THE MECHANICAL PROPERTY CHANGES OCCURRING IN THE SEMITENDINOSUS TENDON POST ACL RECONSTRUCTION WITH A HAMSTRING AUTOGRAFT

by

Stephen M Suydam

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biomechanics and Movement Science

Spring 2015

THE MECHANICAL PROPERTY CHANGES OCCURRING IN THE SEMITENDINOSUS TENDON POST ACL RECONSTRUCTION WITH A HAMSTRING AUTOGRAFT

by

Stephen M Suydam

Approved: ____
C. Buz Swanik, Ph.D.
Director of the Interdisciplinary Program in Biomechanics and Movement Science

Approved: ____
Babatunde A. Ogunnaike, Ph.D.
Dean of the College of Engineering

Approved: ____
James G. Richards, Ph.D.
Vice Provost for Graduate and Professional Education

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: ______________________________________

Thomas S. Buchanan, Ph.D.
Professor in charge of dissertation

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: ______________________________________

Lynn Snyder-Mackler, Sc.D.
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: ______________________________________

Michael J. Axe, MD
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: ______________________________________

Dawn M. Elliott, Ph.D.
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: ______________________________________

Jill S. Higginson, Ph.D.
Member of dissertation committee

ACKNOWLEDGMENTS

I would like to thank a number of people within and external to my lab for their educational, academic and emotional support throughout my doctoral journey. First and most influentially, I would like thank Dr. Thomas S. Buchanan for giving me the opportunity to work in his lab, training me in manuscript writing and storytelling, understanding my goals and providing me with opportunities aligned to my interest and passions. I would like to thank the Buchanan Lab including Dr. Kurt Manal, Amelia Lanier, Ashu Khandha, Dave Olchowski, and Toran MacLeod, for supporting me in data collections and proofreading the many documents that were in need of a critical set of eyes over the years. I would especially like to thank Donald Hume, John Ramsay and Annalisa Na for their additional help with brainstorming and refining study designs to focus them into an impactful direction. I would also like to acknowledge the SMACK Lab for all their clinical views and perspectives on my work to ensure its real world applicability. The ideas of regeneration of the tendon and function, among several other abstracts submissions, were brought about by the MRI tracings of Carlene Meaney. The work performed on echogenicity would not have been possible without the aid in data collection and processing from Nathan McMullen. The nominal Achilles tendon study in the appendix of this document was supported by time dedicated by Elizabeth Soulas who performed data collections, recruited subjects and helped validate a new, emerging metric of continuous shear wave elastography. In terms of collaborations, there was none more important than my collaboration with Daniel H Cortes. Daniel and I were partners in the design, coding, and development of the continuous shear wave technology that was paramount for the research performed in this dissertation. Additionally, I would like to thank Karin Gravare Silbernagel for the opportunity to work with such an established, ambitious and enthusiastic research clinician who helped me craft my first publication and stay excited about my own research. Last, but foremost, my beautiful bride April, who, in addition to countless hours providing feedback on presentations and documents, always reminded me that things are going to work out and to not allow myself to become too worked up over the little bumps along the way.

This research was supported by the National Institute of General Medical Sciences and by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under grants P20-GM103333, U54-GM104941, R01-AR046386, R01-AR050052 and R21-AR067390.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The potential strength asymmetries post anterior cruciate ligament (ACL) reconstruction using a bundled semitendinosus (ST) and gracilis tendon autograft can cause asymmetry in functional tasks measuring return previous level of activity. In order to utilize the regrowth of the ST tendon for the purpose of eliminating asymmetry, it is essential to show that the tendon indeed recovers its functional properties and correlate those to bilateral strength differences. Therefore, the aims of this study are as follows: Develop a non-invasive measurement system for determining tendon functional properties, calculate the elastic properties of regrown ST tendons and correlate those properties to bilateral strength compared to the healthy limb. Continuous shear wave elastography (cSWE) was used on gel samples and compared to published standard measure of MR elastography. cSWE matched the published standard with only 8.2% difference. Echogenicity effectiveness was assessed through the use of B-mode ultrasound and isometric contractions on the Achilles tendon. Ultrasound images were taken to track the soleus junction and collect image brightness during isometric contractions. Tendon stress was then correlated to brightness change. Due to minimal correlation between echogenicity and stress (r^2=0.05), cSWE was used to evaluate the semitendinosus tendon. Sufficient power was reached with 13 subjects, between 6-24 months post-ACL reconstruction, to measure the bilateral difference in shear elastic modulus. To establish the impact of the mechanical property recovery, the same subjects were measured for peak torque during concentric flexion, eccentric flexion, and internal rotation of the leg. Flexion strength was determined at 60º/s and 120º/s and internal rotation strength was determined at 30º/s and 60º/s. The shear elastic modulus was significantly different between the involved and uninvolved legs ($p=0.004$) and was strongly correlated ($r=0.60$) to recovery time. The recovery of the tendon's shear modulus was strongly correlated to the difference in internal rotation strength at both 30º/s and 60º/s ($r=0.70,0.65$). This study is the first to show remaining strength asymmetries previously demonstrated by a semitendinosus resection can be recovered with time. Knowing the tendon recovers its mechanical properties between 6 and 24 months may correlate with the recovery rate within the 2 years following ACL reconstruction.

Chapter 1

INTRODUCTION

1.1 Dissertation Overview

The focus of this dissertation is understanding the implications of a patient's regrowth of the semitendinosus (ST) tendon following an ACL autograft. Tendon regeneration, which has been previously imaged, has yet to be quantified *in vivo* for its mechanical properties. The mechanical properties, i.e., elasticity, dictate the tendon's functionality in terms of the percent of force generated by the muscle transmitted to the joint, which causes motion. While *in vitro* studies have been performed to determine elastic properties of tendon, there has not been a validated *in vivo* measure to date. Therefore, the first aim of this study is to find a measure to characterize soft tissue to be used for tendon property assessment. This aim is discussed in Chapters 2 and 3. Chapter 2 evaluates the metric of echogenicity, which is the increase in acoustical reflective properties with stress. Chapter 3 explores continuous shear wave elastography (cSWE) and compares it to a published standard, magnetic resonance elastography (MRE). In order to use the same material in both cSWE and MRE, the material of interest had to be portable gels rather than biological tissue. An additional study, outside the scope of this dissertation, was performed to ensure this technique successfully functioned *in vivo*, which is referenced as Appendix A.

cSWE was selected as the measure of choice for quantifying elasticity in terms of shear elastic modulus and therefore the second aim was to determine the elastic properties of the resected and uninvolved ST tendons post ACL reconstruction. The second aim is discussed in Chapter 4. Appendix A includes an *in vivo* validation of bilateral symmetry in healthy tendon to ensure any differences found in Chapter 4 were due to resection and healing and not inherent variability. This was included as an appendix due to the fact that it was outside the scope of the work being done for this dissertation. The validation was performed on the Achilles tendon and not on the semitendinosus tendon.

Chapter 4 leads into the correlation between the regrowth of the ST tendon and the strength of the hamstrings, which is important in understanding the impact of recovering the functionality of the ST muscle. Therefore, the third aim focuses on the relationship of ST tendon properties and bilateral strength symmetry. This aim is discussed in both Chapters 5 and 6. Chapter 5 focuses on each of the possible strength asymmetries and addresses implications towards the long term morbidity of the knee. The strength recovery to shear elastic modulus recovery is measured to determine if there are any remaining strength deficits and evaluate the effects the recovery of elastic properties has on them. Chapter 6 is directed at the return to sport function of the knee with regards to level of play and re-injury. The methods between Chapters 5 and 6 are the same but the main difference is Chapter 6 discusses the current deficits and the immediate implications towards sports activity. Chapter 7 summarizes the findings in terms of aims and hypotheses as well as draws conclusions from the outcomes, discusses general limitations of the study and future directions of this work.

1.2 Background

The purpose of this study was to determine the anatomical and physiologic effects of the resection and regrowth of the semitendinosus (ST) tendon, which was harvested for use in an anterior cruciate ligament (ACL) autograft. ACL ruptures are a common sports injury and lead to short and long term physical limitations. The ACL stabilizes the knee joint and provides proprioception aiding in prevention of excessive motion within the joint capsule [Imbert 2014, Relph 2014]. ACL replacements are performed to re-establish joint stability and restore the knee's previous level of functionality post rupture. More than 125,000 ACL reconstructions are performed each year through various reconstruction methods [Mall 2014]. Through proper rehabilitation, function returns and injured subjects can return to high level athletics in less than a year, yet only 64% of those patients return to the same level of play [Ardern 2011]. One of the most common reconstruction techniques is the semitendinosus-gracilis hamstring (STG) autograft. The semitendinosus (ST) autograft requires the resection of the ST tendon from the ST muscle (Figure 1-1). While the STG graft has evidence to support it as the better choice of reconstruction with regards to return to sport, residual pain and knee range of motion, the fact remains that autografts result in the temporary disconnection of their semitendinosus (ST) and gracilis (GRA) muscles after removal of the tendon. Evaluating the morbidity of this tendon resection for graft material for an ACL reconstruction would establish if there are any non-recoverable long term effects and if help in understanding when the patient has recovered from surgery.

Figure 1-1. The semitendinosus and gracilis tendon connecting on the medial surface of the tibia. The sections highlighted in black represents the portion of the tendon that would be removed and used for the ACL graft. Image by MMG.

The resection of these tendons causes initial hamstring weakness following surgery, but much of this strength is regained over time [Lipscomb 1982]. The results of the resection of the ST tendon are weakness during deep knee flexion and internal rotation compared to the contralateral side [Makihara 2006, Nakamura 2002, Carofino 2005, Tashiro 2003, Viola 2000, Ohkoshi 1998]. Kinematic differences manifest in the form of increased external rotation during running, walking, and knee extension [Tashman 2004, Scalan 2010, Nicholson 2011]. These kinematic alterations may lead to the significant loading patterns changes within the knee [Buchanan 2008]. Given the femoral cartilage that protects the knee is not uniform across the contact surface, changes in loading location on the articulating surface creates a non-optimal contact arrangement within the joint [Vincent 2012]. Each of these strength and kinematic changes can be associated with the lack of ST muscle contribution. The ST, because of the tendon's more medial insertion into the tibia, is a major contributor to internal rotation strength. The fusiform muscle type of the ST differs from that of the pennate semimembranosus and biceps femoris and has a different muscle fiber length where peak force generation occurs. The ST resection alters the joint angle to flexion strength profile and leads to flexion strength weakness at increased knee angles.

More than 50% of patients who sustained an ACL rupture have signs of osteoarthritis (OA) within 10 years of injury [Andersson 2009]. The strength changes found may have an impact on increased knee compartmental loading, increased loading frequency, and altered loading surfaces within the knee cartilage, which have been linked to OA [Buchanan 2008, Maly 2008, Andriacchi 2006]. The debilitating disease of OA affects more than 20 million Americans and accounts for more than 100 billion dollars in medical costs per year [Michaud 2006, London 2011]. ACL reconstruction does reduce instability of the knee and decreases the likelihood of future meniscal injuries, which have both been correlated to the progression of OA, but the prevalence of OA remains [Meunier 2007]. Determining if potential strength deficits are recoverable may also reduce the progression of OA.

While there are strength asymmetries linked to the lack of joint torque contribution from the ST, studies have found the ST tendon regrows, leading to the potential for the torque contribution to be restored. Several studies demonstrated regrowth of the ST and gracilis (GRA) tendons within 6 to 18 months [Eriksson 1999, Okahashi 2006, Williams 2004, Ferretti 2002, Leis 2003, Tadokoro 2004, Papandrea, 2000, Rispoli 2001]. Additionally, Ferretti and colleagues showed the recovery of internal rotation strength after ACL reconstruction in which all the subjects recovered their ST tendons and were administered an internal rotation strengthening protocol [Ferretti 2008]. The regrown tendons have been viewed through magnetic resonance and ultrasound imaging techniques but have yet to be directly assessed for mechanical properties; therefore the functional capabilities of the newly formed tissue are unknown. While resected from the attachment point, the ST muscles atrophy [Williams 2004]. If the tendon regrows, the ST muscle will then be providing the necessary resistance for hypertrophy and strength recovery can begin [Kubo 2006]. The strengthening of these muscles will reduce the bilateral asymmetries between legs and aid in returning kinematic function to previous levels. If the mechanical properties of the regenerated tendon were to be known, a strengthening protocol could be designed and implemented at the time of tendon regrowth. Additionally, the mechanical properties of the resected tendons could be incorporated into musculoskeletal models to make them more subject specific. Subject specificity for musculoskeletal modelling increases the accuracy for estimating joint loading conditions and which may aid in detecting residual effects which lead to the progression of OA [Gardinier 2013]. Therefore it is the purpose of this study to determine a technique capable of measuring the mechanical properties of soft tissues, which includes the tendons. Using those techniques, this study aims to compare the mechanical properties of the regrown ST tendons of an ACL reconstructed population to the contralateral limb and determine the relative recovery of the mechanical properties. While the GRA is also harvested for this technique, it is more difficult to scan via ultrasound which may lead to faulty results. Lastly, hamstring strength measurements will be taken of ACL reconstructed subjects in order to relate the regrowth of the ST tendon to any remaining asymmetries.

1.3 Non-invasive Mechanical Property Measure

The viscoelastic properties of tendon, determined through many various techniques, have been correlated to pathology and healing progression [Arya 2010, Schepull 2012]. Techniques involving biplanar fluoroscopy and X-Ray diffraction have been used to determine the elastic modulus of tendon, but given the invasive nature of these tests, ultrasound was also explored as a separate option [Schepull 2007, Sasaki 2012]. The modulus of elasticity, designated as $E = \sigma/\epsilon$ where σ is the stress (force/cross sectional area) and ϵ is strain (Δ length/total length), is determined through tracking the change in tendon length which a known load is applied. This requires the measurement of the overall length of a tendon and tracking the change in length by following the motion of an anatomical landmark attached to the tendon [Maganaris & Paul 2000, Kongsgaard 2011, Joseph 2012]. Tracking the myotendinous junction of a musculo-tendinous unit is only one of the ways of tracking tendon motion. Additional tendon length change has been assessed through speckle tracking, which consists of finding a pattern within an image and using that as a landmark [Okotie 2012, Korstanje 2010]. Though determining the strain has been shown in tendon, it is not always possible to calculate the stress in a tendon. This is due to the redundancy of human anatomy and multiple musculo-tendinous units contributing to a single action. A metric is needed to determine the viscoelastic properties of tendon which is independent of landmark tracking and the stress applied to the tendon. This study focuses on two possible techniques to achieve this goal.

Echogenicity is the measure of a medium's ability to reflect sound. A material's mechanical properties can be linked to its attenuation of sound, specifically the magnitude changes of those mechanical properties when the material is put into tension [Hughes 1953]. Echogenicity could be used to determine the elasticity of tendon by correlating the change in signal intensity reflected from the tissue to the applied stress or strain. Mechanical properties of tissue, in the form of Young's Modulus, have been correlated to acoustical reflection intensity *in vitro* [Duenwald 2011, Crevier-Denoix 2005]. Currently no human studies have performed echogenicity *in vivo* to determine its clinical applicability. The correlation requires tracking the tendon's excursion via ultrasound while a known stress is applied to the tendon. The results are relating the change in signal intensity on the ultrasound, in the form of image brightness, to the increased stress/strain applied. This technique would allow for a single measurement to determine the relative elastic characteristics of a tendon. Therefore the first aim of the first portion of this study is to determine the relationship of the *in vivo* measurements to echogenicity of tendon.

> **Hypothesis 1.1:** The echogenicity of the Achilles tendon will increase with stress. There will be a linear relationship outside of the toe region of tendon stretch between the increasing brightness of the ultrasound image and the stress applied to the tendon.

Elastography is a method of quantifying the mechanical properties of soft tissue, in the form of shear and viscosity modulus, through the use of compressive waves being applied to the medium [Ophir 1991]. Elastography measures the velocity at which shear waves travel through the medium and relates them to the shear modulus. This requires certain assumption of the tissue such as perfect elasticity, isotropy and incompressibility of the tissue. Given those assumptions the shear modulus can be determined through the simplified equation $\mu=\rho c^2$, where c is the speed of the shear wave and ρ is the density of the medium. Preliminary measures used acoustic waves as the source of compression waves within the tissue [Nightengale 2002]. This technique had great success in very soft tissues including liver, breast, prostate, thyroid, and pancreatic tissues [Zhao 2014, Itoh 2006, Saloman 2008, Lim 2012, Janssen 2007]. The use of acoustic waves is limited by the measurable velocity of the compression waves and the single velocity at which the shear wave is generated. Compressive waves travel faster in denser mediums and it was found that tendon causes the available elastography devices which use the acoustic compressive wave method to reach a saturation point [DeWall 2014]. Additionally, tendon is not perfectly elastic material and therefore the simplified equation is not applicable. Tendon has both a viscous and elastic component to it and multiple compressive wave velocities are required to determine the viscoelastic parameters. Multiple shear wave velocities allows for the Voigt mechanical model to be used [Hoyt 2008]. A new method of continuous shear wave elastography (cSWE) using an external actuator and a high speed ultrasound allows for the use of this viscoelastic model. To validate this new method, the actuator would be applied to the medium of interest and the ultrasound would record the shear wave propagation. A map of the propagating shear wave would be created through signal processing from which the wave speed could be calculated (Figure 1-2). The medium of interest will then be assessed using a peer reviewed standard, magnetic resonance elastography [Cortes 2013]. This technique would allow for the viscoelastic properties of tendons to be determined. Therefore, the second hypothesis of the first aim of this study is to determine if elastography is an accurate metric for determining tendon viscoelastic properties *in vivo*.

> **Hypothesis 1.2:** The mechanical properties determined through shear wave elastography will match the mechanical properties determined through a previously established standard (shear wave magnetic resonance elastography (MRE)). The percent error between the mechanical loading and the elastography measure will be less than 10%.

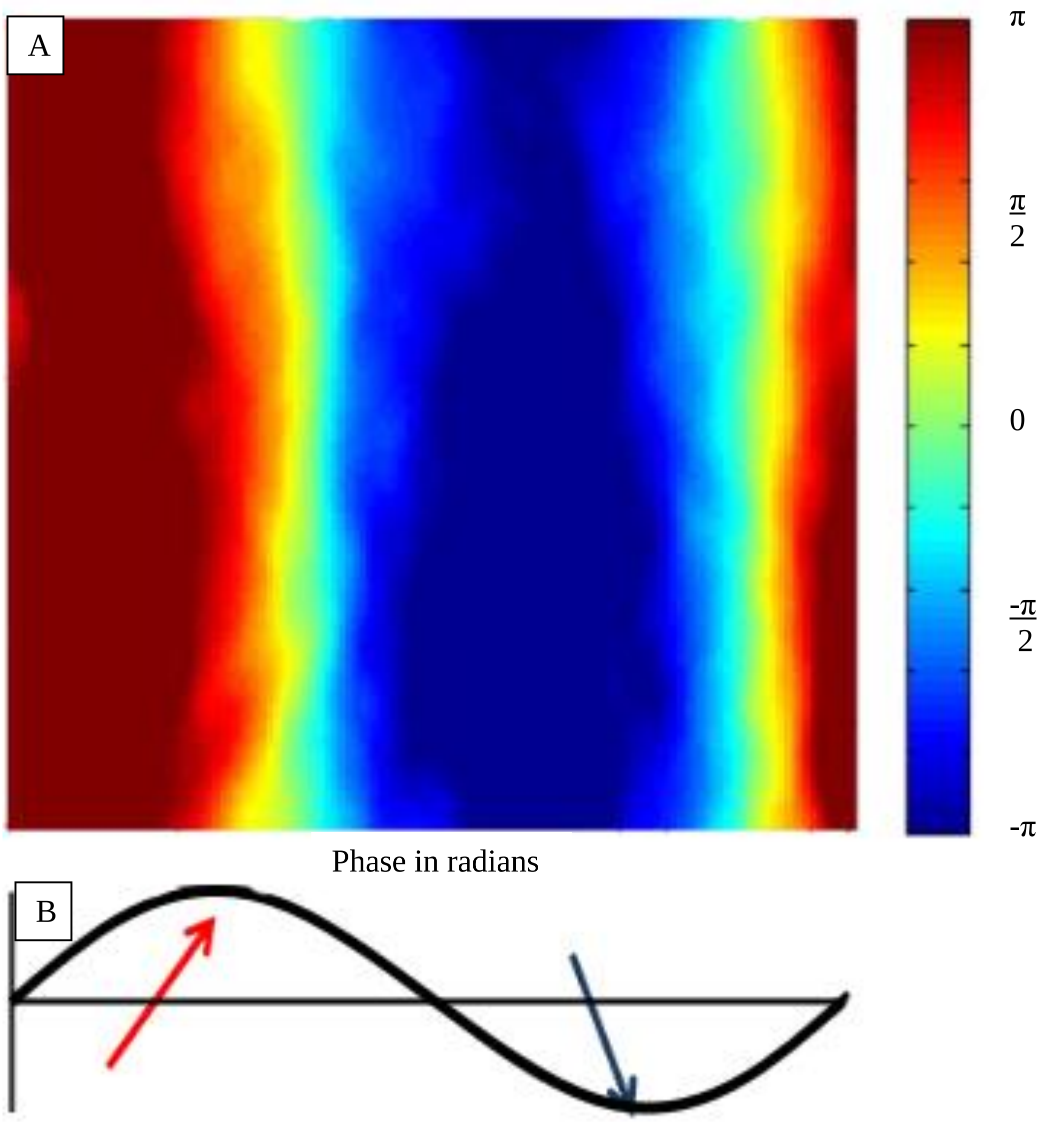


Figure 1-2. The phase map (A) generated through signal processing of the shear wave propagation. The red represents a phase of π radians (180°) and the blue represents -π (-180°) illustrated by the sample sine wave (B) below the phase map.

1.4 Viscoelastic Properties of the Semitendinosus Tendon

The mechanical property of tendon determines the efficacy and efficiency of force transmission from a muscle across the joint to provide torque. After the resection of the ST tendon from the muscle, the muscle is no longer able to transmit force across the knee joint. This causes an initial weakness in leg flexion that is compensated by the hypertrophy of the other hamstring muscles [Snow 2012, Williams 2004]. While peak leg flexion strength recovers, the lack of ST force contribution results in weakness specific to the ST muscles contribution to joint torque including deep knee flexion, eccentric contraction and internal rotation. The tendon has been shown to regrow over time and has the potential to once again generate flexion torque [Williams 2004, Eriksson 1999, Okahashi 2006, Ferretti 2002, Leis 2003, Todokoro 2004, Rispoli 2001]. The neo-tendon starts as a disorganized mass of collagen but over time begins to reorganize and appears to be more functional, though no studies have quantified the properties of this new tendon [Papandrea, 2000]. The ability to determine the mechanical properties of the tendon would establish if the regrown tendons, post resection for ACL reconstruction, are able to transmit force from the muscle across the joint. This has the potential to restore the hamstrings to their previous functional levels, recover strength at the knee and return the knee to its original condylar loading location seen preoperatively. This will be achieved by using the validated echogenicity or cSWE techniques to determine the elastic or viscoelastic parameters of the regrown ST tendon and comparing them to the uninvolved side. Demonstrating the tendon recovers its properties with time would indicate the effects of tendon resection may only be temporary and additional rehabilitation may reduce remaining asymmetries. Therefore, the aims of the second portion of this study are to determine differences in elastic properties of the ST tendon following ACL reconstruction, correlate the recovery of those properties with time post-op and verify the metric used is reliable *in vivo*.

Hypothesis 2.1: The elastic modulus of a ST tendon will have a reduced elastic modulus compared to the contralateral side from 6 to 24 months post-op.

Hypothesis 2.2: A positive correlation will exist between the recovery time and the recovery of elastic properties in relation to the contralateral side.

Hypothesis 2.3: The measures of a single tendon will be repeatable using this novel technique in vivo.

1.5 Recovery of hamstring strength with tendon regeneration

The remaining long term asymmetries of tendon resection are linked to the lack of joint torque contribution from the ST muscle. The remaining internal rotation weakness could be linked to the 11% re-rupture rate or the 55% return to previous level of play for athletes with an ACL reconstruction. These asymmetries could also be contributors to altered kinematics and joint loading which have been linked to the progression of OA. An increase in knee external rotation during running, walking and knee extension can be correlated to a decrease in internal rotation strength [Tashman 2004, Scanlan 2010, Nicholson 2011] and cadaveric studies have shown a shift in knee contact location following ACL reconstruction [Mulcahey 2012]. Internal rotation weakness, along with deep knee flexion weakness, persists from a year to more than 2 years after surgery showing no long term recovery after initial rehabilitation [Viola 2000, Makihara 2006, Nakamura 2002]. Eccentric strength of the hamstrings, which is a primary use for this muscle group during gait, also has remaining asymmetries following ACL STG reconstruction [Novachek 1998, Hiemstra 2000]. In contrast, Ferretti and colleagues demonstrated no internal rotation weakness in subjects with a regenerated tendon having received a surgical technique known to encourage regrowth [Ferretti 2008]. In addition to understanding the recovery of the mechanical properties of the regrown tendon, it is also unknown if the tendon's regrowth is correlated to the recovery of hamstring flexion strength and internal rotation strength. This will be achieved by measuring the strength of the hamstrings in both flexion and internal rotation. Test measures will evaluate the hamstring over the entire flexion range to determine not only if the strength is bilaterally symmetric, but if the location at which peak torque is generated has recovered as well. Additionally, eccentric strength of the hamstring flexors will also be assessed. Showing a correlation between the recovery of the tendon properties and strength would demonstrate the remaining asymmetries can be eliminated after removing the ST tendon as graft material for an ACL reconstruction. Establishing these tendon properties have recovered creates an environment for targeting the hypertrophy and strengthening of the ST muscle. Therefore the aims of the third section are to determine if reported strength asymmetries recover with the recovery of the elastic modulus of the ST tendon.

Hypothesis 3.1: Bilateral knee concentric flexion strength symmetry will be correlated to symmetry of the mechanical properties of the ST tendon.

Hypothesis 3.1a: Bilateral symmetry of the angle at which peak torque is generated during knee flexion will be correlated to symmetry of the mechanical properties of the ST tendon.

Hypothesis 3.2: Bilateral knee eccentric flexion strength symmetry will be correlated to symmetry of the mechanical properties of the ST tendon.

Hypothesis 3.3: Bilateral knee concentric internal rotation strength symmetry will be correlated to symmetry of the mechanical properties of the ST tendon.

1.6 Summary

The use of the ST tendon for the reconstruction of an ACL removes the function of the ST muscle leaving functional asymmetries, changed kinematic patterns and altered joint contact loading. The regrowth of the tendon could lead to the elimination of those asymmetries. A noninvasive metric such as echogenicity or cSWE must be validated to be used to assess the recovery of the ST tendon's functional capabilities. The mechanical properties of the regrown tendon must then be compared to the contralateral healthy tendon to establish return to symmetry. Finally, correlating the strength symmetry to the symmetry of the tendon's mechanical properties will show that functionality of the musculo-tendinous unit has been restored and the ST is now contributing to the hamstring strength.

Chapter 2

IS ECHOGENICITY A VIABLE METRIC FOR EVALUATING TENDON PROPERTIES *IN VIVO*?

2.1 Introduction

The ability to determine the functional status of an injury and the progression of healing is critical for clinicians. Mechanical properties (e.g. elastic modulus, strain) of tissue can be used as indicators of injury severity and recovery progression [Arya 2010, Child 2010, Lichtwark 2005, Schepull 2007]. Due to the unbiased nature of mechanical properties these data provide quantitative measures which can be compared longitudinally within subjects and across populations. Currently, there are several testing measures to acquire these mechanical properties in tendon including the invasive implantation of tantalum beads combined with x-ray imaging [Schepull 2012] and elastography, which requires several large assumptions e.g. tendons are perfectly elastic and isotropic [Kongsgaard 2011]. A simpler, less invasive method for acquiring mechanical properties is needed for general clinical applicability.

Echogenicity is a non-invasive measurement of a material's ability to reflect sound. The attenuation of acoustical signals is characteristic of a material's mechanical properties [Yu 1995] and the magnitude of those properties increases when the material placed into tension [Hughes 1953]. Tendon consists of collagen fibrils which run parallel to the direction of force application and are held together in an extracellular matrix. This structure of linked collagen fibrils allows for tendons to be stretch elastically up to 14% [Wang 2006]. This acoustical attenuation characteristic of materials, when applied to tendon, translates to a greater ability to reflect acoustic signals when the tendon fibers are stretched during contraction. When imaged in vitro via ultrasound, increased tension and tendon strain resulted in less signal absorption and a greater amount of signal reflection, i.e., brightness [Duenwald 2011]. Additionally, mechanical property changes caused by tendon damage decreases echogenicity and has been shown as a precursor to pathology [Duenwald-Kuehl 2012, Malliaras 2008]. In equine studies, diseased tissue was associated with decreased acoustical reflection characteristics [Crevier-Deniox 2005, Garcia 2003]. Therefore it is of interest to determine whether the potential exists for echogenicity of in vivo human tissue to provide meaningful insight into injury mechanisms.

While previous studies working with animal and cadaveric tissue suggest the potential feasibility of using echogenicity for determining in vivo tendon properties, challenges still remains. A major difference between estimating tendon properties in vitro and in vivo is that the mechanical properties may change with loading rates, tendon geometry and the vector of tensile force application. Joint movement within "isometric" contraction in a dynamometer causes a lengthening of the tendon and therefore an erroneous increased strain [Arampatzis 2005]. Additionally, a controlled, uniaxial stress protocol used during in vitro tests are performed with tension being applied to the end of the tendon only, which is unrealistic since, in vivo, muscles connect and produce tension along the length of the tendon and not simply at the end; therefore an in vivo experiment is necessary.

There is a need for a minimally invasive and simple measure to establish the mechanical properties of tendon during the healing progression to be used for longitudinal and cross subject analysis. Therefore the goal of this study is to correlate the minimally invasive technique of echogenicity to the in vivo stress within the Achilles tendon. We hypothesize there will be a linear increase in ultrasound image brightness of the tendon with increased stress which would suggest echogenicity is a functional tool for mechanical property assessment of tendons.

2.2 Methods

Echogenicity and stress measurements were taken from 9 healthy subjects (age 25±5.6 yrs, height 172.0 cm±5.1, mass 68.7 kg±10.8) with no history of Achilles tendon rupture or tendinopathy. Each subject read and signed an informed consent approved by the University of Delaware institutional review board. Retro-reflective markers were placed on the lateral and medial malleoli of the subjects to establish the joint center. The subjects knelt in a Biodex 3 System (Biodex Medical Systems, Shirley, New York) with their ankles and knees fixed at 90^{o} and hips at 0^{o} (Figure 2-1) The moment arm of the Achilles tendon was determined using a method similar to Manal and colleagues [Manal 2010]. A LogiQ P6 ultrasound (GE medical systems, Fairfield, CT) with a ML6-15 transducer was used to measure the distance from the skin's surface to the center of the Achilles tendon. Retro-reflective markers were placed at the center of the transducer and the distance from the markers to the head of the transducer was measured. The transducer was placed on the ankle to align the markers on the transducer with those fixed to the malleoli. 3D motion data (Qualysis Motion Capture System, Gothenburg, Sweden) were recorded during the ultrasound collection and a triggering device was used to establish a time point in the motion capture data at which the ultrasound image was taken. The Achilles tendon moment arm was calculated as the distance between the 2 sets of markers less the distance to the head of the transducer and the distance from the skin's surface to the center of the tendon, measured on the ultrasound images. This was repeated for the opposite leg. The desired forces through the Achilles tendon were 0.5, 1.0, 1.5, and 2.0 body weights. The desired tendon forces and moment arms were used to calculate the torque equivalent for each force, as torque was the measure provided as visual feedback to the subject.

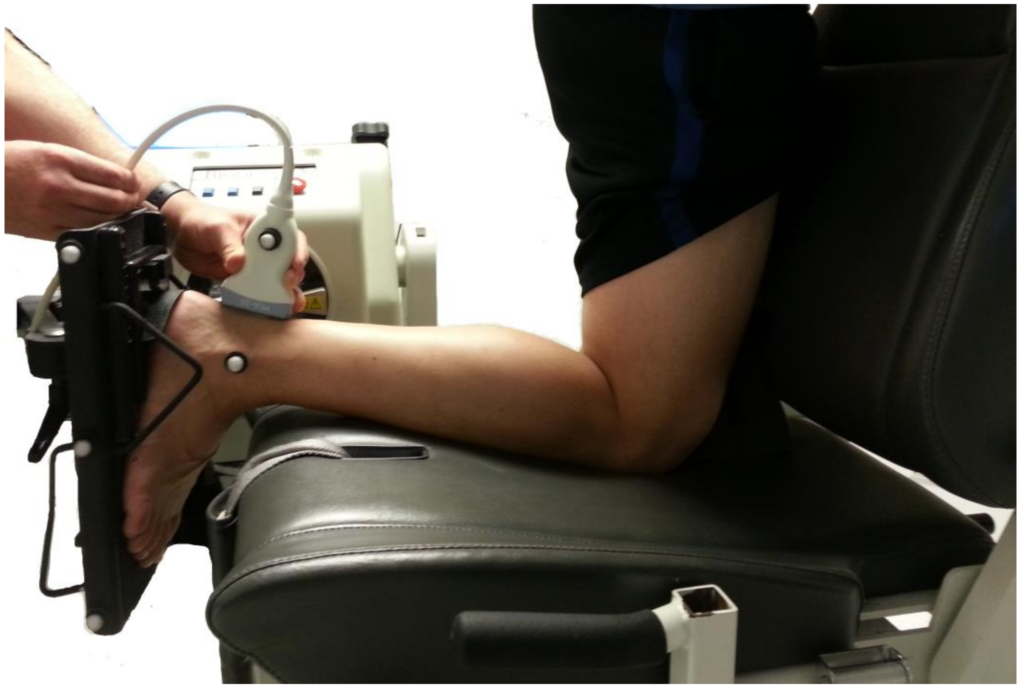


Figure 2-1. The setup for the collection of moment arm data. This is the same setup for the collection of the brightness and stress data with a change in probe position and/or orientation. Note the ankle and knee at 90^{o} and markers placed on the malleoli and at the center of the ultrasound transducer.

