Hamstrings and Quadriceps Muscle Recruitment Following ACL Reconstruction with an Autograft

By

Elizabeth M. Klemm

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Director of Thesis: Zachary J. Domire, PhD

Major Department: Kinesiology

ABSTRACT

Anterior Cruciate Ligament reconstructions (ACLr) are one of the most common knee surgeries. The torn ligament is most often reconstructed with an ipsilateral autograft from the semitendinosus (ST), patella (PT), or quadriceps (QT) tendon. Surgical outcomes are generally positive, but around 50% of individuals develop osteoarthritis in the 15 years after their ACLr. The reasons for this are not completely understood, but reduced and altered knee joint loading have been suggested as the probable causes. It has also been shown that the ST tendon does not restore its pre-surgical properties with reduced tendon stiffness, reduced fibril organization, and increased length common. This study hypothesized that donor site tendon stiffness would also be reduced following ACLr with a BPTB or QT graft. Additionally, it hypothesized that these altered donor site tendon properties would cause altered muscle group force sharing in the hamstrings (ST graft) or quadriceps (BPTB and QT grafts). These deficits would be most pronounced at shorter muscle lengths and lower isometric torque levels and cause a shift of the torque-length curve towards longer muscle lengths.

Data were collected on 15 participants who had an ACLr (5 ACLr – ST; 6 ACLr – PT; 4 ACLr – QT) and 11 healthy matched controls. Participants completed isometric contractions

(knee flexion for ACLr – ST and Healthy – ST; knee extension for ACLr – PT, ACLr – QT, and Healthy – PT) in four different positions that altered muscle length and two different torque levels while active shear wave elastography images were taken of the muscles in the hamstring or quadriceps groups. Active shear modulus was multiplied by muscle PCSA to estimate muscle forces. EMG data were also collected. Donor site tendon shear modulus was also measured. Both limbs were tested, with the unaffected limb serving as an estimate of pre-ACLr behavior. Dependent samples t-tests, repeated measures ANOVAs, and effect sizes were calculated within the ACLr and healthy groups to test for between limb and within limb differences.

The ACLr – ST group had consistently lower ST tendon stiffness on the injured side and produced less force out of the affected ST. Deficits in force production were not affected by contraction level, but they were smaller at longer muscle lengths. The degree of ST tendon stiffness recovery did not impact the size of ST force deficits and there were no consistent shifts in the torque-length curve. Donor site tendon stiffness was not consistently lower in the PT or QT following ACLr with these grafts. As such, there were no large effects on quadriceps muscle force production or load sharing. This improved healing of the PT and QT compared to the ST is likely due to the surgical technique. While the entire ST tendon is used for ACLr, only a portion of the PT and QT are sourced which leaves a scaffold for the tendon to regenerate on.

This study was limited by small sample sizes per each graft type; however, it suggested that donor site tendon recovery is more complete following ACLr with a PT or QT autograft than with a ST graft and that deficits in the ST tendon lead to reduced force production. Future research should examine the timeline of PT and QT recovery, as well as explore rehabilitation techniques that can preferentially activate the ST to improve tendon healing and consequently normalized knee joint loading due to more normal knee flexor force sharing.

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Elizabeth M. Klemm

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Director of Thesis: Zachary J. Domire, PhD

Thesis Committee Members:

Anthony S. Kulas, PhD

John D. Willson, PT, PhD

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Introduction

Anterior cruciate ligament (ACL) injuries are a common orthopedic injury, impacting 100,000-200,000 Americans annually[1]. Teenagers and young adults have the highest incidence rate[2] and are more likely to have an ACL reconstruction (ACLr) than older individuals[1]. Recovery will take an average of 4-12 months[3], but the impacts of an ACL injury extend longer. The annual economic impact of ACL injuries in the US is between $7 and $17.7 billion, with the lifetime burden for individuals an estimated $38,121 when a surgical approach is taken[4]. A large contributor to the economic burden of ACL injuries is the development of osteoarthritis (OA). About 50% of individuals develop OA in the 15 years after their ACL rupture[5]. Since ACL injuries are most prevalent in teenagers and young adults, this means many are developing OA before age 40, despite the disease typically appearing in older populations. Progression of the disease reduces quality of life and can lead as many as 10-15% of individuals who had an ACLr to have a total knee replacement during their lifetime[4]. The high prevalence of ACL injuries makes it pertinent to determine ways to reduce the long-term consequences.

The mechanisms leading to increased risk of OA following ACLr are not known, but recent research has suggested that underloading of the knee following injury and ACLr leads to weakened cartilage and thus OA[6]. In the months after injury and reconstruction, medial knee compartment loading has been shown to be reduced[6] which may be due in part to reduced ST force production[7]. Overtime, this can lead to a degradation of the articular cartilage[6]. By the time joint loading becomes more symmetrical between limbs, the cartilage has likely degenerated and can no longer support typical loads[6]. However, further research needs to be done to determine if medial hamstring force production, particularly in the semitendinosus (ST), is reduced following ACLr.

An ACLr can be completed with several different kinds of grafts, but a ST tendon or ST tendon + gracilis tendon (STG) autograft from the ipsilateral limb has historically been the most commonly used[8]. Alternatively, a bone-patellar tendon bone (BPTB) autograft sourced from the patellar tendon, allografts, or synthetic grafts can be utilized[8]. Recently, there has been an increase in the use of quadriceps tendon (QT) autografts for ACLr, but the long-term impacts of this graft type have not been extensively explored. Generally long-term outcomes are positive, however as many as 14% of those with a ST graft and 8% of those with a BPTB graft will have a graft rupture[9]. In a meta-analysis of 21 studies, QT failure rate was placed at 2.1%[10]. The mean follow up time was not stated but was likely less than the 10-year follow up time that the hamstring and BPTB revision rates were sourced from above. At 2 years post-op, 2 of the 25 participants who had an ACLr with a QT graft had a revision surgery, including one graft failure where the suturing was still intact[11]. Despite the increased failure rates with ST tendon grafts, they have some advantages over other autografts. Individuals with a hamstring graft tend to have better limb-to-limb symmetry in terms of range of motion, single leg hop distance[5], and strength[12,13] than individuals who had BPTB grafts. Additionally, the percentage of patients developing OA after ACLr with a hamstring graft has been shown to be lower (38%) compared to those who had a BPTB graft (58%) at 15 years post ACLr[5,9]. The advantages of the ST tendon graft over other options make it the graft of choice for many, but there are still many long-term consequences that can be difficult to address.

Current rehabilitation methods following an ACLr with ST graft place a large emphasis on restoring knee flexor strength to reduce the risk of reinjury[14]. However, due to the similar function of the three hamstring muscles, it can be difficult to target the ST muscle. To source the ST tendon for the graft, the tendon must be stripped in such a way that it has essentially suffered

a grade 4 muscle-tendon lesion, which is known to cause persistent neuromuscular inhibition[14]. It can take up to 18 months for the muscle-tendon unit (MTU) to heal[14], but rehabilitation programs tend to last less than a year. Before the MTU has healed, it is likely that exercises done with the goal of loading and rehabilitating the ST are producing altered force sharing patterns that load the semimembranosus (SM) and biceps femoris (BF) instead. As this occurs, the ST may be atrophying as the other hamstring muscles hypertrophy to compensate.

Altered force sharing patterns may also be due to ST morphological differences following the reconstructive surgery. The ST tendon has been shown to regenerate in 35-100% of individuals[15–23], but most studies have found morphological differences between a healthy ST tendon and one that was sourced for an ACLr. These differences include increased length[19], reduced fibril organization[24], and altered attachment points[18,23]. Additionally, the muscle cross sectional area (CSA) and volume are reduced, decreasing the force production capabilities of the ST[7,18,20,24,25]. Finally, the tendon is known to be significantly less stiff[22,26], which has a marked impact on a muscle's force production capabilities. The morphological differences seen in a ST sourced for an ACLr all indicate that the ST would likely have altered and reduced force production capabilities.

Existing research generally indicates that there may be altered ST functionality following ACLr with a hamstring graft. However, there are limitations to the techniques utilized, making it difficult to isolate the ST. Dynamometry studies cannot differentiate between the knee flexors, but have determined that knee flexion deficits of 0-14% persist 6-12 months after ACLr[12,13,18,23] and oftentimes persist for years[7]. A reduction in ST functionality that was not compensated for by the other knee flexors may explain these deficits. Surface electromyography (sEMG) studies have found alterations in lower limb muscle activity between an ACLr limb and healthy

controls[27–30]. While these studies are useful, it must be noted that sEMG cannot differentiate between the two medial hamstrings due to their proximity. For this reason, it is not possible to determine if the ST specifically is acting differently. Functional MRI (fMRI) is a non-invasive, but expensive method to qualitatively measure individual muscle use via T2 relaxation time[23]. Tampere et al. and Messer et al. both found significantly smaller exercise induced changes in ST T2 relaxation time in the affected limb compared to the healthy limb, indicating that the muscle was less active[24,31]. However, Takeda et al. did not find any significant differences in ST T2 relaxation time between ACLr affected and healthy limbs[23]. Dynamometry, sEMG, and fMRI provide evidence to suggest that the ST is behaving differently following ACLr. However, these methods have flaws that limit their ability to specifically and quantitatively measure the ST's functional capabilities.

Ultrasound based shear wave elastography (SWE) offers a promising solution to this problem. SWE non-invasively and cost effectively measures soft tissue stiffness or shear modulus via shear wave propagation through the soft tissue[32]. Multiplying the shear modulus by the physiological cross sectional area (PCSA), which can be measured with ultrasound, it is possible to calculate individual muscle forces[32]. Several studies have analyzed the connection between SWE-measured muscle stiffness and force, torque, and muscular activation, and found a linear relationship between these variables with high correlation coefficients[33–35]. For these reasons, it is believed SWE can be used to understand the connection between ST recruitment and tendon morphology following ACLr with a hamstring graft.

Understanding how morphological differences in the ST following ACLr may influence force production patterns will help therapists understand if alternative rehabilitation methods should be employed to improve ST functionality and normalize knee joint loading. It will also

help researchers and clinicians understand if altered hamstring load sharing might be a contributor to the increased risk of early onset OA following ACLr with ST or STG graft. These techniques can also be employed to understand PT and QT healing following ACLr with BPTB and QT grafts, and how morphological differences at the donor site might impact quadriceps load sharing.

Hypothesis

It is hypothesized that there will be morphological differences at the graft site following an ACLr with an ipsilateral autograft, and that this will cause altered muscle group load sharing and reduced voluntary force production capabilities of the affected muscle(s). Support of the overarching hypothesis regarding altered donor site morphology will lead to testing the following four testable sub-hypotheses:

SH1: The effects of a more compliant donor site tendon will lead to a shift of the knee flexion (ST graft) or knee extension (QT or BPTB graft) torque-angle curve towards longer muscle lengths. This is due to the increased strain of the tendon during isometric contractions reducing the length of the muscle fibers at a given joint angle. To reach the optimal fiber length, the resting MTU length must be longer than in a healthy muscle.

SH2: The effects of a compliant donor site tendon will be most pronounced at shorter muscle lengths. At shorter muscle lengths, the increased strain of the tendon upon isometric contraction will lead the muscle fibers to contract to a length that is shorter than their working range.

SH3: The effects of a more compliant tendon will be more pronounced at lower percentages of maximal voluntary contraction (MVC). At lower contraction levels, other muscles in the muscle group will compensate for the reduced functionality of the regenerated muscle-tendon unit, leading to minimal force production. However, at higher

percentages of torque production, the affected muscle(s) will be required to assist to reach the desired torque level.

SH4: Differences between healthy and ACLr ST or affected muscle(s) functionality will be correlated to the percent decrease in affected tendon stiffness. Tendon stiffness plays an integral part in a muscle's force production capabilities. The greater the difference in tendon stiffness compared to a healthy muscle, the greater the likelihood of large differences in muscle behavior and voluntary force production.

Purpose

Based on the long-term morphological differences of the ST tendon following ACLr with a hamstring graft, there is reason to believe that the hamstrings may be functioning differently. While the material properties of the QT and BPTB donor sites have not been explored extensively, due to the degree of lesion suffered, there is reason to believe that similar results would be found. However, this relationship has not been properly explored in any graft type due to the limitations of dynamometry, EMG, and musculoskeletal modeling. The purpose of this thesis is to investigate if an ACLr with an ipsilateral autograft will affect the impacted muscles' abilities to effectively produce force after rehabilitation. This will be explored through SWE, supplemented by EMG, dynamometry, and an exploration of the force-length relationship.

Significance

The significance of this study is to determine if there is reduced ST or quadriceps muscles' voluntary use following ACLr with an ipsilateral autograft. If there are reduced capabilities, this may contribute to altered knee joint loading and contribute to the increased risk of early onset OA. Early onset OA leads to large economic burdens to individuals and society, and reduced quality of life. It has been difficult to measure individual muscle function due to the limitations of dynamometry, EMG, fMRI, and force transducers. Ultrasound imaging and SWE

are an affordable and promising technique to quantitatively and non-invasively measure individual muscle forces. This thesis will investigate if voluntary use of the ST or quadriceps muscles is altered in the affected limb compared to the contralateral limb and healthy individuals during an isometric leg curl, which is known to preferentially activate the ST, or an isometric leg extension. It will test if the working ranges of the affected muscles differ from the unaffected limb. If there is reduced voluntary use of the muscle(s) following ACLr, alternative methods that focus on rehabilitating the donor site and the impacted muscles in its optimal working range should be explored to lower the risk of developing early onset OA.

Delimitations

- All ACL group participants had an ACLr with ipsilateral autograft at least 6 months prior.
- All participants have been cleared to return to sport by a physical therapist or their surgeon.
- All participants are over 18 years of age.

Limitations

- This is a retrospective study and as such cause and effect cannot be inferred.
- Characteristics of the uninvolved limb may have changed since injury but are treated as a baseline measurement.
- This is not a longitudinal study. Because of this, it is not possible to determine if a participant will develop OA.
- Measurements are limited to the reproducibility and accuracy of the equipment and technician operating the equipment.

Assumptions

- The uninvolved limb represents pre-surgical functionality of the involved limb in terms of muscle and tendon size, tendon stiffness, and muscle activation and force production.
- SWE assumes muscle is an isotropic material that behaves linearly. Similarly, it assumes that shear modulus is equivalent to stiffness and as such muscle force can be calculated by multiplying muscle PCSA by the active shear modulus of the muscle. While these are false assumptions, the same assumptions are regularly made during biological material testing[36].
- SWE measures tissue stiffness which will be assumed to be inversely related to tissue compliance.
- Fiber length was estimated from the literature, and was held constant for all individuals for a given muscle regardless of ACLr status.
- All instruments used are accurate and appropriately calibrated.

Operational Definitions

- ACL: anterior cruciate ligament
- ACLr: anterior cruciate ligament reconstruction
- Autograft: tendon tissue from the own patient that is harvested during the ACL reconstruction surgery to replace the ruptured ACL
- B-mode: an ultrasound mode in which two-dimensional images of tissues are displayed from ultrasound echoes
- BF: biceps femoris – the lateral muscle in the hamstring group. It consists of a long head (BFlh) and a short head (BFsh).
- BPTB: bone-patellar tendon bone – a common autograft for ACL reconstructions

- CSA: cross sectional area. In this study, muscle CSA is measured via analysis of B-mode ultrasound images
- Graft: soft tissue, usually tendon, that is placed in the knee during surgery to replace the torn ACL
- MTU: muscle-tendon unit – the entire muscle, including both the muscle and the tendon
- OA: osteoarthritis – a disease in which the articular cartilage wears down resulting in joint pain. The knees are most affected.
- PCSA: physiological cross sectional area; it is the area of the muscle perpendicular to its fibers at its largest point and is calculated as the volume of the muscle divided by the average fiber length.
- QT: Quadriceps tendon – a tendon that is increasingly used as an autograft for ACLr
- RF: Rectus femoris – a superficial quadriceps muscle that is located in the central portion of the anterior thigh
- SM: semimembranosus – a medial hamstring muscle that is deep and medial to the ST
- ST: semitendinosus – a medial hamstring muscle whose tendon is often used for ACL reconstruction grafts
- STG: semitendinosus + gracilis
- sEMG: surface electromyography - a method for measuring muscle activation via electrodes placed on the skin above the muscle
- SWE: shear wave elastography – an ultrasound-based technique to measure real time tissue stiffness
- Ultrasound: imaging technique that uses high-frequency sound waves to produce representations of soft tissue architecture

- VI: Vastus intermedius – a quadriceps muscle that lies deep to the other muscles
- VL: Vastus lateralis – a large quadriceps muscle that is located on the lateral portion of the anterior thigh
- VM: Vastus medialis – a quadriceps muscle that is located on the medial portion of the anterior thigh

Review of the Literature

Introduction to the Review of the Literature

The purpose of this thesis is to investigate if an ACLr with an ipsilateral autograft will affect the impacted muscles' abilities to effectively produce force after rehabilitation. Muscle activation and force production will be analyzed via surface electromyography (sEMG) and ultrasound-based shear wave elastography (SWE) during light and heavy isometric contractions at varying muscle lengths. Additionally, knee flexor and knee extensor strength and basic muscle models will be analyzed to gain an understanding of muscle voluntary use and capabilities following ACLr and rehabilitation. This chapter will review relevant literature by examining 1) ACL injury prevalence, recovery, and long-term consequences, 2) Donor site morphological differences following ACLr, and 3) Measuring semitendinosus activation and force production following ACLr.

ACL Injury Prevalence, Recovery, and Long-Term Consequences

Prevalence and Prognosis

ACL tears are a common musculoskeletal injury, impacting 100,000-200,000 Americans every year[1]. Difficulties obtaining all medical records for a given region can make it hard to define an exact rate of incidence, but the authors of a 21-year population study extrapolated their findings to determine a 68.6 per 100,000 Americans, or 1 per 1457.7, incidence of ACL tear[2]. Over the lifespan, males are more likely to sustain an ACL injury, but the story becomes more complicated when broken down by age and activity[1,2]. Sanders et al. found that females aged 14-18 were at a greater risk of ACL injury than males (227.6 vs 203.0 per 100,000), but then their incidence of rupture drops steeply while the risk increases for males. This is likely due to decreased female participation in organized sports following high school. When comparing injury rates between athletes in similar sports, females have a similar or higher risk of ACL

rupture[37]. ACL injuries are most common amongst teenagers and young adults[2]. This is particularly concerning when the long-term consequences of ACL rupture and subsequent reconstruction are considered.

The prognosis for a ruptured ACL is typically a reconstruction surgery, but some will take a more conservative rehabilitation only approach. Approximately 75% of those that tear their ACL will undergo a reconstruction surgery[1]. Adolescents and young adults are more likely to repair the ACL as they are typically aiming to return to high intensity sports and activities that involving pivoting motions. While Cevallos et al. found an overall ACLr rate of 75% in their analysis of nearly 230,000 ACL tears, 92% of patients aged 10-19 had a reconstruction surgery as did over 80% of those aged 20-39 years. The percentage of those who had an ACLr dropped significantly with age[1]. The recovery period after an ACLr is substantial with typical return to sports timelines ranging from 4-12 months or more [3]. While most are cleared for sports in this timeframe, as few as 33% return to their pre-injury sport level at twelve months postop[38]. In elite athletes, as many as 83% may return to competitive sports with recovery times lasting an average of 6-13 months[39]. This number may be more reflective of the knee's ability to return to competitive sport following ACLr due to the higher commitment levels preinjury of these athletes. Alternatively, the higher rate may be explained by increased resources throughout the recovery process; ACLr and subsequent rehabilitation come with a high price tag,

The median total cost of an ACLr and rehabilitation was $13,403.38 based on an analysis of 229,446 ACL reconstructions from 2005-2013[40]. This number increased throughout the sample period, so it is logical to believe it is higher now. When accounting for income lost, osteoarthritis (OA) development, and other indirect costs, the lifetime burden of an ACL rupture is as high as $88,538 per patient for a rehabilitation only approach and $38,121 per person for an

ACLr[4]. This leads to a substantial annual economic burden of over $7 billion in the United States alone when a surgical approach is taken and over $18 billion for a rehabilitation approach[4]. These numbers support a surgical approach from an economic standpoint, but they also illustrate that the long-term effects of an ACL injury must be addressed to reduce the financial burden.

Development of Osteoarthritis

Around 50% of individuals who rupture their ACL will develop OA in the fifteen years after their injury[5]. The OA will likely be moderate to severe in over a quarter of patients by 20 years after their ACLr[41]. Since ACL injuries most commonly occur in teenagers and young adults, this means many patients are developing OA before age 40, when the disease is typically seen in older populations[42]. It appears that those who have a reconstruction with a BPTB graft (58%) have a significantly higher risk of developing OA compared to those who had a reconstruction with a ST or STG (38%) graft[5,9]. While lower, the risk of developing early onset OA following ACLr with ST graft is still significant. The risk of developing OA following ACLr with a QT has not been explored to the author's knowledge.

The development of OA at a young age causes a decreased quality of life, as well as subsequent surgeries and financial burden. OA in the knee causes a narrowing of the joint space, which can lead to pain, reduced range of motion, and stiffness[43]. Mild OA is estimated to cost individuals an average of $9,801 annually, while the price increases to $22,111 annually for severe OA[4]. As the disease progresses, many sufferers require a total knee replacement. Having an ACLr has been shown to increase the incidence of total knee arthroplasty by as much as seven times[44] with as many as 10-15% of individuals who have an ACLr estimated to have a knee replacement during their lifetime[4]. Knee replacements do not last forever, and the younger an individual is when they receive one, the more likely they are to require a revision surgery. Individuals who have a total knee replacement when they are under 60 years old have as high as

a 33% risk of needing a revision surgery during their lifetime while the risk is under 10% for those who are over 70 years old when they have their arthroplasty surgery[45]. An ACL injury can appear devastating in the short-term to a teenager or young adult, but it is the long-term consequences such as early onset OA that can be detrimental for the rest of the individual's life.

The mechanisms behind early onset OA following ACLr are unknown, but recent research has suggested it may be due to unloading of the medial knee compartment following ACL rupture and reconstruction. A 2016 study followed 22 participants at five separate time marks ranging from after ACL rupture but prior to pre-operative rehabilitation to two years post ACLr[6]. Seven of the participants developed OA, and it was found that these individuals walked with greater frontal plane limb asymmetries, had lower peak knee adduction moments, and lower peak medial compartment contact forces. Interestingly, frontal plane asymmetries were present at baseline prior to preoperative rehabilitation and at 6 months post ACLr, but not at 1- and 2-years post ACLr. Similarly, reductions in knee adduction moment were greatest at baseline, but disappeared until 2 years post ACLr. Peak medial compartment contact forces were only significantly reduced at 6 months post ACLr before stabilizing to match the forces seen in the group that did not develop OA by the two-year post ACLr radiograph[6]. There are two key takeaways from this study. First, it may be possible to detect who is most susceptible to developing OA at the time of injury. Second, medial compartment loading in the first few months after ACLr might have a large impact on the development of OA. The authors theorized that the unloading of the medial knee compartment after surgery led to degradation of the cartilage. Once loading normalized, the cartilage was not strong enough to handle the loads and early onset OA developed[6]. This study unfortunately did not separate findings by graft type, but supports the theory that unloading, not overloading, may be causing OA in ACLr patients. These

changes might be due to decreased axial or transverse loading contributions from the ST, or due to loads being more concentrated on thinner articular cartilage within the joint space than in a healthy limb[46]. Since OA following ACLr is most observed in the medial compartment[5,9,41], it makes sense that differences in medial compartment loading might be occurring following ACL reconstruction, contributing to the development of OA.

