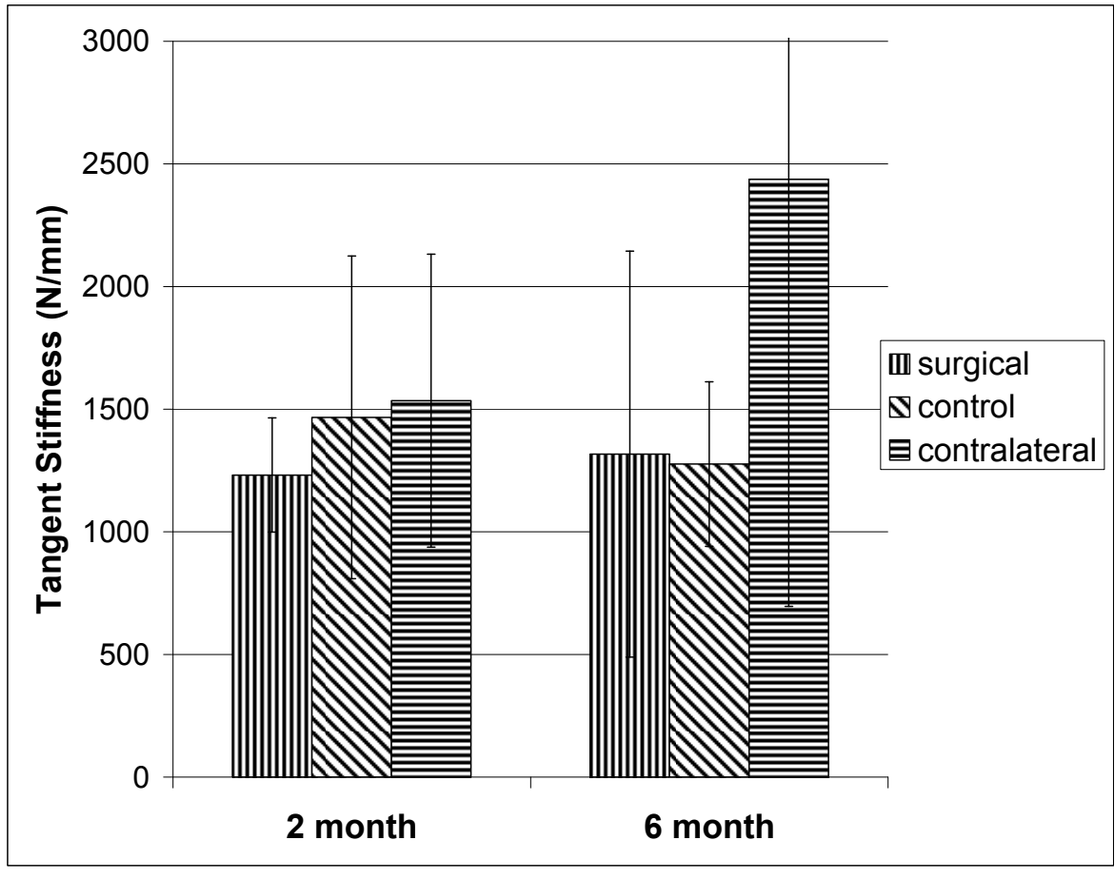

Figure 5.3 Tangent stiffness at the lower Load level of the three groups during the two visits: the stiffness of the surgical tendon was about 80% of the contralateral tendons at the first session and about 86% at the second session. The contralateral group was not included in the paired sign-rank test comparison.

