SEMITENDINOSUS TENDON PREDICTORS FOR SUCCESSFUL OUTCOMES POST-ACL RECONSTRUCTION

by

Clara Amat Fernandez

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Director of Thesis: Dr. Anthony S. Kulas

Major Department: Kinesiology

Abstract: The autologous semitendinosus (ST) tendon is currently the most common type of graft used for Anterior Cruciate Ligament (ACL) reconstructions. Even though most reconstructions enable patients to return to physical activity within ~6-9 months after surgery, long-term outcomes of the surgery must be improved. Previous literature suggests that there may be innate characteristics of the semitendinosus tendon that can potentially predict a negative outcome of the ACL reconstruction surgery. The hypothesis of this thesis is that donor tissue size and stiffness can distinguish between good or poor outcomes post-reconstruction. Specific to this thesis project, we hypothesize that individuals who have had multiple ACL reconstructions will have smaller and less stiff ST tendons compared to individuals with only one reconstruction, as well as, healthy controls. The purpose of this study is to determine if individuals with multiple ACL reconstructions have smaller ST muscle and tendon CSA, length, and lower ST stiffness compared to: 1) individuals who have had a single successful ACL reconstruction and 2) healthy controls.

Data were collected on 20 healthy control individuals, 12 individuals that underwent a single successful ACL reconstruction with an ST autograft, and 4 individuals that had undergone

multiple ACL reconstructions with at least one with an ST graft. Tendon cross- sectional area (CSA), length, and stiffness were measured using ultrasound (US) and shear wave elastography (SWE). In addition, all participants took the Tegner survey to assess sport- and activity-specific knee demands and all ACL reconstructed participants took the KOOS and Hamstring surveys to have an overall impression of their perceived function. One-way ANOVAs were used to assess differences across the three subject groups for ultrasound-based variables and the surveys scores. Moreover, a correlational analysis associating the KOOS scores to the US-based variables of the injured limb was also assessed.

The uninvolved limb of the ACL reconstructed was used as a surrogate of their injured leg prior to ACL reconstruction. The matched limbs of the healthy controls were not statistically different to the uninvolved limbs of the reconstructed individuals in any of the US-based variables or SWE. However, within the reconstructed subjects, ST tendon stiffness of the reconstructed limb was associated with the Quality of Life subscore of the KOOS survey ($r=0.561$, $p=0.024$).

The current thesis had several limitations that could have masked the results obtained: 1) the multiple reconstructed group had a small sample size, 2) the range of time since harvest was highly variable between groups and within individuals of the same groups, 3) it is possible that the US cannot differentiate between good and poor outcomes when using the uninvolved limb as the surrogate and 4) the definition for positive vs. poor outcomes solely based on the number of reconstructions did not take into account other factors that play a large role in determining positive outcomes from ACL reconstruction. Because the harvested ST tendon stiffness does not return to normal levels and because it is positively associated with knee quality of life, stiffness should be further studied in order to know the extent of its role in determining knee joint function post ACL reconstruction.

Semitendinosus Tendon Predictors for Successful Outcomes Post-ACL Reconstruction

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Clara Amat Fernandez

APPROVED BY:

DIRECTOR OF THESIS: ____

Anthony S. Kulas, PhD

COMMITTEE MEMBER: ____

Zachary J. Domire, PhD

COMMITTEE MEMBER: ____

Richard W. Willy, PT, PhD

CHAIR OF THE DEPARTMENT
OF KINESIOLOGY: ____

Stacey R. Altman, J. D.

DEAN OF THE
GRADUATE SCHOOL: ____

Paul J. Gemperline, PhD

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INTRODUCTION

Anterior Cruciate Injuries (ACL) injuries are one of the most common sports injuries in America. About 1 in 3000 Americans suffer an ACL rupture every year.[1] These injuries carry short and long-term consequences for the individual and for society. The life-long economic burden of ACL injuries and its associated consequences for society is estimated to be between $7.6 and $17.7 billion.[2] The worst consequence for ACL injured individuals is the development of early-onset knee osteoarthritis (OA). Knee OA is a debilitating condition that affects 50% of the individuals that have suffered an ACL injury 15 to 20 years after the injury.[3] OA increases the economic burden of ACL injuries because this condition carries its own set of consequences, including total knee replacement.[2] Whereas the normal aging population do not typically develop knee OA until they are 60 to 70 years old, individuals who suffer ACL injuries develop it when they are 30 to 50 years old. Because knee replacements last about 15-20 years,[4] Hence, there is a critical need to lessen the effects of the long-term consequences associated with suffering an ACL injury and the ensuing reconstruction.

Undergoing surgical ACL reconstruction allows injured individuals to resume their normal lives and return to play following a rehabilitation process of approximately 6-9 months. However, outcomes associated with reconstruction are not optimal.[5] Some of the consequences associated with reconstruction include decreased functionality of the knee, anterior knee pain, decreased muscle strength, graft failure, or revision surgery, as well as the already mentioned OA. There are three main types of grafts that are being currently used for the reconstruction: allografts, bone-patella tendon-bone (BPTB) autografts, and ST autografts.[1] Allografts have higher revision surgery rates, higher re-rupture rates, and higher incidence of OA than both groups of autografts.[1,6,7] The BPTB autograft has proved to be slightly superior to the ST autograft regarding revision surgery (2% revision rate for BPTB vs 5% revision rate for ST).[8] The ST autograft is proved to be better than the BPTB autograft regarding anterior knee pain, range of motion deficit, overall functional score of the knee, hop-test results, and incidence of OA.[1,5,9-11] Although both types of autografts have advantages and disadvantages and the literature is not consistent in recommending one type of graft above the other, the ST autograft is the most common graft used by clinicians, per the Norwegian ACL registry.[8] In addition, it would be advantageous to identify which graft is best suitable for individual patients, especially for young and healthy individuals where ST grafts are becoming the most common graft choice yet revision rates (considered to be poor clinical outcomes) are high relative to the other grafts in this population.

Patients younger than 18 years old have higher revision surgery rates.[12] Most of these patients have reconstruction surgery with a ST autograft, which has higher revision surgery rates than the BPTB autograft.[6,8] The reason of the higher revision surgery in this population compared to the normal population is the smaller ST graft size of young individuals. It has been clearly demonstrated that ST grafts with a larger diameter are more likely to survive compared with smaller ST grafts. Specifically, Mariscalco et al. set the threshold for ST graft successfulness at 8mm of intraoperative diameter.[12] In the same article it was also pointed out that individuals younger than 18 years old with a graft diameter of less than 8mm have an 18% revision surgery rate vs. 7% for the overall population. ST tendon graft length has also been correlated with the graft diameter (r=0.477).[13] Given that the tendon is quadrupled-bundled during the harvest, the longer the ST tendon the larger its size, and the better the overall graft is. Patient height and thigh circumference have also been correlated with intraoperative graft diameter (r=0.60).[14]

SWE is a novel and validated tool that has the capability to measure the viscoelastic properties of soft tissues.[15] At first, this technique was mostly used to detect tumors but more recently, this technique has been also applied to musculoskeletal soft tissues, such as muscles and tendons.[16] SWE produces a color-coded elastogram on top of the traditional B-mode US image that can be quantified to obtain the elasticity (or stiffness) of the tissue that is being visualized. This tool is fast, cost-effective and can yield important information about the properties of the tissue.

Most importantly, SWE can correctly differentiate between diseased and healthy Achilles and patellar tendons, which leads us to think that knowing stiffness of the ST is a critical factor to determine graft success.[17] To improve ACL reconstruction outcomes with ST tendon autografts, determining how the donor site size, length, and stiffness collectively influence post-surgical outcomes is a critical step towards this goal. Ultimately, improving ACL reconstruction and potential reconstruction graft choices would improve the revision surgery rates and potentially mitigate, the development of OA in the younger population.

Hypothesis

The overall hypothesis is that the donor ST tendon size, as determined by US, and stiffness, as determined by SWE, can distinguish between surgical success or failure in individuals who are long-term post-ACL reconstruction. Specific to this thesis project, we hypothesize that individuals who have had multiple ACL reconstructions innately have smaller and less stiff ST tendons compared to single-reconstructed individuals and healthy controls.

Purpose

The purpose of this study is to determine if individuals with multiple ACL reconstructions have smaller ST muscle and tendon CSA, length, and lower ST stiffness compared to: 1) individuals who have had a single successful ACL reconstruction and 2) healthy controls.

Significance

The significance of this study is to ultimately utilize a patient-oriented approach to determine optimal graft choices for ACL reconstruction. The use of US imaging technology and SWE to measure the material properties and characteristics of the tendon prior to surgery could have an immediate impact for orthopedic surgeons when deciding which option is the most appropriate for each subject. For instance, a surgeon could choose between patellar tendon or a ST tendon as a graft site based on tissue qualities prior to graft harvesting. Knowing the characteristics of the tendon prior to extracting it would allow for individualized and tailored treatment to the patient, which in turn, would improve the reconstruction outcomes and overall quality of life in the long-term. Compared to MRI, Ultrasound is a quick and inexpensive method that can relay results immediately and potentially determine donor site quality overall. This thesis will provide a first-step towards this goal through retrospectively determining if individuals with poor ACL reconstruction outcomes possess poorer ST tendon characteristics compared to individuals with successful ACL reconstruction outcomes.

Delimitations

- All injured participants had undergone ACL reconstruction with a ST autograft at least 6 months before participating in this study.
- The participants in the multiple ACL reconstruction group had at least two knee surgeries in the same leg to qualify for the current study.
- The participants in the single successful ACL reconstruction group will have had to have only one ACL surgery, the primary reconstruction.
- All participants were 18 years old or older.

Limitations

- This was a retrospective study. Because this is a retrospective study:
    - We cannot infer cause and effect
    - Measurements were taken from the non-operated leg to infer pre-surgical tendon characteristics of the surgical leg.
    - Tendon characteristics and quality might have changed since the reconstruction in both the non-surgical and surgical limbs.
- Ultrasound measurements of size and stiffness were limited to the accuracy and reproducibility of the instrument and the technician operating the equipment.

Assumptions

- The structure and the quality of the non-operative limb's tendon have not changed since the time of the surgery.
- The contralateral leg tendon has similar characteristics as the harvested tendon pre-surgical state.
- All instruments (ultrasound unit, anthropometric calipers, scales, etc) used are accurate and appropriately calibrated.

Operational definitions

- ACL: anterior cruciate ligament
- ACLr: anterior cruciate ligament reconstruction
- Ultrasound: imaging that uses high-frequency sound waves. It is used to measure tissue architecture.

- Shear Wave Elastography (SWE): ultrasound-based technique for real-time visualization of soft tissue stiffness.
- B-mode: A two-dimensional ultrasound presentation of echo-producing interfaces. Also known as 2-D mode.
- Graft: soft tissue, usually tendon, that it is placed in the knee to replace the torn ACL.
- Allograft: tendon tissue from a cadaver that it is used as a graft.
- Autograft: tendon tissue from the own patient that is harvested during the reconstruction surgery to replace the torn ACL.
- ST: semitendinosus tendon
- BPTB: bone-patellar-tendon-bone
- OA: osteoarthritis
- Osirix: medical imaging software that is used to measure the images obtained from B-mode ultrasound.

REVIEW OF LITERATURE

Introduction to the Review of the Literature

The purpose of this study is to determine if individuals with multiple ACL reconstructions have smaller ST muscle and tendon CSA, length, and lower ST stiffness compared to: 1) individuals who have had a single successful ACL reconstruction and 2) healthy controls.

This chapter will review the literature by examining, 1) ACL injuries and reconstruction, and the associated global long-term global health problem, 2) ACL reconstruction treatments and outcomes, 3) ACL reconstruction: review of graft choices, and 4) evidence to support graft architecture and function predicting surgical success.

ACL Injuries/Reconstruction and the Long-term Global Health Problem

1 out of every 3000 people in America suffers an ACL (anterior cruciate ligament) injury every year.[1] Since the implementation of Title IX in America and the increased involvement of women in sports, the incidence of ACL injuries has also increased.[3,18] The primary mechanism of ACL injury is typically considered "non-contact" and occurs during movements with quick changes in direction like landing from a jump or running and cutting.[18] Because of the non-contact mechanism being so prevalent, screening for and correcting how people move has been a focus of injury prevention research.[19-21] Despite injury prevention efforts, 100,000-300,000 ACL reconstructions are still performed annually in the United States alone.[1] While reconstructive surgery allows for athletes to return to play within 6-9 months, the long-term consequences associated with ACL injuries, and the following reconstructions, are still poor.[5]

One of the most devastating long-term consequences highly correlated with ALC injuries is knee osteoarthritis (OA). OA is described as a "common, age-related, group of disorders characterized by a degenerative loss of articular cartilage in a certain synovial joint associated with varying degrees of osteophyte formation, subchondral bone change, and synovitis".[3] OA is usually associated with pain in the affected area, stiffness in the joint, and functional impairments. OA changes the structure of the articular joint affected to such a degree that it can be easily diagnosed with radiography (X-ray). Radiographic signs of this debilitating condition are present within 10 years post ACL reconstruction.[3] After 10 to 20 years of the diagnosis of an ACL rupture with or without concomitant meniscal tear, an average of 50% of the patients develop knee OA with associated pain, functional impairment, and limited quality of life. It is even more significant that the people that develop OA due to a previous knee injury are usually young (between 30 and 50 years old) and, therefore, represent a large portion of the population that develops early-onset OA.[3] Interestingly, there is no difference in OA incidence in patients that underwent a surgical reconstruction vs. patients that were treated with rehabilitation only.[22]

The increased risk of developing OA also increases the total economic burden associated with ACL tears. The mean cost of an ACL reconstruction is $27,452 per patient, whereas the average cost of the rehabilitation strategy following an ACL tear is $32,276 per patient. In the long-term, the average lifetime cost for society is $38,121/patient for ACL reconstruction versus $88,538/patient when a rehabilitation only treatment is chosen. The lifetime burden of ACL injuries with the associated consequences is estimated to be $7.6 billion annually when reconstruction is chosen and $17.7 billion annually when treated with rehabilitation.[2] These estimations illustrate the importance of following a surgical treatment, especially in the long term, when the costs of knee OA or follow-up interventions are needed.