The subject's positioning remained the same from the moment arm collection to the contraction collection. Prior to the subject eliciting any contraction, the passive torque of the subject's ankle at 90° was recorded. This was added onto the calculated desired torques to ensure only active contraction was accounting for the stress in the tendon. As previously stated, the knee was placed at 90°, which increases slack in the gastrocnemius and greatly limits the gastrocnemii's contribution to the ankle torque during plantar flexion. Since the gastrocnemii were not the major contributor to the ankle moment, ultrasound images were taken at the soleus myotendinous junction (MTJ). The ultrasound transducer was held at a fixed angle and position on the skin's surface along the longitudinal axis approximately 6 to 8 cm proximal from the calcaneus. Tendons were imaged at 15MHz and 80% image brightness gain for all trials. An image was first taken with no contraction and then the subject was prompted to contract to a torque of 0.5x BW using visual feedback to match the applied torque to the desired. When that value was reached, an image was recorded and the subject was instructed to contract until the next torque was achieved. This process was repeated for 1.0, 1.5 and 2.0x BW. Three (3) trials of the contraction protocol were performed. The transducer was removed and replaced onto the ankle between each trial. The subject was given sufficient time to recover between trials to avoid fatigue. Axial images were taken with the same protocol described previously but at a distance approximately 3cm proximal from the calcaneus insertion point. Due to the curvature of the skin's surface around the Achilles tendon, an Aquaflex gel pad

(Parker Laboratories, Fairfield, NJ) was used to provide a full field-of-view of the tendon.

Despite efforts to fix the ankle into a particular angle, slight motion of the ankle was inevitable. Using the method described by Arampatzis and colleagues we accounted for any undesired rotation of the ankle. We collected a baseline ultrasound image of the soleus MTJ with the ankle at 90^o. While maintaining the transducer location, we passively changed the angle of the footplate of the Biodex to place the ankle into 92^o of plantar flexion and collected another image. The process was repeated for 94^o, 96^o and 98^o of plantar flexion. The change in MTJ displacement per degree was calculated for each subject. During the contraction collection, the ankle joint rotation was tracked with motion capture. The change in joint angle during the contraction protocol was associated with a MTJ displacement during the passive motion into plantar flexion. The resulting tendon length change caused by the minor foot movement was subtracted from the overall tendon length change [Arampatzis 2005].

The cross-sectional area and brightness of the tendons were determined using ImageJ (National Institutes of Health, USA). The cross-sectional area and longitudinal images of the tendon were manually traced on a hi-resolution touch-screen display (Wacom Technology Corporation, Vancouver, WA). The ultrasound image was collected in grayscale and each pixel had a white count which ranged from 0 to 255. ImageJ provided a histogram of the pixel white count within the area selected, or tendon in the case of this study, along with an overall average white count.

The average value of the tendon area traced in the sagittal plane determined the brightness. Due to brightness being transducer location dependent, the relative change in image brightness between the no contraction image and the subsequent images from the isometric case determined the tendon echogenicity (Figure 2-2). The stress during each isometric contraction was determined using equation Equation 2-1:

Equation 2-1 $$\sigma = \frac{\left(\frac{\tau}{MA}\right)}{A}$$

Where σ is the tendon stress, τ is the torque produced at the joint, MA is the moment arm and A is the cross-sectional area of the tendon. A regression of percent brightness change versus stress determined the correlation between stress and echogenicity. Tracking the movement of the soleus MTJ throughout the contractions not only identified a consistent collection location, but also allowed for the determination of tendon stiffness defined as tendon force (N) divided by change in tendon length, or elongation (mm).

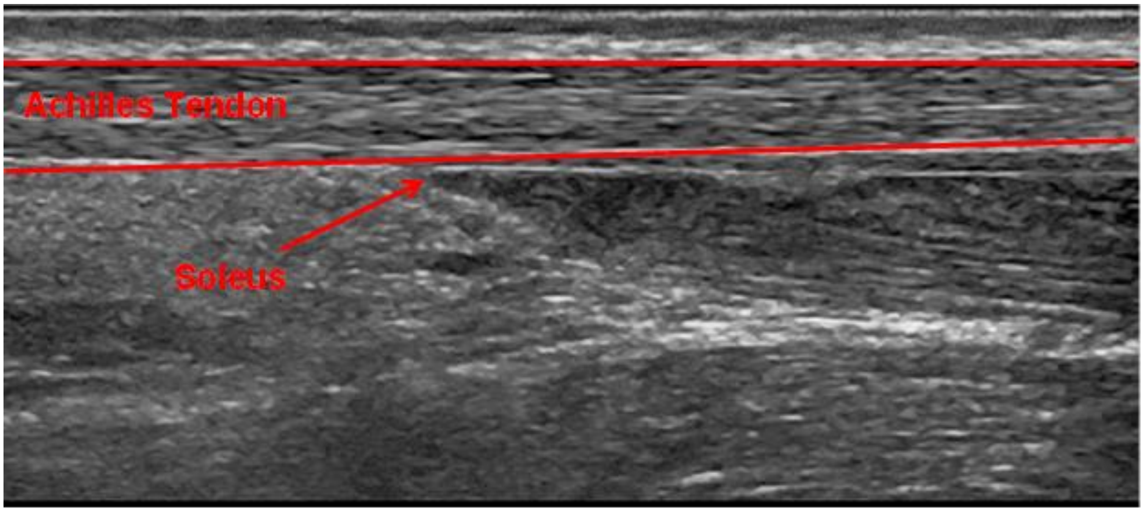


Figure 2-2. The Achilles tendon outlined in red. Note: The soleus MTJ is used as a reference for ultrasound transducer placement.

2.3 Results

A weak correlation exists between the percent change in tendon brightness and stress applied (R = 0.25) (Figure 2-3). Although there was a positive slope associated with the image brightness and the stress (0.14), 7 of the 18 cases decreased in brightness following the baseline case of no contraction. There was a significant motion of the tendon fibers ($p < 0.001$) within the region of interest demonstrated by the tendon elongation, even after the length change of 0.16 (0.08) cm at 2 BW caused by an average ankle rotation of 4.4° (0.82) was taken into account (Table 2-1). Similarly, the stiffness also did not change through the isometric contraction range (p = 0.982).

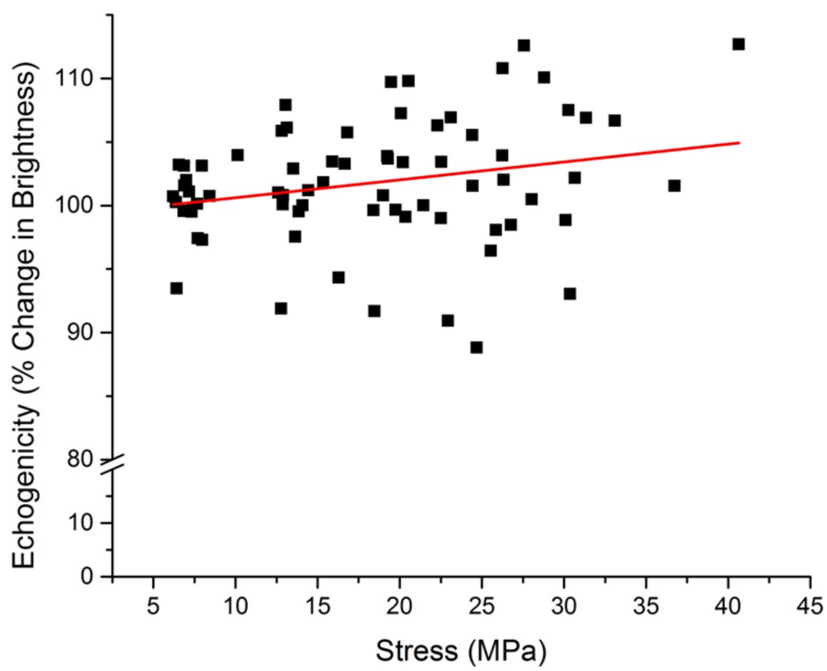


Figure 2-3. The correlation between echogenicity, in terms of % brightness change) and stress on the Achilles tendon. Note: The lack of correlation (R = 0.25) indicates stress does not impact the brightness of the tissue.

Table 2-1. The mean (SD) area, elongation and stiffness of the tendon during isometric contractions. BW indicates the percent body weight being transmitted through the Achilles tendon. Echogenicity is being reported as the absolute brightness (in white count from 0 - 255) at 0 BW and subsequent percent change. Note the lack of significant change in area with increasing force applied to the tendon.

BW (%)	Area (cm^2)	Elongation (mm)	Stiffness (N/mm)	Echogenicity
0	**0.46**(0.07)	-	-	**99.66** (10.03)
50	**0.47**(0.07)	**2.52**(1.23)	**133.7** (42.0)	**100.49%** (2.55%)
100	**0.48**(0.08)	**4.75**(2.10)	**141.9** (49.0)	**101.71%** (4.27%)
150	**0.49**(0.08)	**7.22**(2.39)	**140.1** (64.7)	**102.42%** (5.48%)
200	**0.49**(0.08)	**9.22**(2.05)	**146.2** (100.3)	**102.36%** (6.45%)
p-value	0.683	> 0.001	0.982	

2.4 Discussion

The poor correlation between the change in ultrasound image brightness and the stress within the tendon prevents echogenicity from being a viable metric for determining mechanical properties of the Achilles tendon in vivo. A power analysis with a medium effect size ($\beta = 0.9$) was performed to confirm the number of subjects was sufficient enough to establish the significance of this correlation. While previous in vitro studies demonstrated the feasibility of using increased echogenicity as a measure to track strain within a tissue, these measurements were performed using a uniaxial force (i.e., the tendon is stretched at the ends and without rotation). In vivo measurements are subject to additional variables. The Achilles tendon has spiraling, non-linear, fibers which causes tendon twist under tension [Doral 2010]. The 3D twisting of the tendon fibers while using 2D ultrasound may lead to the imaging of new fibers as the original fibers move out of plane. Tendon elongation also caused different portions of the tendon to be imaged. As the subject contracted, the tendon moved an average of 9.2mm through the region of interest at 2BW. The twisting and elongation add variability disrupting the tendon stress to brightness correlation.

The directional component of force applied to the tendon varied compared to the in vitro cases. The soleus contributed most of the force through the Achilles and the soleus attaches along the inferior portion of the tendon. Rather than pulling from the proximal end along the longitudinal axis, the soleus pulls along the entire attachment and in the line of action of the muscle fibers, which are not parallel to the tendon fibers (Figure 2-4). In some subjects this resulted in a widening of the tendon in the sagittal plane and altered the region of interest imaged, adding variability into the brightness measurement unrelated to fiber tension.

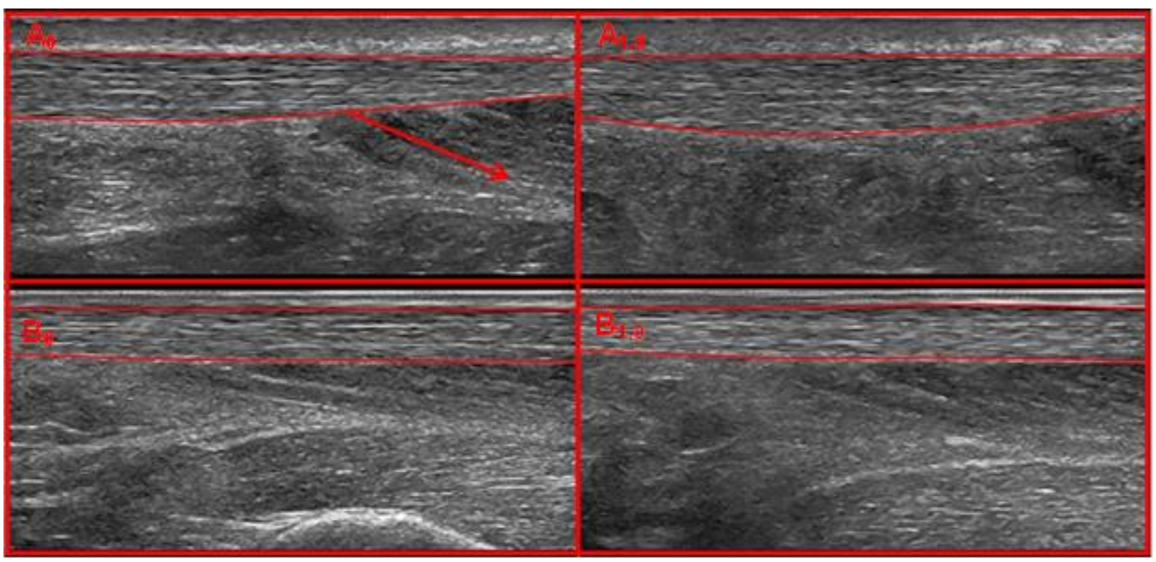


Figure 2-4. Anatomical differences of the Achilles tendon (traced in red) between two subjects at 0 BW and 1.0 BW. The red arrows indicate the line of action of the muscles fibers, which is not in parallel to the tendon fibers. Note the tendon of subject A widens with contraction while the tendon of subject B maintains a similar width.

The mechanical properties of the Achilles tendon in this study were similar to the findings of previous researchers. The tendon stiffness at 2BW was 146.2 N/mm which matched the results of Schepull and colleagues (143 N/mm) and Neugebauer and colleagues (162 N/mm) [Neugebauer 2012, Schepull 2007]. The average stress at 2BW (1349N) averaged 27.7MPa, a similar finding to Magnusson and colleagues reporting 36.5MPa at 2641N [Magnusson 2001]. To better compare the mechanical properties to previous research, the standard error of measurement (SEM) were calculated for the area (0.02 cm^2) and the elongation (0.09 mm). The SEM applied to the values found would lead to a stress to range from 26.1 to 28.1 MPa due to error, but these values are still comparable to previous findings. Similarly, the SEM of elongation accounted for a stiffness range of 144.4 to 147.7 N/mm which also within the range of reported tendon stiffness.

An interesting finding from this study was the incompressible nature of the Achilles tendon at the forces applied. Tendon is typically modeled as transversely isotropic and incompressible [Garcia 2000, Kuo 2001], but these are numerical assumptions. This study showed that as the tendon is put in isometric tension in vivo, the resultant axial stress does not compress it, demonstrated by the axial area not significantly decreasing with increasing tension (Table 2-1). This information can be used in estimates of mechanical parameters using other non-invasive techniques such as ultrasound elastography. These techniques find the shear modulus (μ) of the tendon but desire the more applicable Young's modulus (E), which has been correlated to healing progression [Schepull 2007]. Knowing tendon is incompressible is one key assumption in using the estimation $E=3\mu$ and this finding is limited to the applied force of 2.0 body weights or less..

Movement of the probe during the contractions can vary the brightness measurement. To ensure ultrasound probe movement was not a factor, a reliability test was performed. Longitudinal images were taken with the subject at rest, the subject then contracted and relaxed and an image was taken again to verify the brightness was the same in both cases. This was repeated several times on several subjects to confirm scan repeatability. The ICC (3,1) of brightness yielded a 0.995 indicating the probe location and tendon brightness were not changed by contraction.

This study demonstrated that additional factors associated with in vivo measurements, including tendon fiber arrangement and motion; do not allow for echogenicity as a feasible metric for determining Achilles tendon mechanical properties. Future studies utilizing echogenicity would be advised to be selective in which tendons are measured and ensure the joint involved is able to be immobilized. The twist of the Achilles during contraction and the various attachment points along the tendon led to different tendon fibers being measured, adding variability in brightness. Selecting a superficial tendon, such as the flexor tendons of the fingers, which have fixed uniaxial loading driven by proximal muscle attachments combined with minimal twisting during contraction may yield more favorable results [Lee 2005]. Even if the proper tendon is selected and the joint is immobilized, the tendon will still move relative to the ultrasound transducer due to muscle contraction and this must be a known limitation when using echogenicity.

Despite a weak correlation between brightness and stress, other tendon properties were shown which are applicable to additional, non-invasive measurement techniques. The finding of tendon incompressibility during axially tension provides a key component for translating the shear modulus, found through a non-invasive measure such as elastography, into the more clinically meaningful measure, Young's modulus.

Chapter 3

CONTINUOUS SHEAR WAVE ELASTOGRAPHY: METHODS AND VALIDATION

3.1 Introduction

The characterization of tendon viscoelastic properties has important clinical applications as an objective measure which can be used to assess pathology, track healing progression and evaluate therapeutic practice. Tendon mechanical properties have been related to athletic performance, the progression of injury recovery and the effectiveness of treatment [Arampatzis 2007, Woo 1987]. Tendon healing has been linked to the recovery of the tendon's elastic properties [Lin 2004, Schepull 2007] and the viscosity component of the tendon affects the energy lost during of human performance [Nordez 2008]. A tool which measures both the elastic and viscosity components of tendon is essential for quantifying these properties for use as a metric for clinical evaluation.

Elastography is a technique which utilizes ultrasound or other imaging modalities to determine the mechanical properties of soft tissue. Elastography has been utilized to detect changes in tissue for pathologies including liver fibrosis [Zhao, 2014], breast disease [Itoh 2006], prostate cancer [Saloman 2008], thyroid tumors [Lim, 2012], and pancreatic lesions [Janssen 2007]. Commercially available units have become available that measure the mechanical properties for soft tissues and have been used for characterizing glands [Arda 2011], muscle [Lacourpaile 2012], and relaxed tendon [DeWall, 2014]. Though there have been many studies performed on softer tissue, tensioned tendon has not been studied due to the limitations in previous elastography techniques [DeWall, 2014]. The stiffer tissue requires a data processing speed that has not yet been reached by conventional technology.

Continuous shear wave elastography (cSWE) utilizes the concept of tracking external shear waves traveling along the soft tissue via high frequency ultrasound to determine the shear wave propagation velocity. cSWE has the potential to collect a wide range of external frequencies and therefore is applicable to tissues of many different densities, but the process for cSWE has not been validated. Therefore, the purpose of this study is to compare the output of cSWE with a published method of magnetic resonance elastography [Cortes 2013].

3.2 Methods

3.2.1 Sample Setup

The medium used for validation was Agrose gel with a contrast medium included, 0.5% by weight of calcium carbonate. Three (3) batches of gel were produced at 1.0%, 1.5% and 2.0% concentrations. These batches were used in three arrangements. Each gel was poured into a rectangular container for cSWE examination (Figure 3-1). Samples were prepared for use in an MR scanner by setting the gels in non-ferrous glass containers.

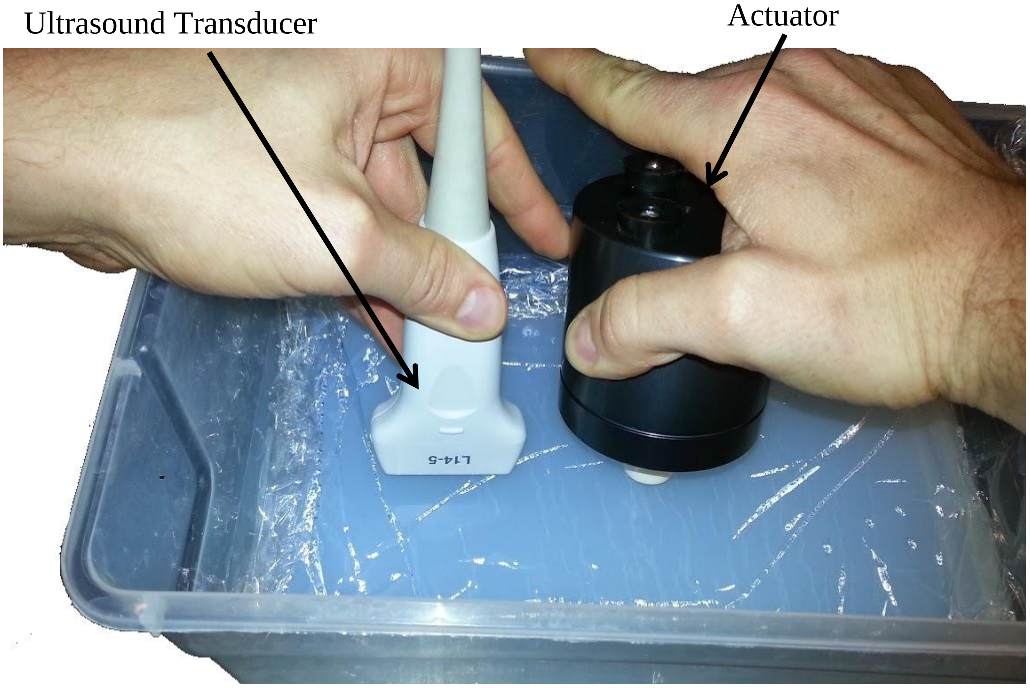


Figure 3-1. The Agrose gel in a rectangular vessel for use during cSWE. Note the ultrasound transducer is aligned with the head of the actuator.

3.2.2 RF Data Collection

The RF data collection rate was 6450 frames per second using an Ultrasonix MDP ultrasound (Ultrasonix, Vancouver, Canada) with an external data acquisition device (DAQ). The additional DAQ allowed for the collection of 128 channels of data simultaneously, each channel representing an element on the transducer. A 3.8mm L14-5/38 linear array transducer was used for the collection. The actuator was connected to a waveform generator which produced sinusoidal mechanical impulses. Each test set the actuator at a specific frequency and enough frames of raw RF data were collected to account for 2 full sine wave cycles to pass across the transducer during the collection. This was repeated five times with actuator frequency increasing from approximately 100 Hz to 600 Hz. The MR elastography was performed on each of the three samples as described by Cortes et al. [Cortes 2013].

3.2.3 Data Processing

The RF data requires remapping and processing as it is being collected in the unprocessed form. A plane wave ultrasound is required for cSWE. The plane wave, without post processing of the raw RF data, causes the signal reflection from a given entity to be received with separate time offsets by each element of the transducer's linear array. This creates a hyperbolic response in the RF signal received by the linear elements (Figure 3-2). A remapping is required to account for the phenomenon. A Fourier transform converts the data into the time domain and the signal response is adjusted using **Equation 3-1**:

Equation 3-1
$$\Gamma(f_x, f_z) = -\pi S(f_x, f_t)\sqrt{\frac{f_t^2}{c^2} - f_x^2}$$

Where: $$f_t = \frac{c}{2f_z}(f_x^2 + f_z^2)$$

c is the speed of light constant and $S(f_x, f_t)$ is the Fourier transform of the signal s(x,t). Following the remapping of the signal, an inverse Fourier transform was performed to convert the data back to the spatial domain.

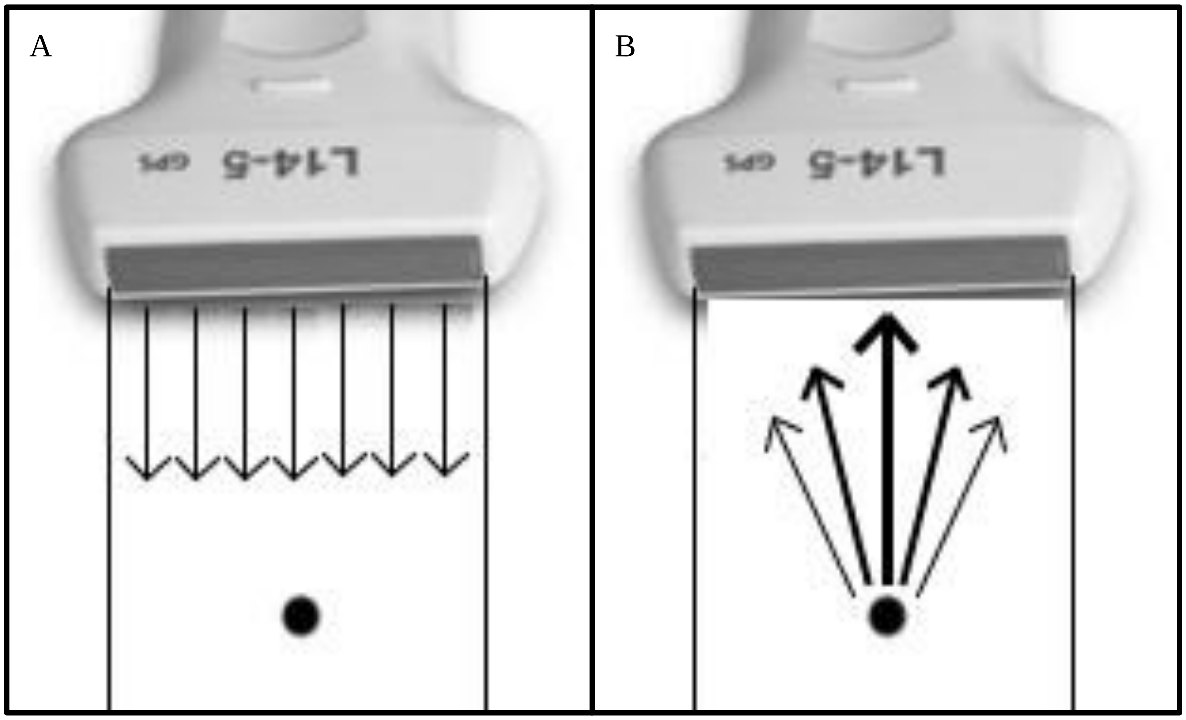


Figure 3-2. An example of a plane wave being transmitted from an ultrasound transducer and the scatter that returns from a single point. The plane wave is seen in **A** and the scattering return signals in **B**. Note the location of each of the vectors at a given time point in **B**, showing the signals will not all reach the subsequent transducer elements at the same time.

In order to calculate the phase velocity, data must be processed in the time domain by performing a Fourier transform on the RF data. Following the Fourier transform, a Hilbert filter was used to find the phase and amplitude of the transmitted sinusoid. This is compared frame by frame, and the calculated phase difference over a known distance yields the transmission velocity of the shear wave through the tissue [Zheng 2007]. The filtering process is sensitive to minor changes in phase and therefore the ideal points to compare along the RF array for the velocity calculation are those are 180° out of phase (Figure 3-3).

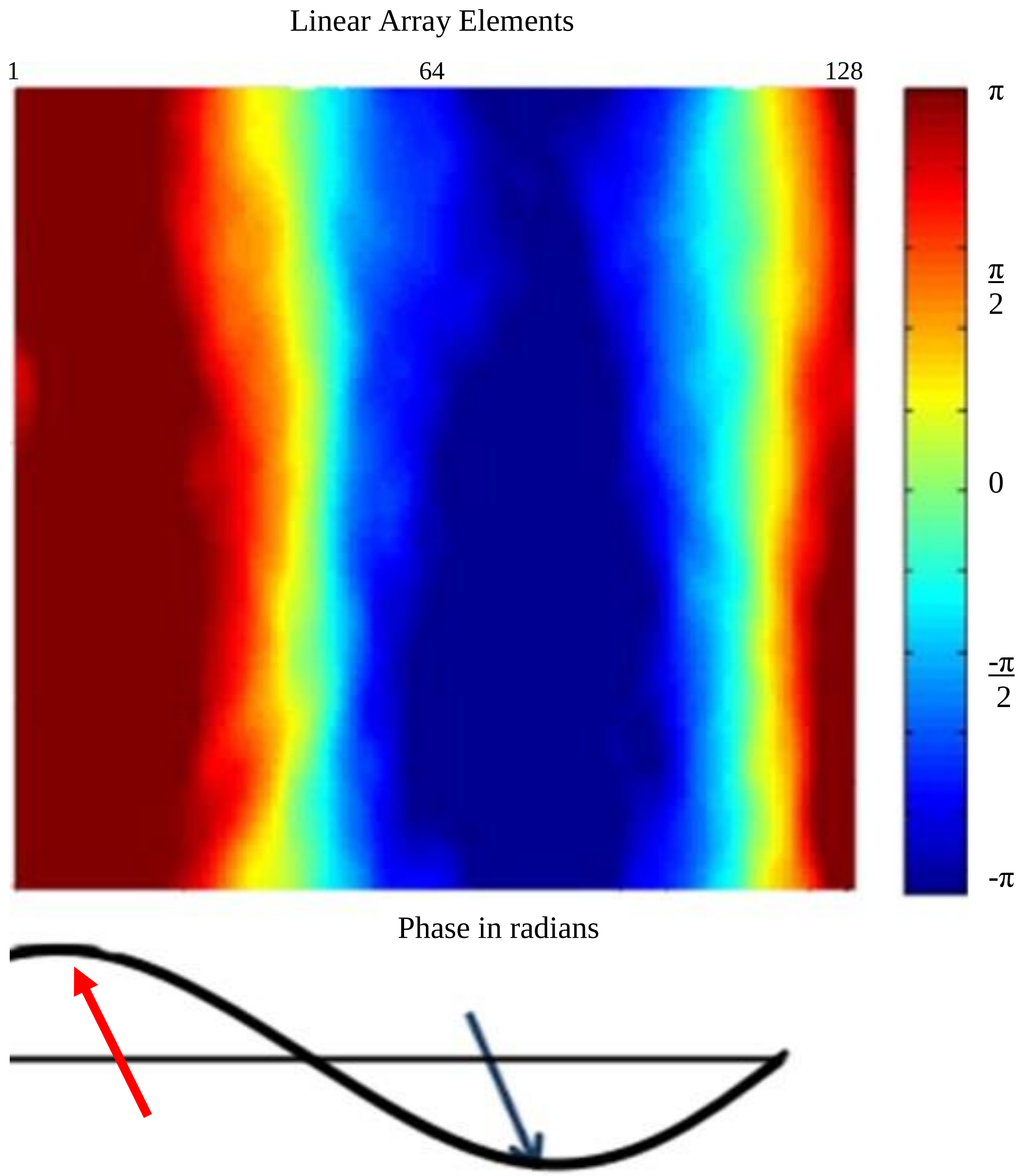


Figure 3-3. The phase map generated through signal processing of the shear wave propagation. The red represents a phase of π radians (180°) and the blue represents -π (-180°). The ideal points for determining wave speed would be the sine wave perfectly out of phase, marked by the red and blue arrows.

A directional filter was also added to the process to prevent reflected shear wave signals from interfering with the primary sinusoidal of interest: the actuator produced wave. A radial band pass filter was applied, which cuts off the very low frequency and very high frequency components. That output is then processed with a spatial filter which has a directional component. The combination of these two filters results in only the signal travelling orthogonal to the transducer as the signal of interest [Manduca 2003].

Each set of frames were processed to determine a velocity map of the gels which led to 6 velocity maps and 6 frequencies. To convert from velocity to shear and viscous modulus, a Voigt equation was used within an optimization algorithm, seen in **Equation 3-2**.

Equation 3-2
$$V_s(\omega) = \sqrt{\frac{2(\mu_1^2+\omega^2\mu_2^2)}{\rho(\mu_1+\sqrt{\mu_1^2+\omega^2\mu_2^2})}}$$

Where Vs is the velocity of the shear wave through the medium, μ1 is the shear modulus, μ_2 is the viscosity modulus, ω is the frequency and ρ is the density of the material (1000kg/m^3, which is the mass of water) [Hoyt 2008]. The moduli were calculated on a pixel by pixel basis creating an overall viscoelastic map for each of the samples. Given the MR elastography was only performed at a single frequency due to equipment limitations, we compared the average wave speed from cSWE to MR elastography.

3.3 Results

The Agrose gels measured by cSWE yielded similar results to those determined by MR elastography. The average velocities of cSWE were **5.46** (0.30), **6.95** (0.24) and **11.00** (1.19) m/s for the 1.0, 1.5 and 2.0% concentrations, respectively. The MR elastography had average velocities of **5.05** (0.1), **7.94** (0.23), and **11.46** (0.45) m/s for the 1.0, 1.5 and 2.0% concentrations, respectively (Figure 3-4). This led to an average difference of 8.2%. Additionally, there was a steady increase in wave velocity with the increase in density of the gel (Figure 3-5)

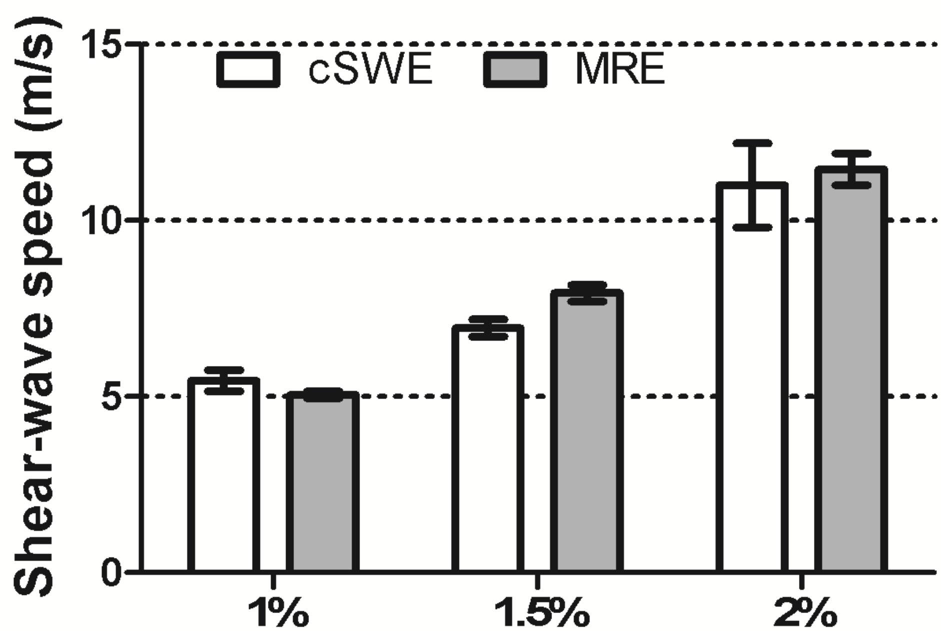


Figure 3-4. The difference in shear-wave speed between cSWE and MR elastography. Note the similar values from both cases.