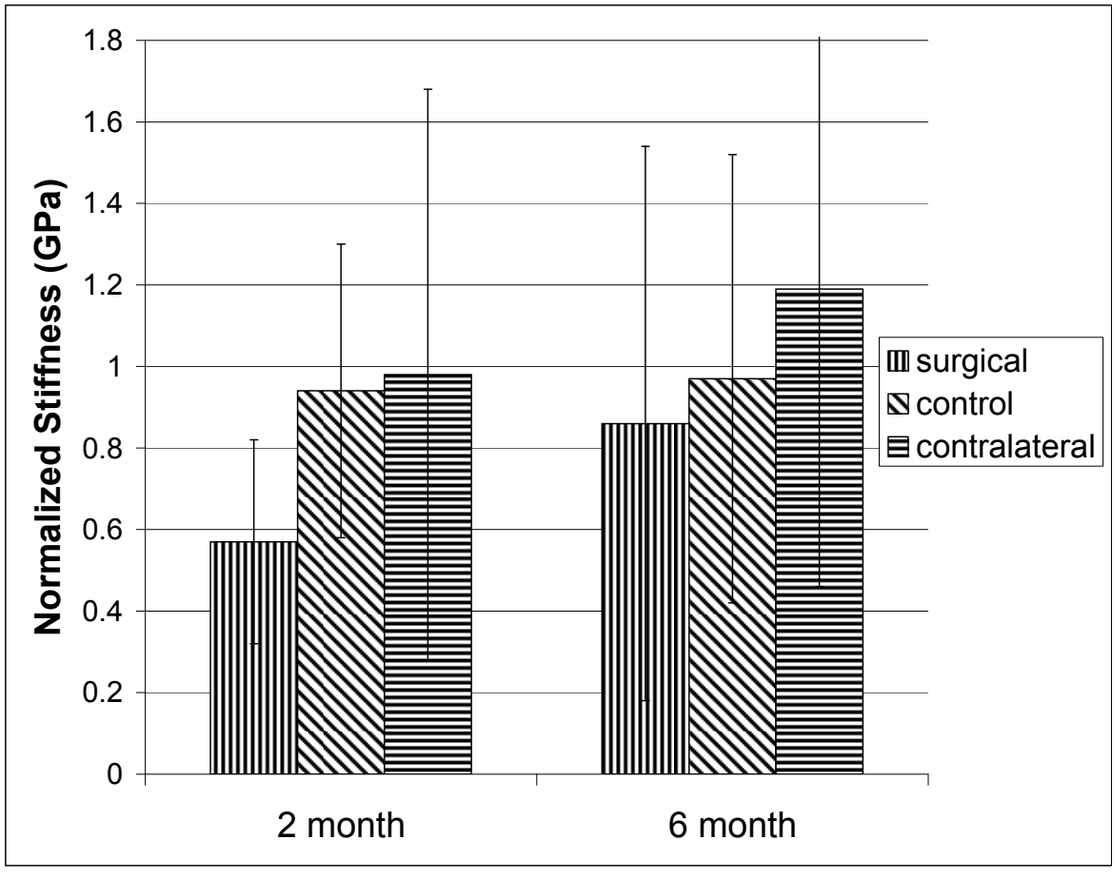

Figure 5.4 Normalized stiffness of the three groups during the two visits: the stiffness of the surgical tendon was about 58% of the contralateral tendons at the first session and about 88% at the second session. The contralateral group was not included in the paired sign-rank test comparison.

Spearman's correlation was performed to examine the correlation between the common clinical evaluation tools, such as VAS, ARS, IKDC scores, maximal knee extension torque and tendon CSA with the tendon stiffness properties measures. Our results showed strong correlation between VAS and IKDC (r=-0.85); strong correlation between IKDC with ARS scores (r=0.83); strong correlation between VAS, IKDC and ARS with maximal knee torque (r=-0.50~ 0.75); and notable correlation between tendon stiffness and maximal knee torque (r=0.51). However, poor correlation was shown between tendon CSA and IKDC, VAS, and ARS (r=-0.36~ 0.3); and poor correlation between tendon stiffness and IKDC, VAS, and ARS (r=-0.23~ 0.11) (Table 5.11).

Table 5.11 Spearman correlation was performed to examine the correlation between IKDC, VAS, ARS scores, maximal knee extension torque and tendon CSA, and tendon stiffness (* P < 0.05).

	IKDC	VAS	ARS	Torque	CSA	Stiffness
IKDC	1.00	-0.85*	0.83*	0.62*	-0.30	0.11
VAS	-0.85*	1.00	-0.61*	-0.50*	0.30	-0.23
ARS	0.83*	-0.61*	1.00	0.75*	-0.43	0.07
Torque	0.62*	-0.50*	0.75*	1.00	-0.69*	0.30*
CSA	-0.30	0.30	-0.43	-0.69	1.00	*-0.63
Stiffness	0.11	-0.23	0.07	0.51	-0.63*	1.00

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 allow for specific treatment strategies to be developed and 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 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 (Hansen *et al.*, 2006; Reeves *et al.*, 2003). 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. The between-day correlation coefficient of secant and tangent stiffness of control subjects of this study was 0.87 and 0.75, respectively, suggesting that the two-scan USD imaging methods in assessing tendon stiffness was repeatable across time.