ACL injuries and their associated costs are worldwide clinical problem. Efforts to improve outcomes after reconstruction are necessary because the economic burden is high even when the

ACL is reconstructed and there are no further complications. Based on the high incidence of knee OA following ACL injuries in reconstructed and non-reconstructed individuals, the research focus should be on improving the outcomes of reconstruction to, ultimately, decrease the number of individuals that develop OA. Decreasing the incidence of OA in ACL injured people would immensely decrease the economic resources that are being destined to treating knee OA and other complications.

While the estimated economic burden associated with ACL reconstruction appears less compared to the rehabilitation only conservative strategy, reconstruction does not provide protection against OA[3]. In a study on soccer female players, at 12 years following ACL injury, in which 62% of the subjects had undergone ACL reconstruction, 75% of the players reported having symptoms that affected their knee quality of life, and 42% were considered to have symptomatic knee OA.[22] Even though, having an ACL reconstruction or choosing the rehabilitation route does not seem to influence the development of OA, it has been reported that maybe graft type does influence the development of OA. In a meta-analysis performed by Xie et al, they concluded that OA incidence was significantly higher in patients with patellar tendon autografts compared to patients with hamstring tendon autografts at least 5 years after reconstruction.[23] While OA affects a large portion of individual's post-ACL injury, regardless of whether the ACL is reconstructed, it is critical to improve outcomes associated with reconstruction to minimize the long-term individual and societal burdens associated with this injury.

ACL Reconstruction Treatments and Outcomes

After an ACL injury there can be two courses of action: perform surgery to repair the ligament or continue with a rehabilitative course of treatment without surgery. The standard surgery to reconstruct a torn ACL is a surgical reconstruction. The aim of the operation is to regain full function and stability of the injured leg after the rehabilitation process by removing the torn ligament and replacing it with a graft. The most widely used autograft tissues are the ipsilateral patellar tendon or the ipsilateral hamstring tendon. Both can be in the form of an allograft (tissue from a cadaver) or autograft (tissue from the own injured individual). This section of the literature review details these surgical techniques as well as the current state of the literature regarding outcomes associated with ACL reconstruction depending on graft type.

Anterior Cruciate Ligament Reconstruction

Common grafts used for ACL reconstruction

There are multiple graft choices to substitute the torn ligament during a primary ACL reconstruction. The graft options are: allografts, which come from cadavers, synthetic grafts, made from materials like carbon fibers or polyester, or autografts, which come from the patient itself.[1] There are also multiple options of autografts. The most commonly used autografts by surgeons currently are the bone-patella tendon-bone (BPTB) and hamstring tendon, more specifically the semitendinosus (ST) muscle tendon.[1,24] The different surgical techniques along with the different graft options to choose from make it difficult to compare what is the best graft option, even when looking at the post-surgical outcomes.

Every patient is different and, in every case the orthopedic surgeon has to make the decision of what type of graft to use based on his own expertise, personal preference, anthropometric measures of the patient, age of the patient, activity level of the patient, patient preference, as well as other considerations.

Autografts

The commonly used graft option is tissue autografts. As explained previously, autografts are tendons harvested from the patient itself at the moment of surgery. There are several options for autografts to reconstruct the ACL.[25] Most orthopedic surgeons use either the ipsilateral semitendinosus (ST) muscle tendon, which is sometimes used in combination with the gracilis muscle tendon, or the ipsilateral bone-patella tendon-bone graft (BPTB).[26]

Both types of autografts have advantages and disadvantages. The most important disadvantage of autografts is that, no matter which one the surgeon decides to use, the patient will have increased morbidity in the area where the graft was harvested. This means that, the patient will have to rehabilitate from the loss of that tendon as well as the already long rehabilitation process ahead for the ACL reconstruction. Having to create a new tendon has, in turn, some disadvantages that contribute when choosing the graft type. For example, increased anterior knee pain for the BPTB graft, or possible long-term decreased hamstring strength for the ST grafts.[1,23]

Outcomes associated with each graft type (BPTB and ST)

The two types of autografts that are most widely used currently are the BPTB graft and the ST graft. Even though they are both used, there has been a shift recently towards ST graft and a decline in BPTB graft.[27] Overall consensus as to which graft type is better is controversial and inconsistent in the literature. One of the reasons why this comparison is so complicated is that it is highly dependable of the subject pool (active vs non-active), age, the numbers of subjects, or the follow-up time to name a few. In the following section we will compare both types of grafts.

Muscle Strength

One of the outcomes of ACL reconstruction, no matter the graft type, is the strength deficit in the short and long-term. Most of the literature is consistent that there is no significant difference between BPTB and ST grafts on lower limb muscle strength deficits, but there is some literature that supports one of the two grafts. Condournet et al. found that there are strength deficits of 10% 2 years post-reconstruction with both graft types. They showed that the type of graft had an influence on the muscle strength deficit: for the extensor muscles the strength recovery was the same for both graft types but for the flexors, the deficits were significantly higher with the ST graft.[28] Aglietti et al. found that, at 2 years follow-up, the peak extension torque with a ST graft was significantly worse than with a BPTB graft.[10] Unlike Aglietti and colleagues, Feller et al. reported larger extension strength deficits with a BPTB graft than with a ST graft. Those deficits were only significant at high-speed movements and showed a trend towards significance in low speed movements.[29] It is important to mention that this study was performed 4 months after the reconstruction unlike most of the other studies, which focus on the long-term outcomes. It can be concluded that, despite the different methods of testing there is not a significant difference in knee extension strength between BPTB grafts and ST grafts but for knee extension strength the BPTB graft is superior to the ST graft.

Pain

It has been shown that ST grafts are better for preventing anterior knee pain or kneeling pain after reconstruction.[1,5] Leys at al, they showed that 74% of the patients with a ST graft had minimal kneeling pain or no anterior knee pain at all 15 years after the surgery, whereas 42% of the patients with BPTB graft reported moderate to greater kneeling pain. 2 years after reconstruction, Shaieb et al reported that, out of the 42% PBTB graft patients had anterior knee pain versus 20% of the ST graft patients.[9] Regarding general pain, 4 months post-surgery the ST graft reported 2.3/10 vs. 3.5/10 in the BPTB graft group.[29] However, this difference was not significant. Given the amount of literature comparing the outcomes of reconstruction using BPTB and ST grafts, it can be concluded that ST grafts are better when evaluating knee pain following ACL reconstruction at, both, short and long-term follow-up times.

Range of motion

Even though there aren't big differences in the literature regarding ROM deficits on the knee after ACL reconstruction, there are some studies that report better ROM on patients that had a ST graft rather than patients that had a BPTB graft.[10,11] Long-term, Leys et al showed that patients with BPTB grafts had greater extension range of motion deficits after 15 years than the patients that had a ST graft (94% of ST group with less than 3° of deficiency versus 79% of BPTB group with less than 3° of deficiency). For flexion, there was no difference between the two groups.[5]

Knee Laxity

The main goal of an ACL reconstruction is to restore stability to the knee. Therefore, stability should be a priority when choosing between a BPTB graft and a ST graft. Biau et al reported no significant difference between both grafts on the Lachman test, and the BPTB graft was reported to have a significantly decreased positive pivot-shift test, which is a clinical measure of knee stability.[30] Spindler reported increased laxity with ST graft in 3 of the 7 studies and no difference in the other 4 studies.[31] Long-term follow-up studies consistently show no difference in stability between BPTB autografts and ST autografts.[32]

Return to sports (frequency and activity level)

Returning to their previous activity level in the shortest amount of time possible is a key factor when deciding what type of graft to choose as the most affected individuals are athletes. Most of the literature is consistent that both graft types are similar in return to pre-injury activity level and percentage of people returning to sports. In a study performed by Gobbi et al. with 100 athletes that underwent ACL reconstruction and played sports, only 65% of the patients returned to their previous level of sport activity, 24% changed sports to a lower activity level and 11% of the athletes had ceased activity. Out of the 11 participants that were not able to return to sports 6 had a BPTB graft and 5 an ST graft.[33] It is important to note the very low percentage of athletes that were able to return to their previous activity level, only 65%, no matter graft type.

Patient Reported Function

There are not conclusive evidence in the literature that show which graft type is better regarding patient reported function. Leys et al., found significant differences between the ST and the BPTB graft in the IKDC Functional Assessment 15 years post-surgery (9.1/10 for the ST vs. 8.5/10 in the BPTB). In the same study, the Lysholm knee score didn't show significant differences between graft types. They did find some significant changes in functionality by measuring the single-leg hop test. In addition, they reported that 92% of the patients with a ST graft achieved ≥90 of the contralateral jump distance vs. 65% of the patients using a BPTB graft and 8% of the ST graft participants vs. 35% of the BPTB graft participants achieved between 75 and 89% of the contralateral jump distance. These findings were significant.[5]

Graft rupture or failure

As we have mentioned in this review of the literature, graft failure or re-injury of the contralateral leg is a very serious complication with people that suffer ACL injuries. It is important to identify if the graft choice can affect clinical failure or a future re-injury of the knee in the future. Leys et al. reported that 15 years post-reconstruction patients with ST grafts had a higher incidence of ACL graft rupture than patients with BPTB grafts (17% (n=15) for the ST graft group vs. 8% (n=7) in the BPTB group), although it wasn't a significant difference.[5] Pinczewski et al. reported no significant differences in rate of graft ruptures between ST graft individuals and BPTB in a 10-year follow-up study (p=0.24).[34] Given the results reported in the literature, it is unclear which graft type is better, although in all studies there were more ruptures in the ST group.

Revision Rates

Revision surgery is often used as a marker of a poor outcome post-ACL reconstruction. Most of the patients that have to undergo revision surgery are young athletes that did not achieve enough stability from the initial surgery and need to be able to achieve full function of their knee in order to return to sports.[6] For these type of patients it is important to know which type of graft is more effective regarding this particular aspect and it is also important to find a way to decrease the rates of revision surgery, especially because the patient-oriented outcomes of ACL revision surgery are worse than primary ACL surgery.[35] In a cohort study that included individuals that underwent revision surgery they reported that out of the patients that had an autograft, 42% of the revisions were from ST grafts and 28% from BPTB grafts.[6] Using the Norwegian Arthroplasty Registry, Persson et al. identified 12,643 patients that had undergone ACL reconstruction with a ST autograft or a BPTB autograft. They found that the rate of revision for ST grafts was 5.1% and 2.1% for BPTB grafts.[8] Herrington et al. reported that patients with ST grafts had more than patients that had received BPTB grafts (10 patients with an ST graft vs. 6 patients with a BPTB graft).[27] Hence, it is clear that BTPB grafts are more effective in this particular aspect than ST grafts.

OA using BPTB or ST grafts

It has been discussed in this literature review that OA is an important long-term negative outcome following ACL reconstruction. Understanding which type of graft has better statistics regarding the onset of OA should be an important factor for the surgeon and the patient when deciding which graft to choose. In a long-term follow-up study that took radiographic images at 2, 5, 7, and 10 years after reconstruction, they found that the BPTB group had significantly higher incidence of OA at 5 and 10 years after surgery. At 2 years post-surgery, 99% of the ST group patients were A-grade (no radiographic signs of OA) on the Kellgren-Lawrence Scale vs. 96% on the BPTB group. At 7 years post-surgery, 91% of the ST group patients were A-grade vs. only 66% of the BPTB group.[36] It has been clearly shown that the long-term outcome regarding OA is significantly better with a ST graft rather than a BPTB graft.

Conclusions

When choosing the type of graft a lot of factors have to be considered and the literature is not consistent in recommending one type of graft. Most studies and review articles conclude that there isn't a significant difference between both graft types although the most used autograft type is the ST graft. In a cohort study by Persson et al. in Norway, out of 12,643 patients, only 27% decided to get a BPTB autograft, whereas 73% opted for an ST autograft.[8] Given that the most used graft type is the ST graft, and that the ST graft also has a higher revision surgery rate, it is crucial that we understand what factors are important to determine possible revision surgeries following an ACL reconstruction.

Outcomes Associated with Allografts

Even though allografts are not currently used as much as autografts, they have some advantages, which make them relevant for discussion. Some of the advantages: they avoid donor-site morbidity, there are many grafts available in many different sizes, decreased surgery time, and shorter recovery time. The disadvantages are that they are more costly: to harvest an allograft, you need a cadaver, whereas to harvest an autograft there are no extra monetary costs, and that there is the potential risk of an immunogenic reaction or a transmitted disease.[25]

Altogether, the outcomes associated with ACL reconstruction are not positive. The failure and revision rates of ACL reconstruction are higher using allografts than using autografts and are also worse in younger populations than older populations. Pallis et al showed that of 120 cadets that entered the US Military Academy, the grafts of the cadets that had an ACL reconstruction with an allograft were 7.7 times more likely to fail than the ones that received autografts (ST or BPTB autografts).[37] Another category in which allografts outcomes are worse than autografts is the rate of revision surgery. Lind et al. found that revision reconstructions performed with allografts were twice more likely than reconstructions performed with autografts.[6] Finally, the incidence rate of OA is also higher in patients with an allograft than with an autograft. On a study done by Tian et al., the grade of OA as determined by the radiological results showed that 45% of the patients with an allografts decreased one or more grades in that classification scale, whereas only 15% of the autograft patients decreased one or more grade in the same classification scale.[7]

Because allografts are not recommended and the better outcome between a BPTB or ST graft is unknown before the surgery, it would be positive if the viability of the autograft could be determined before surgery so that the best possible graft choice could be better determined and maximize outcomes post ACL reconstruction. In addition, improving the outcomes associated with ACL reconstruction using ST autografts is important based on its widespread use in the young and active population. In order to improve the outcomes of the surgery using ST autografts, we need to know more about the intrinsic qualities of the ST tendon that may predispose an individual for poor outcomes.