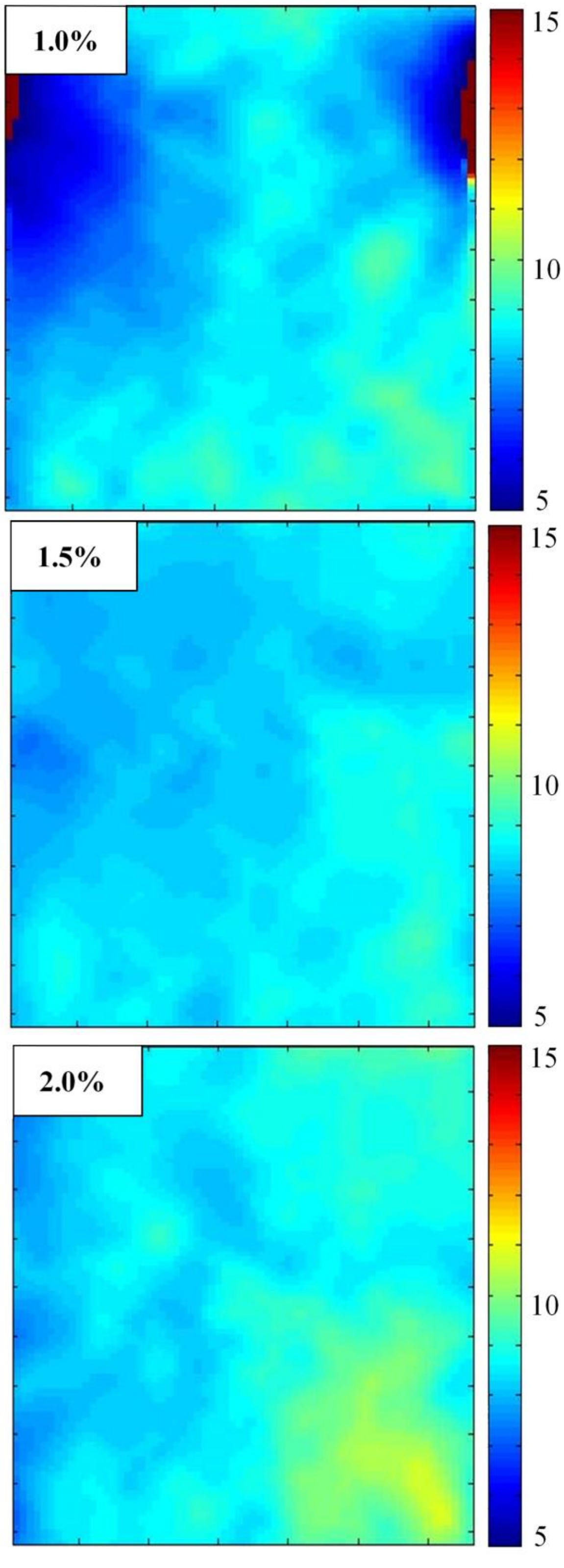


Figure 3-5. The phase change velocity of each of the agrose gel concentrations. Note the increase in velocity as the gel concentration increases.

3.4 Discussion

The technique of continuous shear wave elastography is a viable measure for assessing variations and the mechanical properties of soft tissue. In using this technique, it is possible to determine the shear wave velocity of a medium via high speed ultrasound collection which matched that of the established technique of MR elastography. In previous studies, it was demonstrated that a technique similar to this could be used *in vivo* on tendons [Hoyt 2008]. In a concurrent study, this technique was used in the assessment of Achilles tendons which demonstrated cSWE is a reliable measure, ICC(3,1) = .875, CV = 5.62%, when using fixed anatomical placement of the transducer and actuator [Suydam *in press, Appendix A*].

Other methods of elastography use acoustic radiation force impulse (ARFI) to determine the propagation of an induced shear wave in a soft tissue medium. This method consists of a pulse wave from the ultrasound transducer which excites the medium at the location of interest. This is followed by a "silent" period during which the transducer listens for echoes emitting from the excited medium [Nightingale 2002]. This technique works well for soft tissues such as breast and internal organ tissue, especially for identifying lesions or masses of a different stiffness than the surrounding tissue [Itoh 2006, Saloman 2008, Janssen 2007]. This technique has the limitation of ultrasound collection speed not being sufficient for the wave propagation velocities of stiffer tissue. Additionally, this method produces only a single wave frequency, limiting the ability to use the solid model to predict viscoelastic properties. The estimate for shear modulus can be made using **Equation 3-3**:

Equation 3-3 $$\mu = \rho c^2$$

Where ρ is the density of the medium and c is the velocity of the shear wave propagation. To calculate shear modulus using **Equation 3-3**, several assumptions must be made. It is assumed that the tissue is isotropic, incompressible and purely elastic [Bercoff 2004]. The continuous aspect of cSWE lends itself to being of benefit in creating a map of viscoelastic values, versus a single, averaged value. Since cSWE compares the phase difference pixel by pixel of the wave collected, inhomogeneity in material caused by inclusions are able to be detected. This would be of great benefit in the situation of tendinopathies, tumors, or rupture healing. The musculo-tendinous component also has demonstrated viscous effects ruling out the assumption of a purely elastic material [Fukashiro 2001, Ardt 2012]. Therefore, a non-linear solid model must be used to determine viscoelastic effects in stiffer tissue such as tendon. The model chosen in this study does produce the viscosity value of the material but the application of the viscous component would require validating though a comparable test.

In conclusion, the method of cSWE is a technique that uses an external vibration source to determine the mechanical properties of soft materials and matches with a documented technique. cSWE has the ability to be applied to many mediums including tendon, muscle and superficial structures such as breast tissue or glands. The quantification of these material properties can be used for determining pathology or tracking healing progression in the future.

Chapter 4

SEMITENDINOSUS TENDON HARVESTED FOR ACL RECONSTRUCTION REGROWS AND RECOVERS MECHANICAL PROPERTIES

4.1 Introduction

Anterior cruciate ligament (ACL) tears are common sports injuries that have devastating short-term performance repercussions and the possibility of long-term debilitating effects. A Scandinavian study showed ACL tears occur at average rate of 85 tears per every 100,000 people for those between the ages of 16-39 [Granan 2009]. Short-term ramifications for those receiving an ACL reconstruction include the loss of sport participation and performance with 82% returning to competitive sport and only 64% returning to their pre-injury competitive levels after ACL replacement [Ardern 2011]. Long term effects of ACL rupture include strength asymmetries, residual pain and osteoarthritis. The average quality of life of an individual at 5 years post ACL tear has decreased and more than 50% of people sustaining an ACL rupture have signs of osteoarthritis (OA) 10 years after the incident [Leitgeb 2014, Andersson 2009]. While returning to sport has become more successful, a large knowledge gap exists in returning patients to sport at pre-injury participation levels and preventing long term remaining effects after an ACL tear and reconstruction.

The immediate effects of ACL tear are becoming well known and addressed by the medical community. An ACL tear is characterized by joint laxity and reduced joint proprioception [Imbert 2014, Relph 2014]. These outcomes lead to an increased risk of further ligament damages due to instability [Andersson 1989]. Treatment of

ACL rupture with a surgical reconstruction reduces instability and the occurrence of future related ligamentous injuries, though the recovery is not instantaneous. It requires an average return-to-sport time of 7.3 months post-surgery [Andern 2011]. Unfortunately, even with an ACL reconstruction, the largest risk factor for an ACL rupture is having already sustained an ACL rupture. Within a 10 year time period post-ACL reconstruction, 10% of patients sustain a graft re-rupture and 14% sustained a contralateral rupture in autograft reconstructions [Bourke 2012]. The negative effect of autografts is the graft tissue, typically tendon, must be resected from the patient themselves, leading to possible strength asymmetries additional to those of the traumatic ACL injury.

The primary long term deficit of ACL rupture is the prevalence of OA greater than 5 years after incident. This phenomenon has been linked to meniscal damage and altered loading within the knee [Barenius 2014, Maly 2008, Andriacchi 2006]. ACL reconstruction has reduced the prevalence of meniscal damage after injury which reduces a risk factor for the onset of OA, but ACL reconstruction has not shown a restoration of condylar loading location or joint kinematics [Gardinier 2013, Scanlan 2010, Tashman 2004]. A successful ACL reconstruction restores the anterior/posterior stability in the knee, but transverse plane motion and stability may not be restored nor match to that of the contralateral side during tasks requiring internal/external rotation about the knee [Stergiou 2007]. Though kinematic and loading differences exist, the post-reconstruction research is based on a mix of different graft types including semitendinosus (ST) /gracilis (GRA) autografts, bone patella tendon bone autografts and allografts. The loading and kinematic changes seen post-reconstruction are a result of both the traumatic event which caused the injury and the selected reconstruction graft type. Understanding the impact of the autograft resection creates a better understanding overall asymmetries seen post injury. Determining if the tendon structure returns to pre-operative function will show there is no long term morbidity following tendon resection.

Long term effects and residual strength measures are major concerns when selecting between graft types. While the autograft has been shown to be the superior graft choice for those returning to sport, minimal outcome differences have been shown between the bone patellar tendon bone and the semitendinosus (ST)/gracilis (GRA) tendon graft in terms of re-rupture, stability, function and quality of life [Bourke 2012, Barenius 2010]. The ST/GRA tendon graft has shown a slight advantage with regards to less pain while kneeling and better knee extension [Barenius 2011, Mascarenhas 2012]. Though the ST/GRA autograft demonstrates better outcomes in certain parameters, the resulting musculoskeletal changes that occur with any autograft still remain. The resection of the hamstring tendons removes the function of the corresponding muscles. Though the ST and GRA have smaller cross-sectional areas than the other hamstring muscles, they are still contributors to internal rotation and leg flexion strength. Peak concentric and eccentric flexion strength has been shown to be significantly less for the involved compared to the uninvolved side between 8 and 12 months post-op [Eriksson 2001, Hiemstra 2000, Nakamura 2002]. Other studies contradicted these findings by reporting bilaterally symmetrical peak torque and it has been demonstrated that the other hamstring muscles eventually hypertrophy to compensate for the detached, atrophying ST and GRA muscles [Lipscomb 1982, Ohkoshi 1998, Snow 2012, Williams 2004]. These conflicting studies may be related to post-operative hamstring training, rate of hypertrophy, or another phenomenon of the ST/GRA autograft: the regeneration of the ST tendon. The regeneration of the ST tendon has been shown in between 60% and 100% of patients via ultrasound and MRI for more than two decades establishing this as a common healing mechanism [Leis 2003, Carofino 2005, Cross 1992, Eriksson 2001, Ferretti 2002, Janssen 2013, Nikolaou 2007, Nishino 2006, Okahashi 2006, Papandrea 2000]. The resection of the ST tendon leaves a gap, but imagining evidence shows this gap begins to fill in with tendinous materials around 4-6 months. The regeneration and restructuring of the tendon occurs from the initial identification of the neo-tendon until 24 months post-surgery, assuming the tendon regrows at all [Papandrea 2000, Ferretti 2002].

Continuous shear wave elastography (cSWE) is an ultrasound technique which is capable of evaluating the viscoelastic properties of soft tissue [Cortes *in Press*, Suydam *in Press*]. While the structure of the tendon has been seen with ultrasound and MRI, the tendon's function has not been characterized. A tendon's elasticity is what enables it to pass force from the muscle to boney attachment and create a joint torque. Tendon reorganizes at it heals and this is related to the elastic properties of the tendon [Otoshi 2011, Schepull 2007]. The ability to measure the elastic properties of the ST tendon *in vivo* would establish the functional ability of the tendon.

The ST tendon's recovery its functional characteristics would permit ST muscle to contribute to joint motion. Though the tendon appears to have the characteristics of functional tendon via biopsy and ultrasound, the recovery of the mechanical properties of the tendon is still unknown. Therefore, it is the purpose of this study to determine the viscoelastic properties of the regrown ST tendon, using cSWE, and compare them to the contralateral, uninvolved limb. We hypothesize there will be a significant difference of the shear elastic modulus between the involved and uninvolved ST tendon for subjects following tendon resection. We also hypothesize that the mechanical properties of the tendon will recover with time, becoming more symmetric with the uninvolved limb. Identifying the functional recovery of the ST tendon would aid in the graft type decision, create the opportunity for reduced strength and kinematic deficits and perhaps aid in developing a rehabilitation protocol to take advantage of the regrowth phenomenon.

4.2 Methods

Thirteen (13) subjects who had sustained a unilateral ACL rupture with no other complete ligament tears were recruited from the University of Delaware ACL database. Each patient had received an ACL reconstruction using ST tendon tissue as part of an ACL autograft (21.0±2.5 yrs, height 174.5±9.8 cm, mass 75.7±13.0 kg). These subjects were tested between 6-24 months post-op. Each subject read and signed an informed consent approved by the University of Delaware institutional review board. Subjects lay prone on a plinth with their knee at 45° of flexion to bring the ST tendon more superficial and allow better imaging with ultrasound. The subject's involved tendon was palpated and imaged to confirm it was indeed the ST tendon. Using real time ultrasound imaging, the ST tendon was traced to the muscle in the longitudinal plane. The ultrasound probe, while still imaging the ST tendon, was rotated into the axial plane to confirm the muscle to which the tendon was attached (Figure 4-1). The probe was returned to the longitudinal plane and the subject contracted their hamstrings to verify the regrown tendon was attached, i.e., there is motion of the tendon during contraction. A mark was placed on the skin's surface at the location of the ST tendon and the process was repeated for the contralateral side.

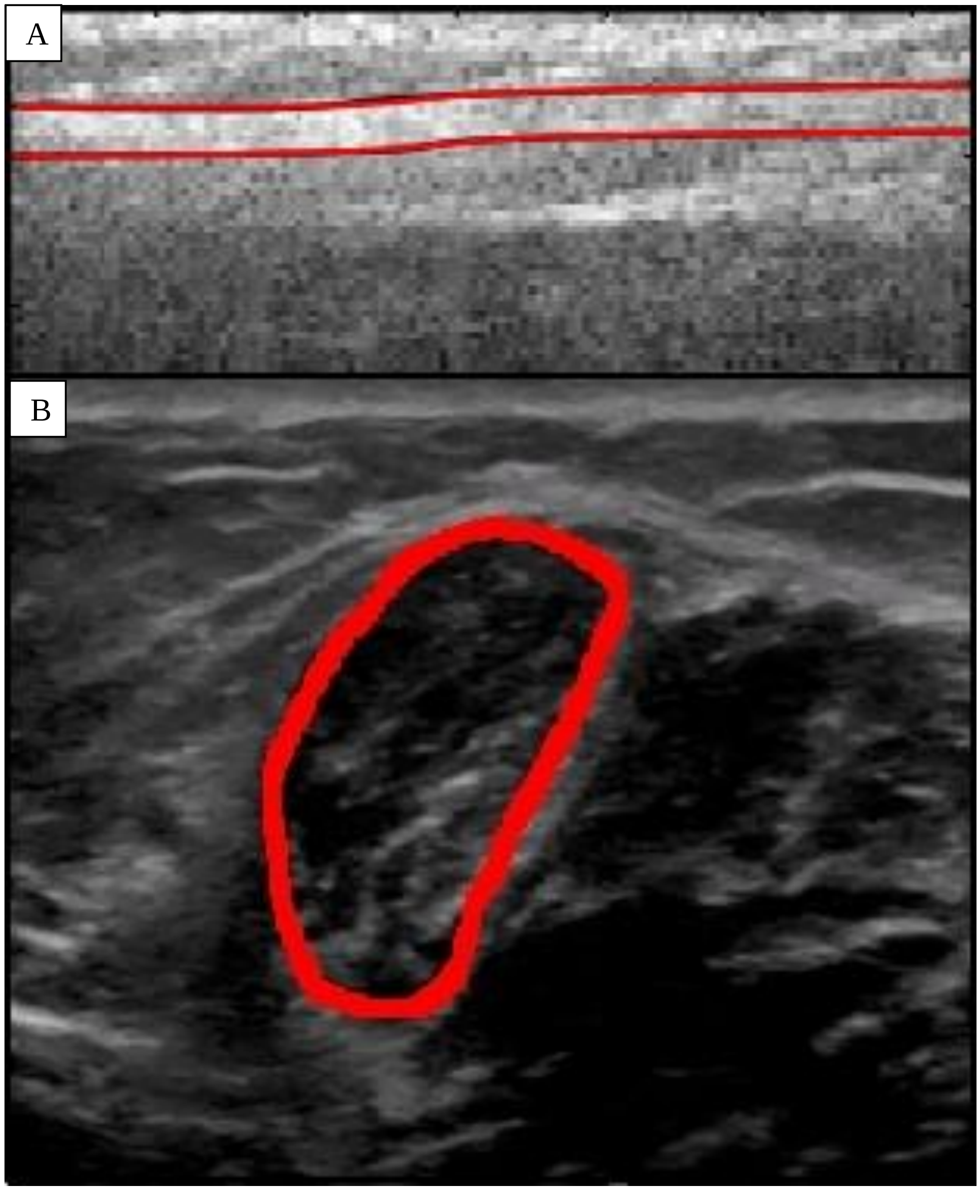


Figure 4-1. The longitudinal view of the semitendinosus tendon (A) and the axial view of the semitendinosus muscle (B). The ST tendon and muscles are traced in red.

cSWE was performed on both the involved and uninvolved ST tendons. The method of cSWE used to determine viscoelastic properties of the ST tendon are described by Cortes et al. [Cortes *in Press*]. cSWE requires a mechanical stimulus to be applied to the tissue of interest and an ultrasound unit to collect the raw RF data. Data were then post-processed using a custom Matlab signal transformation and analysis code. An Ultrasonix MRP ultrasound unit with an attached 128 channel data acquisition unit (DAQ) (Analogic, Peabody, MA, USA) collected the RF data at 6450 Hz. Shear waves were applied to the tendon by means of a Bruel & Kjaer 4810 mechanical actuator (Bruel & Kjaer, Norcross, GA). The ultrasound probe was placed approximately 2 cm proximal to the knee joint along the ST tendon and the actuator was placed 3 cm proximal to the transducer (Figure 4-2). The transducer was set to produce a mechanical impulse along the tendon at 323 Hz. A data set >60 frames was collected. With the transducer and actuator held fixed, 5 more sets of data were taken at 340, 358, 379, 403 and 430 Hz.

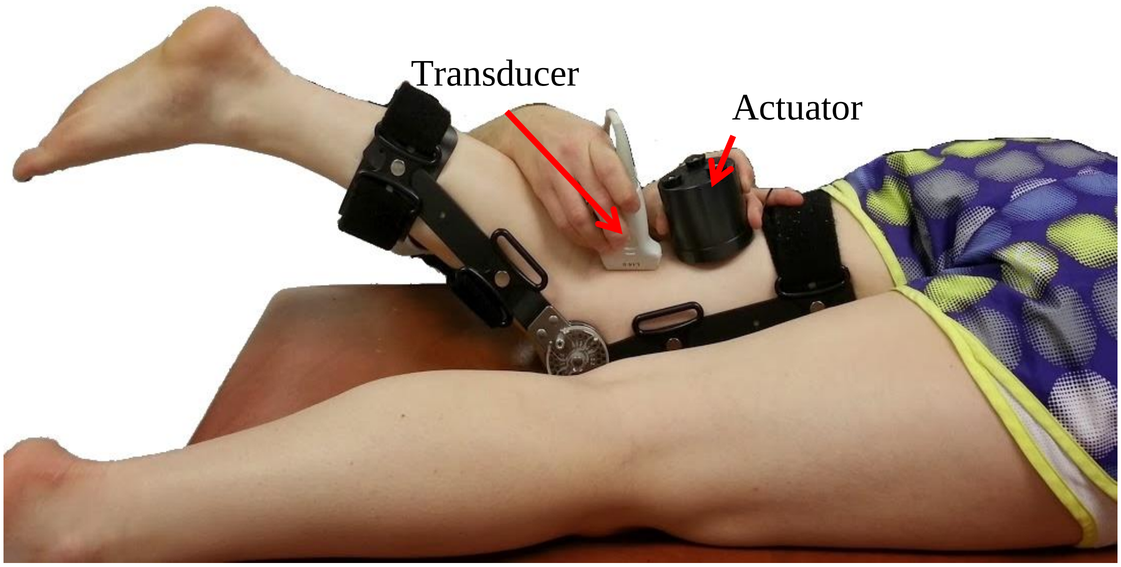


Figure 4-2. The setup for the collection of shear waves propagating through the tendon. The brace was one mean of fixing the knee at a 45º knee flexion angle. Note: The actuator is proximal to the transducer so the shear waves travel from origin to insertion.

Post processing signal analysis yielded the average shear wave speeds for each frequency. Post-processing included a Fourier transform of the signal in order to calculate the phase of the signal. The difference in phase of the shear wave across the length of the transducer allowed for the speed of the wave to be calculated. A Voigt model was used as the minimization criteria of an optimization algorithm to determine the viscoelastic properties by relating the shear speed to frequency (**Equation 4-1**):

Equation 4-1

$$V_s(\omega) = \sqrt{\frac{2(\mu_1^2+\omega^2\mu_2^2)}{\rho(\mu_1+\sqrt{\mu_1^2+\omega^2\mu_2^2})}}$$

Where, the shear modulus is represented by μ_1 (kPa) and the viscosity modulus, μ_2 (Pa-s). V_s is the shear wave speed, ω is the shear wave frequency, and ρ is the tissue density of 1600kg/m^3 for tendon [Hashemi 2005]. The same protocol was collected on 9 healthy subjects as a normative comparison. An ANOVA determined differences between the involved and contralateral tendons ($p<0.05$). A Pearson's correlation coefficient will determine if the elastic modulus is related to recovery time. An inter-class correlation coefficients ICC(3,1) will be used to determine test reliability.

4.3 Results

Patients ranged from 6-24 months post-op with an average of 13.2±5.8 months. 13 subjects were analyzed for ST regrowth and 12 showed recovery of the ST tendon (92%). cSWE produced shear and viscosity moduli maps for each of the tendons of which the average was taken over the entire region of interest to report a single value for each tendon. The average shear modulus of the uninvolved ST tendon was 129.4±55.3 kPa and the average viscosity modulus was 192.6±80.9 Pa-s while the involved side was 73.06±16.7 kPa and 114.3±22.2 Pa-s, respectively. A significant difference between the involved and uninvolved limbs existed for the shear modulus ($p = 0.004$), and the viscosity modulus ($p = 0.007$) (Table 4-1). This displayed a 59.2% shear modulus recovery of the ST tendon and a 65.2% recovery of the viscosity modulus. The shear modulus of the ST tendon had a strong positive correlation with time ($r = 0.60$, $p=0.039$). The viscosity also had a positive correlation, though it did not reach significance (r = ns, $p=0.208$) (Figure 4-3). Height and weight had no significant effect on viscoelastic parameter recovery. The healthy data showed no significant difference bilaterally for μ_1 or μ_2 ($p = 0.875, 0.779$).

Table 4-1. The average viscoelastic properties of the involved (inv) and uninvolved (uninv) ST tendons. Given there is no involved side for the healthy controls, the left are listed as uninvolved for the healthy subjects. Note the greater than 35% difference in shear modulus recovery between the less than 12 months and greater than 12 months groups.

	μ1 (kPa) Inv	μ1 (kPa) Uninv	μ2(Pa-s) Inv	μ2 (Pa-s) Uninv	μ1 Difference	μ2 Difference	Months Post-Op
Mean	135.3	72.6	201.0	113.1	59.2%	65.2%	13.2
Mean < 12 Months	159.4	63.7	228.1	104.8	45.2%	57.1%	*
Mean > 12 Months	106.4	86.0	168.5	125.6	80.2%	77.4%	*
Healthy	135.3	72.6	201.0	113.1	59.2%	65.2%	13.0

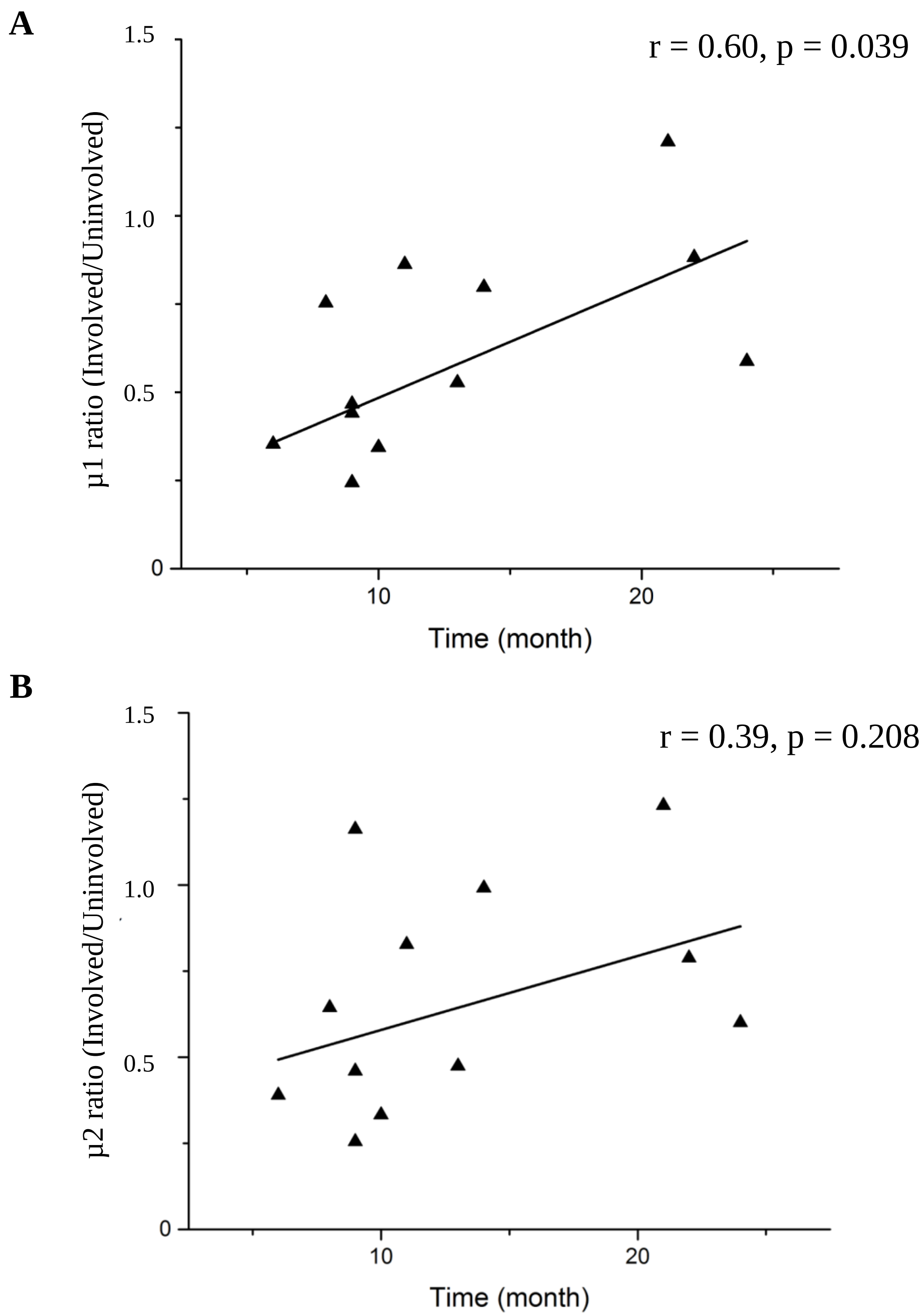


Figure 4-3. The correlation between the shear (A) and viscosity (B) moduli recovery and time in months. Note the positive correlation indicating the recovery of viscoelastic properties with time.

4.4 Discussion

This study determined that the semitendinosus tendon not only regrew in 93% of the subjects, but also that the mechanical properties of the tendon were recovering with time. The significant difference between the shear modulus of the involved and uninvolved tendons demonstrates that there is indeed a reduction in mechanical characteristics even though there is sonographic evidence of the tendon regeneration (Figure 4-4, Figure 4-5). The positive correlation between time and elastic properties of the tendon, through the recovery of the shear modulus, will enable the muscle to increasingly contribute to joint torque with time. The viscosity modulus appears to be less consistent in its regrowth pattern across subjects with an $r = 0.39$, but the correlation is still positive with time. The recovery of these mechanical properties indicates the ST muscle-tendon complex function is being restored with time post ACL autograft reconstruction with a ST tendon.

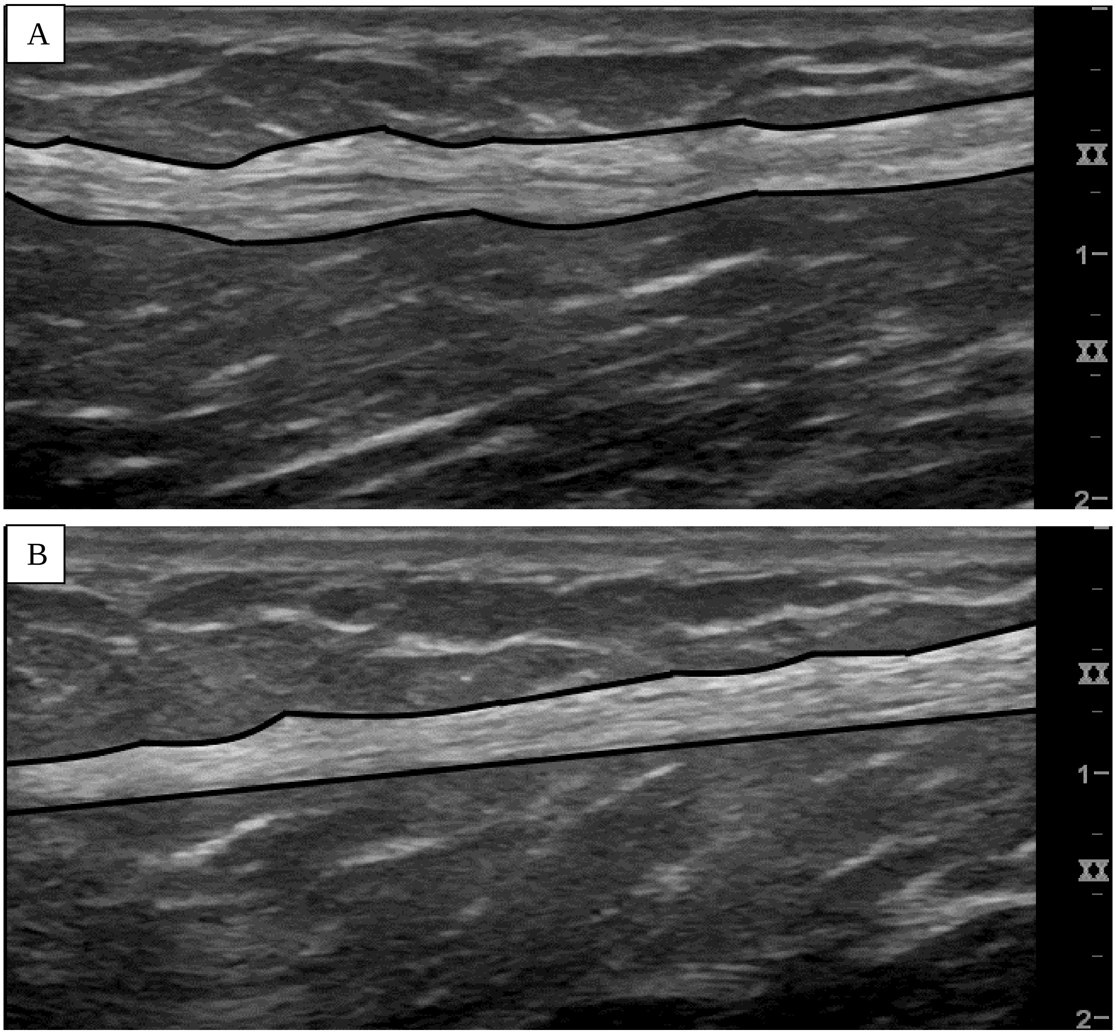


Figure 4-4. Ultrasound images of the regenerating semitendinosus tendon (A) and the healthy semitendinosus tendon (B) from a single patient, 14 months post-op. Note the wavier, disorganized layout of the ST tendon in the healing tendon compared to the healthy, though the thickness appears to be greater.