The force-elongation response for both the contralateral and control tendons displayed a characteristic curvilinear relationship, with relatively larger elongations for a small application of force in the initial concave portion followed by smaller relative elongations as the force increased up to maximal level, while the force-elongation relation for the surgical tendons displayed a more linear response (Figure 5.1). The toe-region is believed to result from the straightening of crimped

collagen fibrils, thus fewer collagen fibers of the remaining tissue in the surgical tendons may have caused a shorter toe-region. Another explanation is that since the toe-region may be a certain percentage of the ultimate load of the tissue, and the higher preload (25N) we used for this study may have removed most of the toe-region of the surgical tendons.

Previous studies on goat and rabbit animal models of healing of the patellar tendon after autograft harvest suggested the ratio of operated limb/contralateral limb patellar tendon CSA at time zero was 54%, while at 3 months and 12 months the ratio was 237% and 184% (Bosch and Kasperczyk, 1992; Leung *et al.*, 2002; Ng *et al.*, 1995; Miyashita *et al.*, 1997). 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 amount of increase in the tissue volume was mainly composed of scar-like connective tissue (Miyashita *et al.*, 1997). However, the cross-sectional USD images of our surgical tendons did not show a large amount of scar tissue formation from 2 to 6 months after surgery. A more carefully performed surgical procedure on humans to minimize injuries on the surrounding tissue may have caused less scar tissue formation as compared to the animals studies. Due to the difference in toe-region of the force-deformation curves between surgical and control tendons, errors may be introduced if we assessed group difference using secant stiffness. Therefore, the tangent stiffness was used to compare between groups. The method we used to obtain the force-elongation curve allowed us to compare the paired surgical subject (both contralateral and surgical tendons) and control subject at the same absolute force level. However, in order to normalize to the weakest subject and time point, we did not include the data from the higher force region, which may have decreased the chance for us to see a difference in the tangential stiffness between groups. In order to prevent this problem caused by the absolute force level

approach, Reeves et al. suggested to calculate patellar tendon stiffness over each 10% interval of maximal tendon force (a relative force level approach), and this approach showed the greatest increase in stiffness with strength training occurred in the higher force level (Reeves *et al.*, 2003). Therefore, it may be useful to apply this relative approach in the future where the tangential stiffness between 90 and 100% of the maximal force level of each group could be used to compare between groups.

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 for the first three months after harvesting a patellar tendon autograft (Linder *et al.*, 1994; Ng *et al.*, 1995; Atkinson *et al.*, 1998; Jackson *et al.*, 1993). Based on the animal studies, we expected to see a significant increase in tendon stiffness in our human subjects. However, our results showed no significant increase in both the structural tendon stiffness and the material properties from 2 months to 6 months after surgery. These results suggest: 1. a longer time may be needed to see the healing effect on human tendon mechanical properties; 2. the surgical subjects compliance with the rehabilitation and exercise programs, which were not recorded in this study, may have caused a confounding effect on the tendon properties and a larger variation between the 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 the rehabilitation program on tendon healing should be further investigated. In the contralateral tendon group, both structural and material stiffness increased ~21% after 4 months, suggesting the increase in weight bearing and activity level also caused some change in the contralateral tendon properties. Reeves et al. (2003) 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.