A musculoskeletal modeling study further determined how decreases in ST muscle cross sectional area (CSA) and volume following ACLr with STG tendon graft may affect medial compartment contact forces. The study's model decreased the ST and gracilis muscle CSAs by 33% and 39% and the muscle volumes by 47% and 35%, respectively, as supported by the literature[7]. Optimization equations in the EMG-based model determined that the semimembranosus (SM), and to a lesser degree the biceps femoris (BF), was able to compensate for most of the ST muscle-tendon morbidity seen by increasing in size, strength, and activation. This stabilized the tibiofemoral joint and led to relatively similar contact forces compared to their healthy model during walking, running, and cutting[7]. While it is possible that contact forces are similar between healthy and surgical repaired knees, there is likely a long-term effect on cartilage health when the share of force production, and thus location and magnitude of cartilage loading, is altered to compensate for a damaged ST. To the author's knowledge, this relationship has not been studied outside of modeling studies.

ACL Reconstruction

The goal of an ACLr is to prevent excessive anterior tibial translation in the sagittal plane[3]. To accomplish this, an autograft from the hamstrings or quadriceps is typically used, although an allograft is occasionally used. An autograft sourced from the patient's ipsilateral ST tendon is used for over 40% of ACLr, and is often supplemented with the gracilis tendon to create a bundled semitendinosus + gracilis (STG) graft[8]. Alternatively, a bone-patellar tendon-

bone (BPTB) graft sourced from the patella tendon can be used; this was the graft of choice for 17% of the patients in the same meta-analysis of 330 studies including ACLr from 1969-2018[8]. Over the past decade, use of the quadriceps tendon (QT) has become a more common autograft choice[47]. When sourcing this autograft from the common quadriceps tendon, a central portion of the tendon is taken to form the graft, but exact surgical procedures vary by surgeon. Typically, the tendon fibers that feed into the vastus medialis (VM) are avoided[48]. Other graft options include allografts or grafts made with synthetic materials. While recovery time is reduced and graft site morbidity is eliminated, the cost of allografts and synthetic grafts are significantly higher and they have been shown to fail at higher rates than autografts[49]. For these reasons, most surgeons and patients tend to choose an autograft for ACLr. However, autografts also have drawbacks.

Independent of graft type, there are frequently short- and long-term strength deficits following ACLr. The location of the most pronounced strength deficits depends on the graft type. Typically, greater knee extensor torque deficits are seen when a BPTB or QT graft is used, while greater knee flexor torque deficits are seen when a graft is sourced from the hamstrings[12]. A study has also found that extensor torque deficits following ACLr with a BPTB graft are more pronounced than the knee flexor torque deficits observed following ACLr with a ST graft[12]. It is intuitive that extensor torque asymmetries are greater following ACLr with a BPTB or QT graft while flexor torques are affected after an ACLr with a ST graft, as the tendons sourced for the autografts affect a knee extensor and knee flexor muscle, respectively. Asymmetries in both the flexor and extensor torque are undesirable and can affect risk of re-injury[14,50], as well as functional performance.

Functional performance deficits, which are likely influenced by the above stated strength deficits, may remain for many years after ACLr. However, these deficits may be less pronounced long term with a ST graft, compared to a BPTB autograft. A 15-year prospective study analyzed single leg hop performance at 2, 5, 7, 10, and 15 years postop in 180 participants[5]. They found 87-90% of patients with a BPTB graft achieved greater than 90% single leg hop distance limb-to-limb symmetry at the first four follow up examinations, but the percentage dropped steeply to 65% at the 15-year follow up. While limb-to-limb symmetry can provide a good idea of affected limb function, it cannot capture changes in the unaffected limb that may inflate or reduce the symmetry index. This suggests that degradation of the knee or graft might have occurred with time. The ST graft group performed similarly to the BPTB graft group for the first four follow up sessions, but significantly better at the 15 year follow up, with 92% of the 90 participants achieving greater than 90% limb-to-limb symmetry[5]. These findings suggest that a hamstring graft may be able to better support the knee's functional performance in the long term.

Range of motion deficits follow a similar pattern to strength deficits: extension deficits are seen with BPTB grafts and flexion deficits are seen with ST grafts, but the deficits are more pronounced with a BPTB graft. Leys et al. found that 31% of participants that had an ACLr with a BPTB graft had 3-5° of knee extension deficit 15 years after their reconstruction surgery[5]. Only 2% of participants in the ST graft group had a flexion deficit greater than 5°. While 6% of those in the study that had an ACLr with ST graft had 3° or more of extension deficit and nobody in the BPTB graft group had a flexion deficit greater than 5°, significantly more individuals in the BPTB group had range of motion deficits than in the ST group. These range of motion results point towards the ST graft having greater outcomes in functional performance. Functional

outcomes in terms of strength, hop, and range of motion deficits point towards a ST graft, but it is far from a perfect option.

ACLr is a reliable surgery, but a non-zero percentage of people do require revision surgeries due to graft failure or rupture. The percentage of people requiring a revision surgery is greatest in those that had an ACLr with a ST graft[5,8]. A meta-regression analysis examining 330 articles published from 1984-2021 related to ACLr revision rates found an overall revision rate of 3.14%[8]. In their analysis, ST grafts had a slightly higher rate of revision surgery (2.71%) compared to BPTB grafts (2.38%). This regression analysis is the most comprehensive study analyzing revision rates, but the studies pooled had a mean follow up time of just 2.3 years. Revision rates for QT grafts have been found to be comparable to the other autograft types[47].

A study that followed 180 patients from the time of ACLr to 15 years post ACLr found that 17% of those that had an ACLr with a ST graft had a graft rupture[5]. 8% of participants in the BPTB graft group had a graft rupture, indicating that the autograft had torn. A 10-year follow up study of 180 participants found a ST graft rupture rate of 14% and a BPTB graft rupture rate of 8 percent[9]. Cevallos et al. found a revision rate of 6% in their retrospective study of 229,296 ACL ruptures from 2010-2017[1].

These rates of graft rupture are significantly higher than the revision rates seen in Liukkonen's meta-regression analysis and most likely are more representative of the true graft rupture rate due to the longer follow up times. They also suggest that a ST graft carries a higher risk of rupture than a BPTB graft.

Rehabilitation after ACLr with a Semitendinosus Graft

Following ACLr with a ST graft, there is a large emphasis placed on restoring knee flexor strength to reduce the risk of re-injury. Since every additional 10% deficit in hamstrings to quadriceps strength ratio has been shown to increase the risk of re-injury 10.6 fold[14], physical

therapists focus on achieving limb-to-limb symmetry. To accomplish this, many knee flexor, hip extensor, and functional exercises are utilized[14]. However, due to the similar functions of the three hamstring muscles, it can be difficult to target the ST muscle specifically.

Exercises may be selected to focus on rehabilitating the donor graft site, but it is unknown if they are successful in rehabilitating the ST or in creating compensatory force production patterns to perform the exercise. During an exercise that preferentially activates the medial hamstrings in healthy individuals such as a Nordic hamstring exercise or leg curl[51], the SM and ST are expected to work together, along with some activity from the BF long and short heads of the lateral hamstring. Following an ACLr with ST graft, the tendon has suffered essentially a grade 4 muscle-tendon lesion, which is known to cause persistent neuromuscular inhibition[14]. It can take upwards of 18 months for the lesion to heal[14]. Before the ST MTU has healed, it is unlikely to be loaded properly.

Before the ST tendon has regenerated, any exercises done with the goal of loading and rehabilitating the ST are likely loading the other hamstring muscles instead while the ST atrophies. Any exercises done also need to be within the working range of the regenerated ST's muscle fibers. This working range may be at different joint angles than observed in a healthy ST due to morphological differences in the ST following ACLr with ST or STG graft. The other hamstring muscles may be doing a good job at compensating as achieving at least 85-90% limb-to-limb symmetry on various tests is typically a prerequisite to be cleared for contact sports[3,14]. However, there might be long-term consequences of changing hamstring activation patterns such as altered knee joint loading which may eventually cause OA.

Semitendinosus Morphological Differences Following ACLr with Hamstring Graft

Following an ACLr with a ST graft, the ST tendon regenerates in most individuals, but there are morphological differences that impact the function of the MTU. Several studies have

estimated that the ST tendon regenerates in 35-100% of individuals[15–23]. However, most studies have found that even tendons that have regenerated have morphological differences compared to their healthy counterparts. These differences include altered attachment points, increased tendon length and area, decreased organization, altered composition, and reduced stiffness.

The large-scale alterations to a regenerated ST tendon such as changes in length, area, and attachment points also affect the muscle and may impact its ability to produce force and torque. The regenerated ST tendon has been found to be significantly longer[19] which consequently decreases the length of the ST muscle[24]. The muscle may reduce in length by 4-10cm, which leads to a proximal shift of the muscular tendon junction by about the same length[20]. The tendon attachment may be more superficial, as observed in a study looking at ST tendon regeneration in rabbits[36]. The increased tendon and reduced muscle length coupled with the more distal attachment point affect the force-length curve of the ST, reducing the spread of optimal fiber lengths for force production.

The differences in the tendon's size may be partially responsible for reducing the muscle area and volume. Smaller ST muscles following ACLr and subsequent rehabilitation have been observed in many studies[18–20,24,25]. The CSA of the regenerated ST muscle can be 20-39% smaller compared to the healthy ST[7,20,24]. The ST volume is also reduced by 25-47%[7,20,24]. These atrophied muscles have a reduced ability to produce high levels of force. This leaves the knee flexors with a strength deficit unless other muscles compensate. Messer et al. and Konrath et al. both found that the SM and BF muscles on the surgical side were larger than on the control side. However, the hypertrophy in these muscles were not enough to overcome the atrophy of the ST, leaving the overall hamstring muscle volume reduced[19,24]. The atrophied regenerated ST leaves

the ACLr limb at a disadvantage. As explained earlier, other muscles may need to compensate for the ST, but this also has short- and long-term consequences.

In addition to alterations in size and attachment point, the regenerated ST tendon is typically less organized. This can reduce its ability to effectively transmit force. Messer et al. found that 11 of the 14 participants in their study had a partial or complete loss of fibrillary patterns in their regenerated ST tendons[24]. Similar results were found in a study looking at ST tendon regeneration in rabbits[36]. The rabbits' regenerated tendons' cells were less organized, the collagen fibrils were significantly smaller in diameter, and the levels of hydroxyproline and proteoglycan in the extracellular matrix were significantly lower compared to normal tendons[36]. The reduced organization of parallel fibers throughout the tendon and altered composition minimized its ability to transmit force. Additionally, it reduced the tensile strength and stiffness of the tendon.

Healed ST tendons are known to be significantly less stiff, reducing their ability to transmit, store, and produce force. In a study with 13 participants who had had an ACLr with a STG graft 6-24 months prior, 12 participants had a regenerated tendon but this tendon was 43.6% less stiff than the uninvolved limb[22]. There was a significant positive correlation between time since ACLr and ST tendon stiffness. Those who had their ACLr 6-12 months prior recovered 39.9% of their healthy limb's ST tendon stiffness compared to 80.6% for those who had their ACLr 12-24 months prior to measurements[22]. In a study analyzing ST tendon regeneration in rabbits, the regenerated tendons were about a third as stiff as native tendons[36]. Amat Fernandez also found reduced ST tendon stiffness in those who had one or more ACLr and underwent subsequent rehabilitation. In line with Suydam's results, she found the reconstructed ST tendon was 40% less stiff than the contralateral healthy limb[26]. Participants had their ACLr(s)

at a large range of time prior to analysis; the range was 6 months to 10 years post ACLr. She did not find an effect of time since ACLr on ST tendon stiffness. Additionally, the healthy limb in the ACLr group had a somewhat stiffer ST tendon than the matched non-reconstruction limb of a healthy control group[26]. This suggests that there may be a compensatory reaction in the contralateral ST causing it to become stiffer, and thus more able to effectively transmit, store, and produce force. While the effect of time since ACLr on ST tendon stiffness is unclear, it is evident that stiffness is reduced in a regenerated ST tendon.

Reduced tendon stiffness is known to have an impact on force production capabilities. A tendon's ability to transmit and store force is positively related to stiffness via Hooke's Law $F_m = k\Delta L_t$ where F_m is equal to muscle force, k is the stiffness of the tendon, and ΔL_t is the change in tendon length from rest to contraction. This relationship is often explained in terms of tendon compliance, or the inverse of tendon stiffness; increased tendon compliance reduces the tendon's ability to transmit force. Because of this relationship, it is logical to believe that a decrease in tendon stiffness, and increase in tendon compliance, will have a significant impact on muscle force. This relationship is explained further in **Appendix C.** No studies have directly measured the effect of a less stiff ST tendon on ST force production capabilities in humans. However, a study has been done in rabbits. In Gill et al.'s study, the rabbits' regenerated ST tendons were only a third as stiff as the native tendons. These regenerated tendons were only able to create and sustain about 25% of the load that the native tendons were able to[36], suggesting that a reduction in ST tendon stiffness might cause an even bigger reduction in force production capabilities. The load was applied to the dissected MTUs via an electrical stimulation machine, so no other muscles were able to contribute to the force production.

In humans, the effect of reduced tendon stiffness has been analyzed in its relation to rate of torque development and force output in the vastus lateralis and the Achilles. Bojsen-Moller et al. found that vastus lateralis tendon stiffness was positively correlated with isometric and dynamic movement performance[52]. The isometric rate of torque development was greater when vastus lateralis tendon stiffness was greater, as were the peak power and mean power in the concentric phase of the counter movement and squat jumps. Hannah and Folland also found that vastus lateralis tendon stiffness is positively correlated with voluntary explosive force, which was defined as the time to achieve 150-300N of force[53]. However, when the authors separated their findings by participant's maximal voluntary force, this relationship disappeared. Their findings suggest that vastus lateralis stiffness affected maximal strength, but not necessarily explosive force production[53]. In the Achilles tendon, reduced tendon stiffness due to tendinosis has been shown to reduce the normalized rate of force development, reduce the amount of stored and released elastic energy, cause longer electromechanical delays in the soleus and gastrocnemius, and increase mechanical hysteresis[54]. These studies show that reduced tendon stiffness in humans leads to many undesirable outcomes including reduced explosive force development capabilities and potentially reduced strength. However, these relationships have yet to be explored in the ST due to the difficulties of collecting such data.

Following ACLr with a BPTB graft, shear wave elastography of the PT found that the shear wave speed increased with time since surgery towards normal values[55]. To our knowledge, SWE has not been used to explore QT material properties following ACLr. It is important to understand these PT and QT donor site tendon properties to determine how quadriceps force production may similarly be impacted following ACLr with a BPTB or QT graft.

Measuring Semitendinosus Activation and Force Production Following ACLr

A variety of techniques have been used to study knee flexor and ST strength following ACLr, but the limitations of these methods make it difficult to isolate the capabilities of the ST. Dynamometers are not able to isolate individual muscles but instead measure the torque development of entire muscle groups such as the knee flexors. EMG attempts to isolate individual muscles but is subject to crosstalk due to the proximity of the ST and SM. Functional MRI can effectively isolate muscles, but it cannot provide an exact amount of force production or activation; instead, it measures changes in T2 relaxation time to make inferences. Force transducers can address the aforementioned issues, but they are highly invasive and as such have not been used to measure ST force production in humans. Modeling can address this issue, but it is using educated guesses to estimate the share of force production between the hamstring muscles. While there are flaws in existing research methodologies, these studies can be used to infer ST functionality after an ACLr with hamstring graft.

Dynamometry

Studies attempting to estimate knee flexor strength have generally found deficits in the affected limb following ACLr and subsequent rehabilitation, but there are limitations to these studies. Dynamometry measures joint torques, which are representative of all the agonists, antagonists, and synergists producing the movement[35]. Because of this, it is not possible to determine the contributions of individual muscles, or muscle groups such as all the agonists.

Most research studies analyzing knee flexor strength after ACLr do so with individuals who had an ACLr 6-12 months after the surgery. At this point, individuals are expected to be far along in their rehabilitation process, or cleared to return to sports[3]. Despite this, peak isokinetic knee flexor strength deficits range from 0-14% for those that had an ACLr with a hamstring graft compared to the healthy contralateral limb[12,13,18,23]. Konrath et al. measured differences in

isokinetic knee flexor force at 2-4 years post ACLr with a STG graft in 20 individuals and found greater strength deficits compared to the studies collected 6-12 months following surgery. When both the ST and gracilis tendons regenerated, isokinetic strength deficits were 20 ± 18%, while they were 25 ± 8% and 38 ± 12% when one or neither of the tendons regenerated, respectively[7]. While regeneration was evident, the stiffness of the tendons was not measured.

Hart et al. also looked at peak isokinetic flexion torque at different angles while participants were in a seated position and found that the differences between the affected and healthy limb differ by joint angle tested at. However, peak knee flexion torque did appear at similar knee flexion angles (64 ± 15° for ACLr limb compared to 62 ± 13° for contralateral limb). Makihara et al. also found that the angle of peak isokinetic knee flexor torque did not differ between ACLr (21 ± 10°) and healthy (17 ± 7°) knees while the participants were in prone[56]. This is a significantly different angle for peak knee flexor torque compared to Hart et. al, but this was likely due to the altered hip flexion angle during testing. Studies have found that differences in knee flexor torque between healthy and ACLr limbs increase significantly as knee flexion angle increases, shortening the ST muscle[56,57]. Similarly, Weaver et al. measured peak isometric force with the knee at 60° of flexion and found that males and females had a 20.31% and 16.4% flexion deficit compared to their healthy limbs, respectively[13]. Using the contralateral limb serves as a convenient comparison, but it cannot factor in potential strength deficits across both limbs compared to pre-injury. While these studies clearly show that knee flexor strength deficits remain after ACLr and rehabilitation, they are not able to determine how and if the ST is contributing. They also cannot determine if there are any compensatory adaptations in the other knee flexor muscles.

Electromyography

EMG studies can isolate muscles in theory, but crosstalk and other issues can make it difficult to obtain accurate data. The SM lays directly medial to and deep to the ST which means the surface electrode cannot differentiate between the two muscles[24]. Most EMG studies recognize this and do not attempt to separate the two, instead grouping them together as the medial hamstrings[7,27–30,58]. Fine wire EMG makes it possible to differentiate between the ST and the SM, but crosstalk is still a potential issue. Additionally, placing the electrodes accurately over the muscle belly can be difficult following ACLr due to movement of the muscle attachment point and changes in the MTU morphology[20]. It must be mentioned that EMG provides a relative value of neural activation and is typically reported as a percentage of a muscle's maximal voluntary isometric contraction (MVIC). However, neural activation is not always linearly related to force production[32]. Changes in muscle length, fatigue, contraction velocity, and contraction type (i.e. concentric compared to eccentric) have been shown to alter the relationship between neuromuscular activation and force production[32]. As the muscle changes length due to the changes in joint angle, the amount of muscle fascicles and motor units beneath the surface electrode change, changing the relative meaning of the EMG signal[59]. Additionally, most studies following ACLr are measuring medial hamstring EMG activity compared to the contralateral limb or healthy controls as a function of each limb's MVIC. A difference in percentage of MVIC is not necessarily fair as the MVIC between the two limbs itself might be very different in terms of its relative meaning. While there is value in EMG measurements to describe the neuromuscular activation strategies of the medial hamstrings following ACLr, these measurements cannot provide information regarding ST functionality compared to the SM.

There are flaws with EMG studies, but they can be a helpful reference of how the ST might be functioning following an ACLr. Due to the accessibility of EMG in most biomechanics

laboratories, many studies have done so with varied findings. Ortiz et al. found that both quadriceps and hamstrings activity was reduced in the affected limb following ACLr with a STG graft during a double leg drop jump task compared to healthy controls[29]. Since both muscle groups had reduced activation, the co-contraction ratio was not altered. However, the reduced hamstrings and quadriceps activation may suggest a lack of knee joint stability, or a reliance on the unaffected limb. The study also analyzed single leg drop jumps and found a significant increase in the quadriceps neuromuscular activity compared to controls, but no difference in hamstrings activity. There was significantly reduced time to maximal neuromuscular activation for the knee flexors and extensors in the single leg drop jumps compared to healthy controls[29]. The authors hypothesize that this is a compensatory action attempting to cushion the landing, but when the additional quadriceps activity is not met with additional hamstrings activity, the knee may be subject to greater anterior displacement[29]. During single leg hops, Rush et al. found greater limb-to-limb asymmetry in medial hamstring activation in 11 ACLr individuals compared to healthy controls (32.38% versus 16.27%), but there were no between limb differences within the ACLr group[30]. This suggests that the differences between the limbs were not in a single direction across all participants. Indeed, of the two individuals who had a hamstring graft in the sample group, one saw a large decrease and the other saw an increase in affected limb medial hamstring activity. The individuals had their surgeries on average 6 years prior to data collection[30]. Briem et al. published a similar study, but analyzed single leg crossover jumps as opposed to single leg jumps. They found similar lateral and medial hamstring activation in the ACLr operated limb and both limbs of healthy controls during the movement. However, the lateral hamstring activity was significantly higher in the unaffected limb of the ACLr individuals

compared to the medial hamstrings[27]. This was surprising as differences are generally expected in the affected limb if they are present.

Electromyography can also be used to analyze tasks other than jumps to gain a fuller understanding of ST functionality. Hansen et al. found that after ACLr, lower limb muscle activation patterns are altered during running. With sEMG, they saw that healthy controls have similar activation patterns while running at 70, 80, and 90% of bodyweight compared to full body weight running[28]. Conversely, at seven months post ACLr with ST graft, activation patterns did not match full bodyweight patterns until 90% of bodyweight. This suggests that even though individuals have been cleared to run and return to sport, they are not using their muscles the same way and as such might be at higher risk of injury. Similarly, using fine wire EMG, Williams et al. suggested that the SM might be acting with less specificity, and therefore more often, six months after ACLr with STG graft during a target-matching protocol. The ST and gracilis muscle activity was not significantly different between the affected and contralateral limbs, but the affected limb's SM was activating more frequently, indicating significantly less specificity in its activation[60]. This finding suggests that it is important to differentiate between the two medial hamstring muscles due to potential compensatory behaviors. The medial hamstring group may be activating normally following ACLr as Briem et al. and Ortiz et al. observed, but there might be differences when studying the ST and the SM individually. It is not possible to measure these differences with sEMG and fine wire EMG is more invasive.