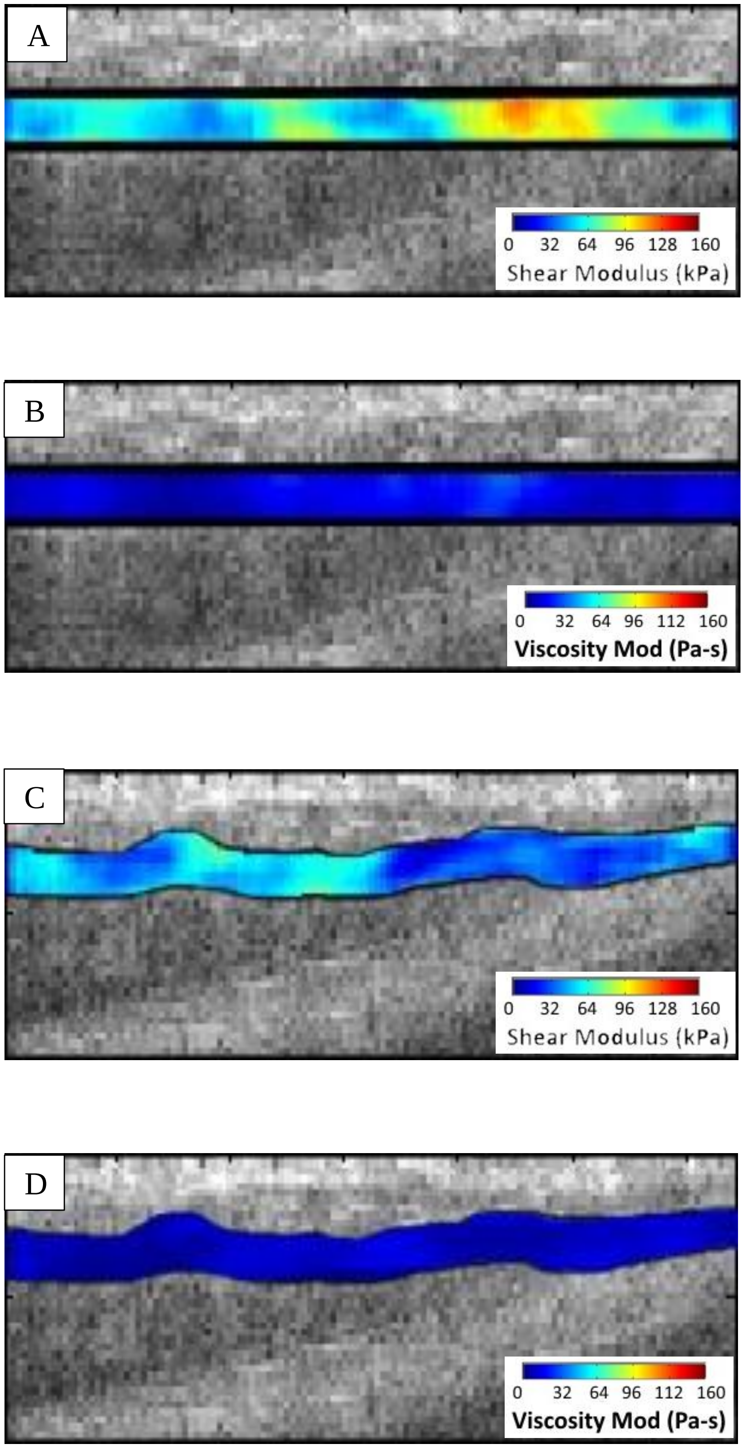


Figure 4-5. The shear modulus (A) and viscosity (B) for the uninvolved tendon as well as the shear modulus (C) and viscosity (D) for the involved tendon of a single subject. Note the lower viscoelastic properties for the involved side as well as the thickness difference. As the tendon heals, it regains its viscoelastic properties.

The recovery of the elasticity of the tendon, demonstrated by the increase in shear modulus, is essential for understanding the strength contributions of the ST muscle. In vitro, it has been shown the shear modulus is linearly related to the elastic modulus within sample [Eby 2013]. If the shear modulus is linearly related to the elastic modulus, and the shear modulus is increasing with time, it stands to reason that the elastic modulus is recovering with time as well. With the understanding that tendon is bilaterally equivalent, the restoration of the shear modulus, compared to the contralateral side, allows us to make the connection to the elastic modulus recovering with the shear modulus. The elastic modulus is important in understand the contribution of the muscle to the joint torque. The area under a stress/strain graph which makes up the elastic modulus is the amount of energy stored per unit volume. In the case of tendon, it is the energy lost in terms of torque contribution. The mechanical properties contribution to joint torque may explain the findings by Jassen et al. which showed the visual presence of the ST tendon, via ultrasound, 12 months post-op does not correlate to a recovery of isokinetic hamstring strength [Janssen 2013]. Our study showed that only 45% of the elastic properties of the tendon have recovered by that time point (Table 4-1). The increased elastic modulus causes the tendon to be stiffer and have less deformation when a force is applied. The reduced elasticity of the tendon results in more energy lost during contraction creating a torque deficit at the joint. It may be that hamstring regrowth is linked to strength recovery, but it is the mechanical property recovery and not just the presence of the tendon.

The mechanisms responsible for the time correlation to viscoelastic property recovery are important to understand for performance recovery optimization. The tendon has a disorganized structure consisting of more type 3 than the stronger type 1 collagen in the initial stages of the tendon healing process after rupture [Best 1993, Eriksen 2002]. The tendon becomes stronger with rehabilitation and the type 1 collagen replaces the type 3. It has been shown that the regrowth and reorganization stem from mechanical forces being placed on the tissue [Wang 2006]. This is supported by the common tendon rehabilitation treatment which shows that early function, while tendon is composed of this unarranged mass of collagen, produces the best outcomes [Kearney 2012]. The recovery time is correlated to the viscoelastic properties, but it may be the tendon not only needs to regrow, but then needs tension put on it in order to facilitate reorganization. The use of b-mode ultrasound can detect the presence of the tendon during rehabilitation or elastography can determine if the tendons mechanical properties have started recovering and this information can be used to help determine an exercise regimen to yield the best results.

The patient population was collected from a number of different surgeons without surgical technique being reported. A variety of techniques of graft harvest exist for the semitendinosus which has a profound impact on the regeneration of the tendon. A technique which reported the harvesting of the distal end of the graft at the periosteum produced a regrowth rate of 82% [Okahashi 2006]. A study that showed the surgical technique which left a 4 cm distal stump for which the regenerating tendon can reattach lead to a 100% regrowth rate [Ferretti, 2008]. The surgeon from which the majority of our patients were recruited (6 of 13) performs a technique which promotes tendon regrowth. The GRA and ST tendons are freed from the original tendon attachment for 10 cm up the thigh to assure ease of stripping without preventing graft resection. The ST tendon sheath is entered and an open stripper is placed around the tendon. The stripper travels proximal until the tendon is released from the muscle. The sheath is then sutured closed, leaving a regeneration path for the tendon. This surgeon, using this technique, had a 100% regrowth rate in this study. There have been no reports of the influence of graft technique on the tendon structure reorganization or mechanical property recovery rate, which may be of interest in the future using cSWE. A variety of techniques exist and each may influence the rate of tendon healing.

The recovery of the mechanical properties of the tendon is beneficial but this study has the limitation of only being from a single time point of several subjects. To affect clinical practice, a longitudinal study of subjects is needed. The graft technique of the surgeon performing the ACL reconstruction determines the effectiveness of the ST tendon regrowth and it is unknown if it affects the regrowth rate. Additionally, given the inherent nature of each subject, variation in rehabilitation protocol and activity following the completion of physical therapy and being cleared for return to sport, patients' recovery rates are most definitely not equal. The rehabilitation protocol and post rehabilitation exercise program may have caused the tendon to reorganize differently depending on the stresses put on the tendon during regrowth. Given that this is the first study of its kind, there were no values of which to compare. There may have been changes of the ST tendon on the contralateral side in addition to the changes within the involved side, though the values seen in the healthy controls were similar to those of the uninvolved side (Table 4-1).

This study showed the majority of patients regrew their tendon which then recovered their mechanical properties with time post-op. It was also shown the tendon requires upwards of 24 month in order to regain the viscoelastic properties.

Chapter 5

HAMSTRING STRENGTH RECOVERS WITH THE MECHANICAL PROPERTIES OF TENDON

5.1 Preface

Chapter 5 reports of all the strength data from this study and correlates it to the shear elastic modulus found in Chapter 4. This chapter reports the outcomes of each of the hypothesis set out to be tested in the third aim of this dissertation. Chapter 6 is similar in the methods but is more of a focus on the return to sport and injury prevention compared to Chapter 5 which is more centered on returning the patient's bilateral symmetry. Chapter 6 is also reporting the same internal rotation strength data (Aim 3.3), but instead of reporting the peak flexion strength, it focuses on the concentric flexion strength at specific angles in order to understand increased flexion strength asymmetries.

5.2 Introduction

An anterior cruciate ligament (ACL) rupture is a common life altering injury that can have drastic implications for the patient in the short and long term. The typical treatment for this injury is surgical reconstruction which restores anterior/posterior joint stability to the knee and reduces the risk to for additional ligament or meniscal damage, especially in running and cutting maneuvers, pivoting and activities requiring extremely dynamic movements [Andersson 1989]. The use of the semitendinosus (ST) tendon as graft material for the reconstructed ACL has demonstrated better range of knee extension and less long term pain while kneeling [Mascarenhas 2012, Barenius 2011]. There is also a reduced prevalence of revision necessary when compared to allografts [Genuario 2012]. Though many advantages exist for the use of the ST tendon as a graft material, the resection of the tendon still disconnects the muscle from the insertion point and removes its contribution to knee flexion torque.

Following ST resection for an ACL reconstruction, patients show a decrease in concentric and eccentric flexion torque [Ardern 2010, Nakamura 2002, Todokoro 2004, Kramer 1993, Hiemstra 2000], internal rotation strength [Viola 2000] and reduced flexion angle at which peak flexion torque is generated [Carofino 2005, Ohkoshi 1998]. The other hamstring muscles hypertrophy to reduce the asymmetry of flexion strength but the knee angle at which peak torque is generated and the internal rotation strength may not be recoverable through the strength gains of other hamstrings due to muscle morphology [Snow 2012, Williams 2004]. The ST tendon has a fusiform muscle fiber type and inserts into the pes anserinus, which is more anterior than the other hamstring tendons insertions [Makihara 2006]. The anterior insertion of the ST tendon allows it to generate a larger torque in the transvers plane and therefore the resection of the tendon directly contributes to the internal rotation strength deficit. The longer, parallel fibers of the ST muscle influences the joint angle at which the ST reaches its optimum fiber length (fiber length of maximum force output). The other fibers being shorter and in a pennate structure causes their joint angle of peak flexion torque to be less. The potential exists for these asymmetries to be recovered with time due to the regenerative nature of the ST tendon post ACL reconstruction [Leis 2003, Carofino 2005, Eriksson 2001, Jassen 2013, Nikolaou

2007, Nishino 2006, Okahashi 2006]. The regrowth of the ST tendon occurs in 60% to 100% of patients, but the attachment point has been shown to be 1-4 cm proximal to its original insertion in some cases [Papandrea 2000, Cross 1992]. This may be linked to several studies showing the lack of correlation between the regrowth of the ST and the recovery of hamstring strength [Jassen 2013, Tadokoro 2004]. Contrarily, Ferretti et al. showed a surgical technique which focused on the regrowth of the ST tendon back to the original insertion site, along with a hamstring strengthening rehabilitation protocol. These methods in concert restored internal rotation and knee flexion strength symmetry. It has also been shown that flexion strength correlates with the muscle morphology of the ST following the regrowth of the tendon [Nomura 2014]. Thus far studies have defined tendon properties by cross-sectional area, length or volume, but none of these features define a characteristic which would permit the tendon to transfer force such as the mechanical properties, i.e., elastic modulus, of the tendon.

Research to date has evaluated tendon properties via imaging techniques, such as ultrasound or MRI, and biopsies. These methods provide muscle and tendon length, cross-sectional area and structure but do not provide mechanical insight into the functional capabilities of the tendon [Okahashi 2006, Leis 2003]. Determination of the mechanical properties of the regenerating tendon would enable more to be known about the contribution of the ST muscle force to knee flexion torque post reconstruction. The imaging technique of continuous shear wave elastography (cSWE) has been shown to evaluate mechanical properties, i.e., the shear elastic modulus, of tendon *in vivo* [Suydam, *in Press*]. Establishing a relationship between the ST tendon’s shear elastic modulus and the recovery of the hamstring strength will aid in understanding how to reduce remaining asymmetries in ACL rupture patients who received a ST tendon autograft. It is the purpose of this study to determine the difference in shear elastic modulus of the ST tendon between the involved and uninvolved sides and compare it to remaining bilateral strength differences. We hypothesize the flexion and internal rotation strength will increase with the shear elastic modulus of the tendon. We also hypothesize the flexion angle at which peak flexion torque is generated will decrease with the recovery of the mechanical properties of the ST tendon. This will show the return of tendon functionality and the restored contribution of the ST muscle.

5.3 Methods

In this study, 13 patients who sustained an ACL rupture and were treated with a ST tendon autograft were recruited from the University of Delaware physical therapy clinic. Each of the 13 subjects was between 6 and 24 months post reconstruction and were cleared for return to sport. Subjects must not have sustained an additional tear requiring surgical reconstruction. Before participating in the study, each subject read and signed an informed consent approved by the University of Delaware's Institutional Review Board.

Prior to evaluating the ST tendon's mechanical properties it was important to establish the regrowth of the tendon. The subject lay on a plinth in the prone position with their leg at 45° of flexion. The tendon assumed to be the ST was manually palpated and, using B-mode ultrasound, was traced in the longitudinal direction up to the muscle belly (Figure 5-1). The ultrasound probe was then rotated, pivoting on the tendon, to image the cross section of the tendon and hamstring muscles. After confirming the tendon being traced was indeed connected to the ST muscle, the probe was returned to the sagittal plane. While imaging in real time, the subject contracted their hamstrings by performing an isometric flexion against resistance to confirm that the tendon moved and/or was stretched by the contraction of the muscle. Marks were placed on the skins surface to identify the location of the semitendinosus tendon for cSWE.

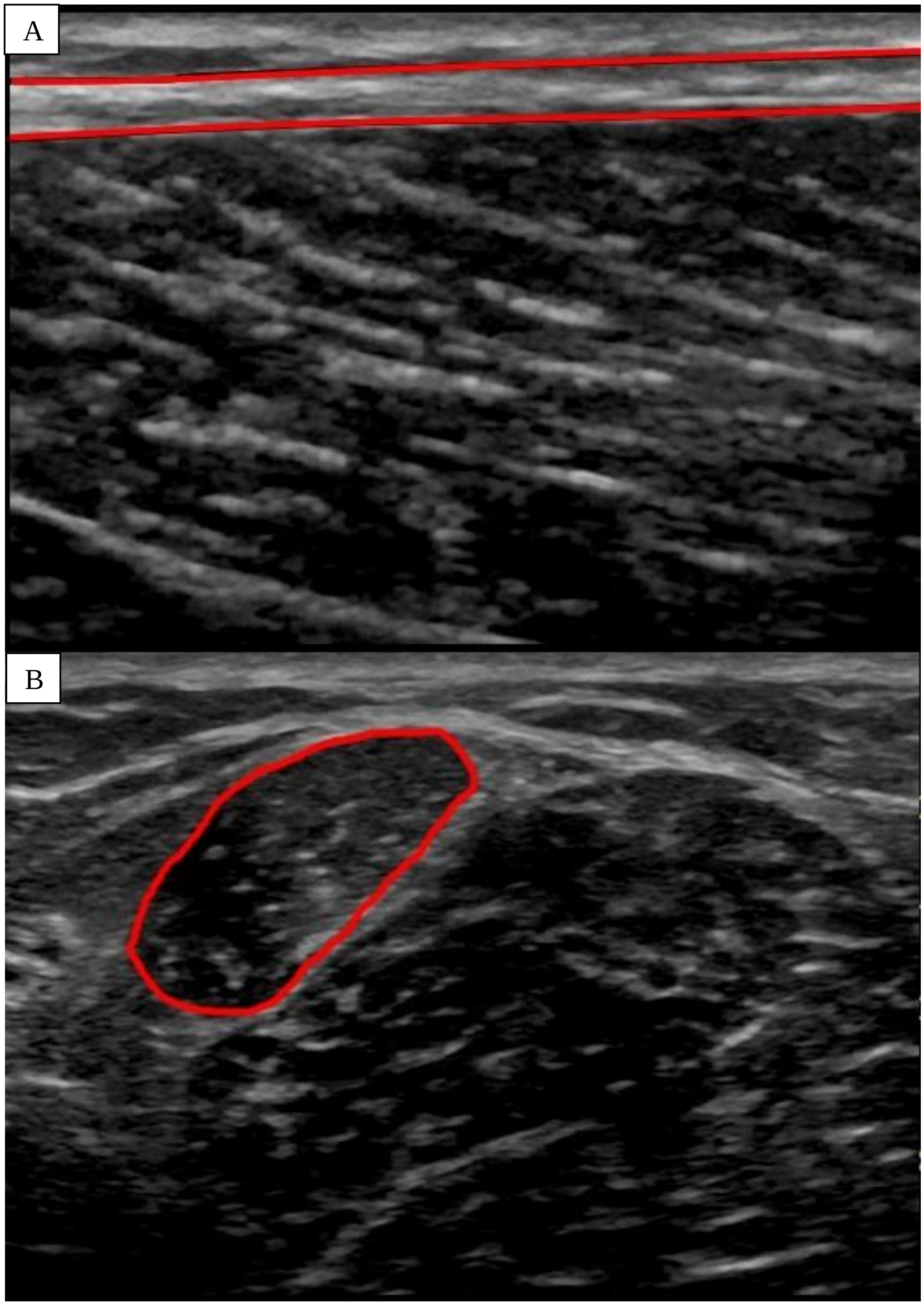


Figure 5-1. Ultrasound images of the semitendinosus tendon (A) and muscle (B) highlighted in red. The tendon is viewed in the longitudinal plane and the muscle is in the transverse plane.

Semitendinosus tendon viscoelastic properties were evaluated using cSWE [Cortes *in Press*]. cSWE requires high frame rate ultrasound collecting raw RF data with an external shear wave generator. An Ultrasonix MDP scanner (Analogic, Peabody, MA, USA) with a linear probe, L14-5/38, and a 128 channel DAQ was used to collect RF data at 6450 Hz. The ultrasound scanner was setup to transmit a plane wave at 10 MHz and at a depth 2 cm. The probe was placed 2 cm proximal to the joint line of the knee. The external shear wave was produced by a Bruel & Kjaer 4810 mechanical actuator (Bruel & Kjaer, Norcross, GA) attached to an external amplifier and an analog to digital waveform generating source. The vibrating portion of the actuator was placed 3 cm proximal to the ultrasound transducer along the ST tendon (Figure 5-2). A continuous sine wave was generated at 323 Hz and greater than 60 frames of RF data were collected. With the transducer and actuator remaining in place, 5 more sets of frames were collected at increasing frequencies of 340, 358, 379, 403, and 430 Hz. Mechanical properties were determined using a constrained nonlinear optimization based on the Voigt model of viscoelasticity (**Equation 5-1**):

Equation 5-1

$$V_s(\omega) = \sqrt{\frac{2(\mu_1^2+\omega^2\mu_2^2)}{\rho(\mu_1+\sqrt{\mu_1^2+\omega^2\mu_2^2})}}$$

Where, the shear modulus is represented by μ_1 and the viscosity modulus, μ_2. V_s is the shear wave speed, ω is the shear wave frequency, and ρ is the tissue density of 1600kg/m^3 [Hashemi 2005]. Viscoelastic properties of the tendon were collected on both the involved and uninvolved limbs.

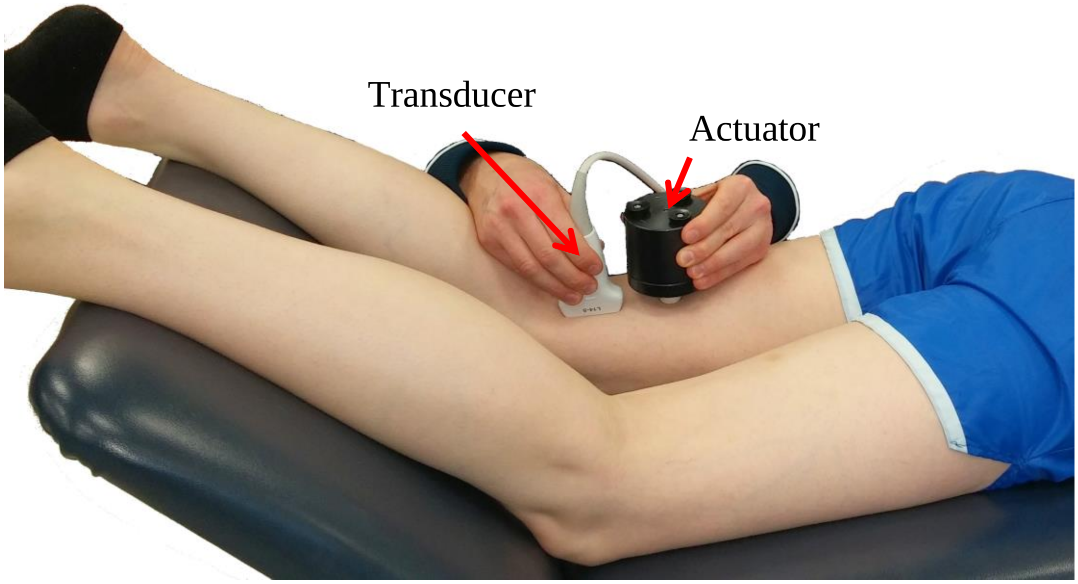


Figure 5-2. The transducer and actuator arrangement for cSWE. The knee was placed at a 45º knee flexion angle. Note: The actuator is proximal to the transducer so the shear waves travel from origin to insertion.

Hamstring strength was determined through isokinetic tests on a Biodex 3 dynamometer (Biodex Medical Systems, Shirley, New York). Subjects were seated with their hips at 90° and knee center of rotation aligned with the axis of rotation of the dynamometer. Subjects performed 3 sets of concentric leg flexion at 60 °/s and 120°/s through their entire range of motion. The subject then performed eccentric flexion tests at 60°/s and 120°/s at 80% of their range of motion. The reduced range of motion was to prevent forced hyper-extension and reduce risk of injury. Subjects also performed internal rotation strength tests by lying on the dynamometer in the supine position with their hips, knees and ankles at 90°. In this position, the subject's tibia was aligned with the dynamometers axis of rotation. Subjects were instructed to keep their legs from falling into hip abduction during the test. Three sets of internal rotation were performed at 30°/s and 60°/s. Trials were performed on each leg. Data were processed using Visual3D (C-Motion, Bethesda, MD) to evaluate the peak flexion torque during each of the tests. Additionally, post-processing determined the joint angle at which peak flexion torque occurs during concentric and eccentric knee flexion.

An ANCOVA with time and the uninvolved leg as covariates was used to establish differences between limbs for each of the strength and position measures (SPSS, IBM Armonk, USA). Pearson's coefficient (r) was used to establish correlations between the bilateral difference in strength (involved/uninvolved) and the recovery of the ST tendon's shear modulus using a partial correlation, again with time included as a covariate.

5.4 Results

Thirteen subjects (10 male, age: 21.0±2.5 yrs, height: 174.5±9.8 cm, weight: 75.7±13.0 kg) were recruited at an average of 13.2±5.8 months post-op. Two (2) subjects were excluded from the results; one subject did not show signs of ST tendon regrowth, the second subject had his contralateral gracilis tendon removed for added graft material and therefore had no uninvolved side. The other 11 subjects showed evidence of tendon regrowth (Figure 5-3). A significant difference existed between legs during the concentric flexion strength at 120°/s ($p = 0.034$). There were no other significant differences between the limbs for strength (Table 5-1). There were also no significant differences between joint angles at which peak flexion torque was generated (Table 5-2).

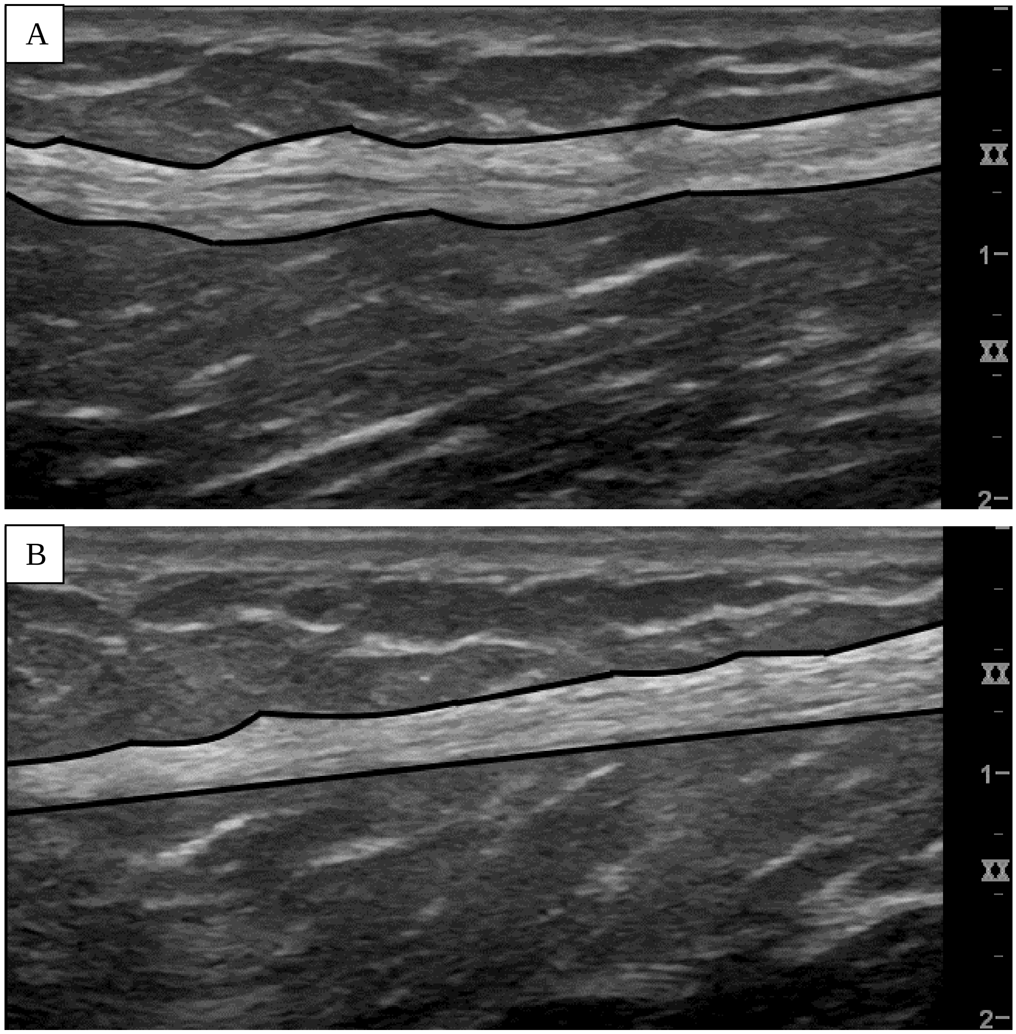


Figure 5-3. Ultrasound images of the regenerating semitendinosus tendon (A) and the healthy semitendinosus tendon (B) from a single patient, 14 months post-op. Note the wavier, disorganized layout of the ST tendon in the healing tendon compared to the healthy, though the thickness appears to be greater.

Table 5-1. The mean hamstring strength for the involved (I) and uninvolved (U) limbs of patient who received an ACL autograft with an ST tendon, including time as a covariate. The (*) denotes a significant difference between the involved and uninvolved sides.

	Speed (°/s)	Peak Moment (I) (N-m)	Peak Moment (U) (N-m)	p - value
Concentric Flexion	60	89.0	98.0	0.145
	120	79.2	87.5	0.034*
Eccentric Flexion	60	99.2	117.6	0.634
	120	104.8	121.1	0.801
Internal Rotation	30	28.0	26.1	0.123
	60	23.4	22.7	0.174

Table 5-2. The mean angle at which peak flexion torque is generated by the hamstring for the involved (I) and uninvolved (U) limbs of patient who received an ACL autograft with an ST tendon, including time as a covariate..

	Speed (°/s)	Angle of Peak Moment (I) (Degrees)	Angle of Peak Moment (U) (Degrees)	p - value
Concentric Flexion	60	40.8	39.5	0.788
	120	43.8	41.2	0.551
Eccentric Flexion	60	40.6	39.1	0.317
	120	41.1	34.6	0.911

Significant correlations existed between the recovery of the shear modulus and the bilateral difference of internal rotation strength at 30°/s and 60°/s (r = 0.70, 0.65, respectively) (Figure 5-4). The correlation of shear modulus and peak flexion strength position at 120°/s of concentric flexion was also significant (r =0.64) (Figure 5-5). The eccentric flexion strength and peak torque joint angle had no significant correlations to the recovery of the shear modulus (Table 5-3).

Table 5-3. The correlations of all the strength data to the shear modulus of elasticity. The (*) represents the significant correlations.

Exercise	Measure	Speed	Pearson's r	p-value
Concentric Flexion	Strength	60	0.42	0.233
		120	0.61	0.060
Concentric Flexion	Peak Torque Angle	60	0.35	0.326
		120	0.64	0.047*
Eccentric Flexion	Strength	60	-0.26	0.464
		120	-0.09	0.803
Eccentric Flexion	Peak Torque Angle	60	0.03	0.935
		120	0.44	0.204
Internal Rotation	Strength	30	0.70	0.026*
		60	0.65	0.043*

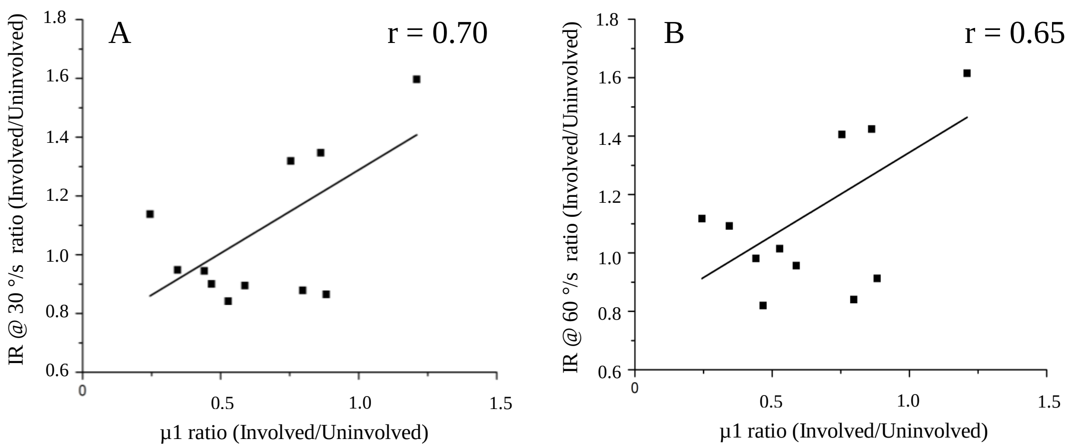


Figure 5-4. The correlations between the differences in internal rotation strength, expressed as the ratio between involved and uninvolved, and the bilateral difference ratio in shear modulus of the semitendinosus tendon (Involved/Uninvolved). Note the recovery of the shear modulus having a strong, significant correlation (p=0.026, 0.043) with internal rotation strength at 30°/s (A) and 60°/s (B).

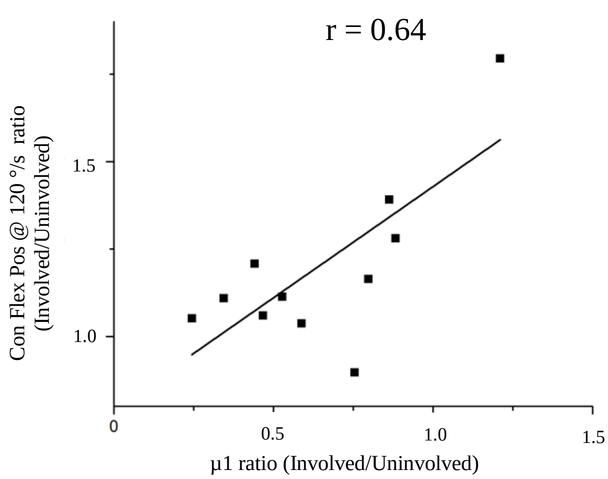


Figure 5-5. The correlation between the difference ratio of peak flexion strength knee angle (involved/uninvolved) and the recovery of the semitendinosus tendon shear elastic modulus (involved/uninvolved). Note the strong correlation with the recovery of the shear modulus and the increasing depth of knee flexion angle for peak torque production.

5.5 Discussion

This study aimed to demonstrate the correlation between the recovery of the ST tendon's shear elastic properties and the strength asymmetries caused by ST resection. There was no bilateral difference of internal rotation strength but there was a strong correlation between the recovery of the shear modulus and the increase of the internal rotation strength which supported our hypothesis. This shows the recovery of the ST tendon elasticity allows force transfer by the ST muscle which leads to rotational torque at the knee. The concentric flexion torque was the only flexion strength measurement with significant bilateral asymmetries of peak torque, though the strength differences were not correlated to the differences of the shear modulus. This lack of correlation held for each of the other flexion strength tests leading to the rejection of the hypotheses predicting strength increases with the recovery of the elasticity of the tendon. There were no bilateral differences in the angle at which peak torque is generated, but the joint angle of peak flexion torque during concentric flexion at 120°/s did correlated with the shear elastic modulus recovery. This partially supports the last of hypotheses stating flexion angle of peak torque should decrease with tendon elasticity recovery. The lack of correlations could be due to the limited strength and angle differences bilaterally or insufficient time for the ST muscle to regain its mass after the initial atrophy that follows resection.