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 shown at the second visit. Strong correlation between the knee torque production and the VAS was also shown in another study (Kobayashi *et al.*, 2004). This correlation suggested that the muscle strength of the knee injured patients improved as symptoms reduced. The knee functional activity score was poorly correlated with the tendon stiffness property measurements, suggesting that the knee functional activity level should not be used as the only indication for tendon healing (Table 5.11). In addition, earlier athletic participation because of the reduced symptoms may put the healing tendons at risk of re-injury and other complications. The higher correlation (r=0.51) between the maximal knee extension torque and the tangent stiffness may be due to the curvilinear nature of the force-deformation response. The correlation dropped to 0.11 when we compared the change of the tangent stiffness and the change in maximal knee torque between across time. Although Muraoka et al. (2005) showed a significant correlation between muscle strength and mechanical properties of healthy tendons, this relation may be different for injured tendons. 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. Off-plane observations will underestimate tendon movements and introduce a geometrical error of 1- cosθ where θ is the angular disparity between the probe plane and the line of bone-tendon action. However, because of the minimal joint rotation during an isometric contraction in the present study, the mediolateral movement of the patella is likely to be negligible (Nagamine *et al.*, 1995; Sheehan and Drace, 1999). Second, because of the larger variation in mechanical properties of the injured tendons from 2 to 6 months after surgery, we 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, 15 paired subjects will 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 there were differential strain across the tendon from lateral to middle. However, at 30° to 110° of knee flexion, previous studies have suggested the difference in length can be ignored (DeFrate *et al.*, 2007). Fourth, elevated noise present in some of the EMG time series data and 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. Lastly, the removal of some trials due to sliding of the USD probe was another limitation for this study.

Summary and Future Directions

From the results of this dissertation, it is concluded:

1. This *in vivo* ultrasonic technique was able to detect the changes in structural properties of tendon due to PFPS.

2. The large variation and amount of tibia deformation during the isometric knee extension can significantly influence the measurement of patellar tendon deformation. The two-scan method could adjust for the tibia deformation at the tibia insertion and provide a more repeatable stiffness value than the single scan measurement.

3. Tendon stiffness measurements of the *in vivo* two-scan USD method were repeatable across time, and thus can be used for a longitudinal study.

4. Due to the relatively short period of time of our study for humans tendon healing and increased variability in the stiffness measure at the 6 month time point, we did not see a significant increase in tendon stiffness. 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.

5. Tendon stiffness measures provide different information from other clinical recovery measures about tendon healing.

Future studies may include:

1. A destructive test in a cadaveric study using both the USD technique and the traditional tensile testing method with a Material Testing System will be helpful to demonstrate the accuracy of the tendon stiffness measurements.

2. A longer-term study over at least 12 months after injury with more than two time points will help to chart the trend of the healing process for the human tendon. Using a longer probe will be helpful to minimize the chance of losing trials because of slippage of the probe. In addition, a relative force level approach might be applied to calculate the tendon stiffness over each 10% of the maximal force level, thus the stiffness measurements at higher force level could be compared between groups.

3. The existing USD technique can also be applied to investigate the efficacy of different treatments and rehabilitation programs on common clinical problems that will alter the biomechanical properties of tendons, such as patellofemoral pain syndrome (PFPS). PFPS is a common diagnosis of knee pain in the United States. However, due to the subjective content of common clinical evaluation methods, the success of the rehabilitative treatments for PFPS remains controversial. Based on results of our previous study, PFPS caused a decrease in both structural property of tendon. The results of our study also showed that the *in vivo* tendon stiffness measurements provided additional information about tendon healing. Therefore, this *in vivo* USD imaging technique can be used to assess the effect of rehabilitative therapies on improving tendon mechanical properties. The results of such studies may help clinicians in designing more effective treatment plans for their patients and help prevent the complications due to prolonged symptoms.

4. The texture correlation technique in measuring tissue strain has the advantage of studying the localized tendon material without fixing one end of the tendon. With higher resolution and higher effective scan rate of the USD images, this technique has the flexibility to monitor the mechanical behavior of tendons during various activities, such as, walking, running, and jumping. Such results would provide valuable information to understand the injury mechanism of tendinopathy, and thus develop more effective exercise programs once an injury has occurred. Therefore, this technique should be re-examined.

In addition, recent studies have shown that exogenous applications of specific growth factors have positive effects on stimulating tissue healing. Other novel technologies, such as gene therapy and tissue engineering, have also been suggested to facilitate tendon healing in animal models. However, some issues surrounding the clinical use of these techniques need to be resolved before their benefit can be determined on a scientific basis. The major problems include; 1. the timing, dose, and location of administration; 2. due to the invasive nature of the traditional method in measuring tendon properties, the researchers were not able to get longitudinal data on human tissue. However, with the development of this ultrasound method, the effects of these technologies on human tendon healing can be assessed *in vivo* in a quantitative manner. Results from such studies would expedite the progress in determining the best protocol to promote tendon healing.