Based on a force-length relationships, it would be expected that the ST would be producing more force at its optimal fiber length. However, several studies have found that ST or medial hamstring EMG activity in healthy individuals is higher at knee flexion angles that result in shorter muscle lengths than those seen when peak isokinetic knee flexor torque is

achieved[56,61–64]. This implies there is not a direct relationship between knee flexor torque and medial hamstring neuromuscular activation. This may mean that the medial hamstrings are more activated at shorter lengths than they are when the knee flexors are producing the most torque, or alternatively there are simply more motor units under the surface electrode at shorter muscle lengths. However, it is also possible that the relationship between neuromuscular activation and torque development is not linear across the muscle length spectrum. At more optimal muscle lengths, the hamstrings can more effectively produce joint torques due to the lengthened moment arm[62]. This means that at shorter muscle lengths there would need to be greater muscle force to produce the same amount of joint torque. Ultimately, the takeaway is there is not a direct relationship between neural activation and joint torques nor between muscle force and joint torques as joint angle changes and alters muscle length.

Functional Magnetic Resonance Imaging

Functional magnetic resonance imaging (fMRI) studies offer an alternative to dynamometry and EMG. While fMRI can isolate muscles and is noninvasive, limitations exist and results from these studies are mixed. Functional MRI assesses T2 relaxation time prior to and immediately following exercise; a muscle that produced more force is expected to have a greater increase in T2 relaxation time from baseline than one that was minimally active[23]. Measuring the T2 relaxation time, which is the "time constant that characterizes the exponential decay of the nuclear magnetic resonance signal after initial excitation[23]," allows researchers to easily determine the proportion of the muscular force produced by each of the muscles. However, this does not provide a quantitative value of force production per each muscle, but instead a comparison between the muscles. This is an advantage over sEMG as it can differentiate between the two medial hamstring muscles, but fMRI remains cost prohibitive for many researchers.

Those that have used fMRI to understand knee flexor force sharing following ACLr with a hamstring graft have found mixed results. Takeda et al. found no significant differences in T2 relaxation times across the hamstring muscles in the ACL reconstructed and healthy control limbs after isometric and isokinetic knee flexion exercises[23]. Participants had their ACLr an average of 12.7 months prior to data collection. Studies completed by Tampere et al. and Messer et al. contradicted Takeda et al., finding significantly smaller changes in ST T2 relaxation time following exercise in the ACLr limb. In 14 individuals who had their ACLr with hamstring graft 1-6 years prior to the study, the affected ST had a third less exercise-related change in T2 relaxation time compared to the contralateral limb[24]. There were no differences in changes in T2 relaxation time pre versus post exercise between groups for the other hamstring muscles. In male soccer players who had an ACLr with a ST graft, there was a significantly reduced change in ST T2 relaxation time and a significant increase in BF T2 relaxation time compared to healthy controls following an intense prone leg curl protocol[31]. This suggests that the ST was not activating as much, and there might have been a compensatory increase in BF activity. This finding is particularly interesting as the prone leg curl is known to preferentially activate the ST compared to the lateral hamstrings[65]. Tampere et al. and Messer et al.'s findings suggest that the ST does not behave the same way following ACLr, but fMRI provides a relative measure; it is still necessary to obtain direct measurements of force to determine how the ST is operating and impacting knee joint loading.

Force Transducers

Force transducers can address many of the problems associated with other techniques, but they are highly invasive. These implantable devices provide a specific value of force produced by individual muscles by measuring the amount the transducer strains; the stiffness of the transducer is known so it is possible to calculate the muscle force from the transducer strain.

However, force transducers must be surgically implanted. For this reason, their use in humans is limited, and published studies tend to be case reports or have very small sample sizes. Studies using force transducers in live humans have typically focused on muscles near the achilles[66–68] or the contact forces associated with joint implants[69]. Force transducers have not been used to measure hamstring forces in healthy or ACLr individuals due to the potential ethical concerns of implantation.

Musculoskeletal Modeling

Musculoskeletal modeling and optimization offer another way to estimate ST muscle forces following ACLr. However, without knowing how forces are typically shared amongst the hamstrings due to the limitations of the other techniques mentioned, it is difficult to know the accuracy and meaningfulness of the results. To create an accurate model, parameters such as muscle, tendon, and fiber length; moment arms; fiber orientations; muscle and tendon cross-sectional areas and volumes; tendon insertion points; tendon stiffness; force-length profiles, force-velocity relationships, and several others must be known. It is very difficult to accurately measure all these factors in live humans, and furthermore to know the cost function applied is accurate. However, if used with caution, muscle models can provide insight into how the hamstrings may be functioning following an ACLr with ST graft.

Konrath et al. used data from 25 individuals who underwent an ACLr with STG graft to create a muscle model to better understand the effect of ACLr on tibiofemoral compartment loading[7]. They collected motion capture, force plate, and EMG data while participants performed various strength and functional tasks. Based on existing literature, the authors reduced the CSA and volume of the surgical ST by 33% and 47%, respectively, and of the surgical gracilis by 39% and 35% due to donor site morbidity. The collected data and the literature were used to produce a model. The results indicated that the SM compensates for the reduced voluntary force production

of the ST and gracilis muscles following ACLr, as well as contributes more to medial compartment loading during running and cutting. The model suggests that there are minimal changes to medial knee compartment loading following ACLr, but the individual muscle contributions are changed[7]. While data was collected to inform the model creation, many informed assumptions were made such as tendon slack length, force-length and force-velocity profiles, and joint moments. It is difficult to verify these assumptions, and as such the findings of this study.

Shear Wave Elastography

Shear wave elastography (SWE) is a relatively new, emerging way to non-invasively measure individual muscle forces. Ultrasound based SWE measures soft tissue stiffness or shear modulus via shear wave propagation through the soft tissue; the propagation velocity of the shear wave is directly related to the shear modulus of the tissue[32]. When muscle fibers are aligned with the probe, it has been shown that the shear modulus strongly and linearly relates to Young's Modulus. It is also possible to use ultrasound to measure a muscle's physiological cross sectional area (PCSA). Given the shear modulus and the PCSA, it is possible to calculate an index of individual muscle forces with the equation

$$F_m = \mu * PCSA$$

Equation 1

where F_m is the muscle force, μ is the active shear modulus, and PCSA is the muscle's physiological CSA. While this may not be exact due to simplifications regarding muscle's anisotropic properties, it provides a strong, simple index of force production. It has also been demonstrated the muscle shear modulus is linearly related to both passive and active muscle force[32]. Unlike EMG, this relationship holds true before and after fatigue, as well as during passive stretching and isometric contractions[32].

Several studies have analyzed the connection between muscle stiffness, force, torque, and activation, and these studies have generally found a linear relationship between these variables. Bouillard et al. analyzed isometric index finger abduction and isometric little finger abduction as these two movements are known to be controlled purely by the first dorsal interosseous and primarily by the abductor digiti minimi muscles, respectively. The 11 participants were told to randomly vary their torque, as well as do so in controlled fashions. The shear elastic modulus and torque were correlated with R^2 values greater than 0.95 for both the muscles, and for all subjects. The EMG and torque data were also well correlated, but R^2 values ranged from 0.847 and 0.992 for the two muscles across the participants[34]. A related study was published in 2015 and found similar results across the entire range of isometric contraction intensity tested (0-70% MVIC)[33]. Since the two movements studied are known to be primarily controlled by the muscles analyzed, the linear relationship between SWE measured shear elastic modulus and torque suggests that SWE can be used to analyze individual muscle force production.

The relationship between torque and shear elastic modulus does not appear to be affected by fiber length nor fatigue. In the tibialis anterior, muscle shear modulus and tetanized muscle force were found to be correlated[35]. These relationships were tested with voluntary and electrically stimulated contractions at different muscle fiber lengths. This shows that values measured with SWE can pick up differences in muscle force production due to the force-length relationship. SWE has also been shown to have the same linear relationship with torque following fatigue, while the EMG-torque relationship changes following fatigue[70]. The adaptability of SWE to different load conditions can be advantageous compared to EMG.

The largest advantage of SWE for studying hamstring use after ACLr is the ability to easily analyze load sharing. In healthy individuals, Pimenta et al. found that the ST had higher

shear modulus values during 20% MVC prone leg curls on a dynamometer compared to the BF, suggesting that the ST was more active during the movement[71]. Similarly, Evangelidis et al. found that the ST was significantly more active than the BF during 20-70% MVC isometric, concentric, and eccentric contractions in prone, as measured via shear wave velocity[72]. The ST was more active, but not significantly more so, than the SM. The stiffness of each of the muscles increased with increasing contraction intensity with the group correlation coefficients being greatest during the isometric contractions (Adjusted $R^2 = 0.810$-0.945). The ST stiffness continued to increase until around 60% of MVIC, while the values tended to stabilize around 30-40% of MVIC for the SM and BF during all contraction types[72]. This suggests that the ST might be most responsible for additional knee flexor torque production at higher contraction intensities. Mendes et al. found somewhat different results than Evangelidis et al., with the BF increasing in stiffness for all isometric contractions from 10%-60% MVIC, and the ST stiffness remaining relatively constant from 20-60% of MVC[73]. Despite the stability of the ST stiffness, it was stiffer than the BF, suggesting that it is more active during knee flexor activities than the lateral hamstring like Pimenta et al. and Evangelidis et al. observed. These results suggest that the ST is vital for knee flexor activity, and as such if it cannot activate following ACLr, this may be problematic. SWE can non-invasively give insight into hamstring force sharing but has yet to be utilized to study force production patterns following ACLr with hamstring graft. While muscle stiffness can indicate individual muscle activity, it is necessary to incorporate muscle PCSA to predict force contributions, and furthermore moment arm to predict joint torque contributions. These three studies suggest that the ST is the most active muscle during knee flexor activities, as denoted by the highest stiffness. However, the ST is also the smallest of the three muscles, indicating that it may not be producing the most force. To calculate muscle force, PCSA would

be needed. The ST does have the longest moment arm, so it may be producing a large proportion of the knee joint torque, particularly at high levels of knee flexion, despite its small size[65].

Translation to Measuring Quadriceps Muscle Force Production

Efforts to measure quadriceps muscle force production have been hampered by similar issues. Dynamometry can only look at the strength of the knee extensors as a muscle group. EMG is not able to differentiate between the vastus intermedius (VI) and the muscles lying above it – depending on location on the leg, this may be the vastus lateralis (VL), rectus femoris (RF), or vastus medialis (VM). Force transducers are too invasive to use widespread in humans, and fMRI is an expensive, relative measure.

SWE of the quadriceps muscles has been shown to be reliable for the VL, RF, and VM muscles. The higher the knee extensor torque is, the higher the shear modulus of these muscles with strong linear relationships indicated[74,75]. Contradicting these findings, Bouvier et al. did not find consistent increases in VL and VM Young's Moduli as quadriceps muscle torque increased[76]. Fewer studies have examined the VI due to its location deep to the other quadriceps muscles impacting signal propagation. However, Deng et al. did so and similarly found that increases in extensor torque led to increases in VI stiffness, indicating increased muscle force production at these higher torque levels[75].

Deng et al. also found that altering hip joint angle from sitting upright at 90° of hip flexion to lying supine only had an impact on passive RF shear modulus. There were no differences in active shear moduli in the RF from 10-30% of MVIC between these two hip joint angles, and no passive or active differences in the VL, VM, or VI[75]. Since the RF is the only biarticulate quadriceps muscle, it makes sense that the passive stiffness changed based on hip angle for only the RF. However, based on this alteration of the RF fiber length, there would also

be an expectation that the active shear moduli between the two positions would also change. If this were to change, there might be changes in the VL, VM, and VI active shear moduli as well due to changes in load sharing.

Bouvier et al. examined the effects of altering knee joint angle from 50° to 100° of flexion on VL and VM shear moduli at 25%, 50%, and 75% of MVIC for the joint position. Similarly to Deng et al., they found minimal impact of joint angle on active Young's Modulus; there were minimal significant differences between a muscle's shear modulus at a given percent of MVIC at the two knee joint angles suggesting that muscle length might have minimal impact on individual muscle stiffness in the quadriceps. The only difference was at 25% of MVIC, where the VL was significantly stiffer at 100° of knee flexion than at 50°[76]. This was a surprising finding, but it may be attributed to the use of normalized joint torques at a given joint position or the force-length curves of the individual quadriceps muscles. This relationship must be explored in greater detail to understand the impacts of joint angle on individual quadricep muscles' force-length curves. In ACL reconstructed individuals with a QT or BPTB graft, it is especially important to understand this relationship between joint position and contraction intensity on load sharing. An understanding of this can lead to more effective rehabilitation protocols.

Summary

ACL injuries drastically increase the risk of developing early onset OA, but the reasons for this mechanism are not well understood. Years after ACLr with ST graft, the donor site tendon has been shown to be less stiff, longer, and less organized. There is reason to believe that these morphological deficiencies may reduce the functionality of the ST, as well as alter load sharing within the hamstrings, specifically at shorter muscle lengths and lower percentages of MVC when the other hamstring muscles can effectively compensate for the ST. The morphological differences of the QT and BPTB graft sites are less well studied. Existing

methods for measuring muscle activation and force production have limitations and cannot truly determine individual muscle behavior. Despite this, studies have generally suggested that there are differences between hamstring load sharing in an affected ACLr limb and the healthy contralateral limb. SWE offers a way to measure a specific muscle's force production in real time, and as such may be able to determine if the ST or specific quadriceps muscles behave differently following ACLr. Knowing this information would help determine if there is altered load sharing following ACLr with an ipsilateral autograft which may be contributing to the increased risk of early onset OA, and as such the knee flexors and knee extensors should be rehabilitated at different muscle lengths following ACLr to focus efforts within the donor site's new working range.

Methods

Study Design

The purpose of this study was to investigate if an ACLr with an ipsilateral autograft will affect the impacted muscles' abilities to effectively produce force after rehabilitation. This was explored with a cross-sectional study aimed at exploring the functionality of the ST following ACLr with an ipsilateral hamstring graft and the functionality of the quadriceps muscles following ACLr with an ipsilateral QT or BPTB graft. The ST tendon has been shown to have decreased stiffness, and as such increased compliance[22,26], following ACLr and subsequent tendon regeneration. The impact of this increased compliancy on ST functionality and hamstring muscle group load sharing at four different joint angles and two different contraction levels was studied. Due to the common QT and PT for all four quadriceps muscles and limited prior research, it was less clear which muscle(s) would be impacted. However, if there were altered graft site material and structural properties, it was reasonable to assume there would be altered force-length profiles, functionality, and quadriceps load sharing profiles as well. Both legs were tested on individuals who had an ACLr with an ipsilateral autograft and on a control group who did not have a history of ACL injury or chronic thigh musculature injuries. Ultrasound based SWE, sEMG, and dynamometry were utilized to gain an understanding of hamstring load sharing and ST functionality following ACLr with a hamstring graft. Similarly, these techniques were used to understand quadriceps load sharing and functionality following ACLr with a QT or BPTB graft.

Participant Characteristics

Fifteen adults who had an ACLr with an ipsilateral autograft were recruited for this study from the Greenville, NC area. All participants in the experimental groups had their ACLr at least

six months prior and had been cleared by their doctor to return to full activity. Additionally, 11 healthy, recreationally active individuals were recruited for the control groups. These participants had no history of knee or thigh musculature surgeries or chronic injuries. The healthy control groups were matched with participants in the ACLr group in terms of age, sex, and physical activity levels to the best of our abilities. A healthy participant who completed the knee extensor protocol could serve as a match to a participant in the ACLr – PT and the ACLr – QT group since the protocols were the same for both graft types. All participants were instructed to refrain from strenuous lower body workouts within 48 hours before scheduled data collection.

Inclusion Criteria

ACLr Groups

1. ACLr 6 months to 10 years prior to data collection with an ipsilateral autograft.
2. Medically cleared to return to full activity.
3. At least recreationally active as self-defined by the participant.

Control Groups

1. At least recreationally active as self-defined by the participant.
2. No current lower limb injuries.
3. No history of knee or thigh musculature surgeries or chronic injuries.

Exclusion Criteria

ACLr Groups

1. History of chronic hamstring or quadriceps strains.
2. ACL injuries on both limbs.
3. Not medically cleared to return to full activity.
4. BMI greater than 30.
5. Had an implanted pacemaker.

Control Groups

1. History of knee or thigh musculature surgeries or chronic injuries.
2. Diagnosed with OA.
3. Not recreationally active.
4. BMI greater than 30.
5. Had an implanted pacemaker.

Equipment

Baseline Measurements

Participant height, weight, and leg length was recorded at the beginning of the data collection using equipment provided in the laboratory. A goniometer was utilized to measure hip angle throughout the collection.

Ultrasound

Ultrasound and SWE images of the graft site (ST tendon, QT, or PT) and the relevant muscle group (hamstrings or quadriceps) were taken with an ultrasound device (Aixplorer MultiWave, SuperSonic MACH 30 S.A., France). Measurements consisted of muscle cross sectional area (CSA), tendon stiffness, and passive and active muscle stiffness. SWE images were taken with the probe positioned longitudinally in line with the fascicles while CSA images were taken with the probe in transverse during a panoramic image. Architectural measurements were taken in B-mode while stiffness measures were taken in SWE mode. For all SWE images, five consecutive images were taken. Throughout the protocol, the 10-2L probe was used. Images were analyzed using the machine's built-in measurement and Q-box features.

Electromyography

Surface electrodes (Ultium, Noraxon, Scottsdale, AZ) were placed on the muscle bellies of the ST and the BF at 50% of hamstring length measured distally from the popliteal crease to

the greater trochanter proximally for the hamstring group. For the quadriceps groups, surface electrodes were placed on the VL, RF, and VM at 33%, 50%, and 20% of distance from the superior edge of the patella to the anterior superior iliac spine. This matched the SWE imaging sites, and was similar to the Noraxon recommendations. Surface EMG measurements were normalized to the participant's maximum voluntary contraction (MVC). Due to variability in muscle architecture, there was some leeway in electrode placement; ultrasound was used to guide placement.

Dynamometry

Participants completed the active portion of the data collection using a dynamometer (Humac NORM Dynamometer, CSMI, Stoughton, MA). Participants in the hamstring group were on a custom-made apparatus firmly affixed to a dynamometer to allow the hamstrings to be exposed for imaging while completing leg curls. The set up allowed for the alteration of hip and knee flexion angles. The participant's knee joint center was aligned to the dynamometer's axis of rotation and the shank was secured to the dynamometer arm. The dynamometer was utilized without the apparatus for the ACLr - QT, ACLr - PT, and Healthy - PT groups. The dynamometer and custom apparatus were used to complete isometric and isokinetic contractions at a range of knee and hip joint flexion angles.

Measurement Protocol

Subjects were recruited from the East Carolina University and greater Greenville, NC population. After participant arrival, they were given the study's Informed Consent Document to review and sign. The study coordinator assured that the subject met the inclusion criteria. The participant height and weight were measured. The participant filled out a brief demographics survey that asked age, sex, foot dominance, and if applicable, details regarding ACL injury or injuries and reconstruction(s). The questions regarding ACL injury and ACLr were side injured,

approximate date of injury, approximate date of ACLr, and graft type(s). This study was completed over two sessions, with one limb being tested per session.

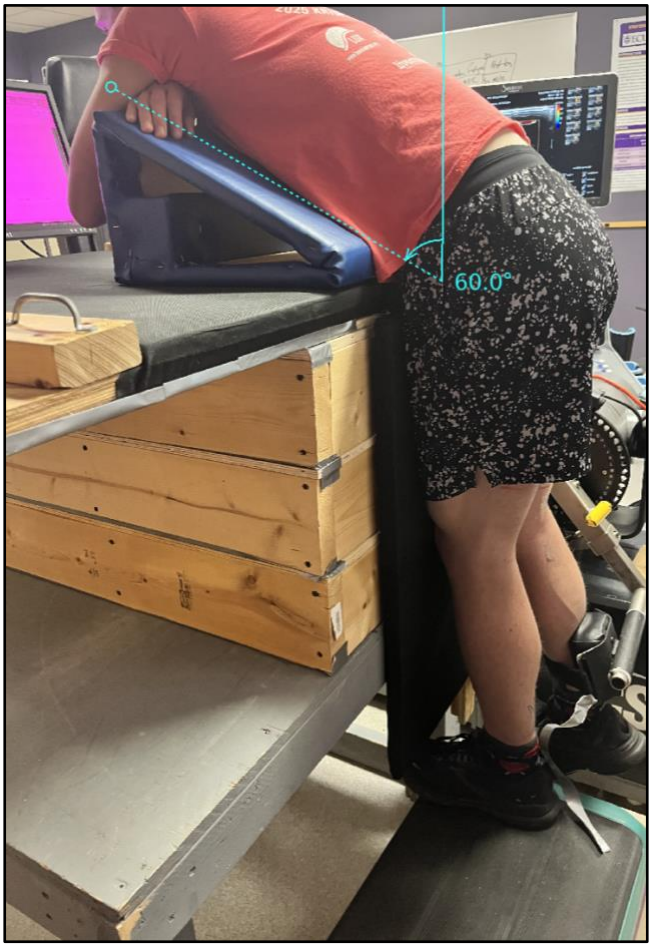
Hamstring group participants transitioned to lying in prone on an exam table with the knee at 15° of flexion and the hip at 0° of flexion. A support was placed underneath the ankles to flex the knee to 15°. Lines were drawn at 30%, 50%, and 60% of femur length measured from the popliteal crease distally to the greater trochanter proximally. Panoramic B-mode images were taken from the BFsh lateral border to the SM medial border at 30% and BFlh lateral border to the SM medial border at 60% of femur length. The BFsh did not extend proximally to 60% femur length. These images allowed for estimating the PCSA of the four relevant muscles to aid in determining force sharing properties, as explained by Hug et al. 2015[77]. To assess SH4, ST tendon stiffness was measured. These measurements were taken on both limbs. These images were taken with the probe directly over the structures of interest. ST tendon images were taken between the medial epicondyle and 50% mark of the tendon, which was defined as 50% of the length from the medial epicondyle and the most proximal site of pure tendon, as Amat-Fernandez did in her study[26]. Also, in this position, the passive, resting stiffness of the ST, BFlh, BFsh, and SM was measured via SWE. Five SWE images were taken of each muscle and the ST tendon with the probe in line with the fibers.

Participants in the QT graft and BPTB graft groups completed a similar process to understand the relevant muscles' and tendons' sizes and passive material properties. While participants were standing, the distance from the superior most point of the patella distally to the anterior superior iliac spine was measured. Lines were drawn over the VM at 20%, the VL at 33%, and the RF at 50% of this distance measured distally to proximally. Additionally, a line was drawn across the thigh at 25% of the distance. Participants sat on the exam table with their hips

flexed and their legs hanging freely off the table. Panoramic B-mode images were taken from the VL lateral border to the VM medial border at 25% and 50% of this distance. Patellar tendon stiffness was measured so that the tibial plateau appeared at the bottom edge of the image. Quad tendon SWE images were taken by tracing the RF distally until it became pure tendon and tracing down an additional centimeter. The images were taken roughly 4-6cm above the superior edge of the patella, dependent on an individual's anatomy. Additionally, the resting stiffness of the VM, RF, VL, and VI was measured at the 20%, 50%, 33%, and 25% lines, respectively.