By having the ability to better understand the intrinsic qualities and properties of the hamstring muscles, and decide when not to choose the ST as graft, we will increase the probability of a better short-term and long-term outcome for those individuals.

Evidence to support graft architecture and function predicting surgical success

Based on evidence supporting that: 1) ST autografts are the most common graft and 2) ST autografts have higher revision rates among young individuals compared to BPTB autografts; we need to determine if there are characteristics associated with individuals with ST grafts that could potentially predict poor outcomes.

Graft characteristics predicts ACL reconstruction outcome

It has been shown by multiple groups that the architecture of the graft, especially the diameter of the graft, is important to determine the viability of the graft and the short and long outcome of the reconstruction surgery[12-14,38]. Mariscalco et al. performed a study in which they used the MOON database to identify patients that had a ST graft or ST graft augmented with the gracilis tendon. They used revision surgery as a marker of graft failure and they also used patient-reported outcome scores to evaluate if the surgery was successful or not. Their major finding was that ST graft (with a gracilis augmentation in some cases) larger that 8mm of diameter are more likely to have a good outcome 2 years after surgery than a graft that was smaller than 8mm. Out of the 263 participants of that study none of the 64 individuals with a graft larger than 8mm had to undergo revision surgery, whereas 14 of the 199 individuals with a graft size of 8mm or smaller had to undergo revision surgery. Also, a 1mm increase in graft size was correlated with a 3.4-point improvement in the patient-reported outcome IKDC score. In addition, they observed an interaction between age and graft size in determining revision surgery rate. The revision rate of patients 18 years-old or younger was 15.3%, while the overall revision rate for the total cohort was 5.3%. It is also important to notice that only 64 of the 263 subjects had a graft diameter of larger than 8mm and, therefore, even though graft size is indicative with better overall outcomes, most of individual's grafts are 8mm or less in diameter (199/263). This is not the only study that revealed that larger grafts are protective of future revision surgeries: Park et al. showed that patients with a graft size larger than 8mm had better results than patients with grafts smaller than 8mm (p=0.043). Magnussen et al. reported that 7% of their subjects underwent revision surgery at an average of 12 months follow-up, and all of those had a diameter length of 8mm or less than 8mm. Their results also match the two studies mentioned: they found correlation between graft diameter size of 8mm and lower to none revision surgeries rates.[38] There does appear to be consensus in the literature regarding graft diameter as a key characteristic of the graft that predicts graft failure.

Anthropometric measures are correlated with final graft diameter

Given that intraoperative graft diameter is such a critical measure for a positive outcome, several groups have assessed how different anthropometric measurements are related to intraoperative gracilis, ST tendon, and graft diameter measurements. Park and colleagues showed that height, weight, BMI, sex and athlete vs. non-athlete are related with the diameter of the graft.[13] Height of the patient, weight and sex are strongly correlated with final diameter graft (0.477, 0.427, and -0.432 respectively).

Because the tendon is bundled during the harvest we can assume that the length of the tendon is critical for the quality of the ST as a graft. Treme and colleagues have shown strong correlations between the height of the patient and leg length and the intraoperative length of the graft (*r*=0.69 and r=0.67 respectively) as well as weight, BMI, and thigh circumference and final graft diameter (r= 0.64, r=0.62, and r=0.60 respectively).[14]

Although anthropometric measurements and basic demographics information are related to graft diameter and length, they are not related to failure rates. According to Park et al, the only factor that is significantly related to failure rates is graft diameter.[13] Therefore, even though anthropometric measurements could predict graft diameter, they can't predict which grafts are more likely to fail and which individuals are more at risk for a revision surgery.

Cross sectional area relating to ACLR success

Final graft diameter is measured in the operating room, when the tendon is being harvested. Having a method to determine graft size preoperatively would provide important information to the medical staff. Measuring the CSA of the ST and the gracilis tendon (GT) preoperatively using MRI imaging has been positively correlated with intraoperative measurements of the ST (0.53), GT (0.56), and graft size (0.53).[39] In a prospective study by Wernecke et al., they found that the total CSA of both, the ST and GT, had to be 26.54 mm^2 to have a diameter of at least 7mm intraoperative.[39] Erquicia et al. assessed MRI and ultrasound (US) with the intraoperative graft diameter. Pearson R correlation of this method was 0.56. CSA of the ST tendon was 9.5±1.7mm^2 and CSA of the gracilis (GR) tendon was 6.2±0.9mm^2 when measured with the US. For the MRI measurements GR+ST were 18.9±2.8 mm^2, and the intraclass correlation was 0.92 (95% CI, 0.82-0.95). Using the US method. Even though the final diameter of the graft cannot be measured until the operation, in this study they were able to predict if the graft would have a >8mm diameter in 80.8% of the patients and in 100% of the cases for patients that had a true graft diameter of <8mm using the ultrasound. The MRI has a sensitivity of 96.2% for those individuals that had a true graft diameter of >8mm and 100% for the grafts <8mm.[40] Galanis et al. obtained similar correlations using both methods, US and MRI. In that study, the correlations were 0.807 for the CSA with the MRI and graft diameter and 0.612 for the same measurement using the US.[41] Surprisingly, Wernecke et al. recently published a study contradicting previous studies that correlated tendon diameter with revision rates. In this study graft diameter was measured during the operation, the patients followed-up two years post-surgery. Number of revisions, and several orthopedic scores and a physical activity score were assessed. There was not relationship between autograft size and revision surgery or between functional scores and ST graft size. However, they did find a negative linear relationship between age and revision surgery.[42] In all, we can confirm that a CSA of $8mm^2$ for the ST tendon is the critical value in which there seem to be no failures. In addition, several studies have demonstrated the relationship between preoperative measurements and operative measurements. All these facts leads us to believe that individualized treatment for ACLR is not only possible but necessary.

Stiffness as a determinant of tendon health.

Evidence suggests that tendon stiffness may predict poor outcomes following ACL reconstruction. Tendon stiffness is traditionally measured *ex vivo* by clamping the ends of the tendon and applying tension to the tendon until such tendon ruptures.[43] However, ultrasound (US) shear wave elastography (SWE) has emerged as a validated novel technology which enhances the qualities of ultrasound (US) to obtain tissue characteristics and properties: it can measure the viscoelastic properties of soft tissues. SWE is a dynamic method that detects the velocity of a transverse wave propagating through a soft tissue then calculating the shear modulus of that tissue.[44,45] Other traditional methods to measure tissue stiffness, use the dynamometer to passively move the limb through a range of motion and calculate the stiffness from the resistance of the muscle group to that movement also known as passive torque.[46] SWE allows to measure the material properties of a very specific region of the structure rather than the whole muscle group.[44]

Most importantly, SWE has the capability to distinguish changes in the viscoelasticity of the material in healthy and diseased tendons.[45]

Aubry et al. showed that Achilles tendons with mid-portion tendinopathies had significantly lower stiffness than normal Achilles tendons (15.75 m/sec for the normal tendons vs. 14.53 m/sec for the tendons with a tendinopathy).[47] Dirrichs et al. also measured the stiffness of the Achilles tendon using ultrasound SWE. They show that symptomatic tendons have lower SWE values than healthy tendons (60.3kPa vs. 185kPa).[17] Most importantly, Zhang et al. found significant differences in Achilles tendon elastography 12 weeks, 24 weeks and 48 weeks after a surgical repair (P=.000). Interestingly, the shear modulus increased between each time point, leading us to think that as the tendon repairs the shear modulus increases.[48] Botangliuou et al. found that the patella tendon stiffness of the operated knee in people that had had closed wedge high tibial osteotomy surgery was higher than in the non-operated leg (74.1kPa±24.7 vs. 47.7kPa±15.3), and both of those were higher than the stiffness measurements of the healthy controls (33.5kPa) [49]. It has been identified that elastography can assess Achilles tendinopathies compared with standard US imaging techniques. In a study performed in Achilles tendons from cadavers by Klauser et al., they found that elastography depicted histologic degeneration better than US did. According to this study, loss of collagen structure, fatty infiltration, capillary proliferation, loss of fiber integrity in Achilles tendon can be detected with elastography.[50] Given the results of these studies, we believe that shear wave elastography can differentiate between a tendon that has or has had a tendinopathy and a tendon that hasn't. To our knowledge, Cortes et al. has been the only one to publish data on SWE in the ST tendon. In that project, they used different methodology than the one that is available in our lab; however SWE clearly differentiated between the new tendon after being harvested for an ACL reconstruction and the contralateral tendon. This leads us to think that healthy tendons have higher SWE values than non-healthy tendons.[44]

Knowing the stiffness of the semitendinosus tendon might be critical to assess the viability of the graft after the reconstruction. By using SWE we could measure the stiffness of the tendon prior to the surgery and avoid future problems with the graft. This approach would reassure the surgeon that the tissue has a good quality, or in the opposite, that it has poor quality and, therefore, other options need to be explored. While the significance of this work points towards prospective screening techniques using ultrasound imaging and ultrasound elastography, a critical first step towards this goal is to determine retrospectively if ST tendon size, length, and stiffness can differentiate between individuals with a poor clinical outcome (i.e. revisions following ACLR) to those with a positive clinical outcome (ACLR without revision).

Hypothesis

The overall hypothesis is that the donor ST tendon size, as determined by US, and stiffness, as determined by SWE, can distinguish between surgical success or failure in individuals who are long-term post-ACL reconstruction. Specific to this thesis project, we hypothesize that individuals who have had multiple ACL reconstructions innately have smaller and less stiff ST tendons compared to single-reconstructed individuals and healthy controls.

Purpose

The purpose of this study is to determine if individuals with multiple ACL reconstructions have smaller ST muscle and tendon CSA, length, and lower ST stiffness compared to: 1) individuals who have had a single successful ACL reconstruction and 2) healthy controls.

Summary

In conclusion, ACL injuries have some negative long-term outcomes, and most times the individuals don’t return at their previous situation and/or activity level. Hence, it is critical that the research focus is centered in improving the outcomes associated with reconstructions. One way in which the outcomes can be improved is by having more information about the graft that will be harvested from the patient so the orthopedic surgeon can make an informed decision on what type of graft is better for each patient. We propose that individuals that have had an unsuccessful graft will have a smaller CSA of the ST, shorter ST, and less stiff ST than individuals that had a successful reconstruction. Knowing this information would be a first step into a prospective study to determine the acceptable ranges for each one of the variables in which the graft is successful or fail.

METHODS

Study Design

This research study was a retrospective study. Because ACL injuries and, even more so, ACL revision surgeries or graft failures are fairly uncommon in a normal population, it was not be feasible to do a prospective study and collect data on the number of patients needed to have a large enough sample size to see significant results. Thus, we collected data after the participants had had an ACL reconstruction or a second ACL surgery. Given the fact that we examined the ST tendons once they have already been removed we collected data on the uninvolved leg and on the regenerating tendon. According to Williams et al. ST and gracilis tendon regeneration occurs at various degrees, whereas it appears some patients regenerate well and show tendon hypertrophy, others show poor tendon regeneration or no regeneration at all.[51] Moreover, Suydam et al., found significant differences in the shear modulus between the injured limb and the uninvolved limb ranging from 6 months to 24 months post-operatively (129.4kPa for the uninvolved vs. 73.0 KPa for the involved limb).[52] Hence, in our present study we cannot assume that the regenerated tendon has the same characteristics as the stripped tendon. We have performed a symmetry analysis of the ST and gracilis tendons to understand the degree of symmetry in a healthy population.

Participant characteristics

12 adult patients (4 men, 8 women) that had suffered an ACL tear and had a single ACL reconstruction with a ST (or ST + gracilis) autograft and, 4 patients (2 men, 2 women) that had suffered an ACL tear and had had revision surgery or a hamstring graft failure in addition to an ACL reconstruction with a ST (or ST+ gracilis) autograft were recruited from the student population. All the ACL reconstructed patients had the primary ACL reconstruction at least 1 year

prior to participating in the study. 20 healthy adults (7 men, 13 women) with no history of knee or hamstring surgeries or injuries were recruited as controls. The healthy controls were matched by age, sex, and physical activity with the ACL participants to the best of our abilities.

Inclusion criteria

Control group

1. Apparently recreationally active healthy adults with no history of knee or hamstring surgeries or injuries.

ACL successful reconstruction group

1. ACL injury in the past 6 months to 10 years.
2. ACL reconstruction with a ST (or ST + gracilis) autograft.
3. Medically cleared by their doctor.
4. Visual regeneration and function of the ST tendon as determined by real time US.

Multiple ACL reconstruction group

1. ACL injury in the past 6 months to 10 years.
2. ACL reconstruction with a ST (or ST + gracilis) autograft.
3. Multiple ACL reconstructions in the ipsilateral knee.
4. Medically cleared by their doctor.
5. Visual regeneration and function of the ST tendon as determined by real time US.