The internal rotation strength recovery correlated to the regeneration of the mechanical properties of the ST tendon is supported by the anatomy of the ST musculo-tendinous unit. The distal portion of the ST tendon inserts into the pes anserinus, which is located on the anterior portion of the knee. This gives the muscle a mechanical advantaged to produce a rotational torque in the transverse plane, which is unlike the more posterior attachment of the semimembranosus or biceps femoris.

The recovery of ST tendon and muscle function increases the internal rotation strength, which adds to the joints ability to resist the increased external rotation of the knee seen from patients post ACL reconstruction during running and walking [Tashman 2004]. This external rotation could cause abnormal loading within the knee joint, not only the magnitude but the location on the tibial plateau. The additional rotational components within the knee have been linked to the progression of OA [Andriacchi 2008]. The rotational change and altered cartilage loading location could progress the early onset OA typically seen in patients post ACL rupture.

Many ACL injuries are resulting from cutting and pivoting tasks during sport. There is a strong recruitment of the medial hamstrings during cutting and crossover step task and the recovery of this ability is paramount to return to play at pre-injury levels. [Hughs 2006]. The demonstration that the internal rotation strength recovers not only has the potential to reduce the progression of OA, but establishes the possibility exists for subjects to return to the symmetry present during previous levels of competition. The finding of strength recovery with tendon regrowth is supported by the finding of Ferretti et al. which showed that there were no differences in rotation strength with the regeneration of the ST tendon [Ferretti 2008]. Given only 64% of ACL injured patients return to their previous level of sport, the knowledge that the ST musculo-tendinous complex can regain its mechanical properties and strength will help in surgical decision making and may influence late term exercise protocols to increase the strength gains of the ST muscle [Ardern 2011].

The angle at which peak flexion torque is generated during a 120°/s contraction was correlated with the recovery of the shear modulus. As the mechanical properties of the ST tendons recovered, the patients were able to produce more torque at a lower joint angle i.e., in deeper knee flexion. The increased deep knee flexion torque will aid patients in returning to sport at a competitive level. Along with internal rotation, deep knee flexion is needed for performing a cut maneuver [Green 2011]. The increase in peak torque production at these lower angles enables the patient to lower their center of mass which allows for a faster change in inertia. The recovery of this deep knee strength at the increased speeds is beneficial as these are the speeds at which dynamic tasks are performed and may help patients return to pre-operative levels of play.

The increase in flexion angle at which peak torque is generated with the recovery of the ST tendon is caused by the differences in optimal fiber length varying for each muscle. Each muscle has a different optimal fiber length and therefore, a different angle at which peak torque is generated over the range of motion [Murray 2000]. In terms of the hamstrings, each muscle's peak torque occurs at different joint angles creating an overall flexion angle strength profile permitting strength to be distributed over a large range of motion. The recovery of the ST tendon, and therefore ST muscle function, leads to one more contributing muscle to alter the joint angle strength profile. The ST muscle contributing to lower joint angle peak torque is a product of its structure. Of the main muscles contributing to leg flexion, the ST muscle is the only fusiform, parallel fiber muscle. The biceps femoris long and short heads, and the semimembranosus are pennate muscles, which have relatively shorter muscles fibers. The shorter fibers reach their optimum fiber length in a shorter range of motion and shallower angle than the longer fusiform type muscle. The longer fibers of the ST muscle do not reach their maximum force output until deeper in knee flexion.

There were only limited strength differences between the involved and the uninvolved legs as well as greater strength on the involved side of several subjects. This phenomenon is most likely a large contributor to the limited differences seen in the correlations to tendon regrowth. The lack of strength differences can be attributed to the patient population from which this study was recruited. Many of the subjects were high level athletes and returned to sport within a year. Following rehabilitation, typically 6-12 months, the subject returned to sport. At that time point, only a small percentage of the tendon's mechanical properties had regrown [Chapter 4]. Since bilateral strength symmetry is a goal of rehabilitation, once symmetry has been achieved and the ST tendon regrows following rehabilitation, the potential exists for the involved hamstring to get stronger with the contribution of the ST muscle following tendon mechanical property recovery.

This study was a cross-sectional study that involved subjects being collected at different time points with a multitude of rehabilitation protocols, post rehabilitation exercise regimens and surgical techniques. The differences in surgical techniques performed by each of the surgeons influences the tendon regrowth and possible strength asymmetries. An additional factor in influencing strength differences is the resection of the gracilis in this surgical procedure. While it was known that each of the subjects regrew the ST tendon, it is unknown if the gracilis regrew like the ST tendon. Due to the difficult nature of accurately imaging the gracilis it was not measured for elasticity. It has been shown that the gracilis regrows in 46-100% of patients adding even more variability into the understanding of strength recovery after surgery [Tadokoro 2004, Janssen 2013]. Though the gracilis does add variability to the strength correlation, it is only half the size, in volume, of the ST muscles. It is smaller than the other hamstring muscles and its contribution to leg flexion is less than that of the other muscles, making it less of a factor. Since this was the first study to use cSWE to measure the mechanical properties of the tendon directly, it was important to take a wide cross-section of ACL patients in order to determine gross findings for future studies to refine and make clear determinations of rehabilitation and surgical effects.

5.6 Conclusion

The internal rotation strength increased with the recovery of the shear elastic modulus of the semitendinosus tendon and the angle at which peak torque was generated during concentric flexion at 120°/s increased with the recovery of the tendon's shear modulus. This shows that strength asymmetries can be recovered with the regrowth of the ST tendon. It is important to consider this when choosing surgical technique and for the determination of rehabilitation protocol.

Chapter 6

DOES HAMSTRING TORQUE OUTPUT INCREASE WITH ELASTIC PROPERTIES OF TENDON?

6.1 Introduction

Restoration of the functional capabilities of the anterior cruciate ligament (ACL) is a primary goal of those receiving ACL reconstructive surgery. More than 125,000 ACL reconstructions are performed each year following ACL rupture and there is a prevalence of those reconstructed to re-rupture their graft within 2 years [Mall 2011] Of those reconstructed, only 64% of patients return to competitive sport at their pre-injury level [Ardern 2011]. While several graft techniques exist for ACL reconstructions, an ACL autograft using the semitendinosus (ST) tendon as graft material has been shown to be superior in long term pain and return to full range of motion of the knee [Barenius 2011, Mascarenhas 2012]. Though reconstructions with a ST tendon graft have positive outcomes, graft harvests results in hamstring potential strength asymmetries due to the resection of the tendon from the muscle causing the inability of the muscle to pass force across the joint. The recovery of these strength asymmetries may lead to reduced ACL re-ruptures after 2 years and help return patients to pre-operative performance levels.

The loss of the ST tendon when use for an ACL autograft results in a loss of internal rotation strength. This loss affects athletic performance as the crossover step recruits the medial hamstrings and requires internal rotation strength [Houck 2003]. The ST tendon has the most anterior connection to the knee of the large hamstring muscles and is a major contributor to internal rotation strength. Following the ST tendon resection, significant reductions in internal rotation strength have been measured [Armour 2004, Viola 2000]. In addition to strength asymmetries, there are reduced internal rotation ranges of motion and increased external rotation during walking [Webster 2012, Scanlan 2010, Tashman 2004]. The recovery of the internal rotation strength would aid in restoring lost run/cut performance and directional change abilities which may also return the increased external rotation to pre-operative conditions and protect the graft.

ST autograft recipients have shown flexion strength asymmetries in increased knee flexion angles. Flexion strength asymmetries include: decreases in peak joint torque over the entire flexion range of motion [Vairo 2014], a reduction of peak flexion torque during isokinetic tests at joint angles of 60° and greater [Makihara 2006, Tashiro 2003], isometric flexion torque at 90° [Makihara 2006, Nishino 2006, Todokoro 2004] and changes in flexion angle at which peak torque was generated [Ohkoshi 1998, Carofino 2005]. The ST musculo-tendinous complex is the third largest muscle of the hamstrings and therefore is a contributor to flexion torque. In addition to flexion strength, the ST hamstring contributes to the prevention of anterior translation of the knee. Reduced hamstring strength has been shown to result in increased loading of the ACL [Wild 2013]. The recovery of concentric hamstring strength would aid in reducing the ACL load and therefore could lead to the prevention of ACL re-ruptures.

These strength asymmetries have the potential to be recovered through the regrowth mechanism of ST tendon following resection. It has been reported that between 60-100% of patients regrow their ST tendons follow reconstruction [Leis

2003, Carofino 2005, Cross 1992, Eriksson 2001, Ferretti 2002, Janssen 2013, Nikolaou, 2007, Nishino 2006, Okahashi 2006, Papandrea 2000]. Morphologic evidence of this phenomenon demonstrates that a collagen like material returns into the tendon gap remaining after surgery, which has been seen by imaging techniques including ultrasound and magnetic resonance imaging. Previous studies have attempted to correlate the regrowth of these tendons with strength recovery of the hamstrings, though no significant findings occurred [Janssen 2013, Todokoro 2004]. Though the tendon morphology, i.e., volume and area, may have returned, it does not demonstrate the functional characteristics of the tendon. The elastic properties of the tendon defines its ability to transmit force from the muscle across the joint. Continuous shear wave elastography (cSWE) is a method to measure elastic properties of soft tissue by transmitting an external compression wave down the medium of interest and using high frame rate ultrasound to track the speed of the wave's propagation [Cortes *In Press*]. The velocity at which a wave travels along based on the frequency at which the wave was broadcast determines the shear elastic modulus of the tendon. Therefore, it is the aim of this paper to determine the effects of the ST tendon's mechanical property recovery on the bilateral hamstring strength differences in concentric flexion and internal rotation. We hypothesize the strength (measured by torque output) will increase with the restoration of the shear elastic modulus of the involved ST tendon, normalizing each variable to the contralateral limb.

6.2 Methods

6.2.1 Subjects

Eleven (11) subjects who sustained an ACL rupture and received an ACL reconstruction using the ST tendon as autograft material participated in the study. Each subject reviewed and signed an informed consent approved by the University of Delaware institutional review board. Subjects were recruited following being cleared for return to sport and between 6 and 24 months post-op. Subjects must not have sustained additional ligament tears requiring surgical repair. Strength measures were not included for those subjects whose ST tendon did not regrow.

6.2.2 Continuous shear wave elastography

cSWE was performed on both legs prior to strength testing to prevent stretching and fatiguing from affecting the results. Each subject lay prone on a plinth with their leg at a 45^{o} angle. B-mode ultrasound was used to evaluate the involved leg to determine if the tendon regrew. The subject's ST tendon was palpated and B-mode ultrasound was used to view the tendon. The tendon was the traced in the sagittal plane to the ST muscle belly. The ultrasound probe was then rotated into the transverse plane to confirm the tendon of interest was indeed attached to the ST verses the semimembranosus or gracilis (Figure 6-1). The ultrasound probe was then returned to the axial plane and the subject performed an isometric contraction to ensure the tendon moved with the contracting muscle. Marks were placed on the skins surface to identify the location of the tendon. The process was repeated for the healthy, contralateral tendon.

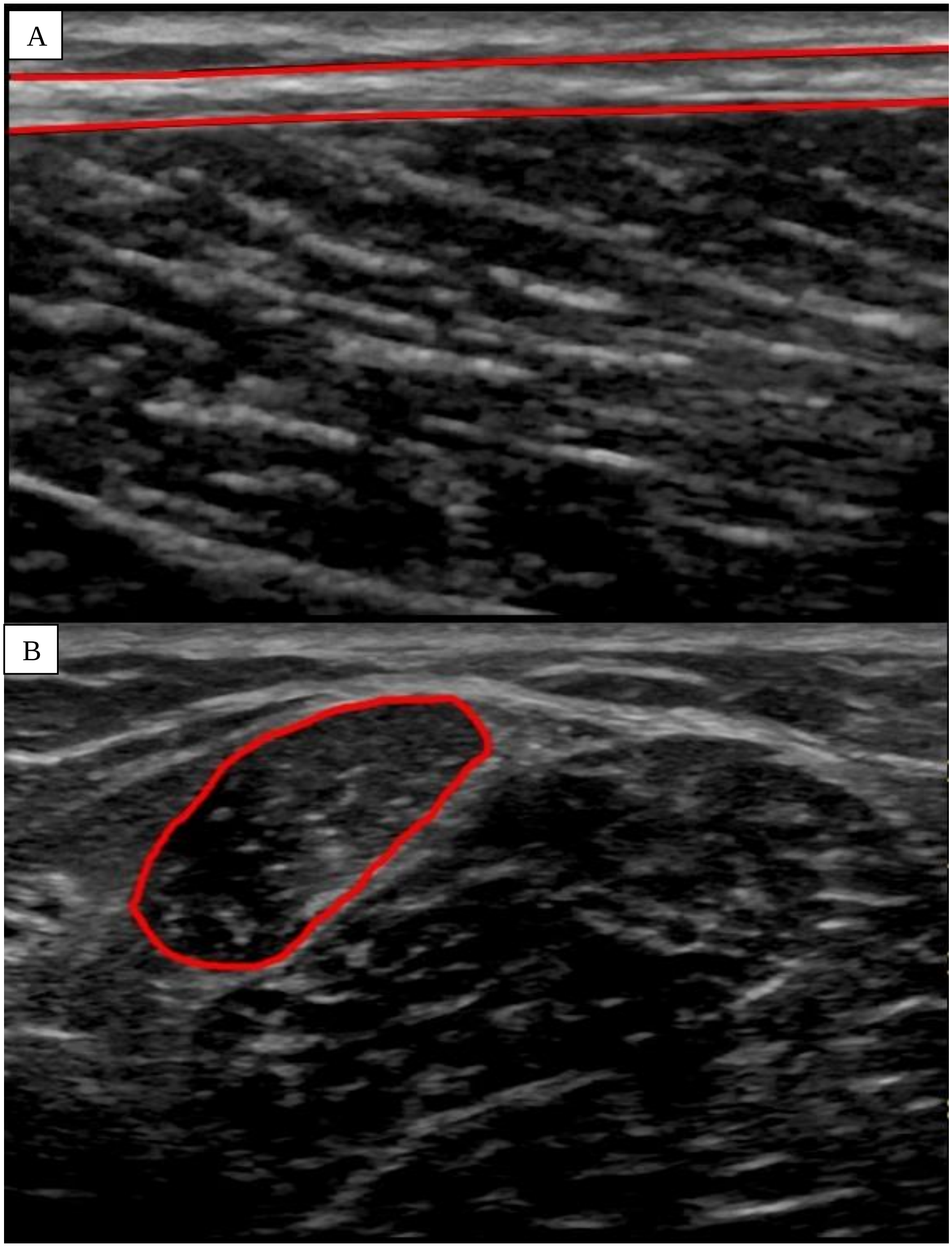


Figure 6-1. Ultrasound images of the semitendinosus tendon (A) and muscle (B), captured mid-thigh, highlighted in red. The tendon is viewed in the longitudinal plane and the muscle is in the transverse plane.

cSWE requires the use of a high frame rate ultrasound and an external vibration source to generate the mechanical shear wave along the tendon. Methods are described in greater detail by Cortes et al. [Cortes *in Press*]. The high frame rate ultrasound collected at 6450Hz using an Ultrasonix MDP scanner (Ultrasonix, Vancouver, Canada). The external vibration source was a Bruel & Kjaer 4810 mechanical actuator (Bruel & Kjaer, Norcross, GA) driven by an external amplifier wave form generator. The high frame rate ultrasound probe was placed on the ST tendon, previously marked, and the actuator was placed approximately 3cm proximal to the ultrasound probe along the ST tendon. The actuator produced a mechanical sinusoidal wave at 323Hz and the ultrasound scanner collected greater than 60 frames of data. Each frame consisted of a plane wave set to a 2cm depth. Without moving the probe or the actuator, the frequency was increased to 340Hz and another set of data were collected. This was repeated for 358, 379, 403, and 430Hz. This measure was repeated on the contralateral limb.

Data were processed using customized signal decomposition software to track the speed of the propagating sine wave. The speed of the sine wave combined with the frequency of the mechanical shear wave was input into an optimization with a Voigt model for solids as the minimization function to determine the shear elastic modulus using **Equation 6-1**:

Equation 6-1
$$V_s(\omega) = \sqrt{\frac{2(\mu_1^2+\omega^2\mu_2^2)}{\rho(\mu_1+\sqrt{\mu_1^2+\omega^2\mu_2^2})}}$$

Where the shear modulus is represented by μ_1, the viscosity modulus is represented by μ_2, the shear wave speed by V_s, the shear wave frequency by ω, and ρ is the tissue density of 1600kg/m^3 for tendon [Hashemi, 2005].

6.2.3 Strength testing

Isokinetic flexion strength was measured on a Biodex 3 dynamometer (Biodex, Shirley, NY). Flexion strength was evaluated over the subject's entire range of motion at 60 °/s and 120 °/s. Each subject was seated in the biodex with their hips at 90° and their knee joint centers aligned with the axis of rotation of the dynamometer. The subject performed 3 trials at each speed over his full range of motion with sufficient rest between trials to avoid fatigue. For the internal rotation strength measure, the subject lay supine in the dynamometer with their hips, knees and ankles at 90° and their tibia aligned with the dynamometer's axis of rotation. Internal rotation strength was evaluated at 30 °/s and 60 °/s. The subject was directed to keep his knee perpendicular to the seat while performing an internal rotation over their entire range of motion (Figure 6-2). Concentric flexion strength (N-m) was evaluated at 30°, 45°, 60°, and 75°. Internal rotation strength (N-m) was reported as the peak torque during the contraction. Each strength test was performed bilaterally.

Figure 6-2. The arrangement for performing internal rotation strength measurements at the knee. Note the hip, knee and ankle at 90° with the lower leg aligned with the axis of rotation of the dynamometer.

6.2.4 Statistics

An ANCOVA with time and the difference between limbs as covariates was run for each of the strength and position measures to establish differences between limbs (α=0.05, SPSS, IBM Armonk, USA). Pearson's coefficient (r) was used to establish correlations between the bilateral difference in strength (involved/uninvolved) and the recovery of the ST tendon's shear modulus using a partial correlation, with time included as a covariate.

6.3 Results

Eight (8) males, 3 females, were recruited (20.3 ± 2.1 years of age, 171.5 ± 9.0 cm, 73.6 ± 13.7 kg) and the average post-op time was 13 months (±6.0). Significant strength differences existed at a flexion angle of 75° during the 60 °/s trial ($p = 0.045$) and at 60° during the 120 °/s trial ($p = 0.029$) (Figure 6-3). There were no differences flexion strength differences at the shallower knee. There were also no peak internal rotation strength differences between legs.

Ten (10) of the 11 subjects showed signs of tendon regrowth and the recovery of the elastic properties of the tendon correlated with time ($r = 0.63$) (Table 6-1). There was a strong correlation between the internal rotation strength and the recovery of the tendon's elasticity at both 30 °/s and 60 °/s ($r = 0.70, 0.65$) (Table 6-2, Figure 6-4). No significant correlation existed between the tendon elastic property recovery and an increase in strength compared to the contralateral side (Table 6-2).

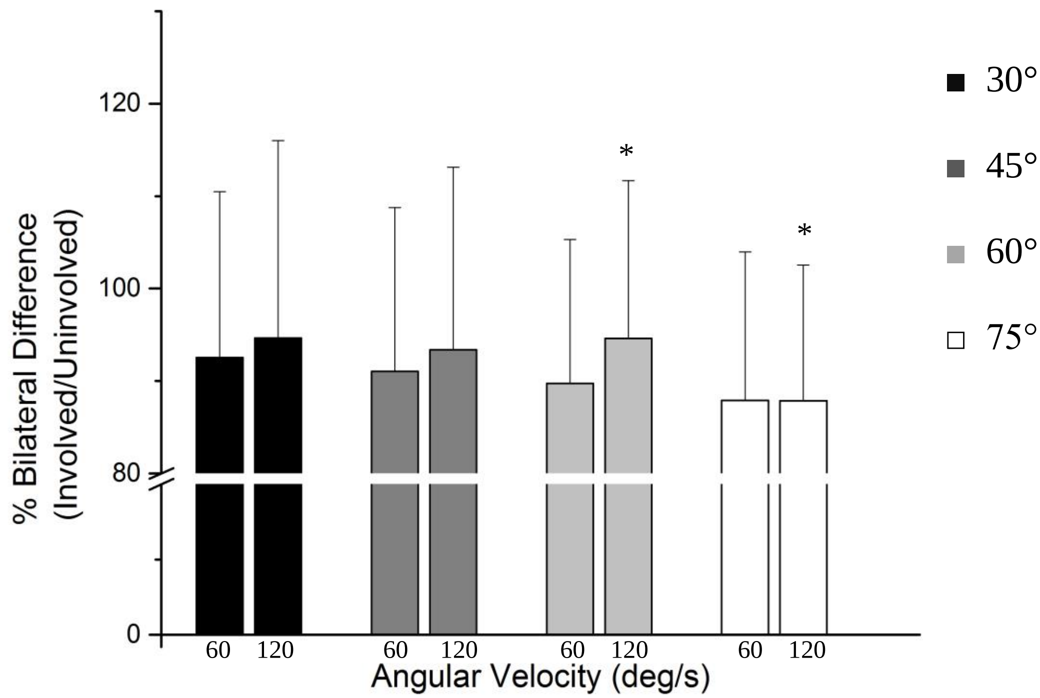


Figure 6-3. Bilateral strength differences during isometric leg flexion at specific knee angles. Note the significant asymmetries (*) during the deeper knee flexion angles at 60° and 75°.

Table 6-1. The shear elastic moduli for the involved and uninvolved semitendinosus tendons post ACL reconstruction. Note the strong correlation between the shear modulus recovery, as a percent of the value to the contralateral side, and the recovery time.

	μ1 (kPa) Uninv	μ1 (kPa) Inv	μ1 Difference	Months Post-Op	Correlation with Time
Mean	138.3	73.5	53.1%	13.00	r = 0.63
Mean < 12 Months	149.5	64.3	49.5%	8.9	*
Mean > 12 Months	120.6	81.7	69.9%	18.3	*

Table 6-2. The correlations between the recovering shear elastic modulus of the ST tendon and the bilateral strength differences at 4 knee flexion joint angles during isokinetic testing at 60 °/s and 120 °/s. Additionally the shear elastic modulus restoration significantly correlated to the bilateral difference of internal rotation strength denoted by the (*) ($p = 0.05$). Note the lack of correlation between the recovery of the ST properties and the concentric flexion strength and the strong correlation between the ST properties and the internal rotation strength.

Exercise	Angle	Speed (deg/sec)	Pearson's r	p-value
Concentric Flexion	30°	60	0.04	0.935
		120	0.29	0.491
Concentric Flexion	45°	60	0.24	0.563
		120	0.57	0.141
Concentric Flexion	60°	60	0.20	0.642
		120	0.45	0.268
Concentric Flexion	75°	60	0.25	0.558
		120	0.34	0.413
Internal Rotation	Peak Torque	30	0.70	0.026*
		60	0.65	0.043*

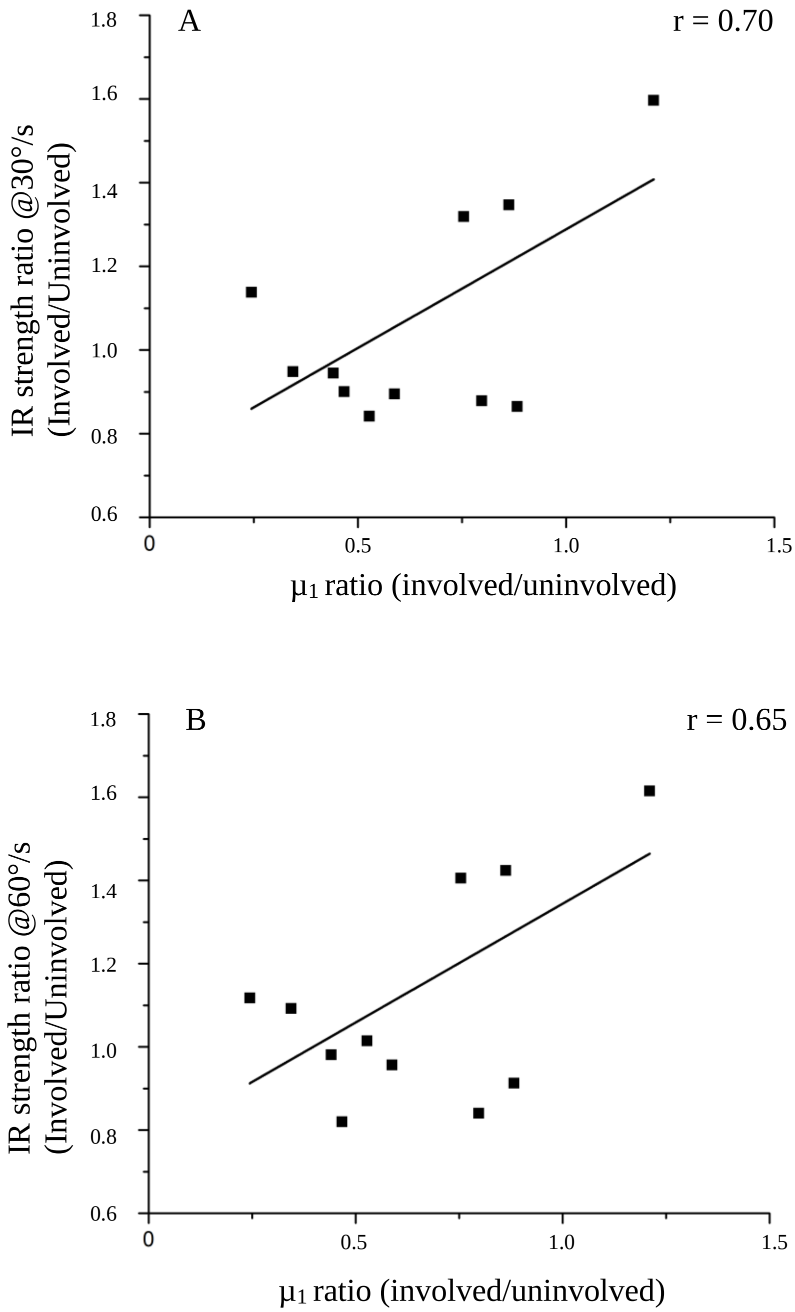


Figure 6-4. The correlations between the bilateral difference, in terms of the ratio of involved/uninvolved, of internal rotation strength and the bilateral difference in shear modulus of the semitendinosus tendon (Involved/Uninvolved). Note the recovery of the shear modulus having a strong, significant correlation (p=0.026, 0.043) with internal rotation strength at 30 °/s (A) and 60 °/s (B).

6.4 Discussion

This study demonstrated the restoration of the elastic properties of the ST tendon with time and the correlation of this recovery to internal rotation strength, which supports our hypothesis. The strong correlation of the tendon regrowth to the increase of internal rotation strength demonstrated that the tendon regains its functionality and contributes to the rotational torque in the transverse plane of the knee. The concentric flexion strength asymmetries existed at the increased flexion angles (60° and 75°). Unfortunately, there were no correlations between the tendon regrowth and the differences in strength between the legs, which is contrary to our hypothesis. These results can be explained based on the hamstring architecture, exercise regimen and surgical choice.

There was no bilateral difference of internal rotation strength but there was a strong correlation between the recovery of the shear elastic modulus of the tendon and the internal rotation strength. This correlation is linked to the anatomy of the ST musculo-tendinous unit. The distal portion of the ST tendon inserts into the pes anserinus, which is located on the anterior/medial portion of the knee. This gives the muscle a mechanical advantaged to produce a rotational torque in the transverse plane, which is unlike the more proximal attachment of the semimembranosus or biceps femoris. This finding is supported by previous research which showed the regrowth and reorganization of the tendon led to no bilateral differences in internal rotation strength [Ferretti 2008]. The recovery of ST tendon and muscle function increases the internal rotation strength, which adds to the joints ability to resist the increased external rotation of the knee seen from patients post ACL reconstruction during running and walking [Tashman 2004]. This increased external rotation puts the knee at a greater risk of re-injury as it causes the ACL graft to bare a greater load. The increased internal rotation strength is required in the cutting maneuver required by high-level athletics [Hughs 2006]. The restoration of internal rotation strength following ACL reconstruction with a ST/GRA graft will aid in injury prevention and return to high level sport, especially after 2 years post-op and full regrowth.

Strength asymmetries remained at deep knee flexion angles and showed no correlation to the recovery of the ST tendon elastic properties. This may be due to the percent contribution of the ST to flexion compared to the other hamstring muscles. The internal rotation strength increases seen with the recovery of a small percentage of the elastic properties of the tendon could be due to the fact that it is one of the few, and largest, contributing muscles to internal rotation strength of the tibia. The ST contributes less to the overall flexion strength since it is the smallest of the 3 primary hamstrings. The return of peak concentric hamstring strength has been shown previously, though at shallower flexion angles than the contralateral limb [Nishino 2006]. This strength recovery is due to the other hamstring flexors hypertrophy in response to the lack of a ST muscle contributing to joint torque [Williams 2004, Snow 2012]. Though the other muscles increase in strength and size, they are not architecturally identical. The fusiform architecture of the ST muscle makes it unique compared to the larger pennate hamstrings. The ST has longer fibers which results in an increased range of travel [Kumazaki 2012]. The increased muscle length at which torque is able to be produced translates into strength at increased knee flexion angles. At increased flexion angles, the ST is able to maintain force production while the other hamstrings are reaching their force generating limits. If the ST tendon is not able to pass the full muscles contribution across the joint, deep knee flexion torque remains reduced compared to the contralateral side. The ST tendon has been shown to regain its mechanical properties with time and therefore, after an increased duration, the deep knee flexion may also return if strengthening measures continue post ST regeneration.

The restoration of the ST tendon's mechanical characteristics for the purpose of strength recovery is essential for ACL protection and performance recovery. The ACL is at a greater risk for re-tear when placed under increased loads. These increased loads occur during stopping and cutting when the knee is at a smaller flexion angle [Yu 2007]. Recovering the deep knee flexion strength would aid in permitting the subject to have an increased squat during stopping and cutting. The deeper squat increases the knee flexion angle, reducing the load on the ACL while also lowering the center of gravity of the subject, allowing them to make faster changes in direction. The recovery of the mechanical properties of the hamstring tendon could benefit from previous techniques used to promote tendon reorganization and recovery. It has been shown that increased activity of the tendon during the healing period helps restores function [Mortensen 1999]. The visualization of hamstring regrowth should encourage rehabilitation protocols to add exercises which target the ST in order for the rate of recovery of mechanical properties to be increased.

There were only limited strength differences between the involved and the uninvolved legs and additionally, there were cases which showed greater strength on the involved side. This phenomenon is most likely a large contributor to the limited differences seen in the strength measurements compared to those demonstrated by previous research. The lack of strength differences can be attributed to the patient population from which this study was recruited. Many of the subjects were high-level athletes and returned to sport within a year. Following rehabilitation, typically 6-12 months, the subject returned to sport. At that time point, only a small percentage of the tendon's mechanical properties had regrown (Table 6-1). Since bilateral strength symmetry is a goal of rehabilitation, once symmetry has been achieved and the ST tendon regrows following rehabilitation, the potential exists for the involved hamstring to get stronger with the contribution of the ST muscle following tendon mechanical property recovery.

This study was a cross-sectional study that involved data from subjects being collected at different time points with a multitude of rehabilitation protocols, post-rehabilitation exercise regimens and surgical techniques. The differences in surgical techniques performed by each of the surgeon's influences tendon recovery. An additional variable in relating the recovery of strength is the harvest of the gracilis needed for an ST-gracilis autograft. While it was known if each of the subjects regrew their ST tendon, it is unknown if the gracilis regrew. The resection of the gracilis would have an effect on the internal rotation and flexion strength. Due to the difficult nature of accurately imaging the gracilis it was not measured for elasticity. It has been shown that the gracilis regrows in 46-100% of patients which adds more variability into the understanding of strength recovery after surgery [Tadokoro 2004, Janssen 2013]. Though the gracilis does add variability to the strength correlation, it is only half the size, in volume, of the ST muscles. It is smaller than the other hamstring muscles and its contribution to leg flexion is less than that of the other muscles, making it a minor factor. Since this was the first study to use cSWE to measure the mechanical properties of the tendon directly, it was important to take a wide cross-section of ACL patients in order to determine gross findings for future studies to refine and make clear determinations of rehabilitation and surgical effects.

6.5 Conclusion

The recovery of the ST tendon's mechanical properties is correlated to an increase in internal rotation strength which will aid in re-rupture prevention and return athletic performance to previous levels. This regrowth should be taken into account when the choice of ACL autograph is being made and when the rehabilitation protocol is being designed for ST autograph patients.

Chapter 7

CONCLUSION

The overall goal of this work was to show, non-invasively, that the semitendinosus (ST) tendon not only regrows following a tendon resection for an ACL reconstruction, but regains its mechanical properties; those mechanical properties are related to the strength asymmetries that have been seen historically in patients who received a semitendinosus autograft. This was done by evaluating two separate methods of *in vivo* tendon evaluation for elastic properties including elastography and echogenicity. The superior of those two methods was used for determining the mechanical properties of the ST tendon within a 6 to 24 month time frame following an ST autograft. This was then used to show that the recovery of hamstring strength correlated with the restoration of the mechanical properties of the tendon. Each of these aims compounds to establish that patients who receive an ST autograft have the anatomical ability to return to pre-injury muscle structure which may reduce the risk of re-rupture and permit them to re-establish symmetry which may return patients to pre-injury levels of sports performance.