Participants then transitioned to the dynamometer. Hamstring group participants used the dynamometer in conjunction with a custom-built apparatus. This testing set up consisted of participants standing at such a height that their knee was aligned to the dynamometer's axis of rotation and their hip was in line with the padded board portion of the custom-made apparatus. The participant's leg that was not being tested was slightly elevated, allowing the tested knee to move through its full range of motion without the foot contacting the ground. By leaning on a 30-60-90° padded triangle, leaning flat against the board, or standing upright while using the triangle for support, four different hip flexion angle conditions, which can be seen in **Figure 1** were tested. This allowed for measuring muscle forces, EMG activity, and knee flexor torque at a wide range of hamstring muscle lengths. The apparatus can be seen in **Figure 1**. Quadriceps group participants sat on the raised dynamometer chair with their knee joint aligned properly to the axis of rotation. The hips were flexed to 30° throughout the quadriceps protocol while the knee angle varied from 30° to 90° of flexion to measure muscle forces, EMG activity, and knee extensor torque across a wide range of quadriceps muscle lengths. Following proper joint alignment with the axis of rotation and the apparatus, and securement of the shank to the dynamometer arm, measurements began.

Figure 1. Custom built apparatus, with knee in line with the dynamometer axis of rotation and hip aligned to the apparatus, which consisted of a board and a 30-60-90 triangle. The triangle was rotated to position the hips in 0°, 30°, and 60° of hip flexion.



At each of the four hip and knee flexion angle conditions, the ultrasound probe was positioned longitudinally and parallel to the relevant muscle's fibers to capture five SWE images of the hamstring or quadriceps muscles, depending on graft type, at rest. These images were taken at 50% of femur length (30% for the BFsh) for the hamstring group. For the quadriceps groups, the VM images were taken at the 20% line, while the VI, VL, and RF images were taken at the 25%, 33%, and 50% lines, respectively[75,76]. The measurements of passive muscle stiffness was subtracted from the active stiffnesses measured during isometric contractions. The participant then completed a slow ramp up MVIC over 3s at each of the four angles to obtain the

peak torque for the given muscle length. The torque-angle data was used to test sub-hypothesis 1 regarding a shift of the torque-angle curve towards longer muscle lengths in ACLr limbs. For the hamstring group, the MVIC during Condition 1H was used to normalize the EMG data, as activation has been shown to be relatively similar between the BF and medial hamstrings when the hip is extended, as well as the position being one that leads to a high flexion torque[62]. For the hamstring group, 20% and 40% of the peak torque at each of the joint angles in **Table 1** was calculated and used for the remainder of the collection. For the quadriceps groups, the MVIC during Condition 1Q was used to normalize the EMG data, as activation was shown to be overall the highest across the RF, VL, and VM in pilot data in this position. The quadriceps group used the calculated 15% and 30% of peak extensor torques for each angle in **Table 2**. for the remainder of the study. Hamstring group participants performed knee flexion isometric contractions while quadriceps groups participants completed knee extension isometric contractions.

Table 1. Combinations of knee and hip joint angles for hamstring groups.

Condition	Knee Flexion Angle (°)	Hip Flexion Angle (°)
1H	15	0
2H	15	30
3H	15	60
4H	15	90

Table 2. Combinations of knee and hip joint angles for quadriceps groups.

Condition	Knee Flexion Angle (°)	Hip Flexion Angle (°)
1Q	30	30
2Q	50	30

3Q	70	30
4Q	90	30

At each of the joint angles in **Table 1** for the hamstring group, participants completed 3s ramp up isometric knee flexor contractions at 20% and 40% of the peak torque. At each of the joint angles **Table *2***, participants in the quadriceps groups completed 3s ramp up isometric knee extensor contractions at 15% and 30% of their peak torque. A screen with the live torque-time feedback was displayed in front of the participant to aid in holding the proper contraction strength. SWE images were taken of the relevant muscles at the locations denoted above. Five successive images were taken of each muscle during the contraction. If a participant was unable to hold a peak torque isometric contraction for sufficient time to capture quality SWE images, adjustments were made (i.e. 25% of peak torque contraction instead of 30%) and recorded in the data sheet.

In addition to SWE data, EMG data was collected separately. Ultrasound and muscle palpation was used to aid in proper surface electrode placement. The skin was prepared for electrode placement by shaving, scrubbing the area with an abrasive cream, and cleaning the site with alcohol wipes. For the hamstrings group, electrodes were placed on the BFlh and the ST at 50% of femur length on both limbs, as suggested by Noraxon. For the quadriceps groups, electrodes were placed on the VM at 20% of the distance from the superior patella to the anterior superior iliac spine measured distally to proximally, 33% of this distance for the VL and 50% for the RF. Then participants completed the maximum, low level, and high level isometric contractions at each of the four joint angles, in the same order as completed for the SWE images. These contractions were held for 5s. Before completing the isometric contractions, the

participant completed a concentric/concentric isokinetic knee flexion/extension throughout the knee's range of motion at a speed of 15°/s while the hip angle was held constant. For the isokinetic contraction, the hip was extended to 0° for the hamstring group. For the quadriceps group, the hip was flexed to 30° as it was for the isometric contractions. This was done to further test SH1 regarding shifts in the torque-angle curve towards longer muscle lengths in ACLr limbs. Due to its extensive use in the existing literature, sEMG data was used as a secondary measure to the SWE based data to aid in assessing the study hypotheses. The SWE and sEMG data collected during the isometric contractions provided the necessary data to test sub-hypotheses 2, 3, and 4 regarding the impacts of a less stiff donor site tendon following ACLr with an autograft and subsequent rehabilitation on muscle force production at different muscle lengths and contraction levels.

The entire protocol was completed on both limbs, with the injured or control matched injured limb always being tested first. The healthy or control matched healthy limb was tested during a second session. The order of the hip angle conditions was randomized to minimize the effects of fatigue on results. To allow for equal comparison between legs, the calculated low and high level torques from the injured or control matched injured limb was used for both limbs.

Data Reduction

Ultrasound

The built-in features of the ultrasound device were used for extracting relevant features from the images to test sub-hypotheses 2-4. The Q-box trace feature was used to extract the mean shear modulus of the tendon or muscle in question. This was done for each of the five images in a condition, with the average of the three middle values being recorded for further statistical analysis. The Q-box contained all fascicles that were in line with the probe and within the tendon or muscle boundaries and excluded any areas that would reasonably alter

measurements (i.e. aponeurotic tendon or areas with clear and obvious variation from the bulk of the image, edges of the image, etc). The trace tool was used to measure the CSA of the BFsh, BFlh, ST, and SM muscles in the panoramic images taken at 30% and 60% of femur length, measured distally to proximally for the hamstring group. The same was done in the panoramic images taken of the quadriceps muscles to determine the CSA of the VL, VM, VI, and RF.

The relationship between the SWE measured muscle stiffness and PCSA, as described by Hug et al. 2015[32], states that an index of muscle force can be calculated by multiplying the two variables together:

$$F_m = \mu * PCSA$$

Equation 1

where F_m is the muscle force, μ is the active shear modulus, and *PCSA* is the physiological cross-sectional area. Using the CSA of the hamstring muscles at 30% and 60% of the distance from the popliteal crease to the greater trochanter measured distally to proximally or the quadriceps muscles at 25% and 50% of the distance from the superior patella to the anterior superior iliac spine distally to proximally, a regression equation was used to estimate individual muscle volumes. Franchi et al. determined that the volume of each of the hamstring muscles can be estimated by

$$Vol = \frac{1}{3} * h * \left(CSA_1 + CSA_2 + \sqrt{CSA_1 * CSA_2}\right)$$

Equation 2

where *Vol* is the individual muscle's volume, *h* is the distance between the two panoramic images in centimeters, CSA_1 is the muscle's CSA in square centimeters at the distal location, and CSA_2 is the muscle's CSA in square centimeters at the proximal location[73]. This truncated-cone formula was also used to estimate quadriceps muscle volume, as it has been done previously[78].

Then, individual muscle PCSA was estimated by dividing the muscle volumes from **Equation 2** by each muscle's fascicle length from the literature:

$$PCSA = \frac{Vol}{fl}$$

Equation 3

where *PCSA* is the muscle physiological cross-sectional area, *Vol* is the muscle volume calculated with **Equation 2**, and *fl* is the muscle's estimated fascicle length based off an average of values reported in the literature. The estimates of fascicle length can be seen in **Table 3**.

Table 3. Estimated fascicle lengths to be used to calculate estimate muscle PCSA.

Muscle	**Fascicle Length (cm)**[74]
BFsh	11.03
BFlh	9.76
ST	19.30
SM	6.90
VM	9.68
VL	9.94
VI	9.93
RF	7.59

The PCSA values calculated in **Equation 3** were then used in **Equation 1** to estimate individual muscle forces at each joint angle and torque level condition. Within this equation, μ is the active shear modulus, or the difference between the muscle's resting shear modulus and the active shear modulus at the specified joint angle. In addition to calculating the muscle force, the

ratio of total force contribution from each knee flexor or knee extensor muscle measured was calculated by dividing the muscle's force by the sum of the total measured muscle force. This ratio was used to determine if there were differences in the hamstring or quadriceps load sharing profile between the ACLr limb and the ipsilateral and control matched limbs at different joint angles and contraction levels to test SH2 and SH3 Additionally, these ratios were compared as a function of donor site tendon stiffness to test SH4.

Electromyography

All sEMG data was normalized to the MVIC from condition 1H (hip 0°, knee 15°) or 1Q (hip 90°, knee 30°). A full wave rectification was applied as was a 100ms RMS smoothing filter. Start and end markers were placed at the beginning and end of the desired torque plateau for each isometric trial. The mean normalized EMG signal for each muscle measured was recorded for each trial. In addition to the MVIC normalized data, raw signals in volts of the MVICs were reported to allow for comparisons between limbs despite potentially varying strength of the neuromuscular signal. Like the SWE data, these ratios were used to further test sub-hypotheses 2-4.

Dynamometry

The torque-angle curves from the isokinetic contractions at 15°/s were exported to create a torque-angle cure for each limb and participant. Additionally for the hamstrings group, the maximal isometric torque values at the four conditions in **Table 1** were analyzed against the hip flexion angle to create another torque-angle curve that represents longer and more variable hamstring muscle lengths compared to the isokinetic contractions. These torque-angle curves were used to test SH1. Additionally, when used in conjunction with the donor site tendon stiffness values, the force-lengths curves were used to test SH4.

Statistical Analysis

Due to the small sample sizes within each of the five groups (ACLr – ST, Healthy – ST, ACLr – PT, ACLr – QT, Healthy - PT), much of the analysis was descriptive in nature. However, dependent samples t-tests were run within each of the groups to compare between the limbs to assess if there were differences in donor site tendon shear modulus, muscle size, strength, muscle force, and neuromuscular activation following ACLr with an ipsilateral autograft. All statistical analyses were run within the groups, comparing the injured limb to the contralateral, unaffected limb in the ACL groups or the dominant limb to the non-dominant limb in the healthy groups. Because there were no between group analyses, no data was normalized to participant's height and weight. The alpha level was set to 0.05 *a priori* and Cohen's d effect sizes greater than 0.80 were considered large effects. Cohen's d values between 0.5-0.8 were set as a moderate effect size while values less than 0.5 were deemed a small effect. Repeated measures ANOVAs were run within the limb to test the impact of muscle length on force production, strength, and load share. The partial eta squared effect sizes were deemed large when η^2 was greater than 0.14 and moderate when η^2 values were greater than 0.06, but less than or equal to 0.14.

A dependent samples t-test was run on the MVIC voltage for each muscle between the injured and control limbs to determine if additional analyses should be run with the normalized values (i.e. no significant difference in MVIC voltage) or the raw voltage values.

Support for the overarching hypothesis regarding reduced donor site tendon stiffness in each ACL group on the ACLr limb was a pre-requisite for running additional analyses related to muscle use. The first four participants in the Healthy – PT group acted as the control group to the ACLr – QT group to properly match to sex and number of participants. To reduce the number of factors analyzed, no analyses were run between the ACLr and Healthy groups. All analyses were

run within the groups (i.e. injured versus control limb for ACLr groups or dominant versus non-dominant limb for the healthy groups), but not between the ACLr and Healthy groups.

Sub-hypothesis 1 regarding shifts in the force-length curve on the injured limb was analyzed descriptively by examining the differences between the injured and control limb (or dominant and non-dominant limb for healthy groups) for each participant. On the group level, dependent samples t-tests were run to compare the isometric and isokinetic peak torques between the injured and control limbs, as well as the angle at peak isokinetic torque. Additionally, a repeated measures ANOVA of the percent deficits between injured and control limbs' isometric peak torque between positions was run. Post-hoc pairwise comparisons were analyzed via dependent samples t-tests.

Sub-hypothesis 2 analyzed the effect muscle length had on deficits in the ACLr limb compared to the contralateral limb and hypothesized that deficits would be larger at shorter muscle lengths. This hypothesis was analyzed descriptively for each participant, as well as by a repeated measures ANOVAs within the limb to test the impact joint position had on load share, muscle force, strength, and neuromuscular activation. The partial eta squared effect size as well as the p-value were reported for the repeated measures ANOVAs. Follow up dependent samples t-tests were run when pairwise comparisons or visual differences warranted it.

Sub-hypothesis 3 examined the effect of torque level on muscle use. In addition to being analyzed on a participant level descriptively, this hypothesis was analyzed with dependent samples t-tests within the limb and between the injured and control limbs for the ACLr groups, or the dominant and non-dominant limbs for the Healthy groups. Muscle forces, neuromuscular activity, and load shares were analyzed.

Sub-hypothesis 4 regarding the relationship between donor site tendon recovery and affected muscle recruitment deficits between the injured and control limbs was assessed descriptively due to the small sample sizes for each graft type.

Results

Demographics

There were 26 participants across the five groups. Demographic information can be seen in **Table 4**. The five participants in the ACLr – ST group had their ACLr with a hamstring autograft an average of 4.4 ± 1.9 years (range: 2.1 – 6.4 years) prior to partaking in the study. Two of the participants in the ACLr - ST group (ACLr – ST2 and ACLr – ST5) had multiple ACLr on the same limb. ACLr – ST2 had her second ACLr with a BPTB graft and ACLr – ST5 said that both of her ACLr were done with hamstring autografts. The four participants in the ACLr – QT group had their ACLr with a QT autograft an average of 2.8 ± 1.6 years (range: 1.2 – 4.9 years) ago. The six participants in the ACLr – PT group had their ACLr with a BPTB autograft an average of 2.3 ± 1.3 years (range: 1.0 – 3.9 years) prior to completing the study. There were no significant differences between the demographics of any of the ACL groups and their corresponding control group. The four females (Healthy – PT1-4) in the Healthy - PT group were used as the controls for the ACLr – QT group to match properly for sex.

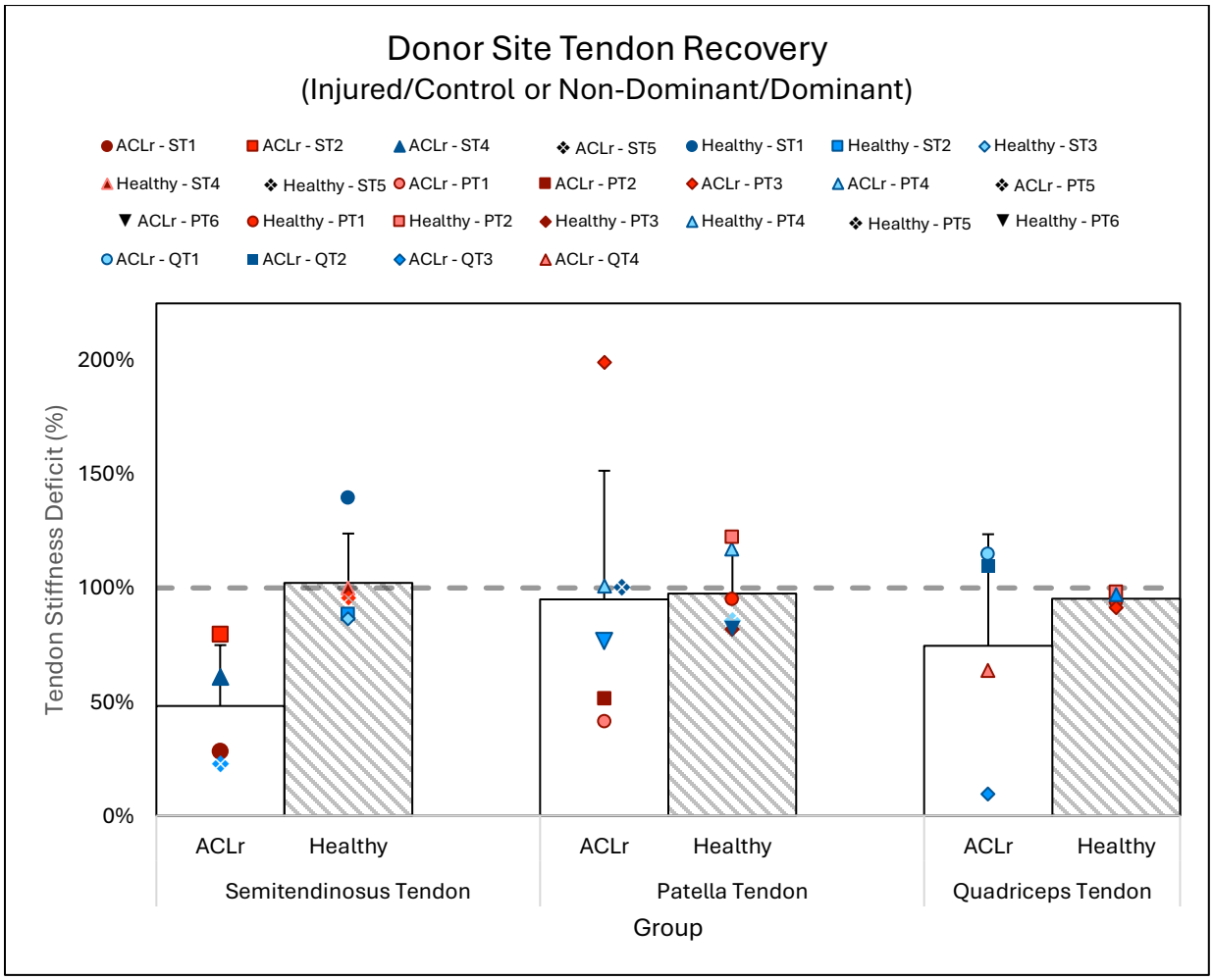
Table 4. Participant demographics

	ACLr – ST *(n=5)*	Healthy – ST *(n=5)*	ACLr – PT *(n=6)*	Healthy - PT *(n=6)*	ACLr – QT *(n=4)*	Healthy – QT *(n=4)*
Sex (M/F)	2/3	2/3	2/4	2/4	0/4	0/4
Age (years)	21.0 ± 0.7	21.4 ± 2.2	20.0 ± 1.1	21.2 ± 4.3	21.0 ± 2.7	20.0 ± 2.2
Height (cm)	170.7 ± 6.8	169.9 ± 6.8	169.1 ± 9.4	168.1 ± 6.7	168.9 ± 4.5	164.8 ± 5.5
Mass (kg)	65.5 ± 10.9	68.9 ± 13.8	79.1 ± 16.5	63.5 ± 10.5	58.7 ± 7.5	58.8 ± 5.8
Injured Limb (Dominant/Non-Dominant)	3/2		5/1		2/2	
Years since ACLr	4.4 ± 1.9		2.3 ± 1.3		2.8 ± 1.6	

Donor Site Tendon Properties

The overarching study hypothesis was that donor site tendon stiffness would be reduced compared to the unaffected limb. Donor site tendon stiffness was calculated for each of the groups. Tendon properties can be seen in **Table 5** and visualized as group averages and for each participant in **Figure 2**. Dependent samples t-tests were run to compare the injured limb to the control limb in the ACLr groups and the dominant limb to the non-dominant limb in the healthy groups.

Figure 2. Tendon recovery for ACLr participants and tendon symmetry for healthy group participants. Each participant is represented by a colored symbol, which will be consistent throughout the presented figures.



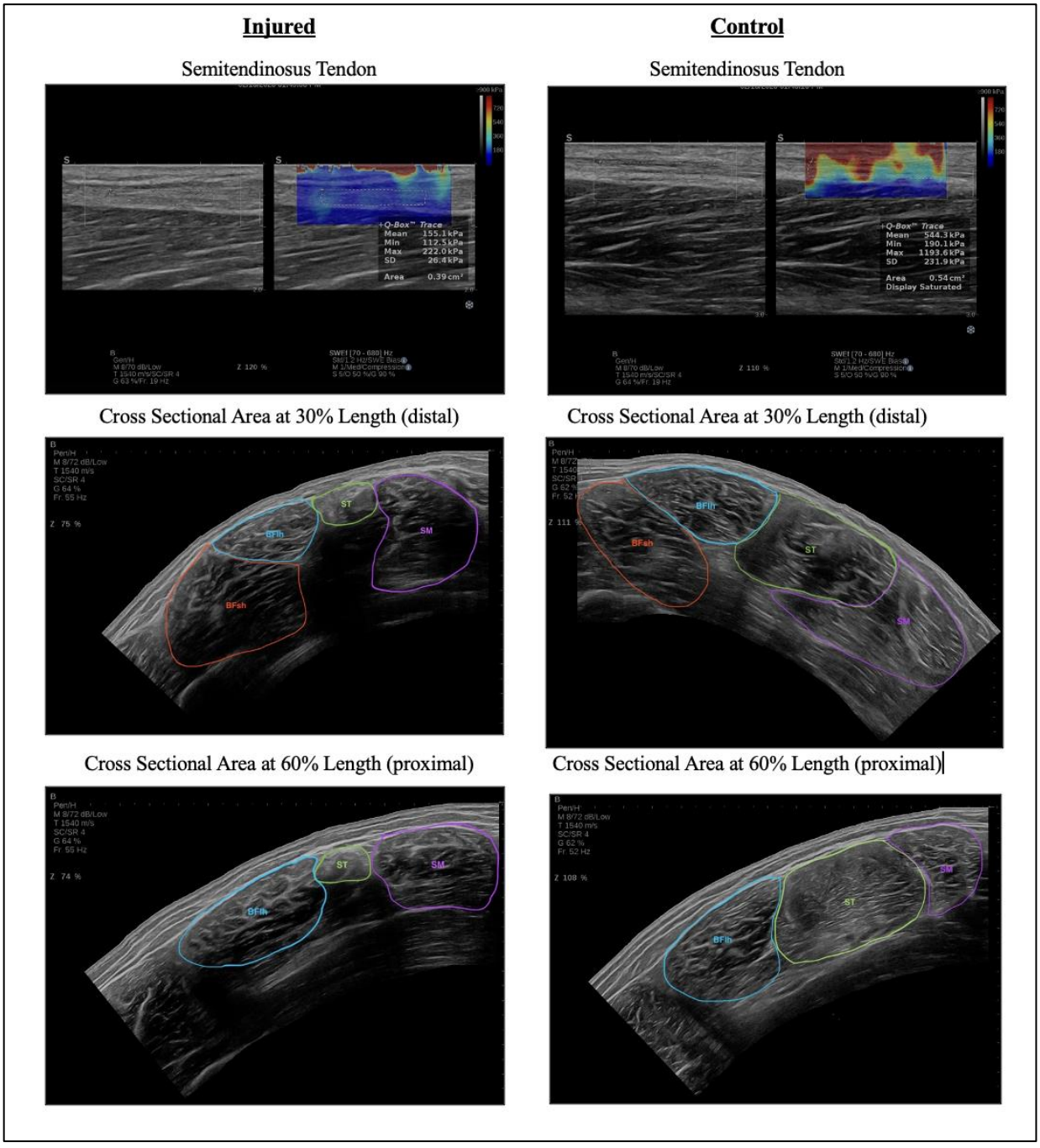
Semitendinosus Tendon Graft

While it did not reach statistical significance (p=.084), the ST tendon on the injured side was consistently less stiff than on the control side in the ACLr – ST group with a large effect size of d=1.277, supporting the overarching hypothesis. Within the ACLr – ST group, one participant (ACLr – ST3) did not have clear regeneration of the ST tendon and thus was not included in this analysis; this participant also did not have a clear ST muscle. ACLr – ST1 did not have a well-defined nor organized ST muscle but did have a clear tendon and as such is included in **Figure *2***. This participant's injured and contralateral limb tendon, as well as panoramic images showing the muscles at both the distal and proximal locations can be seen in **Figure *3*.** Two participants (ACLr – ST2 and ACLr – ST4) recovered over 60% of their healthy tendon's stiffness. There were no differences between the dominant and non-dominant side ST tendon stiffness in the Healthy – ST group (p=.890, d=.066). These findings supported the overarching hypothesis that there is reduced donor site stiffness following ACLr with an ipsilateral autograft.