Exclusion criteria

Control group

1. Previous knee or hamstring injuries or surgeries

2. Lack of physical activity

ACL successful reconstruction group

1. ACL reconstruction with an allograft, synthetic graft or BPTB autograft.
2. Reconstruction surgery less than 6 months prior to the participation of the study.
3. ACL revision surgery.
4. No visible ST tendon regeneration in the US.

Multiple ACL reconstruction group

1. ACL reconstruction with an allograft, synthetic graft or BPTB autograft.
2. Reconstruction surgery less than 6 months prior to the participation of the study.
3. Single ACL reconstruction.
4. No visible ST tendon regeneration in the US.

Equipment

ST tendon measurements were taken with an ultrasound device (AIXPLORER MultiWave, SuperSonic Imagine S.A., France). The measurements were taken using the B-mode, and SWE (Shear Wave Elastography) mode, both using the MSK setting, and the 15-4L probe. Participant height, weight, leg length, and femoral length were measured at the beginning of each data collection using the provided equipment in the laboratory. For data reduction of the B-mode images OsiriX MD™ imaging software (Pixmeo Inc., Geneva, Switzerland) was used to make the measurements for all the variables.

Measurement protocol

Participants were identified from the student population of the East Carolina University. They were divided in three groups: individuals that had a successful ACL reconstruction, individuals that had had multiple ACL reconstructions in one knee, and healthy controls. The ultrasound protocol for all groups was the same. Both ACL groups took the Hamstring Function Survey **(Appendix A)** and the Knee injury and Osteoarthritis Outcome Score (KOOS) survey **(Appendix B)**.

Upon subject arrival, the subject signed the Informed Consent Document (**Appendix C)**, completed the Hamstring Function Survey, the KOOS Survey, and the Tegner Survey **(Appendix D)**. Age, height, weight, leg dominance, resistance training history, leg length, and femoral length of the participant were recorded. Date of last menses and the use of hormonal contraception was recorded from all the women participants. Date of the injury/ies, surgery/ies, injured leg, and mechanism of injury were recorded from all ACL reconstructed participants. All ultrasound based measurements were taken with the participant laying prone on the treatment table with the knee fully extended or with a 30 degree of knee flexion. The variables measured were CSA of the ST tendon at the epicondyle with the leg at 30° of knee flexion, ST tendon CSA at 90° of knee flexion, CSA of the gracilis at the epicondyle, length of the pure ST tendon, stiffness of the ST muscle and stiffness of the ST tendon. For the CSA of the ST at 90°, images were taken placing the probe at the cusp of the calf and then move the probe medially until the ST tendon was in the middle of the field of view. To quantify the material properties of the tendon, SWE mode was used on the ultrasound device, placing the probe on top of the tendon longitudinally just proximal to the medial epicondyle. All CSA images were taken in the transversal plane and the length images of the ST and SWE images were taken longitudinally. This protocol was tested on both legs.

This method had been previously used and validated by using the MRI technique. CSA measurements have been correlated with final graft diameter using ultrasound (US).[40,41,53] Erquicia et al., measured CSA with both, US and MRI. There is a positive correlation between final graft diameter and CSA using both techniques with the correlation using the MRI technique being stronger (0.86) than the correlation from the US technique (0.506). Although the correlation using US is not strong, it predicted graft diameter correctly.[40] The US is a more economically affordable and fast technique than using an MRI and can correctly predict graft size.

Pilot Results: Reliability Estimates from Healthy Controls

Twenty-two healthy adults (Table 1) with no history of knee or hamstring surgeries and/or injuries have been recruited from the student population at East Carolina University to serve as a preliminary control, assess limb asymmetry of the tendon in a healthy population and establish the reliability of the ultrasound measurements and reliability of the user. Reliability and quality of the data collected from the healthy individuals was be used to make a better decision on the variable that will be evaluated on the ACL reconstructed and ACL reconstructed + revised groups.

The protocol for the healthy individuals group was as it follows: upon subject arrival, the subject signed the informed consent and height and weight were taken. All the measurements were taken with the participant lying in a prone position with the leg fully extended or placed at 30° of knee flexion following the protocol that Galanis et al. used in their project.[41] The variables measured for the healthy individuals were: CSA of the ST tendon at the medial epicondyle with the knee fully extended (Figure 1A) and with 30° of knee flexion (Figure 1B), CSA of the ST tendon at the 50% of its length with the knee fully extended and with 30° of knee flexion. 50% of its length was described as the midpoint between the medial epicondyle and the most proximal sight of pure tendon. In addition, CSA of the gracilis was measured at the medial epicondyle and full length of the tendon was measured from the medial epicondyle until the most proximal traceable sight of the tendon fibers (Figure 2). This variable was measured using the panoramic tool of the ultrasound equipment. ST length was measured from the epicondyle until the most proximal traceable tendon tissue. This protocol to measure ST tendon CSA has been previously used in the literature.[40,41]

To quantify the material properties of the tendon, SWE mode was used on the US. The probe was placed on top of the tendon longitudinally between the medial epicondyle and the 50% mark of the tendon (Figure 3A). To test the reliability of ST tendon stiffness, stiffness of the muscle was also tested (Figure 3B). To measure muscle stiffness, the probe was placed longitudinally at the same mark where the tendon ended. Because it has been shown that SWE is a valid and reliable method to quantify stiffness of the muscle, comparing the reliability of the muscle values versus the tendon values can inform us of the capacity of this technique to perform reliable measurements as well as the reliability of the individual that was taking the measurements.[54] The same measurements were taken for both legs and in two separate days to test reliability of the user to correctly use the ultrasound device. Stiffness of the tendon tissue will be measured on a circular area of 2 mm of diameter.

Reliability data of the CSA variables was initially unacceptable (Table 2) hence we performed a pilot study with 6 individuals (Table 3) to assess reliability of CSA variables at different knee positions and ultrasound settings of the measurements (Table 4). We evaluated CSA at the medial epicondyle with the knee flexed at 90° to identify the borders of the tendon with more clarity and obtain better quality images following the protocol of Erquicia et al.[40] In addition we changed the resolution settings of the ultrasound device to get better quality images (Figure 4). Using this protocol the reliability results were significantly better for both variables examined.

Table 1. Participant characteristics of the preliminary study in healthy controls. Mean ± SD.

N	22
Age (years)	21.5 ± 3.54
Weight (kg)	75.4 ±15.91
Height (cm)	175.8 ±10.12
Males	10 (45.5%)
Females	12 (54.5%)

Table 2. Researcher reliability values of the preliminary study. Intraclass Correlation Coefficient (ICC 2,k) and Standard Error of the Measurement (SEM 2,k) of the sample (n=22). Measured variables are CSA of the ST with the knee fully extended (CSA-ST), CSA of the ST tendon with the knee flexed 30° (CSA-ST 30DG), CSA of the gracilis tendon (CSA-GR), and length of the ST tendon (ST length), muscle stiffness and tendon stiffness.

		ICC 2,k	SEM 2,k (mm)	% of Mean is SEM
Right Leg	CSA - ST	0.63	1.68	15.29
	CSA - ST 30DG	0.81	1.23	11.67
	CSA - GR	0.83	0.85	14.50
	ST Length	0.97	0.52	2.92
	Muscle Stiffness	0.57	5.09	23.96
	Tendon Stiffness	0.67	77.25	17.45
Left Leg	CSA - ST	0.35	2.09	18.95
	CSA - ST 30DG	0.34	1.96	18.53
	CSA - GR	0.63	1.06	19.28
	ST Length	0.96	0.65	3.70
	Muscle Stiffness	0.33	5.73	28.56
	Tendon Stiffness	0.69	88.20	19.76

Table 3. Participant characteristics of the pilot group. Mean ± SD.

N	6
Age (years)	26.3 ± 3.27
Weight (kg)	78.3 ±18.18
Height (cm)	178.0 ±12.65
Males	4 (66.7%)
Females	2 (33.3%)

Table 4.Reliability values for the pilot group. ICC 2,k and SEM 2,k of the sample (n=6) Measured variables are CSA of the ST tendon at 30° of knee flexion (ST CSA – 30) and CSA of the ST tendon at 90° of knee flexion (ST CSA – 90).

		ICC 2,k	SEM 2,k (mm)	% of Mean is SEM
Right Leg	ST CSA - 30	0.78	0.583	4.40
	ST CSA - 90	0.93	0.618	4.05
Left Leg	ST CSA - 30	0.99	0.230	1.74
	ST CSA - 90	0.87	1.041	7.04

A **B**

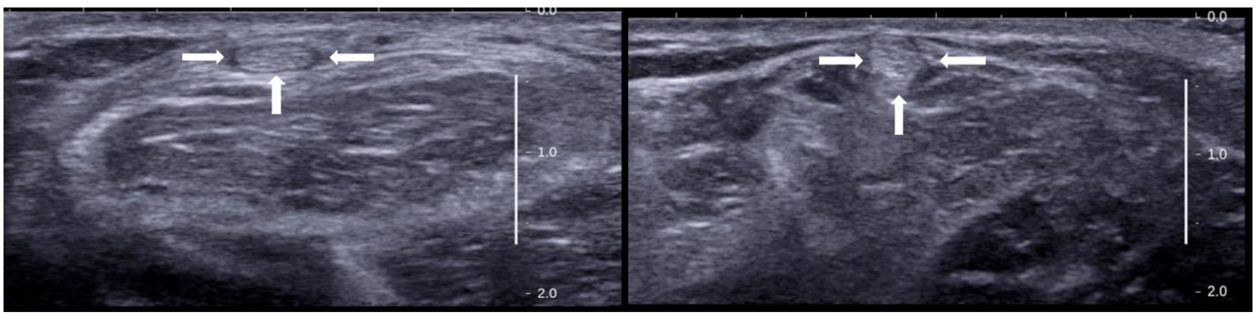

Figure 1. B-mode CSA image of the ST at the medial epicondyle with (A) the leg fully extended and (B) the knee at 30 of flexion.

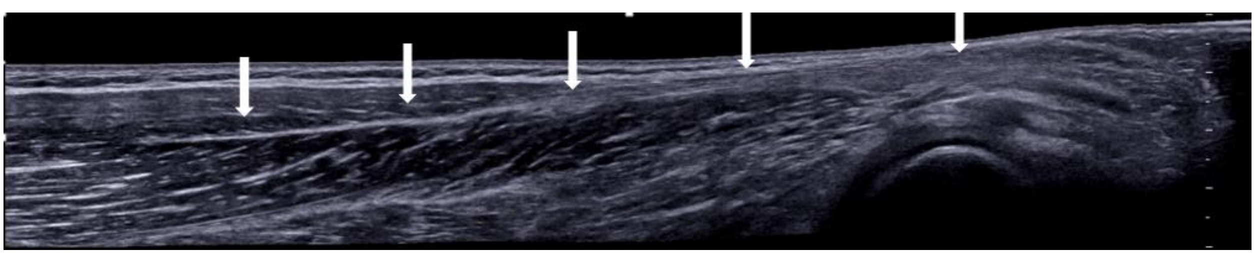

Figure 2. Length of the ST tendon.

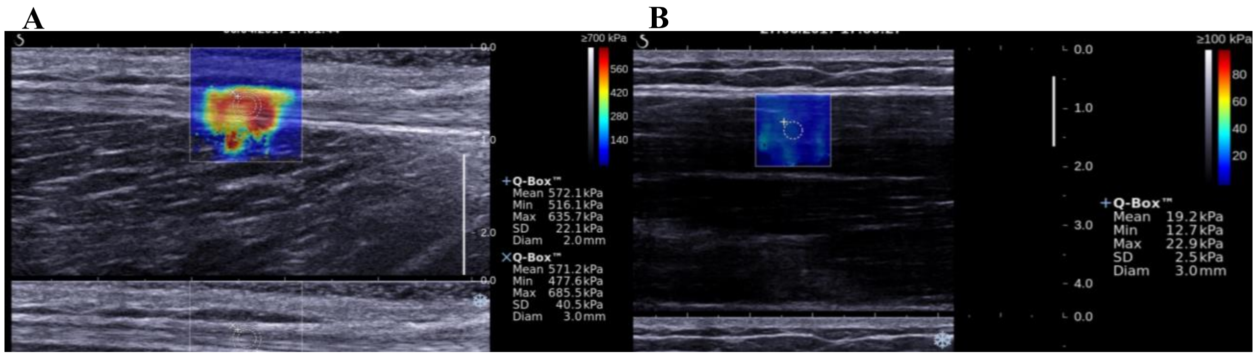

Figure 3. SWE images of (A) ST tendon with the two areas where measurements were calculated and (B) ST muscle belly.

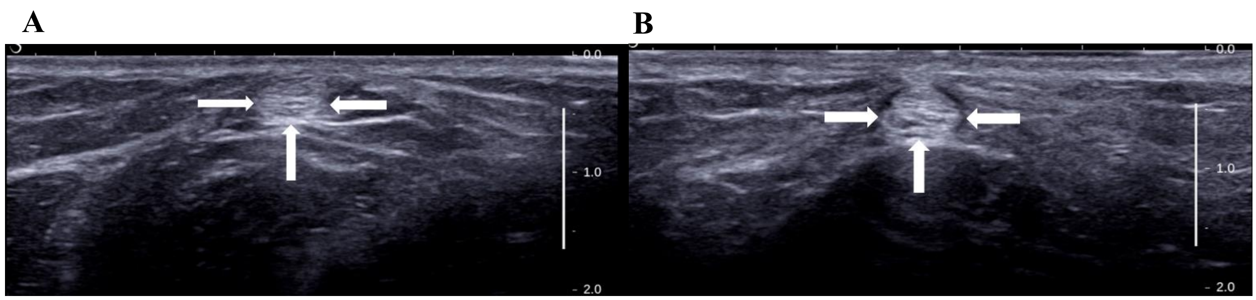

Figure 4. B-mode image of the CSA of the ST tendon with the knee flexed at (A) 30° and higher resolution and (B) 90° and higher resolution.