Appendix A

The Activity Rating Scale:

Please indicate how often you performed each activity since your injury, or since last visit

	Less than one time in a month	**One time in a month**	**One time in a week**	**2 or 3 times in a week**	**4 or more times in a week**
Running: running while playing a sport or jogging					
Cutting: changing directions while running					
Decelerating: coming to a quick stop while running					
Pivoting: turning your body with your foot planted while playing a sport; for example: skiing, skating, kicking, throwing, hitting a ball (golf, tennis, squash), etc.					

Visual Analog Scale

Indicate your greatest level of knee discomfort during the past week

no pain at all

Pain is as bad as it could possibly be

2000 IKDC SUBJECTIVE KNEE EVALUATION FORM

Your Full Name_____

Today's Date: _____/_____/_____ Date of Injury: _____/_____/_____

SYMPTOMS*:

*Grade symptoms at the highest activity level at which you think you could function without significant symptoms,

even if you are not actually performing activities at this level.

1. What is the highest level of activity that you can perform without significant knee pain?

❑Very strenuous activities like jumping or pivoting as in basketball or soccer

❑Strenuous activities like heavy physical work, skiing or tennis

❑Moderate activities like moderate physical work, running or jogging

❑Light activities like walking, housework or yard work

❑Unable to perform any of the above activities due to knee pain

2. During the past 4 weeks, or since your injury, how often have you had pain?

0 1 2 3 4 5 6 7 8 9 10

Never ❑ ❑ ❑ ❑ ❑ ❑ ❑ ❑ ❑ ❑ ❑ Constant

3. If you have pain, how severe is it?

0 1 2 3 4 5 6 7 8 9 10

No pain ❑ ❑ ❑ ❑ ❑ ❑ ❑ ❑ ❑ ❑ ❑ Worst pain imaginable

4. During the past 4 weeks, or since your injury, how stiff or swollen was your knee?

❑Not at all

❑Mildly

❑Moderately

❑Very

❑Extremely

5. What is the highest level of activity you can perform without significant swelling in your knee?

❑Very strenuous activities like jumping or pivoting as in basketball or soccer

❑Strenuous activities like heavy physical work, skiing or tennis

❑Moderate activities like moderate physical work, running or jogging

❑Light activities like walking, housework, or yard work

❑Unable to perform any of the above activities due to knee swelling

6. During the past 4 weeks, or since your injury, did your knee lock or catch?

❑Yes ❑No

7. What is the highest level of activity you can perform without significant giving way in your knee?

❑Very strenuous activities like jumping or pivoting as in basketball or soccer

❑Strenuous activities like heavy physical work, skiing or tennis

❑Moderate activities like moderate physical work, running or jogging

❑Light activities like walking, housework or yard work

❑Unable to perform any of the above activities due to giving way of the knee

SPORTS ACTIVITIES:

8. What is the highest level of activity you can participate in on a regular basis?

❑Very strenuous activities like jumping or pivoting as in basketball or soccer

❑Strenuous activities like heavy physical work, skiing or tennis

❑Moderate activities like moderate physical work, running or jogging

❑Light activities like walking, housework or yard work

❑Unable to perform any of the above activities due to knee

9. How does your knee affect your ability to:

		Not difficult at all	Minimally difficult	Moderately Difficult	Extremely difficult	Unable to do
a.	Go up stairs	❑	❑	❑	❑	❑
b.	Go down stairs	❑	❑	❑	❑	❑
c.	Kneel on the front of your knee	❑	❑	❑	❑	❑
d.	Squat	❑	❑	❑	❑	❑
e.	Sit with your knee bent	❑	❑	❑	❑	❑
f.	Rise from a chair	❑	❑	❑	❑	❑
g.	Run straight ahead	❑	❑	❑	❑	❑
h.	Jump and land on your involved leg	❑	❑	❑	❑	❑
i.	Stop and start quickly	❑	❑	❑	❑	❑

FUNCTION:

10. How would you rate the function of your knee on a scale of 0 to 10 with 10 being normal, excellent function and 0 being the inability to perform any of your usual daily activities which may include sports?

FUNCTION PRIOR TO YOUR KNEE INJURY:

Cannot perform daily activities — No limitation in daily activities

0	1	2	3	4	5	6	7	8	9	10
❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑

CURRENT FUNCTION OF YOUR KNEE:

Cannot perform daily activities — No limitation in daily activities

0	1	2	3	4	5	6	7	8	9	10
❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑

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