7.1 Aim 1: Non-invasive Measure of the Mechanical Properties of Soft Tissue

The purpose of this aim was to develop a tool which can determine the viscoelastic properties of soft tissue, including tendon, in order to be used in the later aims. Echogenicity, which is a materials ability to reflect sound, has been shown to be correlated with stress in tendon at the *in vitro* level. This correlation has then been associated with the elastic modulus of the material. This test had not been performed *in vivo,* therefore:

> **Hypothesis 1.1**: The echogenicity of the Achilles tendon will increase with stress. There will be a linear relationship outside of the toe region of tendon stretch between the increasing brightness of the ultrasound image and the stress applied to the tendon.

The hypothesis was rejected. It was determined that echogenicity, while positively correlated with stress in the tendon, did not reach an acceptable level of correlation ($r^2 = 0.05$). This is in contrast to our hypothesis and prevents the use of echogenicity as the metric to be used in determining the elastic properties of the regenerating ST tendon.

Continuous shear wave elastography (cSWE) uses the speed at which mechanical waves are able to travel along soft materials to calculate elastic and viscous properties. Similar techniques have been used to detect anomalies in phantoms and tumors *in vivo*, though this technique has never been used on tissue with increased stiffness such as tendon. cSWE does not have the stiffness limits of the other elastography techniques. Therefore, the objective of the second part of Aim 1 was to establish cSWE can determine differences in viscoelastic properties of materials with varying stiffness.

> **Hypothesis 1.2:** The shear wave speed determined through shear wave elastography will match the speed determined through a previously established standard, shear wave magnetic resonance elastography (MRE). The percent error between the mechanical loading and the elastography measure will be less than 10%.

The hypothesis was confirmed. It was established that cSWE was able to match the published standard within the 10% range necessary (average difference = 8.2%). This minimal difference between the two methods establishes cSWE as a viable tool for determining the mechanical properties of the tendon following ACL reconstruction.

7.2 Aim 2: Semitendinosus Tendon Harvested for ACL Reconstruction Regrows and Recovers Mechanical Properties

The focus of aim 2 was on the recovery of the mechanical properties of the semitendinosus with time following ACL autograft surgery. The presence of the ST tendon's regrowth has been demonstrated by several previous studies. While structure is known to be present, it has not been shown that the regrown tendon has any functional characteristics, i.e., mechanical properties, which would allow it to transfer force across the joint. Therefore, cSWE was used to determine the difference in mechanical properties between the involved tendon and those of the contralateral side.

> **Hypothesis 2.1:** The elastic modulus of a ST tendon will have a reduced elastic modulus compared to the contralateral side from 6 to 24 months post-op.

The hypothesis was confirmed (Figure 4-3 in Chapter 4). The elastic modulus of the involved tendons indeed had a significant decrease compared to the contralateral side ($p = 0.004$). This established that, while there was a recovery of elasticity of the tendon, there is a decrease of the tendon properties after surgery during the regrowth of the tendon.

The second hypothesis focused on the recovery of the mechanical properties of the ST tendon with time. In the previous studies which showed the presence of tendon, it was established that the tendon fiber reorganizes with time. This reorganization continues until 2 years post-op. Therefore it was important to look as subject from 6 months, when the presence of tendon is first identified, to 24 months, when the tendon is no longer shows signs of reorganization. The tendon fibers reorganize with time and therefore:

> **Hypothesis 2.2:** A positive correlation will exist between the time post-op and the recovery of elastic properties in relation to the contralateral side.

The hypotheses was confirmed (Figure 4-3a in Chapter 4). The elasticity of the tendon was correlated with time ($r = 0.60$, $p = 0.039$). The positive, significant correlation between mechanical properties and tendon shows the tendon regains its functional characteristics with time. It also supports the idea that the reorganization of the tendon is related to the recovery of the elasticity.

cSWE was developed and tested for this study and has not been previously validated, therefore it is necessary to determine if the elasticity measures are repeatable.

> **Hypothesis 2.3:** The measures of a single tendon will be repeatable using this novel technique in vivo.

The hypothesis was confirmed (Section 3 of Chapter 4). The ICC(3,1) for Achilles tendon elasticity was 0.875 and for the hamstring tendon ICC(3,1) = 0.89. This establishes an excellent reliability of the cSWE metric.

7.3 Aim 3: Hamstring Strength Recovers with the Mechanical Properties of Tendon

The resection of one of the major leg flexor muscles undoubtedly causes strength asymmetries. While the muscle is not functional immediately following tendon removal, the recovery of the mechanical properties implies the ST muscle is able to contribute to joint torque. The restoration of the force generated by the ST muscle may be able to eliminate the asymmetries seen by previous literature including concentric flexion, eccentric flexion, flexion angle at which peak torque is generated, and internal rotation strength. The architecture and insertion location of the ST muscle is different than the other hamstring muscles. The fusiform nature of the ST muscle influences the length of contraction before the fibers are at the optimal fiber length for peak muscle output. The medial insertion of the ST tendon allows it to produce more torque in the transvers plane than the other hamstring muscles. Therefore, it is the purpose of this aim to correlate the recovery of the ST tendon's mechanical properties, i.e., shear elastic modulus, with the reported strength deficits.

> **Hypothesis 3.1:** Bilateral knee concentric flexion strength symmetry will be correlated to symmetry of the mechanical properties of the ST tendon.
>
> **Hypothesis 3.1a:** Bilateral symmetry of the angle at which peak knee torque is generated during flexion will be correlated to symmetry of the mechanical properties of the ST tendon.

These hypotheses were not supported (Table 5-3 and Figure 5-5 in Chapter 5). There were no significant correlations between the bilateral difference of the ST shear elastic modulus and the bilateral difference of concentric knee flexion strength at 60°/s or 120 °/s ($p = 0.233, 0.060$). The supplemental hypothesis was partially confirmed.

There was a significant correlation between the shear elastic modulus of the ST tendon and the angle at which peak flexion torque was generated for the 120 °/s case ($r = 0.64$, $p = 0.047$), through there was no significant correlation for the 60 °/s case ($p = 0.326$).

Hypothesis 3.2: Bilateral knee eccentric flexor strength symmetry will be correlated to symmetry of the mechanical properties of the ST tendon.

The hypothesis was not supported (Table 5-3 in Chapter 5). There was no significant correlation between the bilateral difference in shear elastic modulus and the bilateral difference in eccentric flexion strength at 60 °/s or 120 °/s ($p = 0.464, 0.803$).

Hypothesis 3.3: Bilateral knee concentric internal rotation strength symmetry will be correlated to symmetry of the mechanical properties of the ST tendon.

The hypothesis was supported (Table 5-3 and Figure 5-4 in Chapter 5). There was a significant correlation between the bilateral difference in shear elastic modulus of the tendon and the bilateral difference of the internal rotation strength at both 30 °/s and 60 °/s ($r = 0.70, 0.65$, $p = 0.026, 0.043$).

7.4 Limitations

The limitations of this study were begotten in the context of this being a new measurement tool and an inaugural use of cSWE in the quantification of tendon properties. The quantity of subjects was limited in this study. The number of significant correlations to strength may have increased if there were more subjects and a more even distribution of time points from which those subjects were collected. Additionally, the number of subjects limited the correlations to be in a linear model.

Given the strength and viscoelastic values are being compared to the contralateral limb, there is an asymptote of 100% to which the involved limbs is approaching. This implies there may be more of a curvilinear plot. Another limitation of this study was the single time points for single subjects. Given the variability of individuals, rehabilitation protocols, and surgical techniques performed, it would be more conducive for tracking healing progression by establishing a time/elasticity relationship within a subject. The variability of rehabilitation protocol and surgical technique also impacts the strength measures. While there were positive correlations between mechanical properties and some of the strength measures, being able to control for rehabilitation and level of sport the patients returned to would provide greater insight into the strength recovery. Additionally, the gracilis tendon was resected in addition to contribute to the strength of the ACL autograft. It was unknown if these patients had resected gracilis or if it regenerated like the ST tendon. The attachment point of the ST tendon was unknown and is responsible for a large difference in internal rotation torque production. Previous studies have shown that the tendon can reattach at the original site or up to 4cm proximal to the original insertion. The more distal of insertions leads to the tendon being connected to the popliteal fascia and not the pes anserinus. This more proximal connection could reduce the contribution to internal rotation strength given it no longer wraps to the medial side of the tibia. Lastly, the output of cSWE is shear elastic modulus, not linear elastic modulus, which is the measure which allows energy transfer from the muscle to the joint to be calculated. While shear elastic modulus is not a direct measure of energy transfer, it is linearly correlated to Young’s modulus and therefore the recovery of the shear modulus can be equated to a recovery of Young's modulus (Figure 7-1) [Zhang 2013].

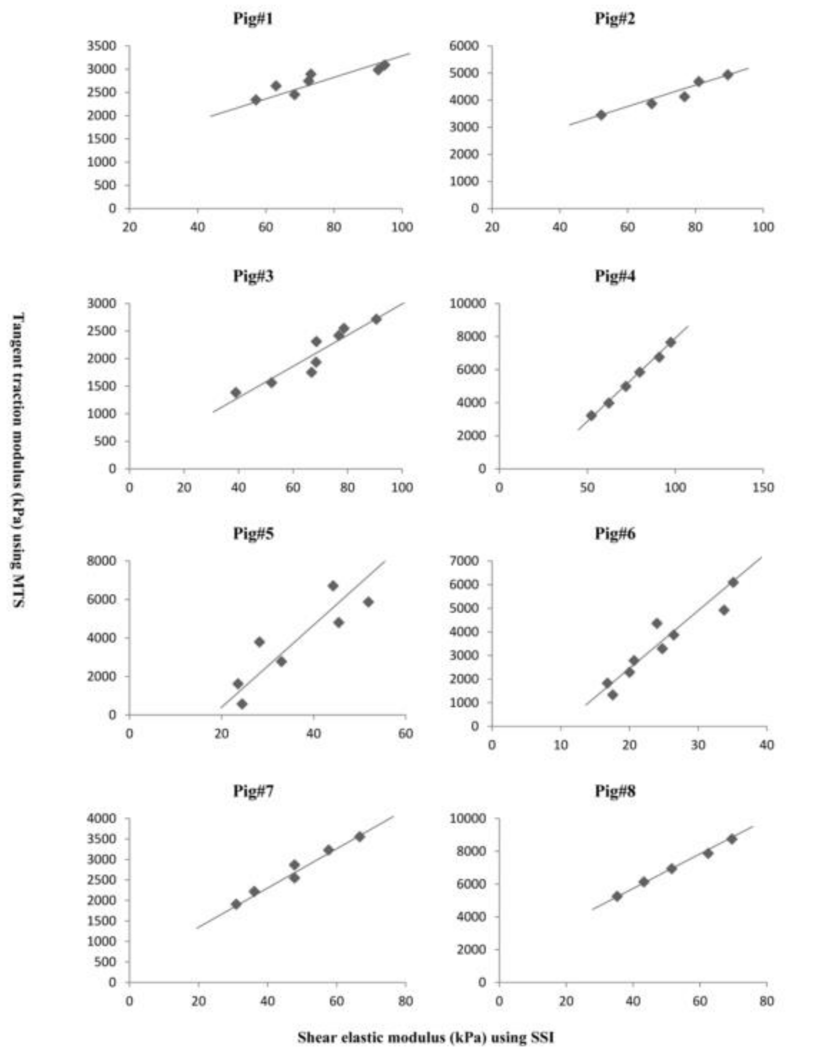


Figure 5. Correlations between shear elastic modulus of tendon captured from a Shear supersonic imaging and tangent traction modulus of tendon computed from a material testing system.
doi: 10.1371/journal.pone.0068216.g005

Figure 7-1. Image from Zhang et al., 2013 showing the linear regression between the elastic modulus and the shear elastic modulus.

7.5 Future Directions

cSWE is a very young technique that has a wide array of applications into tendon assessment following injury. Various tendon healing assessments are needed to determine the best course of action following injury. In the case of ACL injury, the two primary autograft techniques are hamstring, discussed in this dissertation, and quadriceps or patellar tendon. The patellar tendon requires the center third of the tendon to be removed and it is assumed the tendon regrows to fill in the gap. Patients report stiffness and residual pain while kneeling compared to hamstring tendon drafts. The use of cSWE would allow for the quantification of the tendons regrowth and if it truly returns to its pre-op levels as previously assumed. This lessens the concerns of surgeons performing ST autograft ACL reconstructions on the morbidity of the graft site. Additionally this technique can be used for other ruptures occurring in superficial tendons such as the Achilles tendon. Tracking the elasticity of the Achilles tendon allows clinicians to know when it is capable of being weight bearing, rather than waiting a prescribed amount of time. This can reduce healing time by optimizing treatment. cSWE elastography can also be applied to tendinopathy cases. The individualized cases of damage and healing time for patients with tendinopathy can benefit from determining if and when a treatment is having an effect. cSWE can also be used in prospective studies when determining the effects of an exercise regimen. Understanding if tendon laxity increases or decreases due to certain motions, or durations of motion, may lead to reductions in injury rates during fitness training.

In the hamstring reconstruction recovery paradigm, the cSWE technique can be used to evaluate current rehabilitation protocols. Tendon has been shown to regrow at 3-6 months post-op. B-mode ultrasound, which available in most clinics, would be capable of determining when the tendon has started regenerating. This could drive the timing of an exercise protocol to encourage the reorganization of the tendon. Comparing a rehabilitation which focuses on tendon fiber reorganization versus the current standard of care may lead to an optimized recovery rate of the mechanical properties of the tendon. The recovery of the ST tendon mechanical properties may in turn restore the hamstring strength faster which would give the leg more strength to oppose tibial translation and external rotation, two causes of ACL tear.

The robustness of cSWE also has room for improvement. While it has been shown that shear elastic modulus is linearly related to Young's modulus, it still has not been shown *in vivo*. cSWE has also not been evaluated for inhomogeneities of material properties within tendons and at musculotendinous junctions. It has been shown that the aponeurosis has different mechanical characteristics than tendon and it would be of benefit to know how much those properties vary. With the evaluation of those properties, both the tendon and the aponeurosis, these values can be used for not only rehabilitation timing and validation, but for musculoskeletal modelling as well. These values can be input into a biomechanical model to determine values such as joint loading. The adjustments in a musculoskeletal model may make the difference in determining if this recovery has an effect on the progression of OA and if it restores the loading location within the knee.

7.6 Conclusion

The measurement tool of continuous shear wave elastography (cSWE) has been shown to be a viable metric for determining the mechanical properties of soft tissues. cSWE has the implication be being a viable measure for soft tissues in the body including muscle and superficial tendons for use in tendinopathy, muscle stiffness, and rupture to evaluate healing progression and effectiveness of treatment.

cSWE was used to determine the elasticity of the semitendinosus tendon and show the tendon recovers its elasticity with time following a resection for ACL reconstruction. The regrowth of the ST tendon shows patient morbidity can be eliminated over time. This also adds to the return to sport discussion showing when that tendon has recovered following surgery. Knowing the tendon was at an average of 80% recovered at 18 months and only 45% recovered at 9 months makes an argument that a delayed return to sport may help the patient's chances of not re-tearing their ACL. This also can be used for rehabilitation timing. Tendon restructures with stress and understanding when the tendon has started to regrow allows for targeted exercises which encourage the reorganization of the tendon fibers. The recovery of the semitendinosus elasticity was correlated with increases in internal rotation strength and location of peak deep knee flexion strength at increased speeds. This may help patients return to preoperative symmetry. This recovery allows for the patient to participate in activity with confidence in both limbs and a reduced load on the reconstructed ACL. Each of these aims leads to the potential to return to high level sport with reduced risk injury following the regrowth of the semitendinosus tendon.

REFERENCES

Andersson C, Odensten M, Good L, Gillquist J. Surgical or non-surgical treatment of acute rupture of the anterior cruciate ligament. J bone Jt Surg, 1989. 21(7):965–74

Andersson D, Samuelsson K, Karlsson J. Treatment of anterior cruciate ligament injuries with special reference to surgical technique and rehabilitation: an assessment of randomized controlled trials. Arthrosc J Arthrosc Relat Surg, 2009. 25(6):653–85.

Andriacchi TP, Briant PL, Bevill SL, Koo S. Rotational changes at the knee after ACL injury cause cartilage thinning. Clin Orthop Relat Res, 2006. 442:39–44.

Arampatzis A, Karamanidis K. Mechanical properties of the triceps surae tendon and aponeurosis in relation to intensity of sport activity. J Biomech Eng, 2007 40:1946–52.

Arampatzis A, Stafilidis S, DeMonte G, Karamanidis K, Morey-Klapsing G, Brüggemann GP. Strain and elongation of the human gastrocnemius tendon and aponeurosis during maximal plantarflexion effort. J Biomech, 2005. 38(4):833–41.

Arda K, Ciledag N, Aktas E, Aribas BK, Köse K. Quantitative assessment of normal soft-tissue elasticity using shear-wave ultrasound elastography. Am J Roentgenol, 2011. 197(3):532–6.

Ardern CL, Webster KE, Taylor NF, Feller JA. Return to sport following anterior cruciate ligament reconstruction surgery: a systematic review and meta-analysis of the state of play. Br J Sports Med, 2011. 45(7):596–606.

Ardern CL, Webster KE, Taylor NF, Feller JA. Hamstring strength recovery after hamstring tendon harvest for anterior cruciate ligament reconstruction: a comparison between graft types. Arthroscopy, 2010. 26(4):462–9.

Armour T, Forwell L, Litchfield R, Krikley A, Amendola N, Fowler P. Isokinetic evaluation of internal/external tibial rotation strength after the use of hamstring tendons for anterior cruciate ligament reconstruction. Am J Sports Med. 2004;32(7):1639–43.

Arndt A, Bengtsson A-S, Peolsson M, Thorstensson A, Movin T. Non-uniform displacement within the Achilles tendon during passive ankle joint motion. Knee Surg Sports Traumatol Arthrosc, 2012. 20(9):1868–74.

Arya S, Kulig K. Tendinopathy alters mechanical and material properties of the Achilles tendon. J Appl Physiol, 2010. 108(3):670–5.

Ballyns JJ, Turo D, Otto P, Shah JP, Hammond J. Office-Based Elastographic Technique for Quantifying Mechanical Properties. J Ultrasound Med, 2012. 31:1209–19.

Barenius B, Nordlander M, Ponzer S, Tidermark J, Eriksson K. Quality of life and clinical outcome after anterior cruciate ligament reconstruction using patellar tendon graft or quadrupled semitendinosus graft: an 8-year follow-up of a randomized controlled trial. Am J Sports Med, 2010. 38(8):1533–41.

Barenius B, Ponzer S, Tidermark J, Eriksson K. Anterior cruciate ligament reconstruction with semitendinosus graft provided similar stability and knee function and fewer problems with kneeling compared with the bone-patellar tendon-bone graft. J bone Jt Surg, 2011. 93(10):969.

Barenius B, Ponzer S, Shalabi A, Bujak R, Norlén L, Eriksson K. Increased Risk of Osteoarthritis After Anterior Cruciate Ligament Reconstruction: A 14-Year Follow-up Study of a Randomized Controlled Trial. Am J Sports Med, 2014. 42(5):1049–57.

Bercoff J, Tanter M, Fink M. Supersonic shear imaging: a new technique for soft tissue elasticity mapping. IEEE Trans Ultrason Ferroelectr Freq Control, 2004. 51(4):396–409.

Best TM, Collins A, Lilly E, Seaber A, Goldner R, Murrell G. Achilles tendon healing: a correlation between functional and mechanical performance in the rat. J Orthop Res, 1993. 11(6):897–906.

Bourke HE, Salmon LJ, Waller A, Patterson V, Pinczewski LA. Survival of the anterior cruciate ligament graft and the contralateral ACL at a minimum of 15 years. Am J Sports Med, 2012. 40(9):1985–92.

Buchanan TS, Manal K. In vivo estimation of compressive loads in the ACL-recontructed knee. Med Sci Sport Exerc, 2008. 40(5):26–7.

Carofino B, Fulkerson J. Medial hamstring tendon regeneration following harvest for anterior cruciate ligament reconstruction: fact, myth, and clinical implication. Arthroscopy, 2005. 21(10):1257–65.

Cortes DH, Magland JF, Wright AC, Elliott DM. The shear modulus of the nucleus pulposus measured using magnetic resonance elastography: A potential biomarker for intervertebral disc degeneration. Magn Reson Med, 2013. 1–9.

Child S, Bryant AL, Clark R a, Crossley KM. Mechanical properties of the Achilles tendon aponeurosis are altered in athletes with Achilles tendinopathy. Am J Sports Med, 2010. 38(9):1885–93.

Crevier-Denoix N, Ruel Y, Dardillat C, Jerbi H, Sanaa M, Collobert-Laugier C, et al. Correlations between mean echogenicity and material properties of normal and diseased equine superficial digital flexor tendons: An in vitro segmental approach. J Biomech, 2005. 38(11):2212–20.

Cross M, Roger G, Kujawa P, Anderson I. Rengeneration of the semitendinosus and gracilis tendons following their transection for repair of the anterior cruciate ligament. Am J Sports Med, 1992. 20(2):221–3.

DeWall RJ, Slane LC, Lee KS, Thelen DG. Spatial variations in Achilles tendon shear wave speed. J Biomech, 2014. 47(11):2685–92.

Doral MN, Alam M, Bozkurt M, Turhan E, Atay OA, Dönmez G, et al. Functional anatomy of the Achilles tendon. Knee Surgery, Sport Traumatol Arthrosc, 2010. 18(5):638–43.

Duenwald S, Kobayashi H, Frisch K, Lakes R, Vanderby R. Ultrasound echo is related to stress and strain in tendon. J Biomech, 2011. 44(3):424–9.

Duenwald-Kuehl S, Lakes R, Vanderby R. Strain-induced damage reduces echo intensity changes in tendon during loading. J Biomech, 2012. 45(9):1607–11.

Eby SF, Song P, Chen S, Chen Q, Greenleaf JF, An K-N. Validation of shear wave elastography in skeletal muscle. J Biomech, 2013. 46(14):2381–7.

Eriksen H a, Pajala A, Leppilahti J, Risteli J. Increased content of type III collagen at the rupture site of human Achilles tendon. J Orthop Res, 2002. 20(6):1352–7.

Eriksson K, Hamberg P, Jansson E, Larsson H, Shalabi A, Wredmark T. Semitendinosus muscle in anterior cruciate ligament surgery: morphology and function. Arthrosc J Arthrosc Relat Surg, 2001. 17(8):808–17.

Eriksson K, Larsson H, Wredmark T, Hamberg P. Semitendinosus tendon regeneration after harvesting for ACL reconstruction. A prospective MRI study. Knee Surg Sports Traumatol Arthrosc, 1999. 7(4):220–5.

Ferretti A, Conteduca F, Morelli F, Masi V. Regeneration of the semitendinosus tendon after its use in anterior cruciate ligament reconstruction: a histologic study of three cases. Am J Sports Med, 2002. 30(2):204–7.

Ferretti A, Monaco E, Giannetti S, Caperna L, Luzon D, Conteduca F. A medium to long-term follow-up of ACL reconstruction using double gracilis and semitendinosus grafts. Knee Surg Sports Traumatol Arthrosc, 2011. 19(3):473–8.

Ferretti A, Vadalà A, De Carli A, Argento G, Conteduca F, Severini G. Minimizing internal rotation strength deficit after use of semitendinosus for anterior cruciate ligament reconstruction: a modified harvesting technique. Arthrosc J Arthrosc Relat Surg, 2008. 24(7):786–95.

Fukashiro S, Noda M, Shibayama A. In vivo determination of muscle viscoelasticity in the human leg. Acta Physiol Scand, 2001. 172(4):241–8.

Garcia JJ, Altiero NJ, Haut RC. Estimation of in Situ Elastic Properties of Biphasic Cartilage Based on a Transversely Isotropic Hypo-Elastic Model. J Biomed Eng, 2013. 122:1–8.

Garcia T, Hornof WJ, Insana MF. On the ultrasonic properties of tendon. Ultrasound Med Biol, 2003. 29(12):1787–97.

Gardinier ES, Manal K, Buchanan TS, Snyder-Mackler L. Altered loading in the injured knee after ACL rupture. J Orthop Res, 2013. 31(3):458–64.

Genuario JW, Faucett SC, Boublik M, Schlegel TF. A cost-effectiveness analysis comparing 3 anterior cruciate ligament graft types: bone-patellar tendon-bone autograft, hamstring autograft, and allograft. Am J Sports Med, 2012. 40(2):307–14.

Gobbi A, Domzalski M, Pascual J, Zanazzo M. Hamstring anterior cruciate ligament reconstruction: is it necessary to sacrifice the gracilis? Arthroscopy, 2005 21(3):275–80.

Granan L-P, Forssblad M, Lind M, Engebretsen L. The Scandinavian ACL registries 2004-2007: baseline epidemiology. Acta Orthop, 2009. 80(5):563–7.

Green BS, C B, BM C. A comparison of cutting technique performance in rugby union players. J Strength Cond Res, 2011. 25(10):2668–80.

Hashemi J, Chandrashekar N, Slauterbeck J. The mechanical properties of the human patellar tendon are correlated to its mass density and are independent of sex. Clin Biomech, 2005. 20:645-52.

Hiemstra LA, Webber S, MacDonald PB, Kriellaars DJ. Knee strength deficits after hamstring tendon and patellar tendon anterior cruciate ligament reconstruction. Med Sci Sports Exerc, 2000. 32(8):1472–9.

Houck J. Muscle activation patterns of selected lower extremity muscles during stepping and cutting tasks. J Electromyogr Kinesiol. 2003. 13(6):545–54.

Hoyt K, Kneezel T, Castaneda B, Parker KJ. Quantitative sonoelastography for the in vivo assessment of skeletal muscle viscoelasticity. Phys Med Biol, 2008. 53(15):4063–80.

Hughes D, Kelly J. Second-order elastic deformation of solids. Phys Rev, 1953. 92(5):1145–9.

Hughes G, Watkins J. A risk-factor model for anterior cruciate ligament injury. Sports Med, 2006. 36(5):411–28.

Imbert P, Belvedere C, Leardini A. Human knee laxity in ACL-deficient and physiological contralateral joints: intra-operative measurements using a navigation system. Biomed Eng, 2014. 13(1):86.

Itoh A, Ueno E, Tohno E, Kamma H, Takahashi H, Shiina T, et al. Breast Disease: Clinical Application of US Elastography for Diagnosis. Radiology, 2006. 239(2):341–50.

Janssen J, Schlörer E, Greiner L. EUS elastography of the pancreas: feasibility and pattern description of the normal pancreas, chronic pancreatitis, and focal pancreatic lesions. Gastrointest Endosc, 2007. 65(7):971–8.

Janssen RP a, du Mée AWF, van Valkenburg J, Sala HAGM, Tseng CM. Anterior cruciate ligament reconstruction with 4-strand hamstring autograft and accelerated rehabilitation: a 10-year prospective study on clinical results, knee osteoarthritis and its predictors. Knee Surg Sports Traumatol Arthrosc, 2013. 21(9):1977–88.

Janssen RP a, van der Velden MJF, Pasmans HLM, Sala HAGM. Regeneration of hamstring tendons after anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc, 2013. 21(4):898–905.

Joseph M, Lillie K, Bergeron D, Denegar C. Measuring Achilles tendon mechanical properties: A reliable, noninvasive method. J Strength Cond Res, 2012. 26(8):2017–20.

Kearney R, Costa M. Current concepts in the rehabilitation of an acute rupture of the tendo Achillis. Br Editor Soc Bone Jt Surgery, 2012.

Khodabakhshi G, Walker D, Scutt A, Way L, Cowie R, Hose D. Measuring three-dimensional strain distribution in tendon. J Microsc, 2013. 249(3):195–205.

Kongsgaard M, Nielsen CH, Hegnsvad S, Aagaard P, Magnusson SP. Mechanical properties of the human Achilles tendon, in vivo. Clin Biomech, 2011. 26(7):772–7.

Korstanje J-WH, Selles RW, Stam HJ, Hovius SER, Bosch JG. Development and validation of ultrasound speckle tracking to quantify tendon displacement. J Biomech, 2010. 43(7):1373–9.

Kraeutler MJ, Bravman JT, McCarty EC. Bone-patellar tendon-bone autograft versus allograft in outcomes of anterior cruciate ligament reconstruction: a meta-analysis of 5182 patients. Am J Sports Med, 2013. 41(10):2439–48.

Kramer J, Nusca D, Fowler P, Webster-Bogaert S. Knee flexor and extensor strength during concentric and eccentric muscle actions after anterior cruciate ligament reconstruction using the semitendinosus tendon and ligament augmentation device. Am J Sports Med, 1993. 21(2):285–91.

Kubo K, Ohgo K, Takeishi R, Yoshinaga K, Tsunoda N, Kanehisa H, et al. Effects of isometric training at different knee angles on the muscle-tendon complex in vivo. Scand J Med Sci Sports, 2006. 16(3):159–67.

Kumazaki T, Ehara Y, Sakai T. Anatomy and physiology of hamstring injury. Int J Sport, 2012. 33:950–4.

Kuo P, Li P, Li M. Elastic properties of tendon measured by two different approaches. Ultrasound Med Biol, 2001. 27(9):1275–84.

Lacourpaille L, Hug F, Bouillard K, Hogrel J-Y, Nordez A. Supersonic shear imaging provides a reliable measurement of resting muscle shear elastic modulus. Physiol Meas, 2012. 33(3):N19–28.

Lee JC, Healy FJC. Anatomy of the Wrist and Hand 1. RadioGraphics, 2005. 25(6):1577–90.

Leis H, Sanders T, Larsen K, Lancaster-Weiss K, Miller M. Hamstring regrowth following harvesting for anterior cruciate ligament reconstruction; the lizard tail phenomenon. J knee Surg, 2003. 16(3):159–64.

Leiter JRS, Gourlay R, McRae S, de Korompay N, MacDonald PB. Long-term follow-up of ACL reconstruction with hamstring autograft. Knee Surg Sports Traumatol Arthrosc, 2014. 22(5):1061–9.

Leitgeb J, Koettsdorfer J, Schuster R, Kovar FM, Platzer P, Aldrian S. Primary anterior cruciate ligament reconstruction in athletes: a 5-year follow up comparing patellar tendon versus hamstring tendon autograft. Wien Klin Wochenschr, 2014. 126(13-14):397–402.

Lichtwark GA, Wilson AM. In vivo mechanical properties of the human Achilles tendon during one-legged hopping. J Exp Biol, 2005. 208:4715–25.

Lim D-J, Luo S, Kim M-H, Ko S-H, Kim Y. Interobserver agreement and intraobserver reproducibility in thyroid ultrasound elastography. Am J Roentgenol, 2012. 198(4):896–901.

Lin TW, Cardenas L, Soslowsky LJ. Biomechanics of tendon injury and repair. J Biomech, 2004. 37(6):865–77.

Lipscomb A, Johnston R, Snyder R, Warburton M, Gilbert P. Evaluation of hamstring strength following use of semitendinosus and gracilis tendons to reconstruct the anterior cruciate ligament. Am J Sports Med, 1982. 10(6):340–3.

London N, Miller L, Block J. Clinical and economic consequences of the treatment gap in knee osteoarthritis management. Med Hypotheses, 2011. 76:887–92.

Maganaris C, Paul J. Load-elongation characteristics of in vivo human tendon and aponeurosis. J Exp Biol, 2000. 203:751–6.

Makihara Y, Nishino A, Fukubayashi T, Kanamori A. Decrease of knee flexion torque in patients with ACL reconstruction: combined analysis of the architecture and function of the knee flexor muscles. Knee Surg Sports Traumatol Arthrosc, 2006. 14(4):310–7.

Mall NA, Chalmers PN, Moric M, et al. Incidence and trends of anterior cruciate ligament reconstruction in the United States. Am J Sports Med, 2014. 42(10): 2363–70.

Malliaras P, Richards PJ, Garau G, Maffulli N. Achilles tendon Doppler flow may be associated with mechanical loading among active athletes. Am J Sports Med, 2008. 36(11):2210–5.

Maly M. Abnormal and cumulative loading in knee osteoarthritis. Curr Opin Rheumatol, 2008. 50(5):547–52.

Manal K, Cowder JD, Buchanan TS. A hybrid method for computing Achilles tendon moment arm using ultrasound and motion analysis. J Appl Biomech, 2010. 26(2):224–8.

Manduca A, Lake DS, Kruse SA, Ehman RL. Spatio-temporal directional filtering for improved inversion of MR elastography images. Med Image Anal, 2003. 7:465–73.

Mascarenhas R, Tranovich M, Kropf E, Fu FH, Harner C. Bone-patellar tendon-bone autograft versus hamstring autograft anterior cruciate ligament reconstruction in the young athlete: a retrospective matched analysis with 2-10 year follow-up. Knee Surgery, Sport Traumatol Arthrosc, 2012. 20:1520–7.

Meunier a, Odensten M, Good L. Long-term results after primary repair or non-surgical treatment of anterior cruciate ligament rupture: a randomized study with a 15-year follow-up. Scand J Med Sci Sports, 2007. 17(3):230–7.

Michaud CM, McKenna MT, Begg S, Tomijima N, Majmudar M, Bulzacchelli MT, et al. The burden of disease and injury in the United States 1996. Population health metrics, 2006.

Mortensen N, Jensen P. Early motion of the ankle after operative treatment of a rupture of the Achilles tendon. J bone Jt Surg. 1999. 81-A(7):983–90.

Mulcahey MK, Monchik KO, Yongpravat C, Badger GJ, Fadale PD, Hulstyn MJ, et al. The Knee Effects of single-bundle and double-bundle ACL reconstruction on tibiofemoral compressive stresses and joint kinematics during simulated squatting. Knee, 2012. 19:469–76.