Table 5. Donor site tendon stiffness for each group by limb. ST Tendon row represents ST tendon stiffness for ACLr – ST and Healthy – ST group. PT Tendon row represents PT tendon stiffness for ACLr – PT and Healthy – PT groups. QT Tendon row represents QT tendon stiffness for ACLr – QT and Healthy – QT groups. Effect size is the Cohen's d effect size between the limbs within the group.

	ACL Reconstructed			**Healthy**		
	Reconstructed	**Control**	**Effect Size**	**Dominant**	**Non-Dominant**	**Effect Size**
ST Tendon	150.1±51.8 KPa	354.8±151.3 KPa	**1.190**	367.4±82.7 KPa	372.2±89.1 KPa	0.066
PT Tendon	554.6±258.7 KPa	620.1±135.7 KPa	0.207	645.1±234.5 KPa	611.7±176.2 KPa	0.298
QT Tendon	371.6±282.5 KPa	461.0±93.8 KPa	0.445	390.3±71.4 KPa	370.7±59.1 KPa	**1.214**

Figure 3. ACLr - ST1 semitendinosus tendon SWE images and cross sectional muscle area at 30% and 60% of femur length measured distally to proximally from the popliteal crease to the greater trochanter on both the injured and control limbs.



Patella Tendon Graft

The differences between the injured limb and control limb PT tendon stiffness in the ACLr – PT group were inconsistent, and thus did not support the overarching hypothesis. Two

individuals (ACLr – PT4 and ACLr – PT5) had nearly perfect symmetry between the injured and control limb PT stiffness, one participant (ACLr – PT6) recovered about 75% of their healthy PT stiffness, two participants recovered about 50% of their healthy PT stiffness (ACLr – PT1 and ACLr – PT2), and one participant (ACLr – PT3) had nearly double the PT stiffness on their ACLr limb compared to their healthy limb. This participant did re-tear their ACLr less than three weeks after completing the study. There were no significant differences in PT stiffness between the injured and control limb ($p=.634$, $d = .207$). However, if ACLr – PT3 was removed from the sample, the effect size does become much larger at $d=.978$ and the p-value reduced to $p=.094$. There were no significant differences between the dominant and non-dominant PT stiffness in the Healthy – PT group ($p=.499$, $d=.298$). These findings did not provide strong support for the overarching hypothesis that donor site tendon stiffness would be reduced on the ACLr limb.

Quadriceps Tendon Graft

The differences between the QT stiffness in the injured and control limb in the ACLr – QT group were also inconsistent. Two of the participants (ACLr – QT1 and ACLr – QT2) met and exceeded their healthy limb's QT stiffness, one (ACLr – QT4) recovered 63.8% of their healthy limb's QT stiffness, and the last participant (ACLr – QT3) recovered only 9.5% of their healthy limb's QT stiffness at the typical imaging location. Moving more proximally, the tendon did resemble the contralateral limb's tendon stiffness values suggesting the shear modulus varied throughout the tendon. There was no significant difference between the injured and control limbs ($p=.439$), and the effect size was small ($d=.445$). There was not support for the overarching hypothesis that donor site tendon stiffness is reduced following ACLr. There were no significant differences between the dominant and non-dominant QT stiffness in the Healthy – QT group ($p=.094$), however the effect size was large ($d=1.214$). The dominant and non-dominant limb

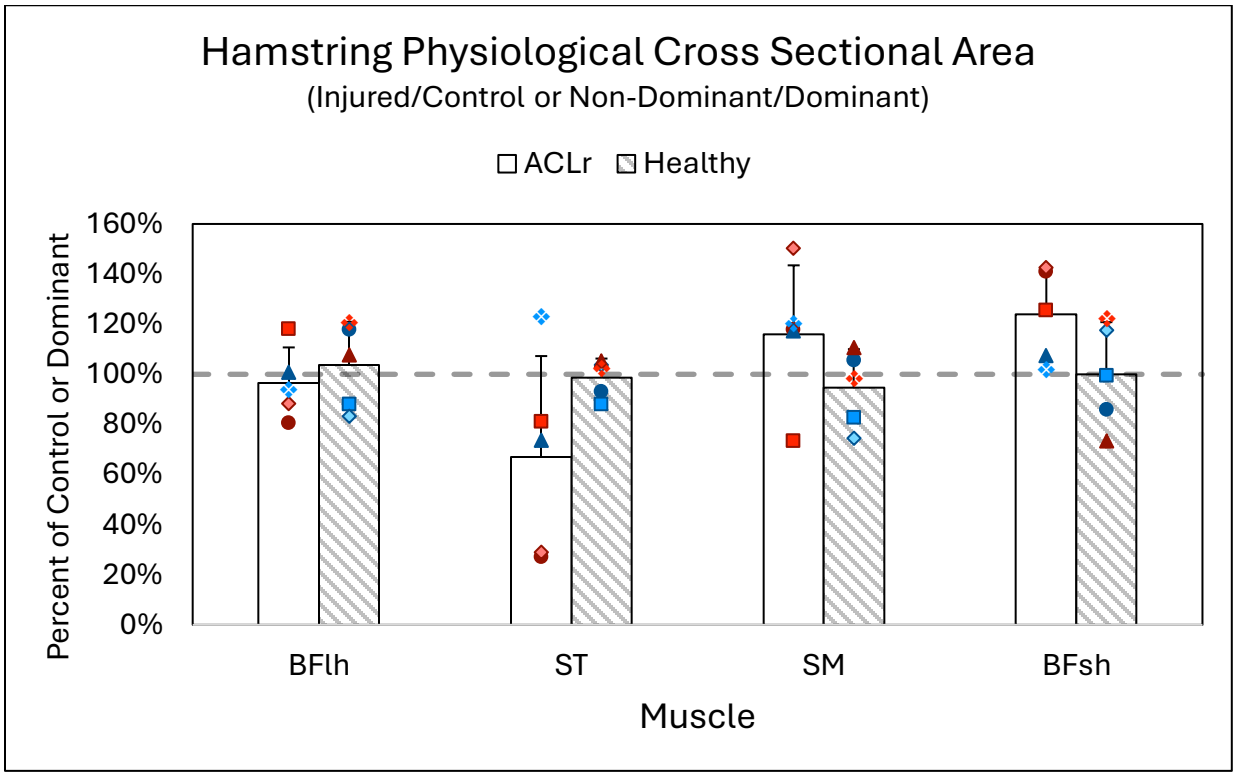
were always within 10% of one another for this control group, but the dominant limb was always slightly stiffer than the non-dominant limb leading to this large effect size.

Muscle Physiological Cross-Sectional Area

Hamstring Muscle PCSA

The estimated PCSA of the four hamstring muscles of interest were compared between the injured and control limb in the ACLr – ST group and the dominant and non-dominant limb in the Healthy – ST group using dependent samples t-tests. There was a moderate effect of limb (d=.688) for the SM in the Healthy – ST group, with the dominant side SM averaging slightly larger than the non-dominant side's SM. However, this was not consistent across the participants. In the ACLr – ST group, the ST was smaller (p=.096, d=1.174) on the injured side by an average of 1.92cm^2 while the SM (p=.253, d=.622) and BFsh (p=.061, d=.755) were larger by an average of 2.11cm^2 and 0.61cm^2, respectively. There was no difference in BFlh PCSA between limbs (p=.444, d=.305). The data can be visualized in **Figure 4**. Each participant has the same symbol for their datapoints as they did in **Figure 2**.

Figure 4. Percent of healthy or dominant limb hamstring muscle PCSA. Error bars represent SD. Points represent individual datapoints with each colored symbol representing a participant.



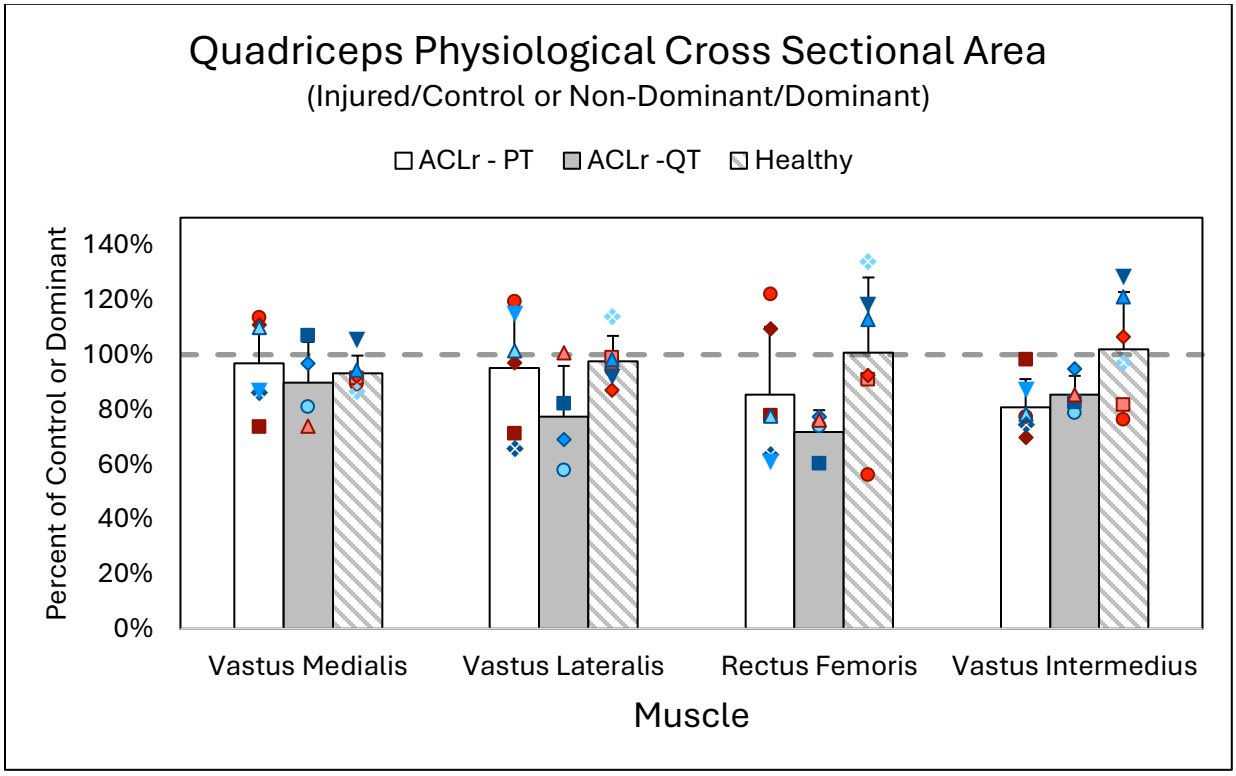
Quadriceps Muscle PCSA

The estimated PCSA of the quadriceps muscles for the ACLr – PT, ACLr – QT, and their corresponding healthy control groups were also computed. Dependent samples t-tests were run within the individual groups, and the data can be visualized in **Figure 5**. For the ACLr – PT group there was a reduction in VI PCSA (p=.006, d=1.882) in the injured limb as well as a moderate effect and reduction of RF PCSA (p=.154, d=.686) on the injured side.

In the ACLr – QT group, there was a large effect of limb on VL (p=.066, d=1.418), RF (p=.029, d=1.969), and VI (p=.028, d=2.007) PCSA, with the injured limb being smaller than the contralateral limb in all individuals; the only exception was the VL for ACLr – QT4, who reached symmetry between sides for this muscle. The VM trended towards being smaller in the injured limb, with a moderate effect size of 0.599.

For the Healthy – PT group there were no significant p-values, but there was a large effect size (d=.868) for the VM, dictating that the muscle was generally larger on the dominant side. For the four females that served as the control group for the ACLr – QT group, the dominant side VM was significantly larger than the non-dominant side with a p-value of 0.004 and effect size of 4.006. The VM was 6.1-12.0% larger on the dominant side across all the females in the control group. The individual datapoints can be visualized in **Figure 5**.

Figure 5. Percent of healthy or dominant limb quadriceps muscle PCSA. Error bars represent SD. Points represent individual datapoints with each colored symbol representing a participant.



Isometric and Isokinetic Strength

Sub-hypothesis 1 hypothesized that the effects of a less stiff donor site tendon would lead to a shift of the knee flexion (ST graft) or knee extension (QT and PT) torque-angle curve towards longer muscle lengths where there is greater hip flexion. Since the participants in the ACLr – QT and ACLr – PT groups did not have consistently less stiff donor site tendons and

thus failed to support the overarching hypothesis, this hypothesis was primarily explored in the ACLr – ST group.

Isometric and Isokinetic Knee Flexor Strength in the Hamstrings Groups

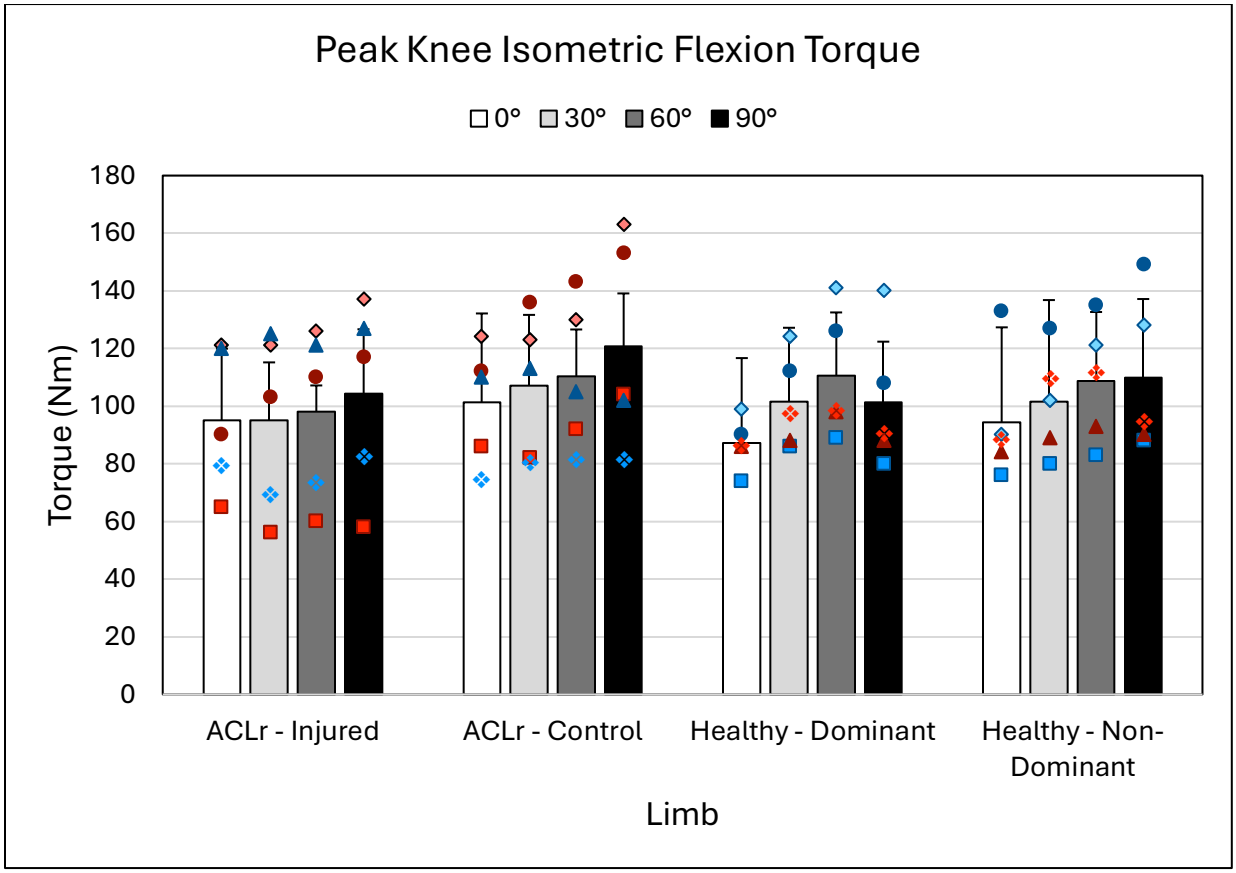
Isometric knee flexion strength was tested in four different positions – 0°, 30°, 60°, and 90° of hip flexion while the knee angle remained constant at 15° for the hamstring groups. Within the ACLr – ST group, the injured limb had significantly lower isometric torque than the control limb with a moderate effect size (p=.016, d=.588). Two of the five participants (ACLr – ST1, ACLr – ST2) had consistently lower peak isometric flexion torque at all positions tested. ACLr – ST4 had consistently higher peak torque on the injured side. This participant is a NCAA DI athlete. ACLr – ST3 and ACLr – ST5 had relatively symmetric isometric strength between their injured and contralateral limbs except at 30° of hip flexion for ACLr – ST3 and 90° of hip flexion for ACLr – ST5. At these angles, the injured limb was over 10% weaker. There was no main effect of limb on peak isometric flexion torque in the Healthy – ST group (p=.356, d=.211).

Within the ACLr – ST group, both the injured and control limbs reached their peak torque at 90° of hip flexion torque, which was the longest muscle length tested. However, when looking at the control limb, there were no significant differences between any of the joint positions. Within the injured limb, the peak isometric torque was significantly greater at 90° than it was at 60° (p=.049, d=1.248) or 30° (p=.037, d=1.374), suggesting a potential preference for longer muscle lengths following ACLr in support of SH1. While not significant, there were also moderate effect sizes between 90° and 0° of hip flexion (p=.187, d=.711) and 60° and 30° of hip flexion (p=.164, d=.761) on the injured limb, with the isometric torque being greater at the longer muscle length. However, these trends were not consistent across all the participants. ACLr – ST2 and ACLr – ST4 had very minimal change in their peak torque between joint positions,

while ACLr – ST1 had the biggest changes between positions. These patterns can be visualized in **Figure 6**. On the control limb of the ACLr – ST group, there were no significant differences between joint positions, but there was a large effect size between the 90° and 0° hip flexion conditions (p=.107, d=.926) with a preference for the longer muscle length. There were no significant differences between the injured and control limbs within a position.

There were no differences between limbs in the Healthy – ST group. However, in both the dominant and non-dominant limb there was an effect of joint position on peak isometric knee flexor torque. Within the dominant limb, the repeated measures ANOVA (p=.004, η^2 =0.658) revealed the main effect of position, with the peak torque occurring at 60° of flexion. Pairwise comparisons found that the torque at 60° of hip flexion was significantly greater than at the three other hip flexion angles. Additionally, the flexor torque at 30° of hip flexion was significantly greater than at 0° of hip flexion. In the dominant limb of the Healthy – ST group, there was a clear force-length curve profile created with 60° of hip flexion appearing to be the optimal joint angle. The non-dominant limb also showed a main effect of joint position on knee flexor torque (p=.048, η^2 =0.469). However, there were no significant differences found within the pairwise comparisons.

Figure 6. Mean peak knee isometric flexion torque by limb as a function of hip flexion angle. Error bars represent SD. Points represent individual datapoints with each colored symbol representing a participant.



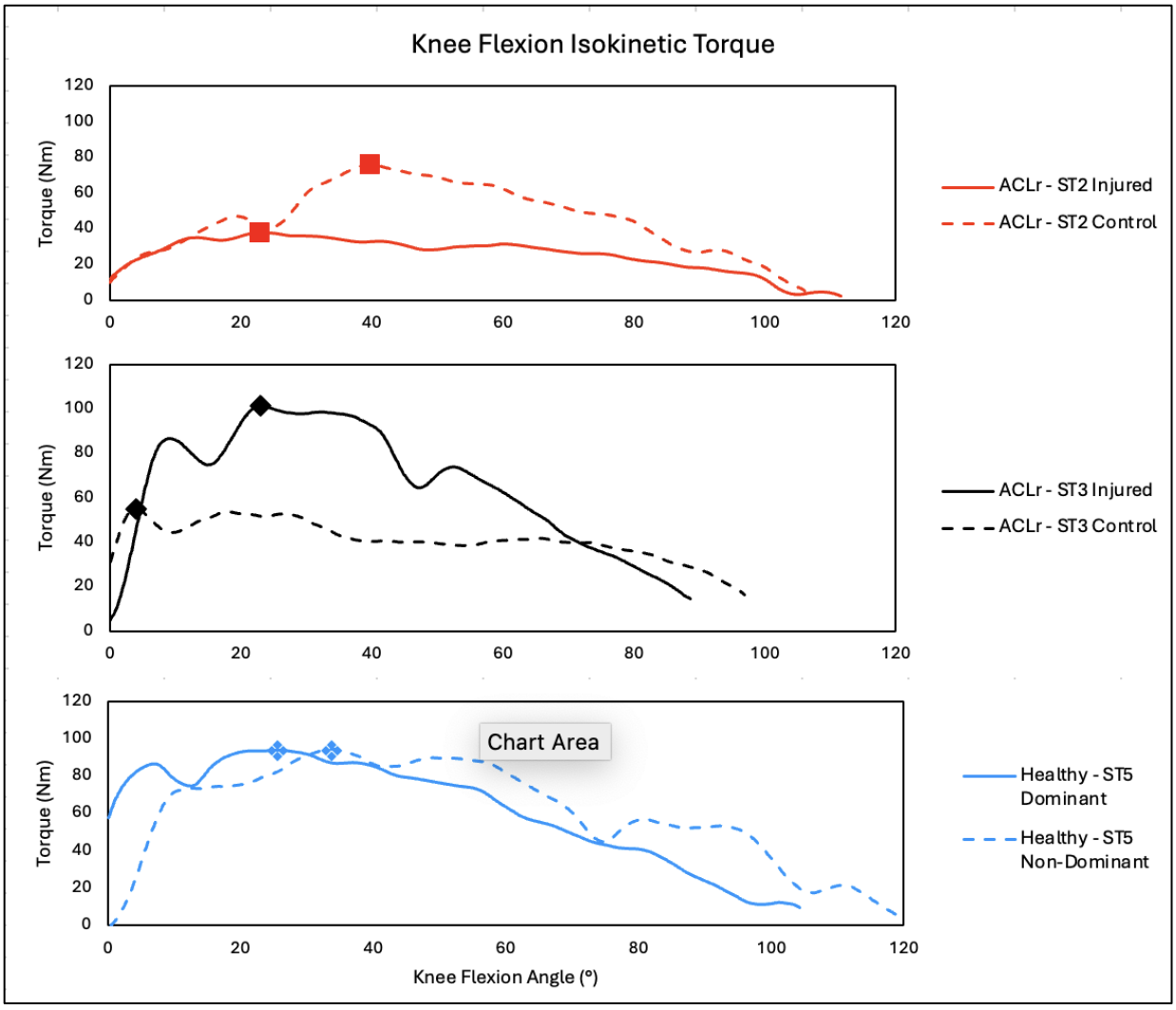
Additionally, isokinetic knee flexor torque was tested while the hips were in 0° of flexion and the knee moved through its range of motion. Dependent samples t-tests showed that there were no differences between the injured and control limbs in the ACLr – ST group nor the dominant and non-dominant limbs in the Healthy – ST group in terms of peak torque or knee flexion angle at peak torque. Support for SH1 would have indicated that the knee flexion angle at peak torque would have been reduced in the injured. Within the ACLr – ST group, three of the participants (ACLr – ST1, ACLr – ST2, ACLr – ST5) had a lower peak isokinetic flexor torque on their ACLr limb but two participants (ACLr – ST3, ACLr – ST4) had a significantly higher torque on their injured limb. Two of the participants (ACLr – ST1, ACLr – ST2) achieved their

peak torque at a longer muscle length on their ACLr limb as hypothesized, but one (ACLr – ST3) preferred a shorter muscle length on the injured leg. Example representative isokinetic knee flexor torque curves can be seen in **Figure 7**.