Statistical analysis

One-way ANOVAs were used to assess differences across the three subject groups for all the ultrasound-based variables using the non-operated limbs of the ACLR subjects and healthy matched limbs. Because tendon characteristics inherently change post-harvest, analyzing non-operated limbs as surrogates for the patient's "normal" tendon characteristics was performed. The hamstring function, KOOS, and Tegner activity scores were compared across both ACL groups to describe the magnitude of patient reported outcomes associated with having a single or multiple ACL reconstructions. Because the intent of this thesis was to determine how semitendinosus tendon characteristics relate to post-surgical outcomes, a correlational analysis associating KOOS scores to the tendon dependent variables, from the involved limbs, was also used. The α level was set at 0.05 and $p \leq 0.1$ values were considered a trend.

RESULTS

General Demographics and Subject Reported Function Scores

The 3 groups were not different in terms of their demographics (Table 5). Time since harvest for the single reconstructed group was 3.3 years (range: 1.0-6.8 years) and for the multiple reconstructed group was 7.0 years (range: 4.1-12.6 years). Physical activity as measured by the Tegner scale was not different between groups. Range of Tegner scores were from 4/10 (n=4), meaning that they reported their physical activity as participating recreational sports such as cycling or jogging on even ground at least twice a week, to 9/10 (n=1), meaning that he/she reported to participate in competitive sports such as soccer, football, wrestling, or gymnastics. Out of all the study participants only 2 were competitive at their activity (1 track and field and 1 lacrosse) and one was in the control group and the other in the multiple reconstructed group. The patient-reported function scores (Hamstring Function and KOOS) were also not different between multiple reconstructed individuals (n=4) and single reconstructed individuals (n=12) except in the Pain category of the Hamstring Function instrument (p=0.028). Single reconstructed individuals showed less hamstring pain in the affected leg than multiple reconstructed individuals. Overall, despite categorizing ACLR groups according to how many surgeries individuals had which was intended to differentiate good vs poor outcomes post-ACL reconstruction, the activity and function results showed no difference between individuals with a single ACL reconstruction versus multiple reconstructions.

Table 5. Participant demographics and Subject Reported Function Scores. Mean ± SD. *p<0.05

		Healthy (n=20)	Single ACLR (n=12)	Multiple ACLR (n=4)	p-value
	Sex (male/female)	7/13	4/8	2/2	N/A
	Age (yrs)	20.20±1.8	20.67±1.2	21.75±2.4	0.367
	Height (cm)	173.23±9.1	173.21±9.2	169.38±6.8	0.739
	Mass (kg)	70.63±13.8	70.53±8.3	80.10±11.8	0.226
	Tegner	5.85±1.1	5.92±0.9	5.50±1.3	0.806
KOOS Subscores	*Symptoms %*	N/A	76.49±18.7	74.11±20.9	0.833
	Pain %	N/A	86.34±11.7	84.72±12.1	0.815
	ADL %	N/A	94.36±5.2	92.28±12.6	0.634
	Sport %	N/A	75.00±14.6	71.25±11.1	0.648
	QOL %	N/A	68.23±22.5	65.63±20.7	0.842
Hamstring Func. Subscores	*Symptoms %*	N/A	64.58±31.0	75.00±20.4	0.545
	Soreness %	N/A	77.08±24.3	92.19±11.8	0.259
	Pain %	N/A	76.56±15.6	92.19±8.3	**0.028***
	Fxn %	N/A	78.65±17.6	90.63±12.0	0.230
	QOL %	N/A	63.54±28.9	81.25±16.1	0.270

Left and Right Limbs are not Statistically Different in the Healthy Individuals

A dependent variables t-test to examine limb asymmetry revealed no significant differences between limbs in healthy, active individuals. Mean difference revealed minimal differences between limbs and the 95% confidence interval (CI) of the difference between limbs showed that that "no difference" or 0 was between the bounds (Table 6). Because there was no significant difference between right and left limbs in healthy individuals, the unaffected limb of the ACLR individuals was assumed to be a surrogate of their "normal" limb to perform statistical comparisons across the healthy, single reconstructed, and multiple reconstructed groups.[55]

Table 6. US-based variables values of the healthy individuals. Mean ± SD, and mean difference between limbs ± SD.

	Left limb (n=20)	Right limb (n=20)	Mean Difference
CSA Gracilis Tendon (cm2)	0.072±0.02	0.067±0.02	0.005±0.02
CSA ST Tendon at 30° (cm2)	0.126±0.04	0.121±0.03	0.005±0.04
CSA ST Tendon at 90° (cm2)	0.129±0.02	0.131±0.04	-0.002±0.02
ST Tendon Length (cm)	7.68±1.71	7.40±1.11	0.28±1.79
ST Tendon Stiffness (KPa)	509.65±85.7	509.62±103.7	0.03±93.6

Differences between Healthy Limbs of Single ACLR, Multiple ACLR, and Healthy Matched Controls

One-way ANOVAs were performed to examine if differences existed between non-operated limbs of the ACLR (12 single-reconstructed and 4 multiple reconstructed subjects) and matched limb healthy controls (n=16) for all dependent variables (Table 7). Healthy limbs were matched to non-operated ACLR limbs based on sex, individual demographics and Tegner activity scores.

There was a trend towards significance between the three groups in the ST tendon CSA at 90° (p=0.058). When comparing healthy to single reconstructed group via follow-up pairwise comparisons, the non-operated limb of the single-reconstructed group limbs had larger ST CSAs compared to healthy matched controls (p=0.021). There was a trend towards significant differences in stiffness between the 3 groups in ST tendon stiffness (Table 7, p=0.100). Follow-up pairwise comparisons showed that the multiple reconstructed group's non-operated limbs exhibited higher stiffness compared to the matched healthy control limbs (p=0.034) and a trend towards significance between multiple reconstructed individuals and single reconstructed individuals (p=0.081). Length of the ST tendon was not different among the 3 groups.

Table 7. US-based variables values of the unaffected limbs of the ACLR groups and matched limb of the healthy group. Mean ± SD.

	Healthy (n=16)	Single ACLR (n=12)	Multiple ACLR (n=4)	One-way ANOVA
CSA Gracilis Tendon (cm^2)	0.07±0.02	0.08±0.02	0.09±0.02	0.212
CSA ST Tendon at 30° (cm^2)	0.13±0.04	0.14±0.03	0.15±0.02	0.243
CSA ST Tendon at 90° (cm^2)	0.13±0.03	0.16±0.03	0.15±0.03	0.058
ST Tendon Length (cm)	7.54±1.59	7.71±1.20	7.21±2.00	0.846
ST Tendon Stiffness (Kpa)	516.44±67.8	532.18±84.7	614.08±100.2	0.100

Association between Knee Functional Outcomes and US-based Measurements

As shown previously, the two ACLR groups were functionally equivalent in terms of patient oriented function scores except for pain for the Hamstring Function Instrument. In addition, the multiple ACLR group only had 4 subjects enrolled which may have masked any meaningful differences between the three groups overall. Therefore, for the following analysis both ACLR groups were combined into one group and correlations between US variables of the involved and the uninvolved limb and the patient reported function scores of all ACLR participants reconstructed limbs were examined to explore if US-based variables can provide insight into good vs poor outcomes based on patient reported knee joint function post-reconstruction. The Quality of Life (QOL%) subscore of the KOOS survey was strongly correlated with stiffness of the previously harvested ST tendon (r=0.561, p=0.024). The rest of the KOOS subscores and US-based variables of both limbs were not significantly correlated (Table 8, 9, Figure 5).

Table 8. Correlations between KOOS scores and US-based variables of the surgical limb. US-based values are from the surgical limb. Correlation are Pearson r's. ADL= Activities of the Daily Living, QOL= Quality of Life. *p<0.05.

	CSA Gracilis Tendon	CSA ST Tendon at 30°	CSA ST Tendon at 90°	ST Tendon Length	ST Tendon Stiffness
Symptoms %	-0.124	-0.132	-0.149	0.185	0.107
Pain %	-0.121	-0.325	-0.257	-0.040	0.098
ADL %	-0.151	-0.011	0.064	-0.032	0.339
Sport %	-0.258	-0.139	-0.071	-0.010	0.221
QOL %	-0.151	-0.025	0.194	-0.171	**0.561***

Table 9. Correlations between KOOS scores and US-based variables of the uninvolved limb. US-based values are from the uninvolved limb. Correlation are Pearson r's. ADL= Activities of the Daily Living, QOL= Quality of Life. *p<0.05.

	CSA Gracilis Tendon	CSA ST Tendon at 30°	CSA ST Tendon at 90°	ST Tendon Length	ST Tendon Stiffness
Symptoms %	0.145	-0.216	-0.018	0.014	-0.025
Pain %	0.392	-0.268	-0.220	0.137	-0.190
ADL %	0.385	-0.107	-0.241	0.344	-0.081
Sport %	0.268	-0.143	0.209	-0.002	0.033
QOL %	0.124	-0.178	-0.377	0.193	-0.139

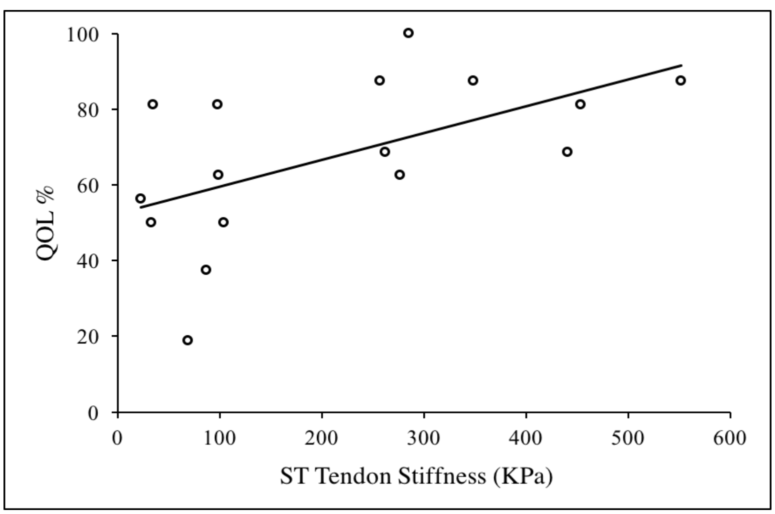

Figure 5. Correlation between Quality of Life score and ST tendon stiffness of the affected leg (r=0.561, p=0.024).

DISCUSSION

Uninvolved Limb Semitendinosus Characteristics Do Not Differentiate between Positive and Negative Outcomes Post-ACL Reconstruction

Donor tissue size and stiffness as measured with US by the unaffected leg post-ACLR cannot distinguish between single reconstructed individuals and multiple reconstructed individuals. However, ST tendon CSA at 90° showed a trend towards significance (p=0.058), due to both ACLR groups having larger contralateral ST tendon CSA compared to the matched leg of the healthy group. Cohen's d for that variable showed a small effect size (0.23) between the multiple and single reconstructed but medium to large effect size (0.71) between the multiple reconstructed and the healthy matched limbs.[56] Similarly, a trend for higher stiffness of the tendon in the contralateral limb of the multiple reconstructed group than in the other two groups existed. Given the novelty of the use of SWE in the ST tendon post-reconstruction, no similar results have been found in the literature for comparison. The current thesis categorized ACLR subjects into "good" vs "poor" outcomes based on whether the patient has a single ACL reconstruction vs multiple ACLR surgeries. There was no significant difference in size or stiffness of the uninvolved ST tendon in individuals that have had a single reconstruction vs. individuals that had multiple reconstructions and compared to healthy matched controls. The reasons for these results have several explanations:

Small sample size

Multiple reconstructions are somewhat common with ACL injuries (~9% for re-injury and ~5% for contralateral rupture) but given our recruiting method, we weren't able to obtain enough individuals that had had multiple reconstructions to potentially observe significant and meaningful differences.[57] A limitation of this study was that our sample size for our multiple reconstruction group was small (n=4), especially because how small the US-based measurements are. It is unknown if the reason why statistically significant results were not obtained was because the sample size masked the true differences, or because there are no true differences between the uninvolved limbs of single reconstructed individuals and multiple reconstructed individuals.

High variability in time since reconstruction

Time since ST tendon harvest was very different between both ACLR groups, and it was highly variable within each of the groups with ranges of 5.8 years for the single reconstructed and 8.5 years for the multiple reconstructed group. This variability in time since tendon harvest might have allowed for adaptations in the ST tendon of the uninvolved limb time since harvest was not controlled for in the current study.[58] Because the possible adaptations in the tendon given the large range since tendon harvest, it is not possible to know if and/or how much the uninvolved limb had changed since ACLR, therefore, not making it possible to establish whether differences truly are present when comparing uninvolved limbs across the groups.

US-based variables do not retrospectively differentiate between good and poor outcomes

It is possible that US cannot distinguish between post-operative good or poor outcomes based on the uninvolved limb retrospectively. There is evidence that sufficient graft size is essential to ensure a successful reconstruction, and reduce the risk for revision or multiple reconstructions. [12,38,59] In turn, there is evidence that MRI tendon measurements of the affected limb pre-reconstruction correlate with intraoperative graft size.[59] Serino et al. that showed an r=0.59 ($p<0.0001$) between graft diameter and tendon CSA measured with MRI. Wernecke et al. (2011) found a correlation of 0.53 between final graft size and pre-operative MRI tendon size.[39,59]

However, Wernecke et al. (2017) have found that increasing graft diameter does not reduce ACL revisions or improve outcomes no relationship.[42] This group of studies show that although graft size is important in avoiding revision surgeries and, therefore, having positive reconstruction outcomes, it might not solely reduce risk for a secondary surgery.