Murray WM, Buchanan TS, Delp SL. The isometric functional capacity of muscles that cross the elbow. J Biomech, 2000. 33(8):943–52.

Nakamura N, Horibe S, Sasaki S, Kitaguchi T, Tagami M, Mitsuoka T, et al. Evaluation of active knee flexion and hamstring strength after anterior cruciate ligament reconstruction using hamstring tendons. Arthrosc J Arthrosc Relat Surg, 2002. 18(6):598–602.

Neugebauer JM, Hawkins DA. Identifying factors related to Achilles tendon stress, strain, and stiffness before and after 6 months of growth in youth 10-14 years of age. J Biomech, 2012. 45(14):2457–61.

Nicholson JA, Sutherland AG, Smith FW. Single bundle anterior cruciate reconstruction does not restore normal knee kinematics at six months: an upright MRI study. J Bone Joint Surg Br, 2011. 93(10):1334–40.

Nightingale K, Soo M, Nightingale R, Trahey G. Acoustic radiation force impulse imaging: in vivo demonstration of clinical feasibility. Ultrasound Med Biol, 2002. 28(2):227–35.

Nightingale K, Nightingale R. Acoustic radiation force impulse imaging of in vivo vastus medialis muscle under varying isometric load. Ultrason Imaging, 2002. 108:100–8.

Nikolaou VS, Efstathopoulos N, Wredmark T. Hamstring tendons regeneration after ACL reconstruction: an overview. Knee Surg Sports Traumatol Arthrosc, 2007. 15(2):153–60.

Nishino A, Sanada A, Kanehisa H, Fukubayashi T. Knee-flexion torque and morphology of the semitendinosus after ACL reconstruction. Med Sci Sports Exerc, 2006. 38(11):1895–900.

Nomura Y, Kuramochi R, Fukubayashi T. Evaluation of hamstring muscle strength and morphology after anterior cruciate ligament reconstruction. Scand J Med Sci Sports, 2014. 1–7.

Nordez A, Casari P, Cornu C. Effects of stretching velocity on passive resistance developed by the knee musculo-articular complex: contributions of frictional and viscoelastic behaviours. Eur J Appl Physiol, 2008. 103(2):243–50.

Novacheck TF. The biomechanics of running. Gait Posture, 1998. 7(1):77–95.

Ohkoshi Y, Inoue C, Yamane S, Hashimoto T, Ishida R. Changes in muscle strength properties caused by harvesting of autogenous semitendinosus tendon for reconstruction of contralateral anterior cruciate ligament. Arthroscopy, 1998. 14(6):580–4.

Øiestad BE, Holm I, Engebretsen L, Aune AK, Gunderson R, Risberg MA. The prevalence of patellofemoral osteoarthritis 12 years after anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc, 2013. 21(4):942–9.

Okahashi K, Sugimoto K, Iwai M, Oshima M, Samma M, Fujisawa Y, et al. Regeneration of the hamstring tendons after harvesting for arthroscopic anterior cruciate ligament reconstruction: a histological study in 11 patients. Knee Surg Sports Traumatol Arthrosc, 2006. 14(6):542–5.

Okotie G, Duenwald-Kuehl S, Kobayashi H, Wu M-J, Vanderby R. Tendon strain measurements with dynamic ultrasound images: evaluation of digital image correlation. J Biomech Eng, 2012. 134(2):024504.

Ophir J, Cespedes I, Ponnekanti H, Yazdi Y, Li X. Elastography: a quantitative method for imaging the elasticity of biological tissues. Ultrason Imaging, 1991. 13:111–34.

Otoshi K, Kikuchi S, Ohi G, Numazaki H, Sekiguchi M, Konno S. The process of tendon regeneration in an Achilles tendon resection rat model as a model for hamstring regeneration after harvesting for anterior cruciate ligament reconstruction. Arthroscopy, 2011. 27(2):218–27.

Owings M, Kozak L. Ambulatory and inpatient procedures in the United States, 1996. Vital Heal Stat, 1998. 13(139):1–128.

Papandrea P, Vulpiani MC, Ferretti a, Conteduca F. Regeneration of the semitendinosus tendon harvested for anterior cruciate ligament reconstruction. Evaluation using ultrasonography. Am J Sports Med, 2000. 28(4):556–61.

Relph N, Herrington L, Tyson S. The effects of ACL injury on knee proprioception: a meta-analysis. Physiotherapy, 2014. 100(3):187–95.

Rispoli DM, Sanders TG, Miller MD, Morrison WB. Magnetic resonance imaging at different time periods following hamstring harvest for anterior cruciate ligament reconstruction. Arthroscopy, 2001. 17(1):2–8.

Salomon G, Köllerman J, Thederan I, Chun FKH, Budäus L, Schlomm T, et al. Evaluation of prostate cancer detection with ultrasound real-time elastography: a comparison with step section pathological analysis after radical prostatectomy. Eur Urol, 2008. 54(6):1354–62.

Samuelsson K, Andersson D, Karlsson J. Treatment of anterior cruciate ligament injuries with special reference to graft type and surgical technique: an assessment of randomized controlled trials. Arthroscopy, 2009. 25(10):1139–74.

Sarvazyan A, Rudenko O, Swanson S, Fowlkes J, Emelianov S. Shear wave elasticity imaging: a new ultrasonic technology of medical diagnostics. Ultrasound Med Biol, 1998. 24(9):1419–35.

Sasaki N, Odajima S. Stress-strain curve and Young's modulus of a collagen molecule as determined by the X-ray diffraction technique. J Biomech, 1996. 29(5):655–8.

Sasaki K, Yamamoto N, Kiyosawa T, Sekido M. The role of collagen arrangement change during tendon healing demonstrated by scanning electron microscopy. J Electron Microsc, 2012. 61(5):327–34.

Scanlan SF, Chaudhari AMW, Dyrby CO, Andriacchi TP. Differences in tibial rotation during walking in ACL reconstructed and healthy contralateral knees. J Biomech, 2010. 43(9):1817–22.

Schepull T, Kvist J, Andersson C, Aspenberg P. Mechanical properties during healing of Achilles tendon ruptures to predict final outcome: A pilot Roentgen stereophotogrammetric analysis in 10 patients. BMC Musculoskelet Disord, 2007. 8(116):1–11.

Schepull T, Kvist J, Aspenberg P. Early E-modulus of healing Achilles tendons correlates with late function: Similar results with or without surgery. Scand J Med Sci Sports, 2012. 22(1):18–23.

Simonian P, Harrison S, Cooley V, Escabedo E, Deneka D, Larson R. Assessment of morbidity of semitendinosus and gracilis tendon harvest for ACL reconstruction. Am J Knee Surg, 1997. 10(2):54–9.

Snow BJ, Wilcox JJ, Burks RT, Greis PE. Evaluation of muscle size and fatty infiltration with MRI nine to eleven years following hamstring harvest for ACL reconstruction. J Bone Joint Surg Am, 2012. 94(14):1274–82.

Stergiou N, Ristanis S, Moraiti C, Georgoulis AD. Tibial rotation in anterior cruciate ligament (ACL) -deficient and ACL-reconstructed knees. Sport Med, 2007. 37(7):601–13.

Sugiura Y. Strength deficits identified with concentric action of the hip extensors and eccentric action of the hamstrings predispose to hamstring injury in elite sprinters. J Orthop Sports Phys Ther, 2008. 38(8):3–9.

Tadokoro K, Matsui N, Yagi M, Kuroda R, Kurosaka M, Yoshiya S. Evaluation of Hamstring Strength and Tendon Regrowth After Harvesting for Anterior Cruciate Ligament Reconstruction. Am J Sports Med, 2004. 32(7):1644–50.

Tashiro T, Kurosawa H, Kawakami A, Hikita A, Fukui N. Influence of medial hamstring tendon harvest on knee flexor strength after anterior cruciate ligament reconstruction. A detailed evaluation with comparison of single- and double-tendon harvest. Am J Sports Med, 2003. 31(4):522–9.

Tashman S, Collon D, Anderson K, Kolowich P, Anderst W. Abnormal rotational knee motion during running after anterior cruciate ligament reconstruction. Am J Sports Med, 2004. 32(4):975–83.

Vairo GL. Knee flexor strength and endurance profiles after ipsilateral hamstring tendons anterior cruciate ligament reconstruction. Arch Phys Med Rehabil, 2014. 95(3):552–61.

Vincent KR, Conrad BP, Fregly BJ, Vincent HK. The pathophysiology of osteoarthritis: a mechanical perspective on the knee joint. PM&R, 2012. 4:S3–9.

Viola RW, Sterett WI, Newfield D, Steadman JR, Torry MR. Internal and external tibial rotation strength after anterior cruciate ligament reconstruction using ipsilateral semitendinosus and gracilis tendon autografts. Am J Sports Med, 2000. 28(4):552–5.

Wang JH-C. Mechanobiology of tendon. J Biomech, 2006. 39(9):1563–82.

Webster KE, Feller JA. Tibial rotation in anterior cruciate ligament reconstructed knees during single limb hop and drop landings. Clin Biomech, 2012. 27(5):475–9.

Wild CY, Steele JR, Munro BJ. Insufficient hamstring strength compromises landing technique in adolescent girls. Med Sci Sports Exerc, 2013. 45(3):497–505.

Williams GN, Snyder-Mackler L, Barrance PJ, Axe MJ, Buchanan TS. Muscle and tendon morphology after reconstruction of the anterior cruciate ligament with autologous semitendinosus-gracilis graft. J Bone Joint Surg Am, 2004. 86-A(9):1936–46.

Yu Z, Boseck S. Scanning acoustic microscopy and its applications to material characterization. Rev Mod Phys, 1995. 67(4):863–91.

Yu B, Garrett WE. Mechanisms of non-contact ACL injuries. Br J Sports Med, 2007. 41 Suppl 1:i47–51.

Zhang, Z. J., & Fu, S. N. Shear Elastic Modulus on Patellar Tendon Captured from Supersonic Shear Imaging: Correlation with Tangent Traction Modulus

Computed from Material Testing System and Test-Retest Reliability. PloS One, 2013. 8(6)

Zhao H, Song P, Meixner D, Kinnick R, Callstrom M, Sanchez W, et al. External Vibration Multi-directional Ultrasound Shearwave Elastography (EVMUSE): Application in Liver Fibrosis Staging. IEEE Trans Med Imaging, 2014. 62(c).

Zheng Y, Chen S, Tan W, Kinnick R, Greenleaf JF. Detection of tissue harmonic motion induced by ultrasonic radiation force using pulse-echo ultrasound and Kalman filter. IEEE Trans Ultrason Ferroelectr Freq Control, 2007. 54(2):290–300.

Zheng Y, Chen X, Yao A, Lin H, Shen Y, Zhu Y, et al. Shear Wave Propagation in Soft Tissue and Ultrasound Vibrometry. 2013.

Appendix A

THE MECHANICAL PROPERTIES OF THE ACHILLES TENDON DETERMINED VIA CONTINUOUS SHEAR WAVE ELASTOGRAPHY

A.1 Introduction

Healing of tendon injuries, such as tears and ruptures, is characterized by large changes in composition and structure during the remodeling process. Previous research showed changes in the elastic modulus with the restructuring of the tendon during healing, post tendon rupture [Schepull 2007]. Therefore, viscoelastic properties can be used as biomarkers to quantify changes in tendon structure after injury and during healing. Continuous shear wave elastography (cSWE) is an ultrasound imaging method recently developed to determine the shear modulus of elasticity and viscosity of a soft tissue [Cortes 2014]. This technique quantifies the viscoelastic properties of soft tissues by measuring the speed at which external shear waves travel through the tissue. In a clinical setting, cSWE has the potential to not only identify pathology, but establish injury severity or healing progression. For shear and viscosity moduli of tendons to be used as a pathologic severity metric, a baseline range of viscoelastic moduli within a healthy population must be reported and compared to other metrics of diagnosis such as tendon structure or loading capability [Sharma 2006, Roche 2013].

Previous studies have demonstrated that the healing time after Achilles tendon rupture correlates to an increased Young's modulus over a 12 week period, and also correlates to the restoration of the subject's heel rise index [Schepull 2007]. The tensile elastic modulus of the Achilles tendon have been measured using invasive and non-invasive techniques including implanted markers scanned by CT or ultrasound [Schepull 2012, Kongsgaard 2011], anatomical landmark tracking [Joseph 2012], and speckle pattern tracking [Farron 2009], but these measurement techniques require loading of the tendon which may cause pain and may not be applicable at early stages of healing from injuries such as ruptures. Ultrasound devices which measure the shear modulus of elasticity for soft tissue have become commercially available and have been used for characterizing relaxed, un-stretched tendon [DeWall 2014]. These devices have been demonstrated to produce viscoelastic maps of soft tissue but the limitation of these devices is they have a maximum detectable shear wave speed [Bercoff 2003]. Softer tissue such as muscle, slack tendon and viscera transmit shear waves well within the capabilities of these devices. Unlike cSWE, commercial systems reach the maximum measurable speed (saturation value) in tensioned tendon, e.g. when the Achilles tendon is stretched in dorsi-flexion [DeWall 2014]. Commercial elastography uses ultrasonic pulses to produce shear wave in the tissue. This wave propagation is not associated to a particular frequency; therefore, the measurement of viscoelastic properties is not possible by known viscoelastic modeling techniques. Additionally, it has been reported that shear wave speed in the Achilles tendon often exceeds the limit of commercial systems [DeWall 2014]. cSWE is capable of assessing tendon at various known frequencies and therefore the analysis of viscoelastic properties is straight forward and flexible enough to measure properties in tissues with high stiffness, such as tendons. This robustness is crucial to clinical applications.

Correlating the viscoelastic properties, measured with cSWE, to standard measurements of tendon function and establishing bilateral symmetry between tendons aids in its application into clinical practice. Strength tests are used as a risk factor for the development of tendinopathy [Mahieu 2006]. Similarly, tendon thickness and area have been used as indication of abnormal structure in tendon [Arya 2010]. In addition to comparing viscoelastic properties to standard clinical measures, it is necessary to establish a range of healthy viscoelasticity properties. In order to use the contralateral tendon as a healthy control, it is essential to determine bilateral symmetry between tendons in a healthy population, the inherent tendon variation and the minimal detectable differences in viscoelastic properties. The objectives of this study are to establish reference values of viscoelastic properties in healthy individuals and to show the mechanical properties of a tendon are bilaterally similar and independent of traditional clinical assessment measures. We will demonstrate this by first determining the viscoelastic moduli of healthy Achilles tendon and establish bilateral symmetry. Next, we will examine correlations between the viscoelastic properties and plantar flexion strength and tendon area, which are commonly used clinical measures.

A.2 Methods

Viscoelastic properties, cross sectional area and plantar flexor strength of the Achilles tendons were measured bilaterally from 29 healthy subjects (age: 29 yrs ± 9.5, height: 173 cm ± 8.8, mass: 70 kg ± 13.2) with no history of Achilles tendon rupture or tendinopathy. Each subject read and signed an informed consent approved by the University of Delaware institutional review board. A licensed physical therapist assessed and screened each subject for tendon health through palpation and

B-mode ultrasound. It was established that none of the subjects had any abnormal swelling, tenderness or hypoechoic regions in the tendon that would allude to tendinopathy.

Cross sectional area measurements of the Achilles tendon were taken just distal to the soleus myotendinous junction. The subject lay in the prone position with their foot relaxed off the edge of a plinth. The junction was found in the longitudinal plane, marked on the skin, and an image was collected after the transducer was placed in the transverse plane. Tendon area was computed via tracing the recorded image in ImageJ (National Institute of Health, USA).

To measure the viscoelastic properties of the Achilles tendon, subjects lay prone on a plinth with their ankle passively flexed at 10° dorsiflexion on a dynamometer (Biodex Medical Systems, Shirley, New York) to remove the slack from the tendon [Hug 2013]. Removing the slack from the tendon leads to the tendon being measured within the linear elastic region, i.e., when the tendon has a constant elastic modulus, and thereby ensuring each subject's individual viscoelastic properties are not affected by their range of motion [Magnusson 2003]. cSWE requires an external shear wave to be generated and a high frame rate ultrasound device to collect the raw RF data [Cortes *in Press*]. An actuator (mini-shaker type 4810, Bruel & Kjaer, Naerum, Denmark) was used to create a sinusoidal mechanical shear wave along the Achilles tendon. The proximity of the actuator does not impact the calculations of mechanical properties as those are determined by the speed at which the wave propagates. The proximity of the actuator to the transducer does have an effect on the post-processing of the signal. The amplitude of the wave is attenuated by the skin's surface and the tissue's viscous effect, therefore, the farther from the actuator the transducer is, the less detectable the wave. The actuator in this study was limited to a peak displacement of 6 mm and a peak output force of 10 N and the ultrasound transducer was kept close to the actuator, 3 cm. An ultrasound scanner (MDP, Ultrasonix, Vancouver, Canada) with a 128 channel external DAQ and 38 mm linear transducer was used to collect raw RF ultrasound data from the free portion of the Achilles tendon, distal to the soleus junction previously identified on the subject (Figure A-1).

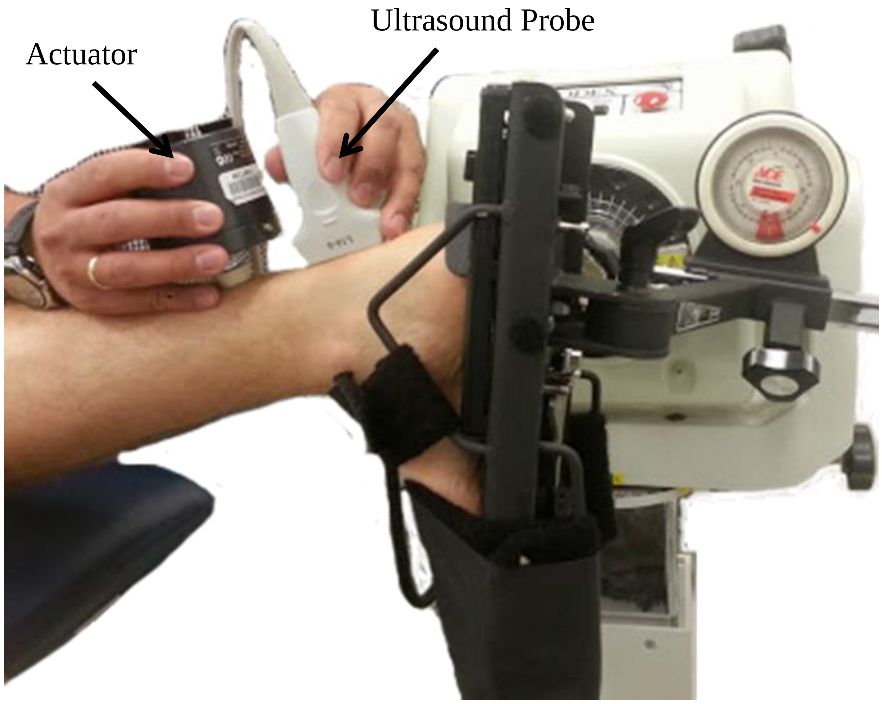


Figure A-1. The elastography arrangement. The ankle is at 10^{o} of dorsiflexion, the probe is placed at the soleus myotendinous junction and the actuator is placed approximately 5cm proximal to that, along the Achilles tendon.

The 128 channels spaced over a 38 mm linear array produced a linear resolution of 0.3 mm. The ultrasound collected at a frame rate of 6450Hz. The initial shear wave was pulsing at 323Hz. With the ultrasound transducer parallel to the Achilles tendon and the actuator located 3cm proximal to the transducer along the tendon, a collection of at least 60 frames of ultrasound data were taken. The actuator and transducer remained in the same locations while data was acquired for five other frequencies (340, 358, 379, 403, 430Hz). The transducer and actuator were removed from the skin's surface after collecting data. Data collections were repeated two more times to evaluate the reliability of this method. This entire procedure was repeated for the opposite leg. Shear wave speed was calculated using custom signal processing software which has been described and validated by Cortes et al. [Cortes, *in Press*]. The viscoelastic moduli were computed through the use of the Voigt model (Equation 1) as the minimization criteria for an optimization algorithm input into commercial software (Matlab, MathWorks, Natick, USA). The Voigt model describes the change in wave speed as a function of frequency using the following relationship:

Equation A-1 $$\mathbf{V}_{s}(\boldsymbol{\omega}) = \sqrt{\frac{2(\mu_1^2+\omega^2\mu_2^2)}{\rho(\mu_1+\sqrt{\mu_1^2+\omega^2\mu_2^2})}}$$

Where, the shear modulus is represented by µ1 (kPa) and the viscosity modulus, µ2 (Pa-s), V¬s is the shear wave speed, ω is the shear wave frequency, and ρ is the tissue density. Based on the density of a similar tendon previously reported, the tendon density was approximated as 1600 kg/m3 [Hashemi 2005]. The Voigt model was selected based on the stability of the model at the increased frequencies being used in the study [Zheng 2011]

The strength protocol was performed at the end of the ultrasound session to prevent stretching and fatiguing affects from creating unknowns in the elastography and cross sectional area data. Each subject was seated on a dynamometer with their hip at 90□ and their knee fully extended to evaluate plantar flexion strength. The subject's foot was affixed to a footplate and their ankle was fixed at 90□. Subjects elicited maximum voluntary isometric contraction (MVIC) into plantar flexion. The contraction was performed for 3 seconds while verbal encouragement was given. This was performed three times on each leg and the average of the three trials for each leg was reported. Nominal and normalized (with respect to body weight) values of strength were used to explore correlations to tendon viscoelastic properties.

As this is a new technique, reliability of cSWE measures was assessed. The intraclass correlation coefficients (3,1) and standard error of measurement (SEM = Stand Deviation * $\sqrt{(1\text{-ICC})}$) were calculated to determine the minimal detectable change (MDC95%). The MDC95% was calculated as follows: at individual level MDC95%= 2.77 x SEM and at group level MDC95%=2.77 x SEM/$\sqrt{n}$. Bilateral variability was assessed with the coefficients of variation (CV). This CV was calculated by determining the standard deviation of the shear (and viscous) modulus for both legs and dividing it by the average modulus of both legs for each subject. A two, one-sided test of equivalence (α=0.05) was used to determine the bilateral symmetry of the viscoelastic moduli [Walker 2011]. Notice that p values lower than 0.05 in the equivalence test means that the two groups have significant similarity, which is the opposite of the regular t-test. The desired detectable difference (δ) for the test of equivalence was determined by adding the possible variation caused by the measure (mean moduli * CV) and the MDC95% for the individual. A Pearson's correlation coefficient (r) was used to establish a relationship between MVIC plantar flexor strength and shear modulus.

A.3 Results

The first aim was to determine the viscoelastic moduli of healthy Achilles tendon. The shear wave speed increased with frequency and ranged from 15.7m/s at 323Hz to 21.0m/s at 430Hz (Figure A-2). cSWE produced shear and viscosity modulus maps yielding nominal shear moduli from 62.1 kPa to 107.8 kPa, with a mean of 83.2 kPa and viscosity from 103.6 Pa-s to 186.3 Pa-s with a mean of 141.0 Pa-s (Table 1 Figure 3). The average bilateral CV was 7.2% for the shear modulus and 9.4% for the viscosity modulus. The ICC(3,1) = 0.875 and 0.876 for the shear and viscosity moduli and the SEM = 3.8kPa and 6.8Pa-s, respectively. The ICC and SEM were used to calculate the MDC95% for the shear and viscosity at the individual level, 4.4 kPa and 7.3 Pa-s, and at the group level, 2.0 kPA and 3.5 Pa-s (Table A-1, Figure A-3). The viscoelastic modulus were found to be bilaterally equivalent ($p_{\mu 1}$ = 0.013, $p_{\mu 2}$ = 0.017) using δ_{u1} and δ_{u2} set at 9.6 kPa and 20.8 Pa-s.

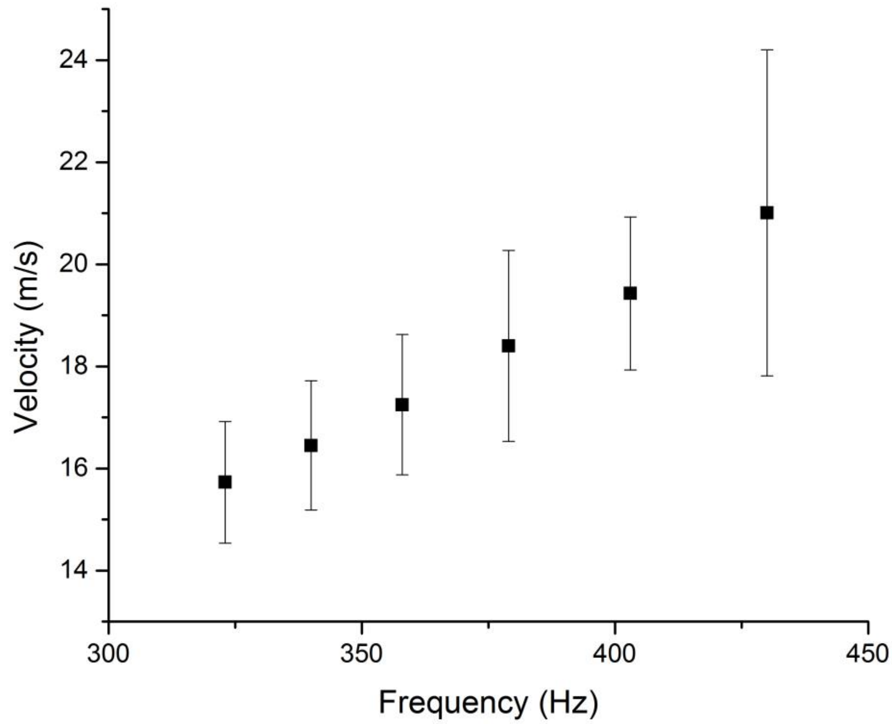


Figure A-2. The average velocity at each of the frequencies applied from the external actuator. Note the consistent increase in velocity with the frequency applied, showing the need for the optimization with a viscoelastic model.

Table A-1. The mean, coefficient of variation (CV), and minimal detectable change (MDC) for the viscoelastic properties, area and plantar flexion strength.

Leg	Right	Left	Legs Combined		
			CV(%)	MDC_{indiv}	MDC_{group}
μ_1 (kPa)	84.0	82.4	7.2	4.4	2.0
μ_2 (Pa-s)	139.0	143.0	9.4	7.3	3.5
Area (cm^2)	0.49	0.48	4.3	0.01	>0.01
MVIC (N-m)	132.2	138.5	8.6	17.2	2.9

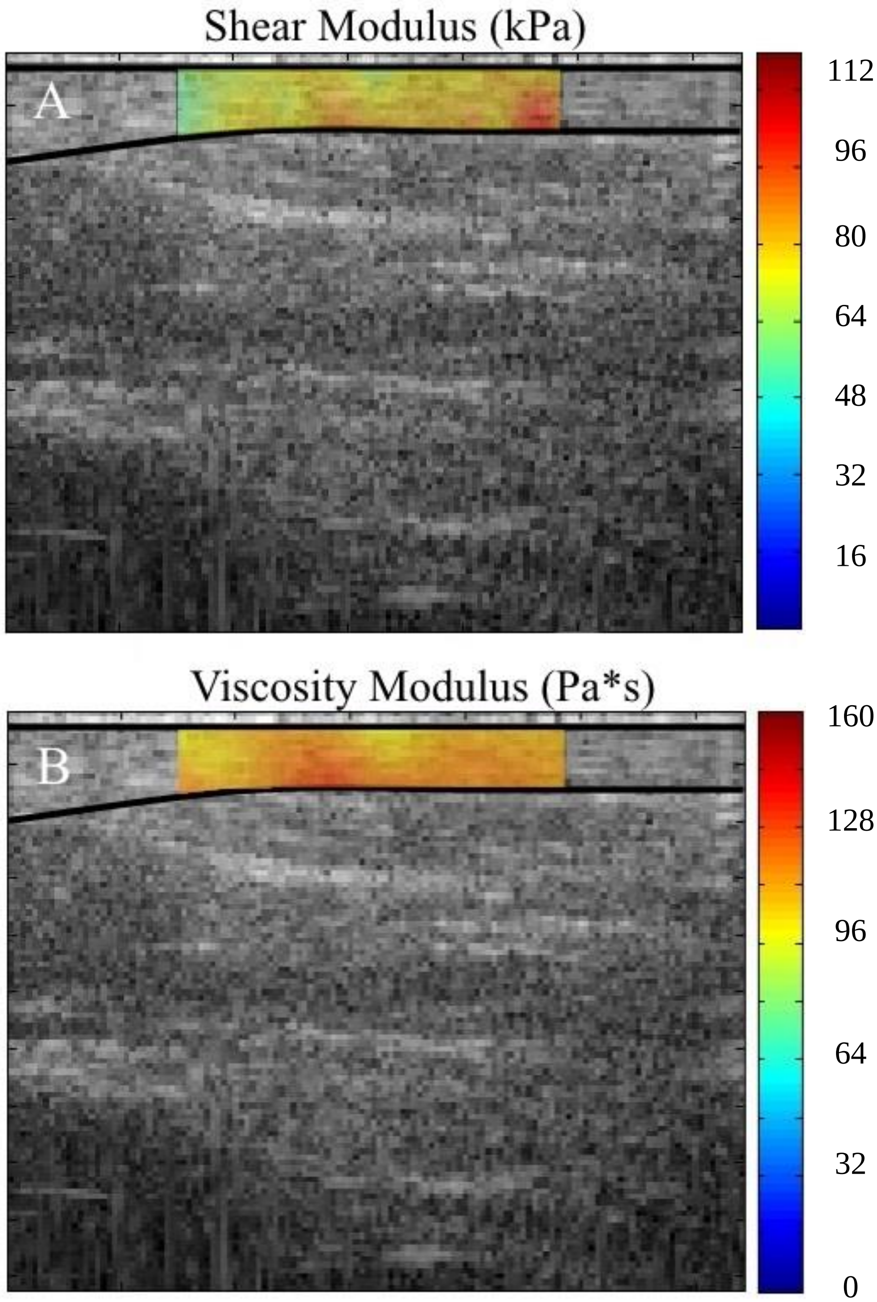


Figure A-3**.** The shear modulus (A) and viscocity modulus (B) maps overlays on the gray scale image of the Achilles tendon. The area evaluted is the center region of the tendon due to the processing technique requiring data before and after the area of interst.

The second aim was to examine correlations between the shear elastic modulus and plantar flexion strength (MVIC) and tendon area. The average MVIC for the right and left were 132.2 N-m and 138.5 N-m. The average traced areas of the tendons were 0.49 cm^2 and 0.48 cm^2 for the right and left, respectively (Table A-1, Figure A-4). No significant correlation was observed between the shear modulus of the Achilles tendons and the MVIC plantar flexion strength ($r^2 < 0.12$) nor were there any significant correlations between either the shear or viscosity modulus and the cross sectional area of the tendon, ($r^2 < 0.06$). Even with strength normalized to body weight and area normalized to height, correlations did not exist in reference to the viscoelastic moduli.

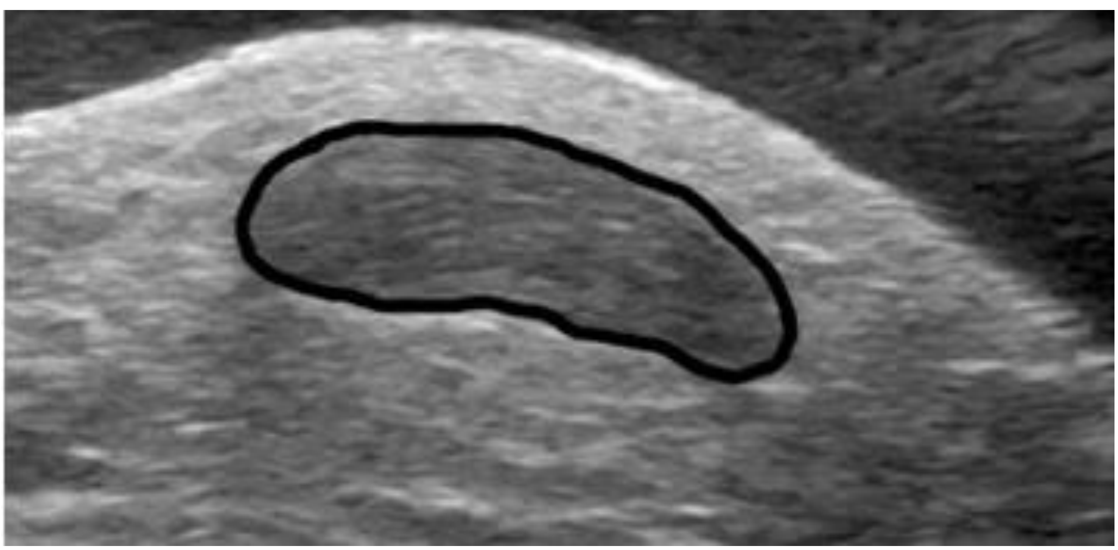


Figure A-4. Tendon Axial Area traced in black at the soleus junction.

A.4 Discussion

Tendon injuries are characterized by substantial changes in composition and structure which lead to altered tendon mechanical properties [Schepull 2012, Alfredson 2011]. Since quantifying compositional and structural changes non-invasively is challenging, measurements of tendon viscoelastic properties can offer an alternative metric to evaluate tendon injuries and recovery. In order to evaluate tendon pathologies using viscoelastic properties, it is necessary to determine the normal variation of these properties in healthy individuals. The objectives of this study were met; the viscoelastic moduli demonstrated no correlation to the MVIC nor area of the tendon. This study also measured reference values and variations of viscoelastic properties in healthy Achilles tendon, calculated the minimal detectable change of viscoelastic properties and established equivalence between bilateral tendons. These findings suggest that viscoelastic properties measured via cSWE can be used as a novel metric to assess tendon pathology and healing using the uninvolved leg as a healthy control.