Table 6. Mean peak isokinetic knee flexor torque and mean angle at peak torque by limb for the hamstring groups. Effect size is the Cohen's d effect size between the limbs within the group.

	ACLr - ST			**Healthy - ST**		
	Injured	Healthy	Effect Size	Dominant	Non-Dominant	Effect Size
Peak Torque (Nm)	75.6±33.1	74.7±11.0	0.027	84.32±16.7	84.1±12.7	0.019
Angle at Peak	20.5°±4.8°	26.4°±18.7°	0.279	28.6°±13.9°	22.4°±8.3°	0.381

Figure 7. Select isokinetic knee flexor torque-position curve for injured/control limbs and dominant/non-dominant limbs. Points represent peak torque and the angle at peak torque for each curve.



Isometric and Isokinetic Knee Extensor Strength in the Quadriceps Groups

There were more consistent strength deficits in isometric knee extensor torque in the ACLr – PT group than there were in knee flexor torque in the ACLr – ST group. The injured ACLr – PT limb was significantly and meaningfully weaker than the contralateral limb ($p<0.001$, $d=1.293$). Within the injured limb, there was also a significant effect of position ($p=.025$, $\eta^2=0.648$). The isometric extensor torque was significantly less at 30° of knee flexion

than it was at all the longer muscles lengths, as was the 50° of knee flexion isometric extensor torque less than that obtained at 70° of knee flexion. Unlike in the ACLr – ST group, there were significant differences between the limbs within individual joint positions. The control limb was stronger than the injured limb at 30° (p=.002, d=2.377), 50° (p=.017, d=1.429), and 70° (p=.002, d=2.533) of knee flexion, but not quite significant at the longest muscle length of 90° of knee flexion (p=.112, d=.786). This provided support for SH1 as the deficits in isometric strength in the between the injured and control limb were smaller at longer muscle lengths. The datapoints for each individual and group averages can be seen in **Figure 8**.

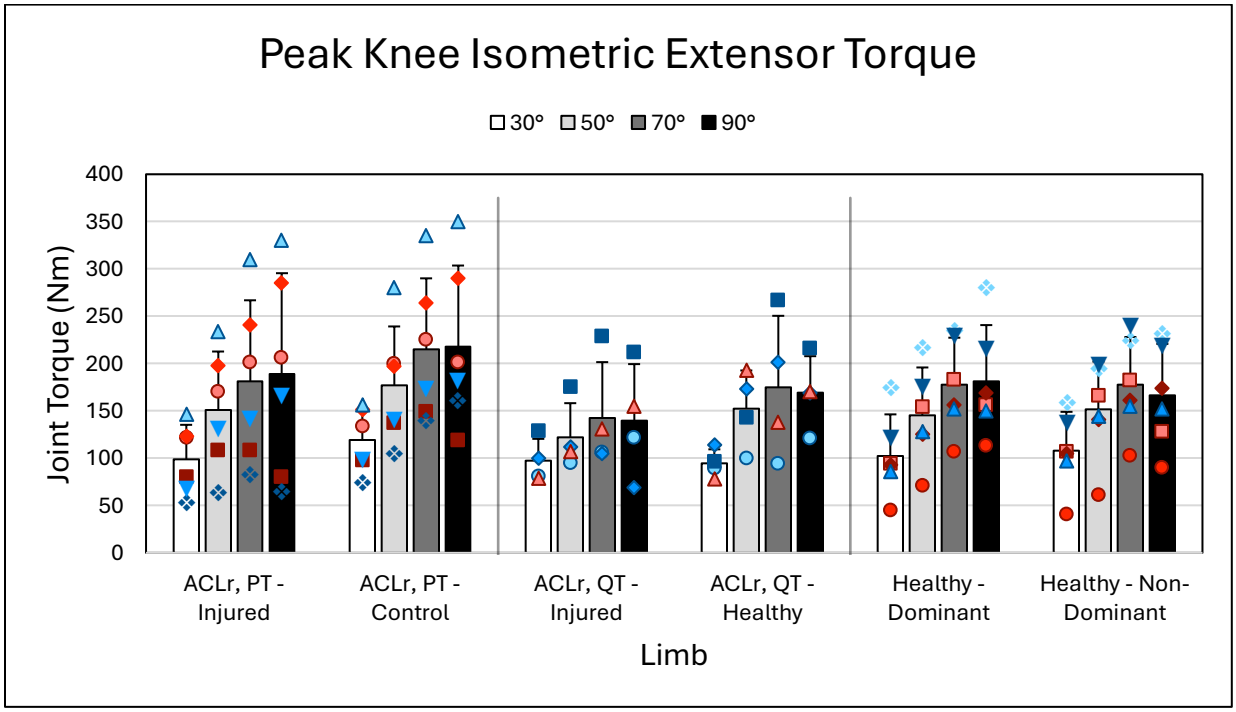
Transitioning to the ACLr – QT group, there were no significant differences in peak isometric extensor torque between the injured and control limbs (p=.053, d=.526). There was a moderate effect size, pointing towards increased isometric knee extensor torque in the control limb. Two of the four participants in this group obtained symmetry between their two limbs within 15% or were stronger on their ACLr limb.

In the Healthy – PT group, there were no differences in peak isometric extensor torque between the dominant and non-dominant limbs (p=.821, d=.047). As expected, within the limbs, there was an impact of position. On the dominant limb, the repeated measures ANOVA was significant and meaningful ($p<0.001$, $\eta^2 =0.902$) with pairwise comparisons revealing that each position was significantly different from the others except for 70° and 90° of knee flexion. The peak isometric extensor torque was at 90° of knee flexion suggesting that in healthy individuals, peak extensor torque is also obtained at one of the two most lengthened positions tested. The findings were similar on the non-dominant limb ($p<0.001$, $\eta^2 =0.905$). However, peak torque was obtained at 70° of knee flexion on average.

While the isometric strength deficits between limbs were evident in the ACLr – PT group, the deficits in peak extensor torque and angle at peak torque during the isokinetic were less consistent. There was no significant difference in peak torque (p=.111, d=.789), but there was a moderate effect size suggesting that the injured limb may be at a deficit. This was the case for four of the six participants in this group. The knee flexion angle at peak torque was not significantly different between the injured and contralateral limb nor did it have a moderate or large effect size (p=.295, d=.478), and thus failed to support SH1. It averaged 78.4°±12.9° of knee flexion for the injured limb compared to 72.1°±12.0° of knee flexion on the control limb.

In the ACLr – QT group, there were no significant or meaningful differences between the injured and control limb in peak isokinetic extensor torque nor the knee flexion angle at peak torque. The group averages for both the injured and control limbs can be seen in **Table 7**.

Figure 8. Mean peak knee isometric extension torque by limb as a function of knee flexion angle. Error bars represent SD. Points represent individual datapoints with each colored symbol representing a participant.

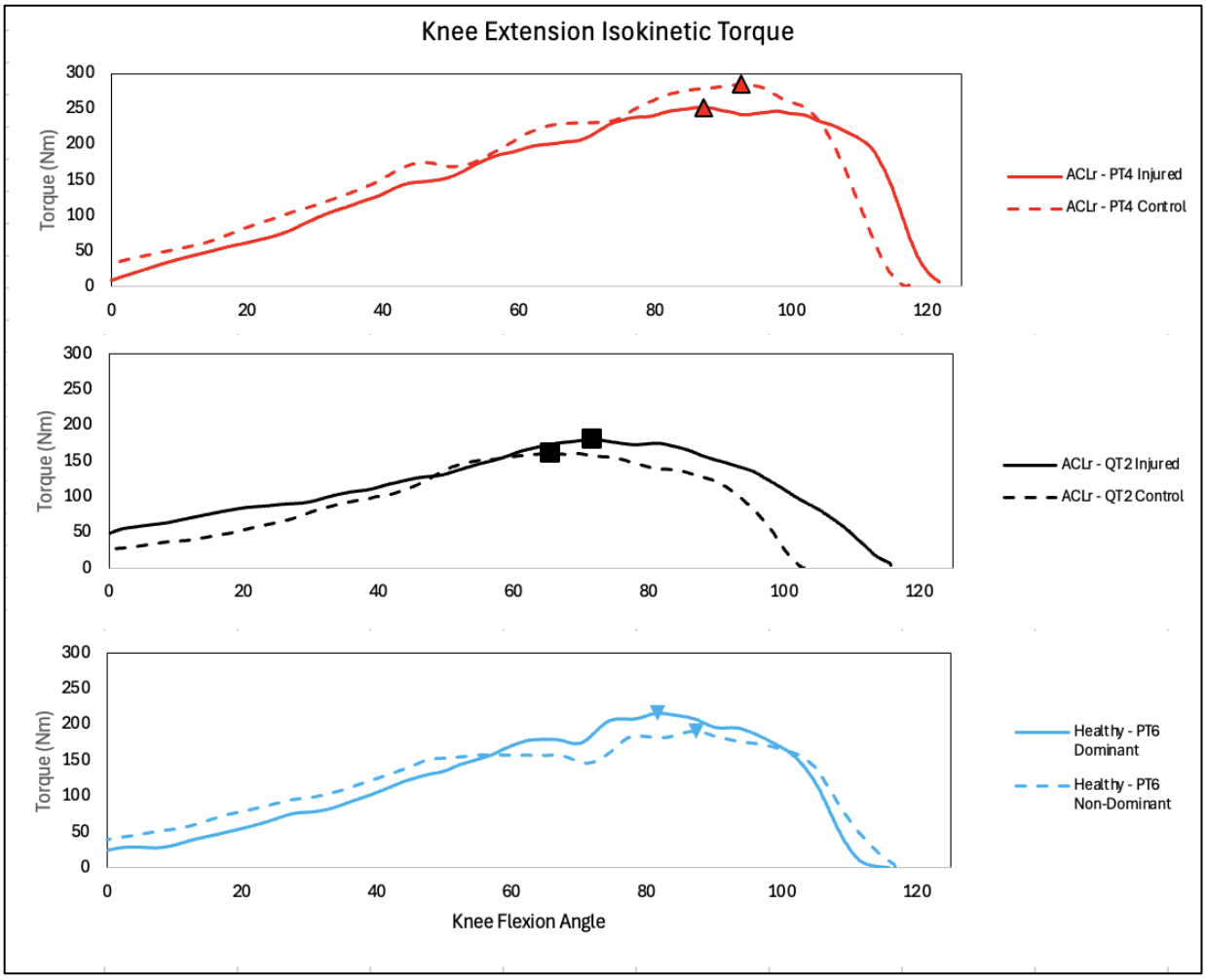


In the Healthy – PT group, isokinetic knee extensor torque was not significantly different between limbs (p=.100, d=.822), but there was a large effect size suggesting that the dominant limb is stronger on average. There was also a moderate effect of limb on the knee flexion angle at which peak torque was obtained (p=.257, d=.522), with the dominant knee obtaining its peak torque at a longer muscle length on average. Examples of isokinetic curves between the injured and control limb, or dominant and non-dominant limb for the knee extensors can be seen in **Figure 9**.

Table 7. Mean peak isokinetic knee extensor torque and mean angle at peak torque by limb for the quadriceps groups. ES is the Cohen's d effect size between the limbs within the group.

	ACLr - PT			**ACLr - QT**			**Healthy - PT**		
	Injured	Control	ES	Injured	Control	ES	Dom.	ND	ES
Peak Torque (Nm)	150.3±74.7	172.0±63.1	.769	108.8±50.2	122.5±27.8	.567	156.8±43.6	147.2±38.0	**.822**
Angle at Peak Torque	78.4±12.9°	72.1±12.0°	.478	65.0±18.6°	69.3±11.4°	.300	77.8±5.2°	72.5±9.1°	.522

Figure 9. Select representative isokinetic knee extensor torque-position curves for injured/control limbs and dominant/non-dominant limbs. Points represent peak torque and angle at peak torque for each curve.



Hamstrings Force Production – Shear Wave Elastography

The ACLr – ST group supported the overarching hypothesis that donor site tendon stiffness would be lower than in the contralateral limb. This led to the investigation of SH2, SH3, and SH4 related to the effect of this compromised tendon on ST recruitment at different muscle lengths and contraction levels, and the impact of tendon recovery on ST recruitment. Since two of the participants (ACLr – ST1, ACLr – ST3) did not have a ST muscle due to atrophy, they are not included in group averages and plots in this section. Likewise, their control matched

participants (Healthy – ST1, Healthy – ST3) are removed from the Healthy – ST group averages and plots. However, these participants will be analyzed in the discussion section to understand how these participants compensated for the lack of a ST muscle. ACLr – ST2, ACLr – ST4, ACLr – ST5, and Healthy – ST2, Healthy – ST4, and Healthy – ST5 are included in this analysis of ST force production.

Main Effect of Limb on Semitendinosus Force

Across all participants in the ACLr – ST group, the injured ST produced less force and contributed less to the total load share. Averaging across the different hip flexion and contraction levels, the injured side ST in the three participants that had a clear ST muscle produced an average of 71.2±25.2N while on the healthy limb, these participants produced an average of 192.6±96.7N from their ST. The dependent samples t-test determined this to be statistically significant ($p<.001$) as well as meaningful ($d=1.207$) with a large effect size. The load share of total knee flexor force coming from the ST was 12.6±3.6% in the injured limb, compared to 25.8±12.4% in the contralateral limb. This was also statistically significant and a large meaningful difference ($p<.001$, $d=1.073$). In the Healthy – ST group, there was a significant difference in ST force production between limbs ($p=.046$) with more ST force on the non-dominant side, but the effect size was small ($d=.431$). There was no difference in ST load share between limbs in the Healthy – ST group ($p=.201$, $d=.269$).

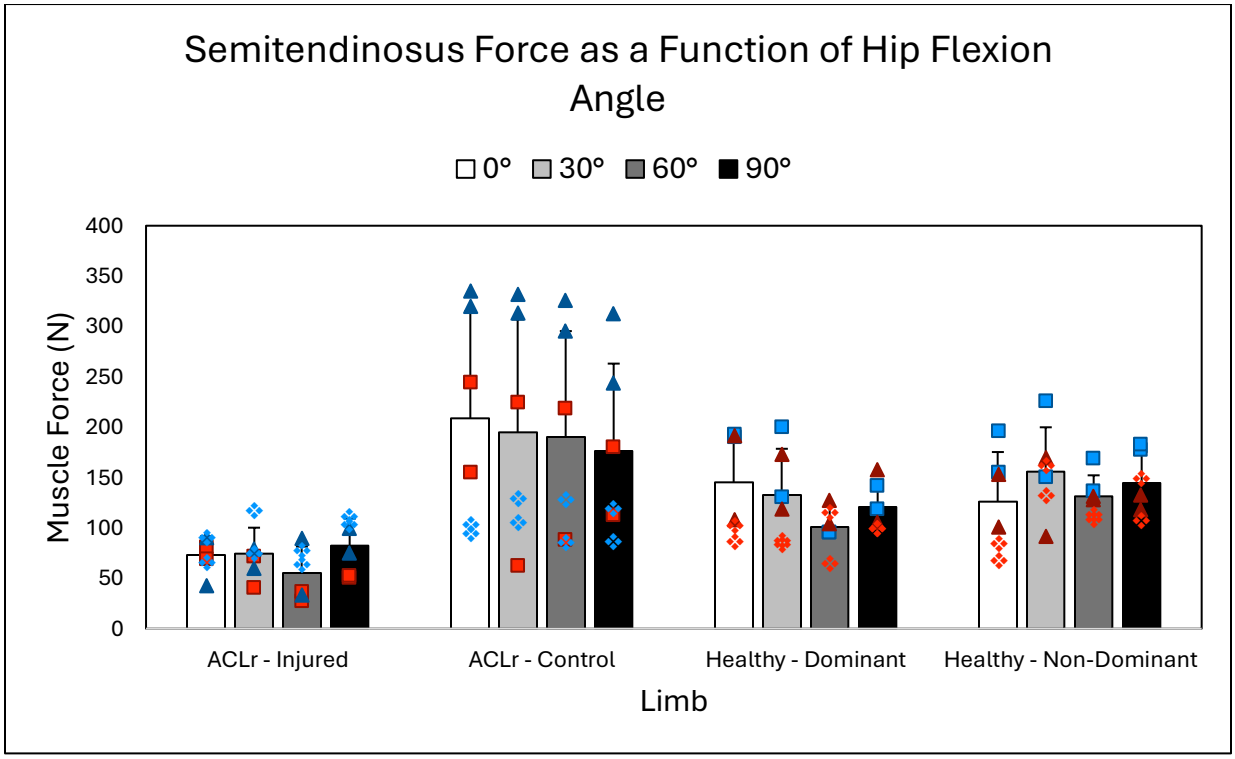
Effect of Muscle Length on Semitendinosus Force

Sub-hypothesis 2 hypothesized that deficits in ST force production between the injured and contralateral limb would be smaller at longer muscle lengths. The effect of joint position on ST force was not consistent. A repeated measures ANOVA within the ACLr injured limb revealed a significant impact of joint position on ST force ($p=.049$), with a large effect size (η^2

=0.398). ST force was significantly less at 60° of hip flexion than at 90° of hip flexion (p=.006, d=1.862). Looking at **Figure 10**, the magnitude of differences in ST force between hip flexion angles is quite small in the injured limb and is not consistent between participants. In the healthy limb in the ACLr – ST group, there is a clearer effect of joint position on ST force. As the muscle length increases, the force produced by the ST decreases in ACLr – ST2 and ACLr – ST4, despite the target isometric flexion torque being higher in Newton-meters at these longer muscle lengths. This was not statistically significant (p=.155), but did have a large effect size (η^2 =0.287). The Cohen's d effect size between the shortest muscle length and longest muscle length in ST force production in the healthy limb was large at d=.936. ST force production remained relatively constant across the muscle length range tested in ACLr – ST5's healthy limb.

In the Healthy – ST group there was not a clear impact of hip flexion angle for any participant for either limb. However, there was a significant impact of position in the dominant limb (p=.008, η^2=0.349) suggesting that ST muscle force may be highest at the shortest muscle length.

Figure 10. Semitendinosus muscle force within each limb as a function of hip flexion angle. Error bars represent SD. Points represent individual datapoints with each colored symbol representing a participant. Data from both torque conditions are compiled in the chart.

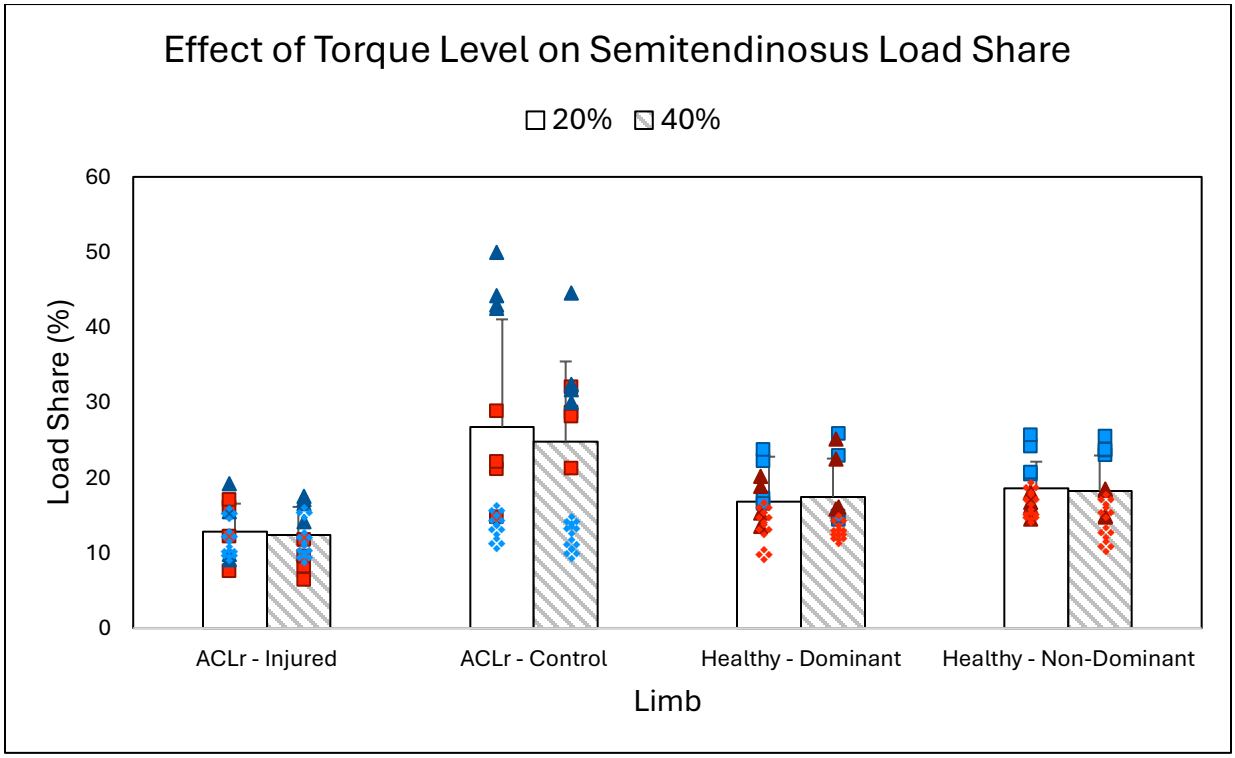


SH2 hypothesized that the deficits between the injured and control limb in the ACLr – ST group would be smaller at longer muscle lengths due to the affected ST's optimal working range being shifted towards longer muscle lengths because of increased tendon compliancy. This was tested through a repeated measures ANOVA that compared the difference in injured and control limb ST force at each position. While there was not a clear impact of muscle length on ST force production on the ACLr injured limb, there was a relatively consistent preference for shorter muscle lengths in the contralateral limb. The hypothesis was supported ($p=.019$, $\eta^2=.477$); paired sample t-tests found raw ST muscle force deficits were meaningfully smaller at 90° of hip flexion than at 0° ($p=.049$, $d=1.059$), 30° ($p=.207$, $d=.592$), and 60° ($p=.025$, $d=1.289$) of hip flexion.