It is unknown if the ST tendons of both limbs were symmetric even before the injury, which could predispose individuals to ACL rupture in the first place. Although this explanation is unlikely and highly difficult to prove, it could be possible that one of the reasons that ACL injury occurs in the first place is limb asymmetry. It is known that healthy individuals have about 3% of limb asymmetry with a 95% Limits of Agreement (LOA) of 11% in the whole hamstring muscle group volume.[60] Similarly, the mean ST muscle volume asymmetry was also 3% with a 95% LOA of 14%. Even though, the mean asymmetry is not high, the LOA analysis show that healthy individuals can have up 14% of asymmetry in the ST muscle. Although, this research is done in the hamstring muscles, tendon CSA is a function of muscle CSA, given that higher muscle volume will create more pull to the tendon, increasing its size as a result.[61] Altogether, it is clear that, even in healthy individuals both limbs are not symmetric and the contralateral limb should not be used as a surrogate. Moreover, it is not well understood the level of limb symmetry in individuals that undergo an ACL injury prior to the reconstruction, and how that possibly affects injury risk.

Moreover, as already mentioned, adaptations to uninvolved tendon could have occurred. Konrath et al. showed that strength deficits, lower ST muscle volume, and lower ST muscle CSA persist in the injured limb over 2 years post-ACLR, indicating that the ST muscle of the involved limb atrophies and it is related with the strength deficits.[62,63] Because of these strength deficits in the involved limb, the uninvolved limb has to compensate in both, strength and morphological characteristics, to achieve the desired performance once the patient goes back to play.[58,64,65]

Because of this contralateral compensation, volume of the hamstring muscles increases and, in turn, the hamstring tendon has to adapt to the higher forces and strains by increasing its size.[58] These likely adaptations to the uninvolved tendon could have confounded our ability to distinguish between good and poor outcomes based on the analysis of the uninvolved limb.

Correlation analysis between the KOOS subscores and US-based variables of the uninvolved limb showed no association, as none of the US-based variables correlated with none of the KOOS subsocres. This is contradictory to some literature that showed a correlation between graft size and knee joint function as measured by the KOOS 2 years post-ACLR. They found significant correlations between graft size and the Pain, Activities of the Daily Living, Sports subscores of the KOOS.[12] According to Mariscalco et al., if using the contralateral limb as a surrogate was a good measure to evaluate the ST tendon post-ACLR, the correlations between the uninvolved limb and the KOOS subscores would have been stronger given the present state of the literature. Contrarily, Wernecke et al. (2017) did not find a correlation between graft diameter and any of the KOOS subscores.[42] Given the literature and the current research, it is unclear if graft diameter can predict KOOS scores post-ACLR. In conclusion, the lack of association or trends between variables indicates that the uninvolved limb was not an appropriate surrogate.

To our knowledge, there haven't been any studies that have used SWE to assess the viability of the graft retrospectively. Stiffness of the uninvolved limb in the multiple reconstructed group was larger than in the other two groups. Although this difference did not achieve statistical significance, it did show a trend towards significance (p=0.100). Effect size for this variable as shown with Cohen's d was large (0.88) between single and multiple reconstructed and also large (1.18) between multiple reconstructed and healthy matched limbs. Even though this comparison was not significant, the large effect size suggests that, prospectively measuring SWE of the ST tendon pre-surgery could potentially lead to important information about the viability of the future graft and should be further studied.

Positive/Negative outcomes were defined based on single or multiple reconstructions solely

ACLR have many possible negative clinical outcomes such as development of knee OA, revision surgery, re-injury of the same ACL or the contralateral ACL or return to previous activity level. Revision surgery or multiple reconstructions is one the most important negative outcomes along with development of knee OA.[3,5,66] Because some of the severe ACLR outcomes can take a long time to develop, defining poor outcomes as multiple reconstructions and successful reconstruction as a single was feasible and objective. This definition has two important limitations: 1) multiple reconstructions is not the sole determinant for a poor post-operative outcome. A poor outcome is typically defined by multiple measures (knee laxity, knee OA development, return to previous activity level, and strength), therefore, there is a high likelihood that some of the participants in the single reconstruction group would be classified under the category of poor outcome if the classification system for poor vs positive outcomes would have been different or more comprehensive. 2) The time since harvest in the single reconstructed group had a range from 1 to 6.8 years since reconstruction. Given that most revisions and re-injuries happen within the first two years post-reconstruction and the range since harvest of our sample, it is possible that some of the participants in the single reconstructed group could have a re-injury in the future and should be classified as multiple reconstructed.[67-69] Because of the study design, possible future reconstructions of the single reconstructed group could not be taken into account for this study.

Furthermore, another important limitation of this study is that Tegner scores pre-ACLR were not recorded. Recording this information would have provided the change in physical activity from pre-surgery to the time data was collected. The data shows that the 3 groups (2 ACLR and healthy controls) were the same at collection, however it is unknown if the single reconstructed group had originally higher Tegner scores than the multiple reconstructed. This information could be informative of positive or negative outcomes of the reconstruction, as well as return to sports information. For example, it could be the case that the two reconstructed groups did not have the same Tegner score prior to surgery, or the reason they had multiple reconstruction was that they returned to their previous activity level whereas the single reconstructed group did not return to the previous physical activity level. It is also possible that, because the reduced physical activity, there wasn't enough loading on the tendon and, therefore, affect stiffness of the tendon. In addition, this information would have allowed for further analysis irrespective of number of reconstructions.

Overall, using the uninvolved leg as a surrogate leg of what the injured leg was like prior to the reconstruction did not yield any significant results because of the small sample size, the time since ST tendon harvest was large among both groups and among individuals of the same group, the possibility of adaptations to the uninvolved limb over time, and because the definition that was used for good vs. poor outcomes did not englobe other variables that define the post-surgical success.

US-Based Measurements of the Surgical Limb are Retrospectively Informative of Post-Operative Success

Surgical limb semitendinosus characteristics are associated with patient reported outcomes post ACL reconstruction

Function of the knee post-ACLR has been widely investigated because the high incidence rates of knee OA following reconstruction.[3] It is surprising that, given the importance of the hamstring muscles in preventing ACL injuries, the relationship between the health of the regenerating ST tendon and function of the knee have not been studied.[70-72] The Quality of Life KOOS subscore was moderately correlated with stiffness of the ST tendon of the harvested tendon of when all reconstructed individuals were combined (r=0.561, p=0.024, n=16). After controlling for time since harvest, this correlation was relatively unaffected (partial r=0.560, p=0.03). As mentioned earlier, previous literature is unclear regarding the effect that graft size has on function of the knee long-term post-ACLR.[12,42] However, in the present research it is shown that patient-reported quality of life of the knee is also impacted by the regeneration of the tendon post-ACLR. Regardless of the time since harvest of the ST tendon, if the stiffness is not sufficiently high, the perceived quality of life based on knee joint function seems to be also negatively affected. Because the quality of the regenerated tendon might have an impact in the function of the knee, the secondary analysis assessing the surgical limb is presented. For this analysis all the reconstructed individuals were grouped together as there is no significant differences between the single reconstructed and the multiple reconstructed.

Regenerated tendon characteristics are not recovered compared to healthy values

A 2x2 mixed model ANOVA was performed to evaluate the difference between the harvested/regenerated ST tendon and the contralateral tendon of ACLR individuals and the tendons of the matched healthy control in all the US variables (Table 10). This analysis revealed interactions in the ST tendon CSA at 30° and 90° and, ST tendon stiffness, and a trend in the gracilis tendon CSA. To explain the location of the interactions, independent t-tests were performed. CSAs, at both 30° and 90°, showed that the surgical limb was significantly larger than the non-surgical limb (p=0.027 and p=0.040 respectively) and the healthy matched limb (p=0.003 and p=0.004 respectively). ST tendon stiffness of the surgical limb was significantly lower than the non-surgical limb (p<0.0001) and the healthy matched limb (p<0.0001). Gracilis tendon CSA of the surgical limb was smaller than the non-surgical limb (p=0.06) and the healthy matched limb (p=0.325). In addition, there were no differences between healthy control limbs in none of the variables examined. The results from this analysis at an average of 4.2 years post-reconstruction show that the characteristics of the regenerating ST tendon and its stiffness are far from the levels of both, the uninvolved limb and the healthy control matched limb. Overall, the CSAs were larger in the ACLR limb compared to the non-operated limbs whereas the CSAs were no different among the healthy control limbs. Moreover, ST tendon stiffness was significantly lower in the ACLR limb compared to the contralateral limb and both sides of the matched individuals, and stiffness did not significantly differ among the non-operated limbs.

Table 10. Interactions between US-based variables of both reconstructed limbs of the reconstructed individuals and the matched limbs of the healthy controls. Mean ± SD. *p≤0.05

	ACLR (n=16)		Healthy Control (n=16)		2x2 ANOVA
	Recon	Nonrecon	Matched recon	Matched Nonrecon	
CSA Gracilis Tendon (cm^2)	0.065±0.03	0.080±0.02	0.073±0.02	0.071±0.02	0.069
CSA ST Tendon at 30° (cm^2)	0.183±0.08	0.145±0.03	0.115±0.03	0.126±0.04	**0.019***
CSA ST Tendon at 90° (cm^2)	0.205±0.09	0.153±0.03	0.124±0.03	0.127±0.03	**0.031***
ST Tendon Length (cm)	8.24±1.2	7.58±1.4	7.57±0.9	7.54±1.6	0.258
ST Tendon Stiffness (KPa)	213.84±169.6	552.66±92.8	490.39±96.0	516.44±67.8	**0.000***

Previous literature has shown that the regenerated ST tendon does not appear to have the same characteristics as the contralateral ST tendon or the same tendon pre-reconstruction. Konrath et al. have shown that the regenerated ST tendons were longer, and had larger CSA compared to the contralateral side.[62] Although in that study, the tendons were evaluated with MRI, in the present study similar results were found with significant increases in ST tendon CSA and non-significant increases in tendon length. Choi el al. regeneration of the ST and gracilis tendons were correlated with knee flexor strength. They also found that ST musculostendinous retraction was correlated with knee flexor strength deficits.[63] Contradictorily, Janssen et al. also correlated ST tendon regeneration to knee flexor strength but found no relationship.[73] Williams et al. found that although tendon regeneration was found in most if their sample, it was not complete six months post-reconstruction, which is when most patients start returning to physical activity.[51] Altogether, it is clear that the morphological characteristics of the ST tendon are altered post-reconstruction and the data presented here supports it. Since physical characteristics of the ST tendon have already been evaluated in the literature and because these variables have not shown any correlation with functional scores, the rest of this discussion will focus on investigating regeneration of ST tendon stiffness and its potential role in knee function.

Stiffness of the tendon does not regenerate to healthy levels

To our knowledge, this is the second study examining stiffness of the ST tendon post-reconstruction, and the first one with a wide timespan post-reconstruction, as well as the first one to establish a relationship between ST tendon stiffness and quality of life (Figure 5).[52] Stiffness of the ST tendon did not return to normal after an average of 4.2 years after reconstruction (Figure 6). There is a group*limb interaction with no significant differences between limbs of the healthy controls but ACLR individuals had significantly lower stiffness in their reconstructed limb than their non-reconstructed limb (Table 10). The bilateral stiffness ratio of reconstructed/non-reconstructed ST tendon stiffness for ACLR individuals was also significantly lower than for the healthy controls (0.39±0.3 vs. 0.95±0.2, $p<0.0001$). Overall, there is significant stiffness deficits from the previously harvested ST tendon 4.2 years post-ACLR (Figure 6, 7). In addition, stiffness of the regenerating tendon is highly variable compared to the non-reconstructed tendon or the matched controls.

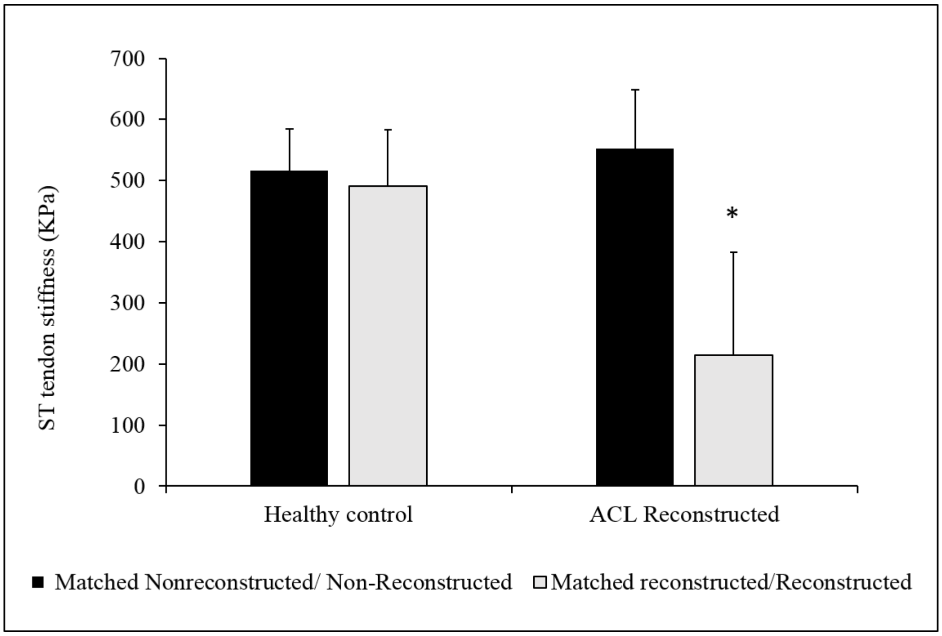

Figure 6. Mean ST tendon stiffness. Error bars represent SD. Significant group*limb interaction, p<0.0001.