The application of cSWE requires the variability of the test metric to be reported and the establishment of control values for comparison. The MDC95% is the amount of change necessary before a difference in measured values can be considered meaningful. In reference to this study for an individual, it was determined that a difference greater than 4.4 kPa would be considered a true difference in shear modulus and a change greater than 7.3 Pa-s would be considered a true difference for viscosity. These values are important to determine differences in viscoelastic properties in longitudinal studies. Similarly, the MDC95% for shear and viscosity modulus at the group level were 2.0 kPA and 3.5 Pa-s which can be used in the context of a controlled study looking for differences in population means. The CV indicates the normal difference between both tendons in a healthy subject, which was 7.2% for the shear modulus and 9.4% for the viscosity modulus. While we determined an average variation of 7.2% in shear modulus, a concurrent study of semitendinosus regrowth post-resection has demonstrated a much larger difference (59%) between the regrowing and healthy sides [Cortes *in Press*].

Previous studies have reported bilateral differences in Young's modulus between tendons due to limb dominance [Bohm 2014]. Bohm et al. report differences in the Achilles tendon Young's modulus using the displacement of the gastrocnemius myotendinous junction [Bohm 2014]. These elastic properties include the stress and strain of both aponeurosis and tendon. Our study showed bilateral equivalence of the mechanical properties at the free tendon, distal to the soleus junction, and did not include the aponeurosis. The aponeurosis and tendon do not have the same mechanical properties and should be evaluated separately [DeWall 2014]. The training response of the aponeurosis and tendon differ as well, which may explain the equivalence in our study and the differences seen by others. Eccentric training exercises lead to increased stiffness in the combined tendon and aponeurosis system, but no training effects were seen within the free tendon alone [Bohm 2014, Maganaris 1999, Duclay 2009]. Additionally, Rosagers et al. showed an increase in free tendon cross sectional area with training, which was not found in the myotendinous junction of the gastrocnemius [Aubry 2013, Bohm 2014]. There are large variations in mechanical properties and training effects between structures and there is a need to investigate the tendon and aponeurosis in a more isolated environment which can be performed with cSWE.

Lower shear-wave speeds than those found in this study have been previously reported. Values were reported with the ankle at a resting angle and at 90o using a fixed shear wave frequency (12.0 m/s and between 11.7 and 15.5m/s, respectively) [DeWall 2014, Aubry 2013, Brum 2014]. Though these numbers were less than the velocity values demonstrated in our study, it must be considered that in our study, the ankle at 10° of dorsiflexion would cause an increase in the tendon stiffness due to the tendons elastic nonlinearity. In a relaxed state, tendon fibrils have a wavy configuration which leads to a low elastic modulus and slower shear wave speeds. Once a load is applied, the fibrils are pulled tight which causes the elastic modulus and the shear wave speeds to increase. Our study also used increasing shear wave frequencies yielding speeds which reflected those increases (Figure 2). The frequency-speed relationship has been shown in previous research for other soft tissues and establishes the need for using the Voigt dynamic model to describe the viscoelastic nature of the tissue [Hoyt 2008].

The viscosity parameter is a measure that has been studied on a limited basis, but may have large clinical implications if it were to become a clinical biomarker. Previous studies have described the viscosity of a musculo-tendinous complex as a means of energy dissipation [Nordez 2008, Fukashiro 2001], but no studies have used tendon viscosity as a biomarker. This is due to the previous inability to separate the muscle tendon complex into its individual components. cSWE uses localized measurements of the tendon to determine both the shear and viscous components. The viscosity of tendon acts as the dampening mechanism [Babic 2004], which means a larger viscosity leads to an increased resistance to lengthening (i.e., increased stiffness) with an increased loading rate. This effect may act as a defensive mechanism by reducing the amount of tendon stretch; on the other hand, increased viscosity enables the tendon to transmit larger forces which may increase the risk of tendon injuries. Limited information is available on the effects of viscosity in tendon function and therefore future studies are needed to determine whether increased viscosity is a positive or negative attribute in order to apply it as an assessment or treatment measure.

The demographics and sample size were a limitation of the study. The age range of the population used in this study is slightly younger than the population more prone to Achilles tendon pathologies. Another limitation of this study was the location of the ultrasound with respect to the insertion site. Since the ultrasound probe was placed next to the myotendinous junction, the anatomical differences in tendon length cause the probe to be closer or farther from the calcaneal insertion. Since a previous study showed that wave speed changes along of the length of the tendon [DeWall 2014], the proximity of the probe to the calcaneal insertion may introduce variability in the measured viscoelastic properties. However, the evaluation of bilateral differences in viscoelastic properties may not be affected since the tendon length is similar in right and left tendons for each subject [Silbernagel 2012]. Another limitation is that average values of viscoelastic properties were calculated from the portion of the tendon present in the field of view. This approach ignores heterogeneities in the tendon, though only minor heterogeneities of viscoelastic properties are expected in healthy tendons. This approach does not find Young's modulus and therefore the direct application to energy transmission is not yet possible, but shear elastic modulus has been linearly correlated to the longitudinal elastic modulus [Zhang 2013]. This allows for the recovery of the shear elastography to be related to the recovery of tendon function.

The viscoelastic parameters are new, unique measures which can now be used to complement other clinical metrics including self-reported outcomes and clinical assessments. The relationships between tendon compositions, structure and mechanics suggests that viscoelastic properties may provide the ability to measure healing progressions and, of more clinical importance, evaluate the effectiveness of therapy for given pathologies. A case of interest is Achilles tendinopathy. Several techniques such as exercise, injections, and surgery have been used to alleviate pain and restore normal function [Silbernagel 2011, Yelland 2011, Longo 2008]. However, the optimal clinical pathway is still debated [Sussmilch-Leitch 2012]. An unbiased, non-subjective rating system of tendon recovery, such as cSWE, provides a means of assessing each of these techniques and timing for administration of rehabilitation.

A.5 Conclusion

In conclusion, both the shear modulus and viscosity modulus of healthy tendon were bilaterally equivalent and therefore cSWE has the potential to determine pathologies through side to side deviations in viscoelastic properties. It was found that the expected difference between legs was 7.2% and 9.4% for shear and viscosity modulus. Lastly, the viscoelastic properties are independent measures unrelated to the strength and area of the tendon providing a unique metric for tendon assessment and healing progression.

Conflict of interest

Stephen M Suydam and Daniel H Cortes are pursuing a patent for the Continuous Shear Wave Elastography protocol. Daniel H Cortes and Karin Gravare Silbernagel are submitting an R-21 grant to the National Institute of Health to study the viscoelastic properties of the Achilles tendon after tendinopathy.

Acknowledgements

Research reported in this publication was supported by the National Institute of General Medical Sciences and by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under grants P20-GM103333, U54-GM104941, R01-AR050052 and R21-AR067390. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Appendix B

INFORMED CONSTENT FORMS

B.1 Elastography Consent Form

UNIVERSITY OF DELAWARE
POSTURE AND MOVEMENT BIOMECHANICS LABORATORY
INFORMED CONSENT FORM

Study Title: Tissue characteristic change with contraction.

Principal Investigators: Thomas S. Buchanan PhD, Stephen Suydam, MSc

PURPOSE AND BACKGROUND

You are being asked, along with approximately forty other subjects, to participate in a study that will establish soft tissue properties using ultrasound imaging during a static contraction. Ultrasound images of your leg muscles and tendons will be recorded while you contract your upper and lower leg muscles up to their maximum. The results of this study may provide a new technique for establishing muscle and tendon properties to aid clinicians in diagnosis and healing progression.

Participation in this research study is entirely voluntary and refusal to participate will involve no penalty. You may withdraw from the study at any time. You must be between the ages of 18 and 70 years of age to participate in this study and either; have sustained an Achilles tear and have been non-operatively treated, be 12 weeks post ACL reconstruction, or have no previous injuries to your knee, lower leg, and ankle. In addition, you must have little to no pain your leg muscles or tendons during contraction.

All testing will take place in the Posture and Movement Biomechanics Laboratory at the University of Delaware. The entire testing session will take approximately 1 hour to complete.

PROCEDURES

An ultrasound device will take images of your leg muscles and tendons with your leg fixed in a device which measures how much force you can produce. You will maximally push against the device prior to imaging as a base line of your maximum torque. You will then relax and an ultrasound image will be taken of your muscle or tendon with no contraction. You will then elicit and hold a mild contraction, between 10% - 30% of your maximum. The torque will be recorded and an image will be captured. Collections will continue with you increasing your contraction and images being collected (approximately 6 – 8 steps) until you are not able to increase your contraction intensity (i.e., until maximum voluntary contraction). This process will be repeated across several muscles and tendons and will be repeated for the opposite leg.

Risks/Discomforts

The risks associated with participation in this study are minimal. If you have an allergy to any gels or lubricants, you may be excluded from the trial. Following testing your muscles may feel as if you exercised.

Page 1 of 4 Subject's Initials_______

Benefits

The results of this study may allow for clinicians to determine muscle and tendon parameters through a new analysis technique; however there will be no direct benefit to the current participants.

Compensation

You will receive no compensation for participation in this research study

Confidentiality and Records

All subjects will be identified by number only. Only the investigators will have access to the data. Neither your name nor any identifying information will be used in publication or presentation resulting from this study. A statistical report which may include pictures that do not identify you may be disclosed in a scientific paper. Photographs are optional and can be refused with no consequence to the subject or the data. Data will be electronically encrypted and archived indefinitely. There will be no identifying information of you stored with the data.

Subject's Initials_______

Study Title: Tissue echogenicity change with contraction

Principal Investigators:

Thomas S. Buchanan, PhD
Stephen M. Suydam, MSc

If you have any questions concerning the rights of individuals who agree to participate in research, you may contact the Chair of University of Delaware IRB, at (302) 831-2137.

Further questions regarding this study may be addressed to:
Thomas S. Buchanan, PhD
(302) 831-2401
Delaware Rehabilitation Institute
Department of Mechanical Engineering
Center for Biomedical Engineering Research

Subjects Statement:

I have read this consent form and have discussed the procedure described above with the principal investigator. I have been given the opportunity to ask questions regarding this study, and they have been answered to my satisfaction.

In the case that I am injured or experience an acute medical emergency during the study, I will be provided with immediate first aid. Any additional care will be at my own expense. I have been fully informed of the above described procedures, with its possible risks and benefits, and I hereby consent to the procedures above.

__________________________ ______________________________
Subject's Name Subject's Signature Date

____
Witness Date

Page 3 of 4 Subject's Initials_______

Delaware Rehabilitation Institute
201 Spencer Lab
Newark, DE 19716
302-831-3466

PHOTO RELEASE FORM

By signing this form, you agree to the use and publication of your photograph(s) in whole or in part in any Delaware Rehabilitation Institute media and publications. These photograph(s) may be published, reproduced, exhibited, copyrighted, and used in and published anywhere in the world in connection with The Delaware Rehabilitation Institute without further consent from or payment to you. You hereby waive any such uses of publications, including claims relating to rights of privacy, rights of publicity, confidentiality, copyright, and any other claims, against The Delaware Rehabilitation Institute, its licensees, and assigns, or any other person or entity.

____________________	______________________________
Signature	Description of Pictures or Videos Taken
____________________	______________________________
Print Name	Signature of Individual Taking Picture
____________________	__________________
Date	Date

Page 4 of 4 Subject's Initials_______

B.2 Semitendinosus Assessment Consent Form

UD IRB Approval from 08/12/2014 to 09/11/2015

UNIVERSITY OF DELAWARE
POSTURE AND MOVEMENT BIOMECHANICS LABORATORY
INFORMED CONSENT FORM

Study Title: Tissue characteristic change with contraction.

Principal Investigators: Thomas S. Buchanan PhD, Stephen Suydam, MSc

PURPOSE AND BACKGROUND

You are being asked, along with approximately forty other subjects, to participate in a study that will establish soft tissue properties using ultrasound imaging and a vibrating device. Ultrasound images of your leg muscles and tendons will be recorded while the device vibrates along the tissue. Additionally, you will be asked to perform seated tasks to evaluate your leg strength to compare to the soft tissue properties. The results of this study may provide a new technique for establishing muscle and tendon properties to aid clinicians in diagnosis and healing progression.

Participation in this research study is entirely voluntary and refusal to participate will involve no penalty. You may withdraw from the study at any time. You must be between the ages of 14 and 70 years of age to participate in this study and either; have sustained an Achilles tear and have been non-operatively treated, be 12 weeks post ACL reconstruction, or have no previous injuries to your knee, lower leg, and ankle. In addition, you must have little to no pain your leg muscles or tendons during contraction.

All testing will take place in the Posture and Movement Biomechanics Laboratory at the University of Delaware. The entire testing session will take approximately 1 hour to complete.

PROCEDURES

An ultrasound device will take images of your leg muscles and tendons with your leg fixed at a specific angle while an actuator vibrates on top of the tissue being measured. To evaluate strength, you will elicit a maximum flexion and extension contraction of your leg at various angles and angular velocities. Strength tests will be repeated across several muscles and will be repeated for the opposite leg.

Risks/Discomforts

The risks associated with participation in this study are minimal. If you have an allergy to any gels or lubricants, you may be excluded from the trial. Following testing your muscles may feel as if you exercised. If you are injured during research procedures, you will be offered first aid at no cost to you. If you need additional medical treatment, the cost of this treatment will be your responsibility or that of your third-party payer (for example, your health insurance). By signing this document you are not waiving any

Page 1 of 4 Subject's Initials_______

rights that you may have if injury was the result of negligence of the university or its investigators.

Benefits

The results of this study may allow for clinicians to determine muscle and tendon parameters through a new analysis technique; however there will be no direct benefit to the current participants.

Compensation

You will receive $50 for participation in this research study

Confidentiality and Records

All subjects will be identified by number only. Only the investigators will have access to the data. Neither your name nor any identifying information will be used in publication or presentation resulting from this study. A statistical report which may include pictures that do not identify you may be disclosed in a scientific paper. Photographs are optional and can be refused with no consequence to the subject or the data. Data will be electronically encrypted and archived indefinitely. There will be no identifying information of you stored with the data.

Subject's Initials_______

Study Title: Tissue characteristic change with contraction.

Principal Investigators:

Thomas S. Buchanan, PhD
Stephen M. Suydam, MSc

If you have any questions concerning the rights of individuals who agree to participate in research, you may contact the Chair of University of Delaware IRB, at (302) 831-2137.

Further questions regarding this study may be addressed to:
Thomas S. Buchanan, PhD
(302) 831-2401
Delaware Rehabilitation Institute
Department of Mechanical Engineering
Center for Biomedical Engineering Research

Subjects Statement:

I have read this consent form and have discussed the procedure described above with the principal investigator. I have been given the opportunity to ask questions regarding this study, and they have been answered to my satisfaction.

In the case that I am injured or experience an acute medical emergency during the study, I will be provided with immediate first aid. Any additional care will be at my own expense. I have been fully informed of the above described procedures, with its possible risks and benefits, and I hereby consent to the procedures above.

______________________________ ________________________________
Subject's Name Subject's Signature Date

______________________________ ________________________________
Witness Date Parental Guardian's Signature

 Subject's Initials_______

UD IRB Approval from 08/12/2014 to 09/11/2015

UNIVERSITY OF DELAWARE

Delaware Rehabilitation Institute
201 Spencer Lab
Newark, DE 19716
302-831-3466

PHOTO RELEASE FORM

By signing this form, you agree to the use and publication of your photograph(s) in whole or in part in any Delaware Rehabilitation Institute media and publications. These photograph(s) may be published, reproduced, exhibited, copyrighted, and used in and published anywhere in the world in connection with The Delaware Rehabilitation Institute without further consent from or payment to you. You hereby waive any such uses of publications, including claims relating to rights of privacy, rights of publicity, confidentiality, copyright, and any other claims, against The Delaware Rehabilitation Institute, its licensees, and assigns, or any other person or entity.

____________________	______________________________
Signature	Description of Pictures or Videos Taken
____________________	______________________________
Print Name	Signature of Individual Taking Picture
____________________	__________________
Date	Date

Page 4 of 4 Subject's Initials_______

B.3 Semitendinosus Assessment Assent Form

UD IRB Approval from 08/12/2014 to 09/11/2015

Minor Assent Document

Project Title: Tissue characteristic change with stimulus
Investigator: Stephen M Suydam

We are doing a research study about *knee injury and recovery*. A research study is a way to learn more about people. If you decide that you want to be part of this study, you will be asked to have an ultrasound measurement taken, push on a pad with the back of your leg and rotate your foot as much as you can. *This study should take about two hours.*

There are some things about this study you should know. First we will measure your height and weight. Then, using an ultrasound device, will take pictures of your leg tendons with your leg fixed at a specific angle while an small rubber shaker vibrates your leg. To measure leg strength, you will push as hard as you can on a pad that controls how fast your leg moves. Also to measure strength of a different muscle, we will Velcro your foot to a plate and have you turn it at a couple different speeds. All tests will be repeated for the other leg.

A benefit means that something good happens to you. There is no direct benefit for the participants of this study, but we believe this study will benefit society. We think the benefits of this study include helping us understand how the knee joint works after injuries which may help physical therapists create better treatments.

When we are finished with this study we will write a report about what was learned. This report will not include your name or that you were in the study.

If you are injured during the study, you will be offered first aid at no cost to you. If you need additional medical treatment, the cost of this treatment will be your responsibility or that of your insurance. By signing this document you are not waiving any rights that you may have if injury was the result of negligence of the university or its investigators.

You do not have to be in this study if you do not want to be. If you decide to stop after we begin, that's okay too. Your parents know about the study too. During the course of this study we may find more information that could be important to you. This may include information that may cause you to change your mind about participating in the study. We will notify you as soon as possible if any new information becomes available.

If you decide you want to be in this study, please sign your name.

I, ______________________________, want to be in this research study.

______________________________ (Sign your name here) ______ (Date)

B.4 Semitendinosus Assessment Parental Consent Form

UD IRB Approval from 08/12/2014 to 09/11/2015

Parental Informed Consent Document: Tissue characteristic changes with stimulus

Primary Investigator: Stephen M Suydam

INTRODUCTION
Your child is being invited to join a research study to look at knee muscle recovery after anterior cruciate ligament injury. Please take whatever time you need to discuss the study with your family and friends, or anyone else you wish. The decision to let your child join, or not to join, is up to you.
In this research study, we are investigating knee joint stability after ACL injury and reconstruction.

The goal of this study is to understand how leg flexion muscles change over time after ACL reconstructive surgery. ACL injuries are very common and costly injury. If the surgery was done with a hamstring autograft, then the tendon for one of the hamstring muscles was removed and, initially, that muscle no longer provides the ability to flex the leg. It has been shown that tendon grows back and the muscle can contribute to flexing the leg. This study is aimed to measure the amount of which that tendon has regrown. Additionally, strength measurements are collected to determine if the regrowth of that tendon leads to a stronger leg.

STUDY PROCEDURES
Your child will be asked to have ultrasound images taken of their hamstring tendon while a mechanical device is shaking on their leg. Additionally, they will be asked to perform leg flexion tasks and rotation tasks with their feet at a fixed speed. We think this will take him/her around 2 hours. This study will take place at the *bioengineering biomechanics movement lab at the STAR Health Sciences Complex.*

For this study we will first measure your child's height and weight. An ultrasound device will take images of his/her leg muscles and tendons with his/her leg fixed at a specific angle while an actuator vibrates on top of the tissue being measured. To evaluate strength, he/she will elicit a maximum flexion and extension contraction of his/her leg at various angles and angular velocities. Strength tests will be repeated across several muscles and will be repeated for the opposite leg.

The investigators may stop the study or take your child out of the study at any time they judge it is in your child's best interest. They may also remove your child from the study for various other reasons. They can do this without your consent. Your child may be removed from the study if he/she has a skin allergy to the ultrasound gel.

Your child can stop participating at any time. If your child stops he/she will not lose any benefits.

RISKS
The risks associated with participation in this study are minimal. If your child has an allergy to any gels or lubricants, he/she may be excluded from the trial. Following testing his/her muscles may feel as if he/she exercised.

If your child is injured during research procedures, he/she will be offered first aid at no cost to you. If he/she need additional medical treatment, the cost of this treatment will be your responsibility or that of your third-party payer (for example, your health insurance). By signing this document you are not waiving any rights that your child may have if injury was the result of negligence of the university or its investigators.

BENEFITS
While there is no direct benefit to the participant (your child) it is hoped that his/her participation in this project will improve our understanding of knee flexor recovery after knee injuries and subsequent reconstructive surgery. Information gained from this study may inform clinical rehabilitation and practice which may or may not be a direct benefit for participants but we believe will be a benefit to society.

UD IRB Approval from 08/12/2014 to 09/11/2015

CONFIDENTIALITY
Your child's name will not be used when data from this study are published. Every effort will be made to keep clinical records, research records, and other personal information confidential.

To keep information confidential and protected your child's personal information will be coded as an ID number. Any computer files that contain both ID number and personal information will be stored on a password protected computer. Only the investigator will have access to this computer and will be stored within an encrypted file. Any paperwork containing your child's personal information will be stored in a locked filing cabinet within our locked offices.

INCENTIVES
Your child will receive $50 for this study as compensation for their time and travel expenses.

RESEARCH PARTICIPANT RIGHTS
Participation in this study is voluntary. Your child has the right not to participate at all or to leave the study at any time. Deciding not to participate or choosing to leave the study will not result in any penalty or loss of benefits to which your child is entitled, and it will not harm his/her relationship with the University of Delaware Physical Therapy Clinic. During the course of this study we may find more information that could be important to you. This may include information that may cause you to change your mind about participating in the study. We will notify you as soon as possible if any new information becomes available.

If your child decides to leave the study please contact the primary investigator (see information below).

CONTACT INFORMATION
Call **Stephen Suydam** at 314-600-0396 or email **Stephen Suydam** at suydam@udel.edu if you have questions about the study, any problems, if your child experiences any unexpected physical or psychological discomforts, any injuries, or think that something unusual or unexpected is happening.

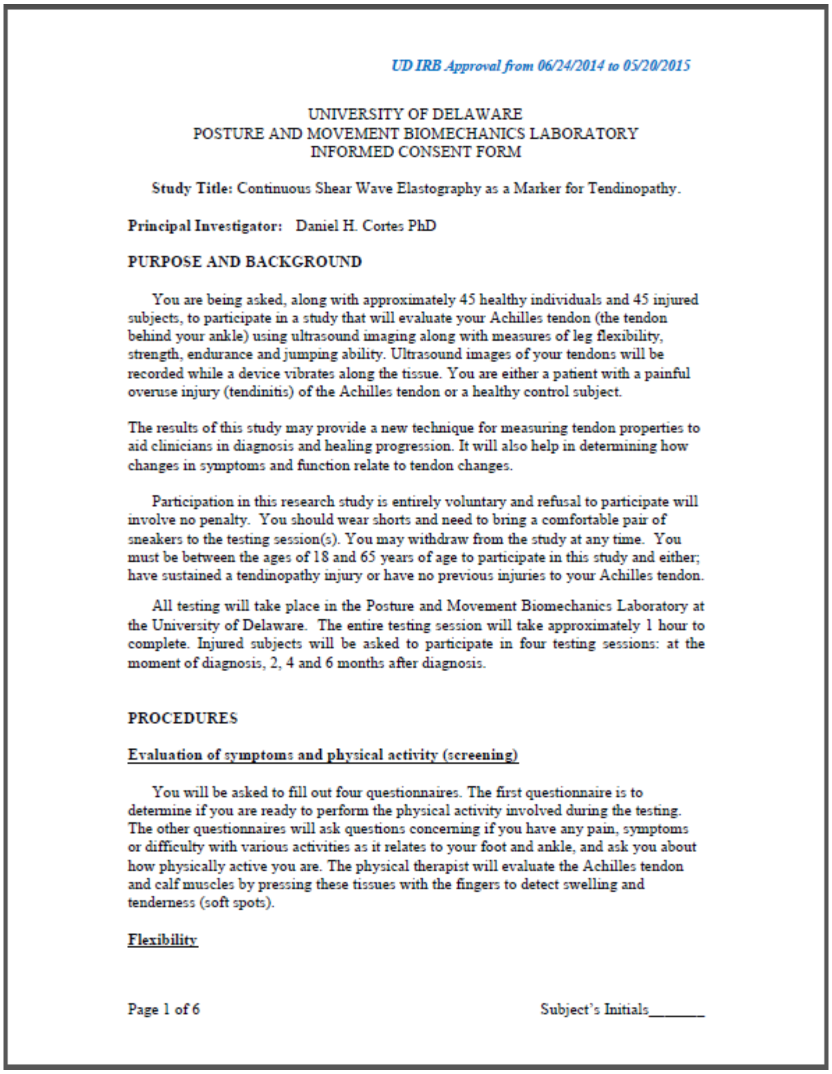
Permission for a Child to Participate in Research

As parent or legal guardian, I authorize ______________________________ (child's name) to become a participant in the research study described in this form.

Parent or Legal Guardian's Signature Date

____

B.5 Healthy Achilles Assessment Consent Form



UD IRB Approval from 06/24/2014 to 05/20/2015

UNIVERSITY OF DELAWARE
POSTURE AND MOVEMENT BIOMECHANICS LABORATORY
INFORMED CONSENT FORM

Study Title: Continuous Shear Wave Elastography as a Marker for Tendinopathy.

Principal Investigator: Daniel H. Cortes PhD

PURPOSE AND BACKGROUND

You are being asked, along with approximately 45 healthy individuals and 45 injured subjects, to participate in a study that will evaluate your Achilles tendon (the tendon behind your ankle) using ultrasound imaging along with measures of leg flexibility, strength, endurance and jumping ability. Ultrasound images of your tendons will be recorded while a device vibrates along the tissue. You are either a patient with a painful overuse injury (tendinitis) of the Achilles tendon or a healthy control subject.

The results of this study may provide a new technique for measuring tendon properties to aid clinicians in diagnosis and healing progression. It will also help in determining how changes in symptoms and function relate to tendon changes.

Participation in this research study is entirely voluntary and refusal to participate will involve no penalty. You should wear shorts and need to bring a comfortable pair of sneakers to the testing session(s). You may withdraw from the study at any time. You must be between the ages of 18 and 65 years of age to participate in this study and either; have sustained a tendinopathy injury or have no previous injuries to your Achilles tendon.

All testing will take place in the Posture and Movement Biomechanics Laboratory at the University of Delaware. The entire testing session will take approximately 1 hour to complete. Injured subjects will be asked to participate in four testing sessions: at the moment of diagnosis, 2, 4 and 6 months after diagnosis.

PROCEDURES

Evaluation of symptoms and physical activity (screening)

You will be asked to fill out four questionnaires. The first questionnaire is to determine if you are ready to perform the physical activity involved during the testing. The other questionnaires will ask questions concerning if you have any pain, symptoms or difficulty with various activities as it relates to your foot and ankle, and ask you about how physically active you are. The physical therapist will evaluate the Achilles tendon and calf muscles by pressing these tissues with the fingers to detect swelling and tenderness (soft spots).

Flexibility

Page 1 of 6 Subject's Initials_______

A hand held measuring tool will be used to measure your ankle flexibility. To measure your ankle flexibility you will be asked to sit with your leg straight out in front of you and you will move your ankle so that your toes go towards your head. You should move your ankle as far as you can. You will also be asked to perform this in standing similar to when stretching your calf.

Ultrasound imaging

You will be lying on your stomach while this is performed. An ultrasound device will take images of your Achilles tendons with your leg fixed at a specific angle. For some of the images a small pad will be placed on the skin to vibrate the tendon.

Strength Testing

Strength will be measured while you are seated in a device that controls your motion and measures how much force you can produce. Strength will be measured during dynamic and static tests.

Heel-rise Test (Going up and down onto your toes)

Your ability to get up onto your toes will be evaluated by having you stand on one leg with your foot on an incline. A thread will be attached to your heel with adhesive athletic tape. You will be asked to get up on to your toes as many and as high as you can until fatigued. You will hear a tic-toc sound that will help you keep the appropriate pace during the test.

Jump Testing

This testing protocol has been used in previous studies in healthy individuals and patients with Achilles tendon injury. You will attempt to perform three jump tests. All the evaluations will be performed on both your injured and uninjured leg. This is so we can have the healthy leg as comparison.

For the jump testing you will first perform a single-leg jump where you try to jump as high as you can and then land on the same leg. We would like for you to perform 3 jumps on each leg but you can stop at any time. For the second test you will jump down from an 8-inch box followed by jumping up again as high as you can. For the last jump test you will jump 25 jumps on one leg just like skipping rope. For this test we would like you to do this once on each leg. However you can stop at any time. For these tests our equipment will measure how high you jump on each leg. We will also ask you if you have any pain or discomfort during the testing. You should only perform as many jumps as you feel comfortable.

Risks/Discomforts

You may experience local muscle soreness or fatigue during testing. Following the testing your muscles may feel as if you have exercised vigorously. If you experience

Subject's Initials_______

excessive pain during the testing just let the investigator know and the test will be stopped immediately. If you have an allergy to any gels or lubricants, you may be excluded from the trial. The evaluator will pay close attention to avoid falls during the jumping tests. A first aid kit will be available in the room to attend minor injuries.

What if you are injured during your participation in the study?
If you are injured during research procedures, you will be offered first aid at no cost to you. If you need additional medical treatment, the cost of this treatment will be your responsibility or that of your third-party payer (for example, your health insurance). By signing this document you are not waiving any rights that you may have if injury was the result of negligence of the university or its investigators.

First aid procedures:
Basic first aid procedures will be provided by trained clinician(s) who will be present in the building during the test sessions. Specifically, in case of injury to the lower extremity elastic bandage compression and ice will be applied if appropriate based on a trained clinicians assessment. In case of fainting, the person will be laid down on their back and make sure he/she has plenty of fresh air. If the person vomits, he/she will be rolled his/her side to keep the windpipe clear. The incidence will be reported the person's doctor. In case of unconsciousness, the response of the person's will be evaluated by tapping the shoulder and asking, "Are you okay?" If there is no response, 9-1-1 will be called immediately. If the person is not breathing and has no pulse, CPR will be administrated by a trained clinician.

What if new information becomes available about the study?
During the course, we may find more information that could be important to you. This may include information that may cause you to change your mind about participating in the study. We will notify you as soon as possible if any new information becomes available.

Termination Criteria (Injured subjects only)
Your participation in the study will be terminated if you do not attend two consecutive evaluations or if you choose to have surgical treatment to the tendon after being recruited in the study.

<u>Benefits</u>

The results of this study may allow for clinicians to determine muscle and tendon parameters through a new analysis technique; however there will be no direct benefit to the current participants.

<u>Compensation</u>

You will receive no compensation for participation in this research study.

<u>Confidentiality and Records</u>

 Subject's Initials_______

All subjects will be identified by number only. Only the investigators will have access to the data. Neither your name nor any identifying information will be used in publication or presentation resulting from this study. A statistical report which may include pictures that do not identify you may be disclosed in a scientific paper. Photographs are optional and can be refused with no consequence to the subject or the data. Data will be electronically encrypted and archived indefinitely. There will be no identifying information of you stored with the data.

Subject's Initials_______

Study Title: Continuous Shear Wave Elastography as a Marker for Tendinopathy.

Principal Investigator:

Daniel H. Cortes, PhD

If you have any questions concerning the rights of individuals who agree to participate in research, you may contact the Chair of University of Delaware IRB, at (302) 831-2137.

Further questions regarding this study may be addressed to:
Daniel H. Cortes, PhD
(302) 831-7135
Biomedical Engineering

Subjects Statement:

I have read this consent form and have discussed the procedure described above with the principal investigator. I have been given the opportunity to ask questions regarding this study, and they have been answered to my satisfaction.

In the case that I am injured or experience an acute medical emergency during the study, I will be provided with immediate first aid. Any additional care will be at my own expense. I have been fully informed of the above described procedures, with its possible risks and benefits, and I hereby consent to the procedures above.

_____________________________ _________________________________
Subject's Name Subject's Signature Date

____
Witness Date

Delaware Rehabilitation Institute
540 S College Ave
Newark, DE 19713

PHOTO AND VIDEO RELEASE FORM

Photographs and videos may be taken during the test. These photographs and videos may be used for publications, presentations in scientific meetings and educational purposes.. Regardless the use, your identity will not be revealed. If you accept photographs or videos to be taken during your test session, please sign below.

____________________ Signature	______________________________ Description of Pictures or Videos Taken
____________________ Print Name	______________________________ Signature of Individual Taking Picture
____________________ Date	__________________ Date

Appendix C

INDIVIDUAL RECOVERY INFORMATION

SWE-ID	Age	Gender	Time Post Op	Surgeon	Recovery
SWE001	22	M	13	1	52.7%
SWE002	24	M	24	2	58.8%
SWE003	25	M	9	3	24.4%
SWE004	22	M	6	4	35.3%
SWE005	23	F	22	5	88.3%
SWE006	20	M	21	3	121.0%
SWE007	18	M	9	3	44.1%
SWE008	20	M	9	6	46.7%
SWE010	21	F	10	2	86.2%
SWE011	19	F	10	3	34.4%
SWE012	23	M	16	7	-
SWE013	27	M	8	3	75.4%
SWE014	18	M	14	3	79.8%