Effect of Isometric Flexor Torque Level on Semitendinosus Force

SH3 hypothesized that at higher levels of joint torque, the effects of a less stiff ST tendon would be less pronounced. ST contribution to entire knee flexor torque was compared at the 20% and 40% torque levels for each limb. As seen in **Figure 11**, there was not a clear impact of torque level on ST load share for any of the participants except for ACLr – ST4 on the control limb. She generally decreased her ST load share at higher torque levels by about 10%. Within the ACLr injured limb, the average ST load share at 20% contractions was 12.9±3.7% while it was 12.4±3.7% at the 40% torque level. Dependent samples t-tests were run and found this was not a significant or meaningful result ($p=.730$, $d=.102$). There were also no significant group level differences in load share between the torque levels in the ACLr control limb ($p=.434$, $d=.234$), the healthy dominant limb ($p=.419$, $d=.243$), or the healthy non-dominant limb ($p=.680$, $d=.122$). SH3 was not supported, and the findings suggest that the ST load share may not differ between isometric knee flexion torque levels in healthy or ACLr limbs.

Figure 11. Semitendinosus load share to total knee flexor forces within each limb as a function of torque level. Error bars represent SD. Points represent individual datapoints with each colored symbol representing a participant. Data from all joint angles are compiled in the chart.



Effect of Donor Site Tendon Recovery on Semitendinosus Recruitment

SH4 hypothesized that the magnitude of the deficits between the ACLr and contralateral limbs would be correlated to the percent decrease in the donor site tendon. Due to the small sample size, this hypothesis was analyzed descriptively. The percent of healthy limb tendon stiffness recovered was compared to the average difference in ST load share for each participant as well as the average percent difference in ST force, peak isometric knee flexor torque, peak isokinetic knee flexor torque, and ST PCSA. The percent difference, or in the case of load share the difference, was calculated for each condition before finding the average deficit for the participant across the different muscle length and torque level conditions. The deficits are summarized in **Table 8**.

Table 8. ACLr - ST participants' injured limb deficits compared to ST tendon stiffness recovery.

TP	*ST Tendon Recovery (%)*	*ST Load Share Deficit*	*ST Force Deficit (%)*	*Isometric Peak Torque Deficit (%)*	*Isokinetic Peak Torque Deficit (%)*	*ST PCSA Deficit (%)*
ACLr – ST1	23.04	N/A	N/A	-22.63±2.05	-14.46	N/A
ACLr – ST2	79.52	-13.52±7.42	-62.59±15.61	-33.78±8.21	-50.00	-18.77
ACLr – ST3	N/A	N/A	N/A	-5.77±6.81	+71.82	N/A
ACLr – ST4	61.04	-24.94±10.12	-77.00±10.12	+14.86±6.94	+28.56	-26.15
ACLr – ST5	23.52	-1.02±3.06	-16.35±20.55	-3.86±3.06	-23.08	+23.69

There was no relationship between ST tendon recovery and ST function in this small sample size. ACLr – ST5 had the worst tendon recovery of the three participants who had a clear ST muscle, but she had the most symmetrical use of her ST, as well as the most symmetrical isometric strength at the four angles tested. Additionally, despite not having a ST muscle on the injured side, ACLr – ST5 had symmetrical isometric strength within 10% and greater peak isokinetic strength on the injured side. ACLr – ST2 and ACLr – ST4 had relatively strong recovery of the ST tendon, but they still used their ST less on the injured side in terms of both load share and raw force production. ACLr – ST2 demonstrated significant strength deficits while ACLr – ST4 was stronger on her injured side. In this small sample, there was not a clear relationship between tendon recovery and ST functionality so SH4 was not supported.

Hamstrings Neuromuscular Activation – Electromyography

In addition to SWE, EMG was used to explore SH2-SH4 related to deficits of the ST muscle at different muscle lengths and torque levels, and the relationship between donor site tendon recovery and the size of any deficits between limits. For these analyses, all participants were included as surface EMG typically picks up signal from the SM in addition to the ST. To assure that it was accurate to use the normalized data for comparisons, the RMS MVIC voltage for the injured and contralateral limbs were compared within the ACLr – ST group. This was

done in case reduced ST voluntary recruitment in the injured limb led to a reduction in peak neuromuscular activation and signal compared to the contralateral limb. The injured limb averaged 253.06±48.06μV for the MVIC while the control limb averaged 329.74±113.13μV. This difference produced a moderate effect size (d=.710) so the voltages and normalized values will be reported to allow for fair comparisons between limbs. In the Healthy – ST group, there was also a moderate effect size (d=.607) between the dominant (219.68±34.26μV) and non-dominant limb (302.82±165.83μV) RMS MVIC voltage.

Main Effect of Limb on Medial Hamstring Neuromuscular Activity

In the ACLr – ST group, there was not a moderate or large effect of limb on medial hamstring neuromuscular activity suggesting that there were no meaningful differences in medial hamstring neuromuscular activity following an ACLr with a ST graft in our sample. The Cohen's d for the normalized RMS medial hamstring signal between limbs was d=.109 while it was d=.421 when comparing the RMS medial hamstring voltage between limbs. There were also no moderate or large main effects between limbs in the Healthy – ST group.

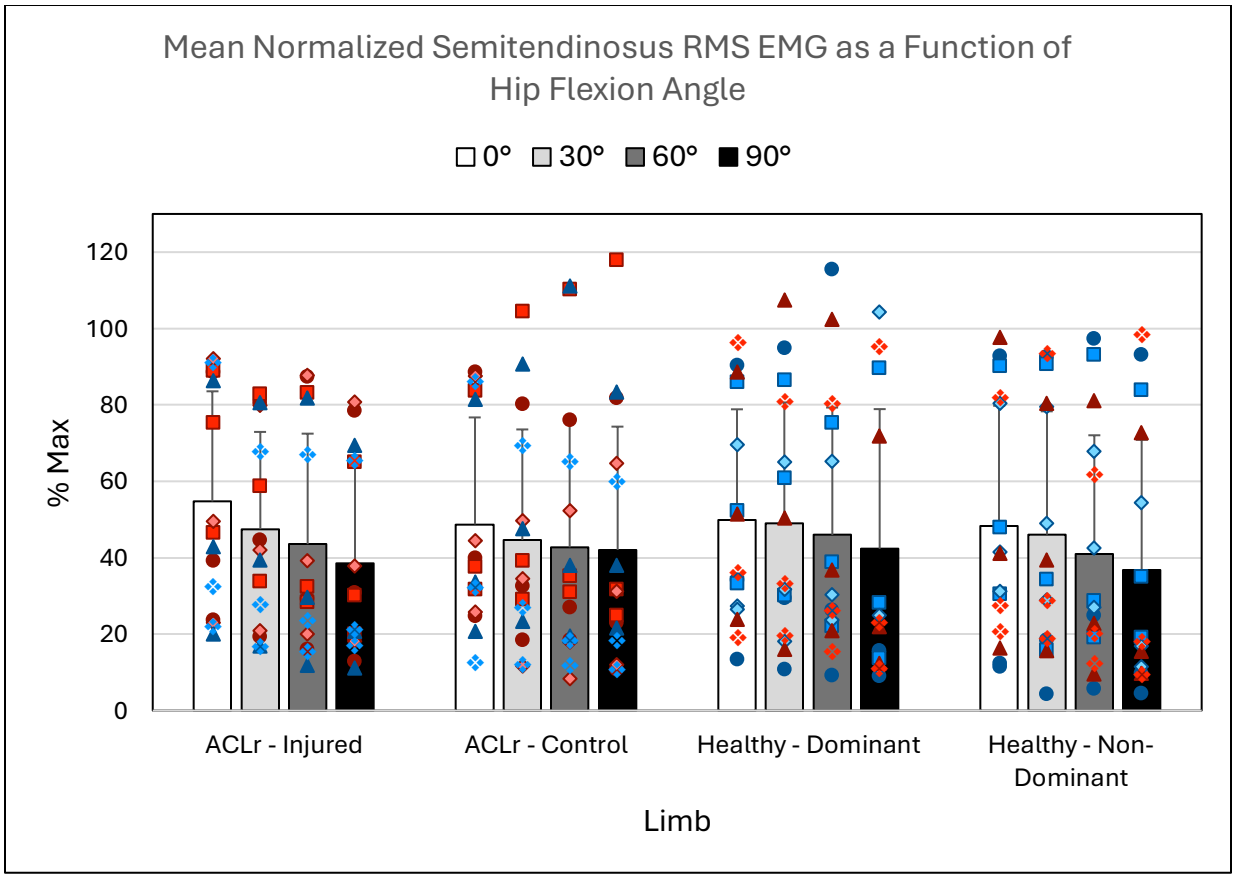
Effect of Hip Joint Position on Medial Hamstring EMG Signal

Sub-hypothesis 2 hypothesized that deficits in EMG signals between the injured and contralateral limb would be reduced at longer muscle lengths. The effect of muscle length on EMG signal was explored within each limb, and then between the limbs within the ACLr group and the healthy group.

There were clear effects of hip flexion angle on medial hamstring neuromuscular activity in the injured limb. As the muscle length increased, the medial hamstrings EMG signal decreased. This can be seen in **Figure 12**. There was a slight decrease in normalized RMS EMG

signal in the other limbs, but it was most pronounced and consistent in the ACLr injured limb. A repeated measures ANOVA found a significant and meaningful impact of hip joint position on the RMS normalized EMG signal ($p<.001$, $\eta^2=0.516$) in the ACLr injured limb. Post-hoc dependent samples t-tests found that the normalized signal was significantly and meaningfully different between each position except for 30° to 60° of hip flexion. For all other comparisons, p-values were less than 0.005 and Cohen's d effect sizes were larger than 0.900. The one other exception was 60° to 90° of hip flexion where the effect size was moderate (d=.613).

Figure 12. Semitendinosus normalized RMS EMG signal within each limb as a function of hip flexion angle. Error bars represent SD. Points represent individual datapoints with each colored symbol representing a participant. Data from all torque conditions are compiled in the chart.



When analyzing the effect of position within the ACLr – ST control limb, there was not a significant effect of position on normalized RMS medial hamstring activity but there was a moderate effect size (η^2=0.111). Within the dominant limb of the healthy group, there was a large effect size due to position (η^2=0.173), but the repeated measures ANOVA was not significant (p=.102). The only moderate Cohen's d effect size between positions was between the shortest and longest muscle length (d=.574). In the non-dominant limb of the Healthy – ST group, there was a significant and meaningful effect of hip flexion angle on medial hamstring muscle activity (p<.001, η^2=0.582). Much like the injured ACLr limb, the muscle activity reduced as the muscle length increased with effect sizes ranging from d=.273 to d=.980. The bigger the difference in muscle lengths, the larger the effect size was.

As there were no significant deficits between the ACLr limb and the contralateral limb, joint position did not play a role in reducing any deficits and SH2 was not supported.

Effect of Torque Level on Medial Hamstrings EMG Signal

As expected, there was an effect of torque level on the EMG signal for both limbs in both groups (p<.001). There were no meaningful deficits in neuromuscular activity between the ACLr limb and the contralateral limb, so torque level did not play a role in reducing any deficits and there was not support for SH3 within the EMG data in the hamstrings groups.

Quadriceps Force Production - Shear Wave Elastography

Since there was no consistent deficit in QT or PT material properties between the ACLr knee and the healthy knee, this data will not be analyzed extensively. It was difficult to obtain quality, active SWE images of the quadriceps muscles due to participants across the three knee extensor groups (ACLr – PT, ACLr – QT, Healthy – PT) having trouble holding the isometric

contractions steady; potential reasons for this are explored in the discussion section. However, the main effect of limb was analyzed for each of the quadriceps muscles' forces and load shares in the ACLr – PT, ACLr – QT, and Healthy – PT groups to see if there were any specific muscles impacted by the harvesting the graft.

In the ACLr – PT group, there were no large effect sizes between the injured and contralateral limb for any of the muscle forces or load shares. However, there was a moderate effect of limb for the VI muscle force. The injured VI averaged 400.2±122.8N of force produced across the different conditions while the contralateral VI averaged 472.4±150.6N. This difference resulted in a Cohen's d effect size of d=.519. All other muscle forces and load shares had minimal differences between the limbs.

Likewise, in the four participants in the ACLr – QT group there were no large effect sizes between the injured and contralateral limb. However, there were more moderate effect sizes, including increased VM load share (d=.589), reduced VL force (d=.727) and load share (d=.515), decreased RF force (d=.558), decreased VI force (d=.517), and decreased summed forces (d=.700) in the injured limb.

In the Healthy – PT group, there were also no large effects of limb on muscle force or load share. There were moderate effect sizes, though; there was a moderate decrease in VM force (d=.549) and load share (d=.602) and a moderate increase in RF force (d=.545) in the dominant limb.

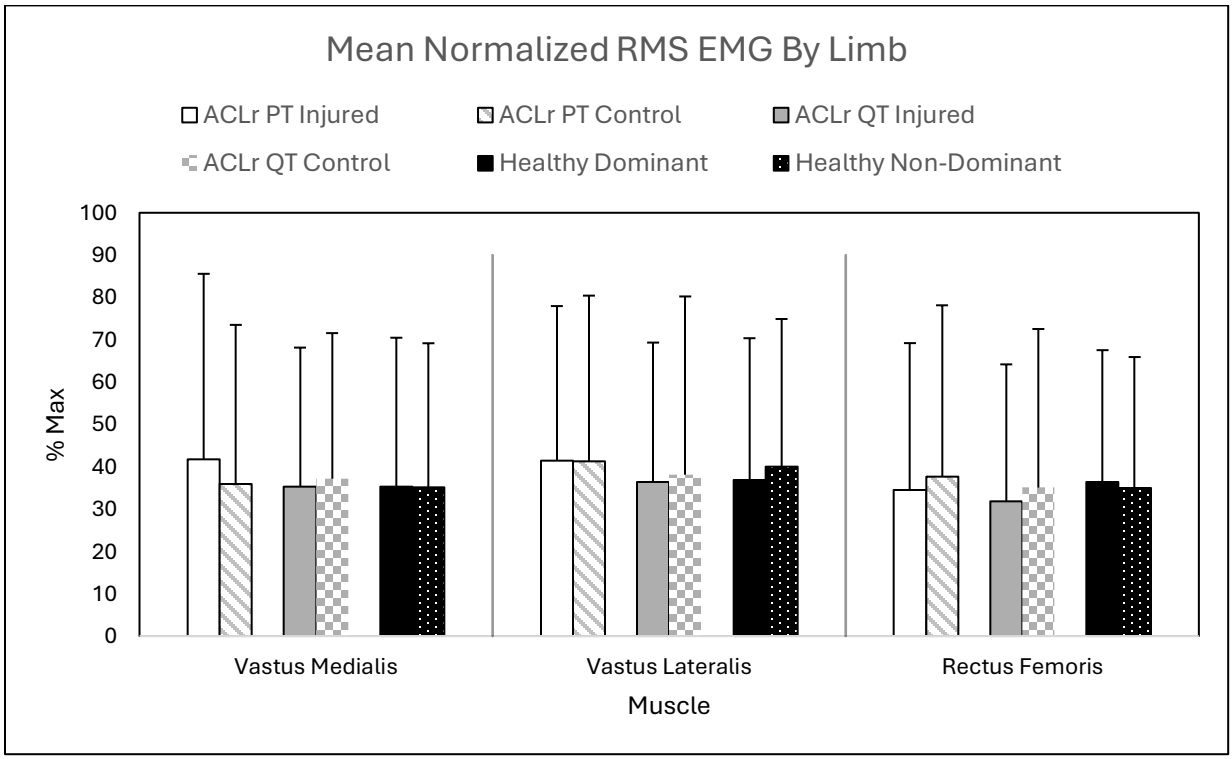
Table 9. Summary statistics for quadriceps muscle force by group and limb. ES is the effect size between the limbs within the group.

	ACLr – PT *(n=6)*			**ACLr – QT** *(n=4)*			**Healthy – PT** *(n=6)*		
	Injured	**Control**	**ES**	**Injured**	**Control**	**ES**	**Dominant**	**Non-Dominant**	**ES**
VM Force (N)	312.1±181.6	309.8±168.0	.018	293.0±147.0	280.6±147.4	.098	222.0±120.7	318.2±199.4	.549
VM Share (%)	25.7±10.5	23.7±10.5	.211	30.2±6.5	24.0±8.7	.589	18.5±6.3	23.7±8.7	.602
VL Force (N)	352.5±162.8	371.7±168.0	.116	224.4±135.0	336.2±250.8	.727	273.4±147.2	328.8±202.9	.304
VL Share (%)	29.3±7.7	29.4±7.4	.010	22.3±6.2	25.9±7.6	.515	23.0±8.2	24.7±6.9	.217
RF Force (N)	105.2±94.9	97.7±76.9	.105	60.1±56.2	111.1±94.1	.558	140.8±80.9	123.6±98.6	.172
RF Share (%)	9.4±8.7	8.0±6.6	.210	5.8±5.0	9.9±8.4	.453	12.7±7.3	9.6±5.8	.545
VI Force (N)	400.2±122.8	472.4±150.6	.528	368.2±113.5	504.9±307.4	.517	530.5±210.2	548.9±243.4	.111
VI Share (%)	35.6±9.0	38.9±9.1	.303	41.6±9.1	40.3±14.4	.080	45.8±12.1	41.9±13.9	.365
Summed Force (N)	1170.0±356.8	1251.6±406.0	.335	942.8±369.6	1216.9±652.6	.700	1166.8±385.6	1319.5±580.7	.339

Quadriceps Neuromuscular Activity – Electromyography

The main effect of limb was explored in each of the quadriceps groups. There were no moderate or large effects of limb in the ACLr – PT or Healthy – PT groups, with all EMG measurements being comparable between the injured and control limbs. In the ACLr – QT, group there was a moderate effect (d=.676) of limb for the VM voltages. The voltages were slightly higher in the injured limb, but the normalized values were very similar to one another as seen in **Figure 13**.

Figure 13. Mean normalized RMS EMG signal for the quadriceps muscles, separated by limb and group. Error bars represent SD. Data from all torque conditions are compiled in the chart.



Discussion

This study explored donor site tendon properties following ACLr with an ipsilateral autograft and the impact tendon stiffness deficits may have on strength and muscle force production. ST tendon stiffness was found to consistently be lower in the affected limb than in the contralateral limb in those who had an ACLr with a ST graft. This was coupled with a decrease in ST force production in the injured limb, across all muscle lengths and contraction levels. PT and QT stiffness were not consistently compromised in those who had an ACLr with a BPTB or QT autograft. As such, there was not a large effect of limb on any quadriceps muscle forces following ACLr with these autografts.

Statistical analysis choices had minimal impact on the overall story.

Due to the variability between graft types, and small, variable samples within each graft type, analyses were done within each group as opposed to between the ACLr and Healthy matched group. It was not possible to run powered factorial ANOVAs between the groups and different muscle length and torque level conditions. Within the healthy groups, analyses were done between the dominant and non-dominant limbs as opposed to between the injured matched and control matched limbs. These choices were made to focus on differences within the injured participants due their ACLr and expected differences within healthy individuals as opposed to differences between the participants across the groups. However, this may have had minor impacts on the story being told by this data. Basic analysis of the main findings found that there were no differences in the story being told in terms of donor site tendon properties or ST muscle force production regardless of the analysis style, but there were differences in isometric strength deficits.

The ST tendon was meaningfully less stiff following ACLr with a ST tendon compared to both the contralateral limb within the ACLr injured group (p=.084, d=1.277) and the injured matched limb (p<.001, d=4.760) while the PT and QT stiffness were not meaningfully different from their contralateral limbs (ACLr – PT: p=.634, d=.207; ACLr – QT: p=.439, d=.445) or their injured matched limbs (ACLr – PT: p=.548, d=.359; ACLr – QT: p=.965, d=.033). Regardless of statistical analysis method, ST muscle force and its contribution to total knee flexor force were reduced in the ACLr – ST injured limbs, and deficits were smaller at longer muscle lengths. Within the healthy groups, differences between the injured matched and control matched limbs were minimal, and comparable to or smaller than those seen in the dominant versus non-dominant limb comparisons in terms of tendon properties and ST force production.

While peak isometric flexor strength was found to be significantly lower in the ACLr – ST injured limbs compared to the contralateral limb (p=.016, d=.588), it was not meaningfully different from the injured matched limbs (p=.859, d=.056). Additionally, the ACLr – ST control limb isometric flexor strength was not significantly different from the control matched limb (p=.708, d=.119). Within the Healthy – ST group, the control limb was stronger than the injured limb in terms of isometric strength (p=.004, d=.742) while there were no differences between the dominant and non-dominant limbs (p=.356, d=.211). This does show that the analysis method can have an impact on the interpretation of the study results. However, it appears that major findings regarding muscle recruitment were consistent regardless of statistical analysis method.

Muscle PCSA results were similar to past studies.

Like other studies in the ACL space, this study estimated knee flexor and knee extensor muscles' PCSA. In this study, muscle PCSA was estimated by calculating muscle volume using a truncated cone formula from two cross-sectional images of the muscle– one distal and one

proximal. Then, the muscle volume was divided by fiber length values from the literature to estimate PCSA[79]. The same fiber lengths were used for all participants for a given muscle, regardless of ACLr status. This makes the calculated PCSA an estimate based on measurements as well as values from the literature.

The hamstring muscle PCSA changes observed in the ACLr – ST group were consistent with Messer et al. who found a 45% reduction in ST volume, paired with a significant increase in SM and BFsh volume based off of MRI scans[24]. Likewise, we found a large effect size of limb for ST PCSA (d=1.17), with the affected ST averaging 37% smaller. There were also moderate increases in BFsh (d=.755) and SM (d=.623) PCSA on the injured limb. Three of the five ACLr – ST participants injured their dominant limb. The dominant limb tends to have slightly larger muscle sizes at baseline, so the differences in PCSA between sides may have been more pronounced if we had controlled for this. However, our estimation of fascicle length might have particularly underestimated injured ST PCSA as muscle length has been shown to be reduced following ACLr with a ST[24]. This decrease in muscle length may also be met with a decrease in ST fascicle length. An underestimation of ST PCSA would have led to an underestimation of ST force production.