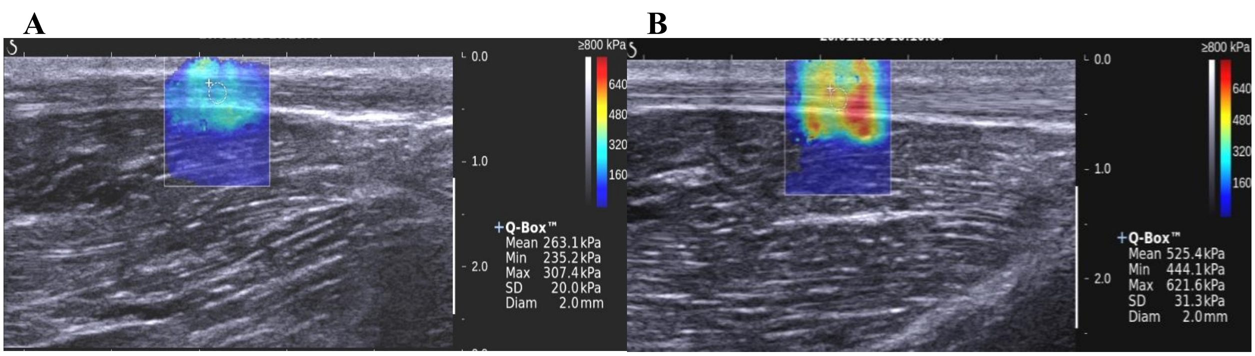

Figure 7. ST tendon stiffness color map of (A) the harvested tendon and (B) the contralateral tendon of the same individual.

Given the wide range of time since tendon harvest in the sample, a correlation between absolute stiffness values and time since harvest was done to assess if the high variability of the ST tendon stiffness values can be explained because of time (Figure 8). The correlation is moderate and it does not reach statistical significance. In addition, the r =0.432 (p=0.094). This correlation shows that, although there is a moderate relationship between the two variables, time alone does not explain the variability of these values. Moreover, these data shows that the stiffness of the tendon may never return to normal levels and, in the individuals that is does, it takes a long time. Therefore, there are other factors that play a role in determining the recovery of the ST tendon stiffness to pre-reconstruction values.

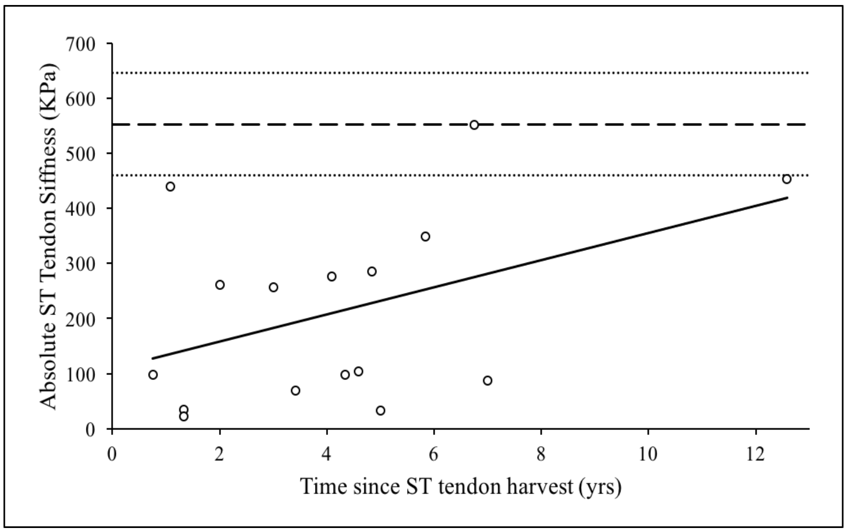

Figure 8. Correlation between ST tendon stiffness and time since harvest (r=0.432, p=0.094). Horizontal lines symbolize the mean ST tendon stiffness of the matched healthy limb and SD of upper and lower bounds.

Suydam et al. showed that after 12 months post- ACLR 80.6% (involved /uninvolved) of the shear modulus (named stiffness in this thesis) was regained (Figure 9). These results differ from the present ones (39% at 4.2 years post-ACLR). However, some differences may be explained by the different equipment used by Suydam et al. (continuous SWE), different methodology (the knee angle was at 45° vs. at full extension in our study), the sample size, the different time frame (all their subjects were under 2 years post-reconstruction), and the fact that there is a data point that heavily drives that correlation (~1.2 ratio and ~2 years post-reconstruction). In addition, it is unknown the physical activity level of those subjects, which is known to have a big impact in stiffness of the tissue.[52] The present study adds a longer time frame to the literature which demonstrates that material properties of the ST tendon are not recovered in the long-term and should be studied more to further understand how it affects knee joint function.

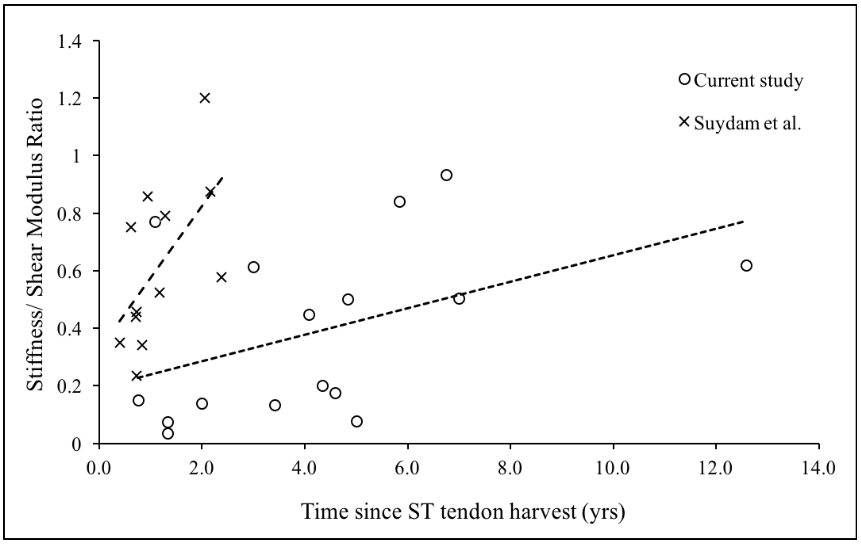

Figure 9. Correlation between ST tendon stiffness/shear modulus ratio (involved/noninvolved) and time since tendon harvest of the current study (r=0.454, n=16) and Suydam et al. (r=0.596, n=13).

Quality of the regenerating ST tendon is poor as observed by increased CSA and decreased stiffness

CSA of the ST tendon of the regenerating tendon is larger than the CSA of the ST tendon of the uninvolved limb and healthy matched controls. This is consistent with several reports in the literature that show larger CSA, volume and length of the ST tendon post-ACLR compared to the contralateral side. In addition, these deficits are usually accompanied by reduced ST muscle volume, and correlated with reduced knee flexor strength.[58,62] It is clear that the ST tendon regenerates in most individuals post-ACLR but this tendon is larger in size and has decreased stiffness implying the quality of regeneration is insufficient. Gill et al. performed a study with rabbits that showed that the ST tendon had physically regenerated after harvest. The composition of the tendon tissue was Type I collagen, and the ability of the tendon to transmit force was restored, although it was only capable to transmit 25% of the force than the native tendon calculated through electrophysiologic force transmission across the musculotendinosus junction.

However, the tendon was characterized by the poor organization of the collagen fibers, thinner collagen fibrils, increased cellularity, increased vascularity, and had significantly different maximum load to failure, and sitffness.[74] Ferreti et al. performed a study in 3 humans that were undergoing further surgery in the same area and were able to obtain a biopsy of the tissue. The biopsies revealed fibroblastic proliferation in the regenerated ST tendon, but poor tissue organization with only a few well-oriented fibers of collagen.[75] In the present study we were able to evaluate the change in ST tendon CSA and the decreased quality of the material quantified as stiffness that has previously reported in the literature (Figure 10). As shown on Figure 10, US can identify the larger CSA (Figure 10 A, B), and poor tissue organization as shown on the longitudinal images (Figure 10 C, D). It is still unknown what the effects are of the decreased quality of the tendon material and how long these effects can last. However, the present data shows large deficits in material quality over 4 years post-ACLR.

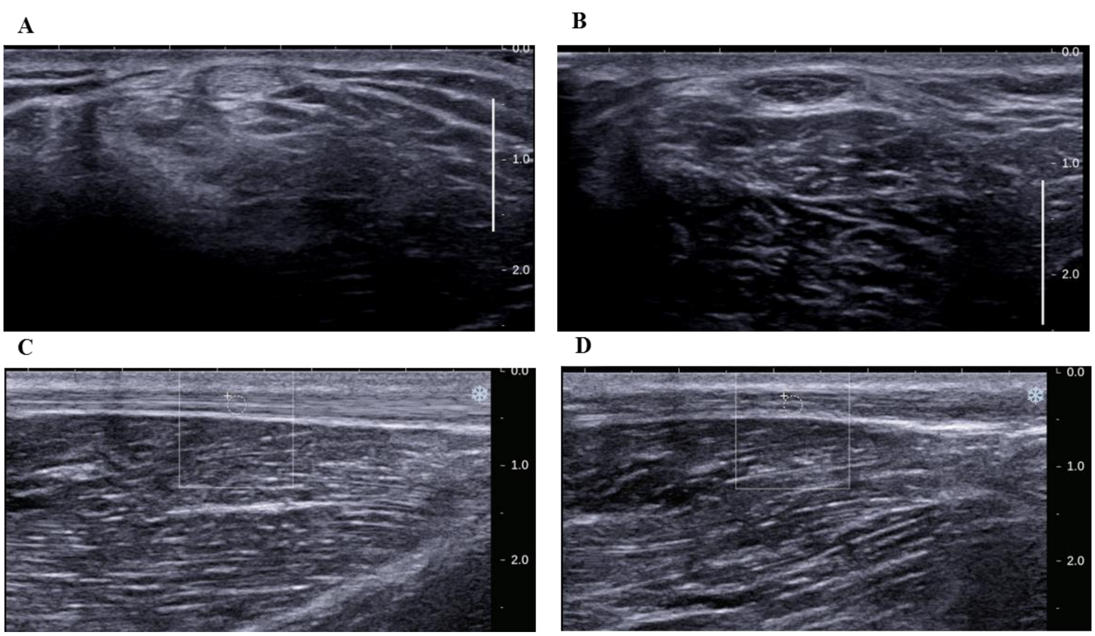

Figure 10. ST tendon CSA of the uninvolved limb (A) and involved limb (B), and longitudinal image of the ST tendon of the uninvolved limb (C) and involved limb (D) of the same individual at 1 year post-tendon harvest.

Clinical importance

It is not clear what the exact role ST tendon stiffness on knee function is because it has not been investigated extensively yet. However, this study provides evidence that stiffness of the tendon may have important clinical significance. As shown previously, ST tendon stiffness and quality of life of the knee joint, as measured by the KOOS survey, are related. This shows that ST tendon stiffness has severe implications besides what can be measured through the US. Interestingly, the same positive correlation between stiffness and perceived joint function has also been observed in the Achilles tendon (r=0.916).[76] In the present study, it is also important to note that, although only one participant was within 1 SD of the healthy stiffness values, all of them had returned to activity and were physically active, but were not participating is sports that put their knee at risk anymore (highest Tegner score was 7/10). It is unclear if lower stiffness has an effect in return to play and/or return to previous activity level but it is clear that it has a clinical and functional significance that should be studied. It is also unclear what the long-term effects of reduced ST tendon stiffness are. Because reduced tendon stiffness could potentially change the force output of the ST muscle, and the relative force output of the hamstring muscles, it could change how the load is distributed in the tibiofemoral joint, causing the development of knee OA.

Conclusion

Assessing the unaffected semitendinosus tendon post-ACL reconstruction did not provide any answers about graft success using US and SWE technology. Evaluating the affected semitendinosus tendon does offer information about the health of the tissue, most significantly tendon stiffness does not seem to return to normal levels even on the long-term. Moreover, semitendinosus tendon stiffness is related to patient-reported outcome, which suggests that tendon stiffness post-surgery has an impact on long-term function on the knee. The current study had the limitation that it was retrospective but given the results obtained, a prospective longitudinal study evaluating the viability of the ST tendon and its regeneration from pre-reconstruction to post-reconstruction and return to play is warranted.

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Appendix A. Hamstring Function Survey

Hamstring Function

INSRTUCTIONS: This survey asks for your view about your hamstrings. This information will help us keep track of how you feel about your hamstrings and how you function in training and daily life.

Please respond to every question by ticking the appropriate box; only one box for each question. If you are unsure about how to answer a question, please give the best answer you can. Remember to answer both for the left & the right hamstrings.

Symptoms

These questions should be answered thinking about the symptoms from your posterior thigh/hamstrings during the **last week**.

1- Have you experienced soreness/stiffness/had complaints from your posterior thigh/hamstrings?

Left side:

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

Right side:

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

Soreness

The following questions cover soreness in the posterior thigh region. Report the degree of soreness that you have experienced from your posterior thigh/hamstrings during the **last week**.

2 – How sore is your posterior thigh?

Left side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

Right side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

3 – How sore is your posterior thigh during training?

Left side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

Right side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

4 – How sore is your posterior when you wake up in the morning?

Left side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

Right side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

5 – How sore is your posterior thigh if you have been sitting still for a while during the day?

Left side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

Right side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

Pain

6 – How often do you experience pain from your posterior thigh?

Left side:					Right side:				
Never	Rarely	Sometimes	Often	Always	Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

7 – Do you often sustain small strains in your posterior thigh that resolve quickly?

Left side:					Right side:				
Never	Rarely	Sometimes	Often	Always	Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Report the degree of pain that you have felt from your posterior thigh/hamstrings during the **last week** when performing the following activities:

8 – Stretching the posterior thigh/hamstrings

Left side:					Right side:				
No pain	A little	Moderate	Considerable	Very painful	No pain	A little	Moderate	Considerable	Very painful
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

9 – Walking up a ladder/stairs (double steps)

Left side:					Right side:				
No pain	A little	Moderate	Considerable	Very painful	No pain	A little	Moderate	Considerable	Very painful
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

10 – Jogging

Left side:					Right side:				
No pain	A little	Moderate	Considerable	Very painful	No pain	A little	Moderate	Considerable	Very painful
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

11 – Changing direction while running

Left side:					Right side:				
No pain	A little	Moderate	Considerable	Very painful	No pain	A little	Moderate	Considerable	Very painful
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

12 – Accelerating

Left side:					Right side:				
No pain	A little	Moderate	Considerable	Very painful	No pain	A little	Moderate	Considerable	Very painful
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

13 – Breaking speed after sprinting

Left side:					Right side:				
No pain	A little	Moderate	Considerable	Very painful	No pain	A little	Moderate	Considerable	Very painful
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Function, daily living, and sports

The following questions concern your physical function. For each of the following activities, please indicate the degree of difficulty you have experienced in the **last week** due to your posterior thigh/hamstrings.

14 – Running

Left side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

Right side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

15 – Jumping

Left side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

Right side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

16 – Accelerating

Left side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

Right side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

17 – Braking sped after sprinting

Left side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

Right side:

Not at all	A little	Moderate	A lot	Very much
☐	☐	☐	☐	☐

Quality of life

The following questions concern how problems from your hamstrings restrain you during physical activity. Report the degree of difficulty you have experienced during the **last week** due to your posterior thigh/hamstrings.

18 – In what degree do you trust your hamstrings during physical activity?

Left side:

Totally	A lot	Moderate	To some degree	Not at all
☐	☐	☐	☐	☐

Right side:

Totally	A lot	Moderate	To some degree	Not at all
☐	☐	☐	☐	☐

19 – Do you sometimes keep from performing 100% due to concerns of sustaining a hamstring strain?

Left side:

Totally	A lot	Moderate	To some degree	Not at all
☐	☐	☐	☐	☐

Right side:

Totally	A lot	Moderate	To some degree	Not at all
☐	☐	☐	☐	☐

Appendix B. Knee injury and Osteoarthritis Outcome Score Survey

KOOS KNEE SURVEY

INSTRUCTIONS: This survey asks for your view about your knee. This information will help us keep track of how you feel about your knee and how well you are able to perform your usual activities.
Answer every question by ticking the appropriate box, only one box for each question. If you are unsure about how to answer a question, please give the best answer you can.

Symptoms
These questions should be answered thinking of your knee symptoms during the **last week**.

S1. Do you have swelling in your knee?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S2. Do you feel grinding, hear clicking or any other type of noise when your knee moves?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S3. Does your knee catch or hang up when moving?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S4. Can you straighten your knee fully?

Always	Often	Sometimes	Rarely	Never
☐	☐	☐	☐	☐

S5. Can you bend your knee fully?

Always	Often	Sometimes	Rarely	Never
☐	☐	☐	☐	☐

Stiffness
The following questions concern the amount of joint stiffness you have experienced during the **last week** in your knee. Stiffness is a sensation of restriction or slowness in the ease with which you move your knee joint.

S6. How severe is your knee joint stiffness after first wakening in the morning?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

S7. How severe is your knee stiffness after sitting, lying or resting **later in the day**?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Pain

P1. How often do you experience knee pain?

Never	Monthly	Weekly	Daily	Always
☐	☐	☐	☐	☐

What amount of knee pain have you experienced the **last week** during the following activities?

P2. Twisting/pivoting on your knee

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P3. Straightening knee fully

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P4. Bending knee fully

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P5. Walking on flat surface

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P6. Going up or down stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P7. At night while in bed

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P8. Sitting or lying

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P9. Standing upright

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Function, daily living

The following questions concern your physical function. By this we mean your ability to move around and to look after yourself. For each of the following activities please indicate the degree of difficulty you have experienced in the **last week** due to your knee.

A1. Descending stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A2. Ascending stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

For each of the following activities please indicate the degree of difficulty you have experienced in the **last week** due to your knee.

A3. Rising from sitting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A4. Standing

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A5. Bending to floor/pick up an object

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A6. Walking on flat surface

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A7. Getting in/out of car

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A8. Going shopping

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A9. Putting on socks/stockings

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A10. Rising from bed

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A11. Taking off socks/stockings

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A12. Lying in bed (turning over, maintaining knee position)

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A13. Getting in/out of bath

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A14. Sitting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A15. Getting on/off toilet

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

For each of the following activities please indicate the degree of difficulty you have experienced in the **last week** due to your knee.

A16. Heavy domestic duties (moving heavy boxes, scrubbing floors, etc)

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A17. Light domestic duties (cooking, dusting, etc)

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Function, sports and recreational activities

The following questions concern your physical function when being active on a higher level. The questions should be answered thinking of what degree of difficulty you have experienced during the **last week** due to your knee.

SP1. Squatting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP2. Running

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP3. Jumping

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP4. Twisting/pivoting on your injured knee

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP5. Kneeling

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Quality of Life

Q1. How often are you aware of your knee problem?

Never	Monthly	Weekly	Daily	Constantly
☐	☐	☐	☐	☐

Q2. Have you modified your life style to avoid potentially damaging activities to your knee?

Not at all	Mildly	Moderately	Severely	Totally
☐	☐	☐	☐	☐

Q3. How much are you troubled with lack of confidence in your knee?

Not at all	Mildly	Moderately	Severely	Extremely
☐	☐	☐	☐	☐

Q4. In general, how much difficulty do you have with your knee?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Thank you very much for completing all the questions in this questionnaire.

Appendix C. Informed Consent Document

East Carolina University

Informed Consent to Participate in Research

Information to consider before taking part in research that has no more than minimal risk.

Title of Research Study: Hamstring Characteristics and ACL Reconstruction Outcomes

Principal Investigator: Dr. Anthony Kulas
Institution, Department or Division: East Carolina University Department of Kinesiology
Address: 249 Ward Sports Medicine Building
Telephone #: 252-737-2884

Researchers at East Carolina University (ECU) study issues related to society, health problems, environmental problems, behavior problems and the human condition. To do this, we need the help of volunteers who are willing to take part in research.

Why am I being invited to take part in this research?
The purpose of this research is to determine whether or not individuals that have had revision surgery after an ACL reconstruction have different tendon characteristics than individuals that have not had a revision surgery. You are being invited to take part in this research because you either have had an ACL reconstruction with a hamstring graft, you have had an ACL reconstruction with a hamstring graft and also a consequent revision surgery, or you are healthy with no history of knee joint injury at all. The decision to take part in this research is yours to make. By doing this research, we hope to learn if individuals that have a revision surgery after an ACL reconstruction have smaller, shorter and less stiff tendons than individuals that had a successful ACL reconstruction and healthy controls.

If you volunteer to take part in this research, you will be one of about 80 people to do so.

Are there reasons I should not take part in this research?
I understand I should not volunteer to be in this research study if I have had an ACL reconstruction and the reconstruction was not performed with a hamstring graft. I also should not volunteer to be in this research study if I have any known allergies to hypoallergenic gel commonly used with ultrasound imaging.

What other choices do I have if I do not take part in this research?
You can choose not to participate.

Where is the research going to take place and how long will it last?
The research will be conducted in the Ward Sports medicine Building at East Carolina University. You will need to come to the Biomechanics Lab located in room 332 in the Ward Sports Medicine Building one time during the study. The total amount of time you will be asked to volunteer for this study is approximately 1.5 hours.

What will I be asked to do?
- You will be asked to do the following: You will be asked to lie on your stomach on a standard padded table so that we can image your hamstring muscles and tendons with ultrasound while you are relaxed. We will also take several standard anthropometric measurements i.e. height and weight. If you are a participant that has

had an ACL reconstruction, you will also be asked to complete some standardized questionnaires related to your perceptions of your hamstring muscles and knee joint.

What might I experience if I take part in the research?
We don't know of any risks (the chance of harm) associated with this research. Any risks that may occur with this research are no more than what you would experience in everyday life. We don't know if you will benefit from taking part in this study. There may not be any personal benefit to you but the information gained by doing this research may help others in the future.

Will I be paid for taking part in this research?
We will not be able to pay you for the time you volunteer while being in this study.

Will it cost me to take part in this research?
It will not cost you any money to be part of the research.

Who will know that I took part in this research and learn personal information about me?
ECU and the people and organizations listed below may know that you took part in this research and may see information about you that is normally kept private. With your permission, these people may use your private information to do this research:

- Any agency of the federal, state, or local government that regulates human research. This includes the Department of Health and Human Services (DHHS), the North Carolina Department of Health, and the Office for Human Research Protections.
- The University & Medical Center Institutional Review Board (UMCIRB) and its staff have responsibility for overseeing your welfare during this research and may need to see research records that identify you.

How will you keep the information you collect about me secure? How long will you keep it?
If you elect to enroll in this study by signing this informed consent document, you will be assigned an alphanumeric code. Only this alphanumeric code, not your name, will appear on the saved ultrasound images or any other electronically saved measurements or paper questionnaires. All data collected from you will only have this alphanumeric code associated with it and this data will be backed up on a network server in this lab. The only person to have access to the master list of names which link your name to your alphanumeric code will be the researchers identified above, Ms. Clara Amat Fernandez and Dr. Anthony S. Kulas. All paperwork and forms linking you to the study will be kept in the PIs office (Ward Sports Medicine – room 249) which remains locked except when in use. Your ultrasound images collected in this study may be used for manuscript/presentation purposes. If used for these reasons, no information identifying you (your name or alphanumeric code) will be on any images/figures used for research purposes.

What if I decide I don't want to continue in this research?
You can stop at any time after it has already started. There will be no consequences if you stop and you will not be criticized. You will not lose any benefits that you normally receive.

Who should I contact if I have questions?
The people conducting this study will be able to answer any questions concerning this research, now or in the future. You may contact the Principal Investigator at (252) 737-2884 (days, between 8am-5pm).

If you have questions about your rights as someone taking part in research, you may call the Office of Research Integrity & Compliance (ORIC) at phone number 252-744-2914 (days, 8:00 am-5:00 pm). If you would like to report a complaint or concern about this research study, you may call the Director of the ORIC, at 252-744-1971.

I have decided I want to take part in this research. What should I do now?
The person obtaining informed consent will ask you to read the following and if you agree, you should sign this form:

- I have read (or had read to me) all of the above information.
- I have had an opportunity to ask questions about things in this research I did not understand and have received satisfactory answers.
- I know that I can stop taking part in this study at any time.
- By signing this informed consent form, I am not giving up any of my rights.
- I have been given a copy of this consent document, and it is mine to keep.

____

Participant's Name (PRINT) **Signature** **Date**

Person Obtaining Informed Consent: I have conducted the initial informed consent process. I have orally reviewed the contents of the consent document with the person who has signed above, and answered all of the person's questions about the research.

____

Person Obtaining Consent (PRINT) **Signature** **Date**

____

Principal Investigator (PRINT) **Signature** **Date**
(If other than person obtaining informed consent)

Appendix D. Institutional Review Board Approval

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board Office
4N-70 Brody Medical Sciences Building· Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office **252-744-2914** · Fax **252-744-2284** · **www.ecu.edu/ORIC/irb**

Notification of Initial Approval: Expedited

From: Biomedical IRB
To: Anthony Kulas
CC:

Date: 6/5/2017
Re: UMCIRB 16-001915
Hamstring Tendon Characteristics and ACL Reconstruction Outcomes

I am pleased to inform you that your Expedited Application was approved. Approval of the study and any consent form(s) is for the period of 6/1/2017 to 5/31/2018. The research study is eligible for review under expedited category #4, 7. The Chairperson (or designee) deemed this study no more than minimal risk.

Changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. The investigator must submit a continuing review/closure application to the UMCIRB prior to the date of study expiration. The Investigator must adhere to all reporting requirements for this study.

Approved consent documents with the IRB approval date stamped on the document should be used to consent participants (consent documents with the IRB approval date stamp are found under the Documents tab in the study workspace).

The approval includes the following items:

Name	Description
Hamstring Function Survey	Surveys and Questionnaires
Informed Consent_revised	Consent Forms
IRB Protocol_revised	Study Protocol or Grant Application
KOOS survey revised	Surveys and Questionnaires
Minor Assent	Consent Forms
Parent Permission_revised	Consent Forms
Recruitment flyer revised	Recruitment Documents/Scripts
Tegner Activity Score	Surveys and Questionnaires

The Chairperson (or designee) does not have a potential for conflict of interest on this study.

IRB00000705 East Carolina U IRB #1 (Biomedical) IORG0000418
IRB00003781 East Carolina U IRB #2 (Behavioral/SS) IORG0000418