The ultrasound aided estimated PCSA for the Healthy – ST group, the ACLr – ST control limb, and the unaffected muscles in the ACLr – ST injured limb were on par with values reported in the literature for healthy individuals, except for the BFsh. The BFsh PCSA reported in this study were lower than the values Ward et al. measured from cadaveric samples[79]. This was likely due to our methods – we used a truncated cone equation based on two CSA measurements taken at 30% and 60% of femur length measured distally to proximally. Franchi et al. also used this method, but they took panoramic B-mode ultrasound images at 30%, 40%, 50%, and 60% of

femur length and used the sum of the truncated cone formula run between each pair of adjacent panoramic images to estimate muscle volume[78]. Since the BFsh does not extend to 60% of femur length proximally, we only had one measurement of BFsh CSA. This likely led to a significant underestimation of BFsh PCSA. SM PCSA was also likely impacted by just taking two images as this muscle is usually starting to tendon off by 60% of femur length. Ultimately, this may have led to an underestimation of knee flexor group PCSA, as well as an underestimation of BFsh and SM force. The group average PCSA for each hamstring muscle in each limb can be seen in **Table 10**, as can the literature reported values.

Table 10. Hamstring muscles and muscle group PCSA by limb and compared to literature values.

	BFlh (cm^2)	ST (cm^2)	SM (cm^2)	BFsh (cm^2)	Summed PCSA (cm^2)
ACLr – ST Injured	12.02 ± 1.64	3.31 ± 1.78	15.17 ± 4.34	2.98 ± 1.00	33.48 ± 3.87
ACLr – ST Control	12.63 ± 2.30	5.24 ± 1.51	13.06 ± 2.04	2.37 ± 0.55	33.29 ± 2.34
Healthy – Dominant	10.89 ± 2.07	5.16 ± 1.22	14.03 ± 1.66	2.20 ± 0.66	32.28 ± 3.38
Healthy – Non-Dominant	11.12 ± 1.73	5.04 ± 0.91	13.08 ± 1.01	2.06 ± 0.42	31.35 ± 1.49
Literature Values[79]	11.33 ± 4.75	4.82 ± 2.01	18.40 ± 7.53	5.06 ± 1.69	40.1 ± 13.6

Summed quadriceps PCSA was meaningfully reduced in the ACLr – PT Injured limb (d=1.021) and the ACLr – QT Injured limb (d=4.426) compared to the participants' unaffected limbs. For the ACLr – PT group, these deficits came primarily from reductions in the RF and VI PCSA on the injured limb. In the ACLr – QT group, deficits were meaningful in all the muscles except the VM. Quadriceps tendon graft harvesting typically tries to avoid fibers that lead to the VM so it makes sense that this muscle would be the least affected[48]. Many studies have described reduced quadriceps muscle volume and PCSA following ACLr, but far fewer have examined muscle atrophy as a function of graft type.

Macron et al. found that the quadriceps group muscle volume in the injured limb obtained 91.6% of the volume seen in the contralateral limb following ACLr with a BPTB graft[80]. This was very comparable to our findings where we found an 89.5% average limb to limb PCSA symmetry. When broken down by muscle, Hunnicutt et al. found average limb symmetry indexes for CSA following ACLr with a BPTB graft of 77.4% for the VM, 78.4% for the VL, 79.8% for the VI, and 90.3% for the RF[81]. Our data were comparable for the RF and VI muscles, but the ACLr – PT group had better limb to limb symmetry for the VM and VL muscles than Hunnicutt et al. reported. The same study also examined limb to limb CSA symmetry following ACLr with a QT group. They found no significant differences in limb-to-limb CSA symmetry indexes between the graft types[81]. In this study, the ACLr – QT group had overall worse PCSA symmetry than the ACLr – PT group despite having similar tendon regeneration and fewer strength deficits.

When comparing the quadriceps group PCSA values to established values in the literature[79], the summed muscle group PCSA was on par as were the VM and RF sizes. However, the VL PCSA was smaller in this study while the VI PCSA was larger. This was likely due to the rater's delineation of the border between the VL and VI. This border can be difficult to differentiate, and it appears that the rater consistently under-estimated VL PCSA in favor of increased VI PCSA. This would lead to an overestimation of VI muscle forces and an underestimation of VL muscle contribution to extensor torque.

Table 11. Quadriceps muscles and muscle group PCSA by limb and compared to literature values.

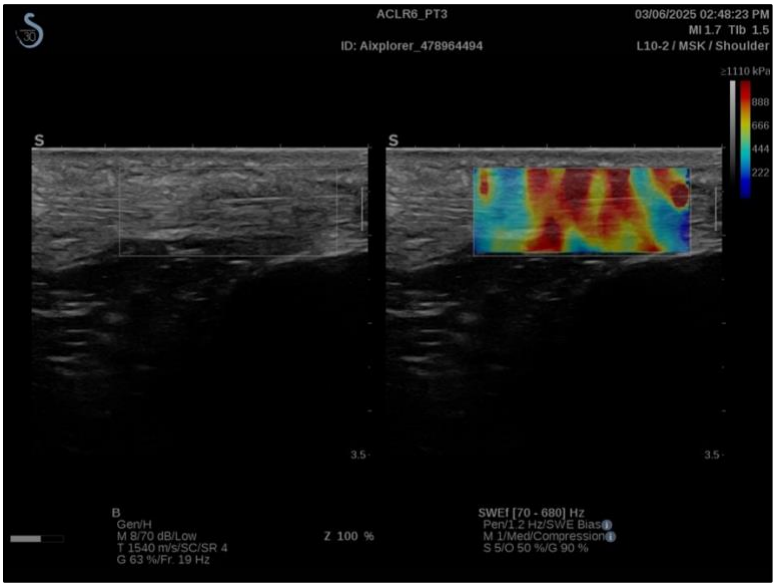
	Vastus Medialis (cm^2)	Vastus Lateralis (cm^2)	Rectus Femoris (cm^2)	Vastus Intermedius (cm^2)	Summed PCSA (cm^2)
ACLr - PT Injured	22.47 ± 8.92	24.70 ± 6.30	10.38 ± 2.26	21.17 ± 4.78	78.09 ± 16.79
ACLr - PT Control	22.61 ± 6.08	25.94 ± 2.88	12.43 ± 1.86	26.32 ± 5.75	87.30 ± 11.93
ACLr - QT Injured	17.28 ± 6.29	18.77 ± 8.27	9.63 ± 2.26	24.44 ± 3.68	70.13 ± 17.65
ACLr - QT Control	18.85 ± 3.97	23.94 ± 8.83	13.57 ± 3.61	28.73 ± 4.85	85.09 ± 18.40
Healthy - Dominant	22.80 ± 7.70	24.78 ± 5.79	13.08 ± 2.21	26.97 ± 7.93	87.63 ± 21.64
Healthy - Non-Dominant	21.16 ± 6.57	24.28 ± 6.92	13.33 ± 5.00	27.71 ± 9.55	86.48 ± 26.61
Literature Values[79]	20.58 ± 7.17	35.09 ± 16.14	13.51 ± 4.97	16.74 ± 6.91	88.4 ± 30.5

Donor site tendon recovery was worse following ACLr with ST graft compared to BPTB and QT grafts.

The overarching theory of this thesis was that donor site tendon recovery is not complete following ACLr with an ipsilateral autograft, and that these deficits in the tendon would impact muscle force production. This idea of reduced tendon stiffness had been previously supported following ACLr with a ST graft[22,26], but had not been explored following ACLr with a BPTB or QT graft. Ultrasound based SWE was used to measured donor site tendon stiffness, which is the inverse of tendon compliance. Increased tendon compliance impacts a muscle's ability to produce force. In this study, the ST tendon was found to be consistently less stiff in the ACL limb following ACLr with a hamstring graft with percent of the contralateral limb stiffness recovered ranging from 4.9% to 79.5%. The Cohen's d effect size for this difference was d=1.277. This was in line with previous findings. Amat-Fernandez found an average recovery of 39% of healthy ST tendon stiffness while Suydam found participants to regenerate an average of 80.6% of tendon stiffness[22,26]. However, this theory was not supported in the ACLr – PT and ACLr – QT groups. In these groups, recovery of the donor site tendon was inconsistent.

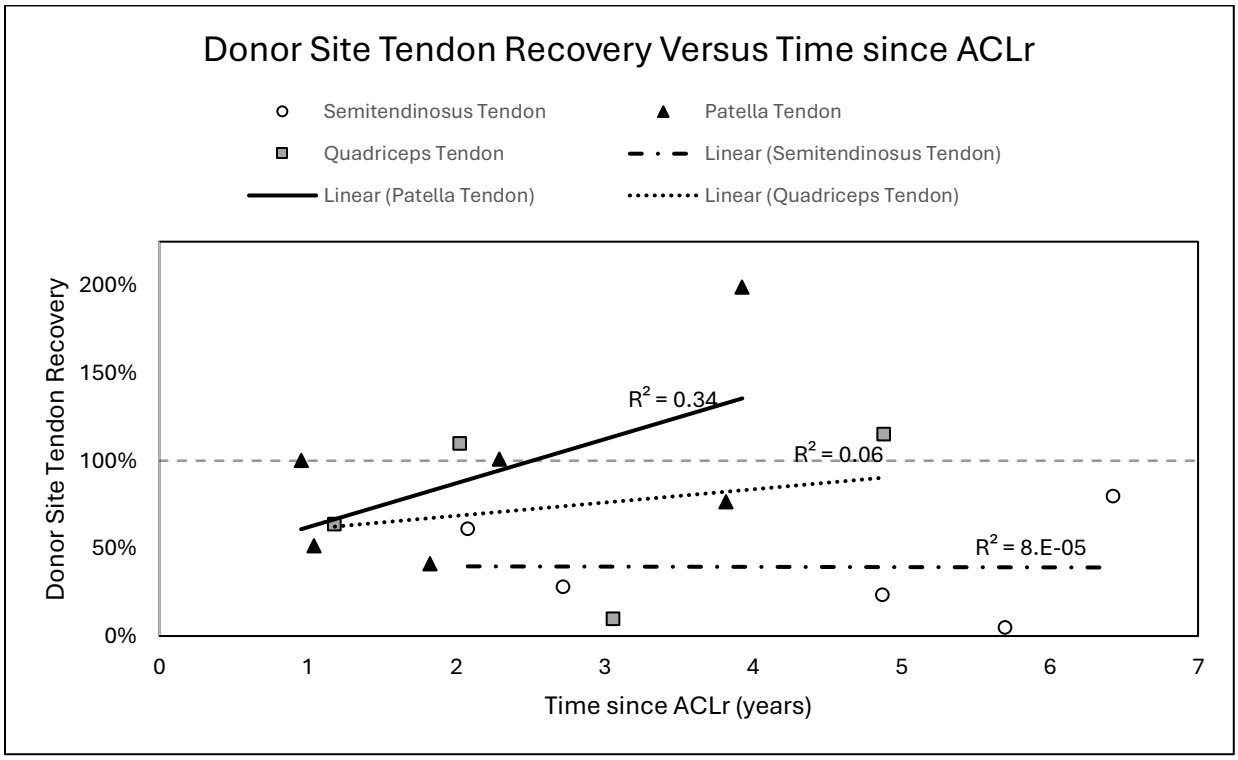
Of the six participants in the ACLr – PT group, two recovered about half of their healthy PT's stiffness, one recovered 75% of their healthy PT stiffness, and two participants had nearly perfect symmetry between the two limbs at 100% and 101% of the contralateral limb's PT stiffness. The last participant in this group had double the stiffness of their unaffected limb's PT stiffness in their ACLr limb; this participant re-injured their ACL three weeks after participating in the study and retrospectively complained of patellar tendon pain in the months prior to the second injury. This value was likely indicative of a pre-injury mechanism in addition to the presence of scar tissue as seen in **Figure 14**. There was a link between time since ACLr and tendon recovery, however this was driven by ACLr – PT3 who had the abnormally high tendon stiffness. Previous research has shown a link between PT stiffness recovery and time, but the findings have shown conflicting results with Gulledge et al. suggesting that PT shear wave speed increases over time while Ito et al. have suggested that the stiffness of the PT is higher in the injured limb at one month post-op before normalizing by 9 months[55,82].

Figure 14. ACLr - PT3 injured side patella tendon, with scar tissue evident superficially.



In the ACLr – QT group, two of the four participants had a slightly stiffer QT on their injured side, one recovered 63% of their unaffected limb's QT shear modulus, and ACLr – QT3 only recovered 9.5% of the healthy tendon's stiffness at the typical imaging site. QT stiffness following ACLr has not been previously studied. For all graft types, the amount of time since ACLr did not play a role in the recovery of tendon material properties, as seen in **Figure 15**.

Figure 15. Donor site tendon stiffness recovery over time for each graft type.



While ACLr – QT3 only recovered 9.5% of her healthy tendon's stiffness, she may offer a good basis for why tendon recovery might be superior following ACLr with a PT or QT autograft. When her tendon was traced more proximally, it had nearly identical stiffness to the contralateral limb (370.67 KPa Injured vs 391.87KPa Control). This proximal portion of the injured limb's tendon was likely not harvested for the ACLr and thus remained intact. While exact surgical methods vary, only a portion of the QT width is used to create the autograft[48,83]. The QT is 2.5-3cm in width on average and generally has a trilaminar structure formed by

components from each of the four quadriceps muscles. The RF fibers lay most superficial while the VI fibers are deepest. The QT graft may be partial or full thickness, but surgeons will not utilize the full width of the tendon; typically a 10-12mm wide central part of the tendon is harvested[48]. The remaining parts of the QT may provide a scaffold or blueprint for the tendon to recover along. This scaffold might explain why the ACLrr – QT participants generally had strong recovery of their donor site tendon stiffness.

Similarly, the BPTB graft is sourced from the central third of the tendon[84] and thus there is a remaining tissue that can be used to help guide healing of the tissue. This might explain why three of the six participants in the group were able to meet or exceed their unaffected limb's PT's stiffness. The remaining three participants still recovered 76.7%, 51.5%, and 41.3% of their healthy limb's PT stiffness. While this was strong recovery, the presence of scar tissue at the donor site may have artificially inflated the donor site tendon stiffness recovery. On the contrary, three of the five participants in the ACLr – ST group regained 30% or less of their unaffected limb's ST tendon stiffness, and the maximum recovery of tendon stiffness was 79.5%. The ST tendon is much smaller than the QT and PT, and thus the entire tendon must be stripped from the muscle to make an autograft with at least the 8mm diameter that has been deemed critical for a successful ACLr[48,84]. Since there is no scaffold to guide healing of the ST tendon, regeneration may not occur or be inferior. Additionally, there are other hamstring muscles that may be able to compensate for the ST. The QT and PT are both common structures that affect muscle contraction in not one muscle, but each of the four quadriceps muscles.

Nevertheless, donor site tendon recovery is not the only marker of a superior graft choice. Metrics such as patient reported outcomes, graft rupture rates, donor site morbidity, strength recovery, and the rate of OA development are also important considerations when selecting a

graft type. BPTB autografts have been considered the gold standard due to the low rates of graft rupture[5,8,10]. This research study would support that they are a better choice than the ST tendon graft in terms of donor site recovery. However, patients often complain of donor site morbidity and persistent knee range of motion and extensor strength deficits following ACLr with a BPTB autograft[8,10]. At nine months post-ACLr with a BPTB graft, Schwery et al found an average isometric extensor strength deficit of 16%[85] compared to this study which observed an 18% deficit at 2.3±1.3 years after the ACLr. The rate of OA development is also higher with a BPTB graft than it is with a hamstring graft[5,9].

ACLr with a ST graft has issues as well including reduced knee flexor strength, higher rates of graft rupture than after ACLr with a BPTB graft, and higher than ideal rates of OA development in addition to reduced donor site tendon stiffness. That said, range of motion, strength symmetry, and functional performance have been shown to be better following ACLr with a ST autograft than a BPTB graft, as well as comparable to what has been observed following ACLr with a QT graft [5,9,10,12,13]. One meta-analysis found that functional outcomes were better following ACLr with a QT graft than with a ST graft[10], but another more recent meta-analysis found no differences in functional outcomes between the two graft types[47]. Our observed knee flexor isometric strength deficits following ACLr with a ST autograft were variable, ranging from a 34% deficit to a 15% increase on the injured limb, but they were in line with what has been reported in the literature. An average of 95% symmetry has been reported at five years post-op[86], as have deficits ranging from 3-27% in another study at an average of 32 months post-op[87].

Research on ACLr with a QT graft has been very positive. Functional and clinical outcomes have been shown to be like those seen with BPTB and ST grafts. The main advantage

of the QT is reduced donor site morbidity such as pain, tenderness, and numbness[10,47]. Since this graft type has only gained significant popularity in the last decade, it remains to be seen if there are any long-term issues that may arise. Future research should include 10-15 year prospective studies to track revision surgery rates, rates of OA development, and functional and clinical outcomes over time as has been done with the other graft types[5,9]. While our exploratory study in four participants found donor site tendon recovery to be 74% on average, and isometric strength recovery to be within 10% for three of the four participants, there are long term outcomes that need to be explored before claiming the QT autograft to be the premier choice.

Activity level and baseline semitendinosus use might mediate ST tendon recovery.

Within the ACLr – ST group, there was a wide range of ST tendon stiffness recovery. While we cannot say with certainty why some participants had better healing of the donor site tendon, there were some key similarities between the participants who had the best recovery. To our knowledge, no studies have analyzed factors that contribute to tendon recovery. ACLr – ST2 and ACLr – ST4 both continued to participate in organized sports with substantial cutting at a high level (club level lacrosse and NCAA DI soccer) following their ACLr and subsequent rehabilitation. They recovered 79.5% and 61.0% of their tendon stiffness, respectively. This may suggest that continued participation in functional activities that load and stress the hamstrings may be necessary to rehabilitate the ST tendon. ACLr – ST2 and ACLr – ST4 also produced the most force out of their ST on their unaffected limb with an average load share of 24.6±5.7% and 39.8±7.4% across the different position and torque level conditions, respectively. ACLr – ST1, ACLr – ST3, and ACLr – ST5 had average ST load shares of 18.5±5.78%, 15.6±5.5%, and 13.0±1.9% on their unaffected limbs, respectively. Assuming the unaffected limb is reflective of

the injured limb prior to ACL rupture, this potentially suggests that a natural preference for ST use may encourage the MTU to recover more completely.

Future research should also explore the impact of electrical stimulation on ST tendon recovery and voluntary muscle recruitment. Electrical stimulation preferentially activates a muscle by sending electrical pulses directly to the MTU through electrodes that elicit contraction of the muscle in place of a neural signal. This would be able to activate the ST preferentially to illicit tendon healing before it could be done so voluntarily. Snyder-Mackler et al. did a study using electrical stimulation following ACLr and found that isokinetic quadriceps strength increased significantly compared to a volitional exercise program alone[88]. There were also increases in hamstrings isokinetic torque, albeit not significant, suggesting that there might have been improved voluntary use of the ST MTU following the three-week exercise program. It would be interesting and clinically important to understand how this type of intervention directly impacts donor site healing and muscle use.

Semitendinosus force is reduced in the injured limb but met with additional biceps femoris short head and occasionally semimembranosus force.

Each of the ACLr – ST participants had reduced ST force production and load share on their ACLr limb, as expected due to the increased tendon compliancy. This was consistent with Messer et al. who found significantly reduced ST T2 relaxation time in the ACLr – ST limb following eccentric knee flexion exercise[24]. Since healthy ST fascicle lengths reported in the literature were used for the ACLr – ST Injured limb PCSA calculations, it is possible that ST force production was underestimated on the injured limb; the ST muscle tends to be shorter with shorter muscle fascicles following ACLr with a hamstring graft. However, the deficits in ST force production were driven not only by reductions in PCSA, but also by reductions in SWE

measured ST muscle stiffness during activity. Across the different torque and muscle length conditions, the ST had an average active shear modulus of 160.8±67.2KPa on the injured limb compared to 357.5±108.0 KPa on the control limb. A dependent samples paired t-test found this to be statistically significant as well as a large meaningful difference ($p<.001$, $d=1.721$).

However, greater recovery of the ST tendon did not reduce the size of the ST force deficit as hypothesized in SH4. ACLr – ST5 only recovered 23.5% of her ST tendon stiffness but was the closest to reaching ST force and load share symmetry; the ST load shares were only off by 1% while the ST force on the injured side was 89.3±20.8N compared to 108.0±17N on the contralateral limb across the different conditions. On the contrary, ACLr – ST2 and ACLr – ST4 had 67% and 77% deficits in their ST force on their injured compared to their unaffected limbs.

While a more compliant tendon would presumably lead to a shorter than optimal muscle for a given MTU length and applied force (see **Appendix C**), it appears that other principles may have also been at play, preventing the ST from producing more force after ACLr. Perhaps force within the ST tendon was being transmitted through other neighboring connective tissues and muscles such as the SM as opposed to the ST muscle. Maas and Sandercock have shown that muscles do not work independent of one another, and that intermuscular force transmission may be substantial in a damaged MTU such as the ST following ACLr[89]. This idea proposes that force may be produced in the tendon, but it may not move to the muscle in a series fashion like traditionally assumed. Another potential explanation for reduced ST force production regardless of the degree of tendon recovery is a neuromuscular inhibition to protect the compromised ST. This may be more likely since there appears to be a cap in ST force production regardless of muscle length. Future research would be needed to explore why the ST is not producing force as expected despite satisfactory donor site recovery.

Compensations for reduced ST force were typically seen in the BFsh and occasionally the SM, but not the BFlh. The exception to this was ACLr – ST5 who was able to reach nearly symmetrical ST force. This may be because, while her ST tendon only recovered 23% of her healthy tendon's stiffness, the estimated injured side PCSA was 0.75cm^2 bigger on the injured side. PCSA is critical for determining how much force the muscle is able to produce[32], and may explain why ACLr – ST5 had relatively similar load sharing between her injured and unaffected limb. If there is an inhibitory component to ST force production, it is also possible that she has found a way to overcome this.

For the rest of the ACLr – ST participants, they generally relied on the BFsh to attempt to produce more force. Including ACLr – ST1 and ACLr – ST3 who did not have clear ST muscles, the participants produced an average of 76.1±35.3N with their ACL reconstructed limb's BFsh compared to 51.8±24.9N on their unaffected limb. This was statistically significant ($p<0.001$), with a moderate Cohen's d effect size (d=.717). This pattern was most evident in ACLr – ST1, ACLr – ST2, and ACLr – ST3 who produced 117%, 562%, and 85% more force out of their injured limb's BFsh, respectively. However, this is a very small muscle, and it was not able to make up the difference in force.

Compensations occurring in the BFsh is a curious choice and does not appear to be optimal from a biomechanical standpoint. It is the smallest of the four hamstring muscles, so force production is minimal, and it is the only uniarticular hamstring muscle. This means that any additional torque production from the BFsh can only assist with knee flexion movements as opposed to knee flexion and hip extension. It would be interesting to see if these compensatory effects in the BFsh would develop if rehabilitation programs focused more on hip extension

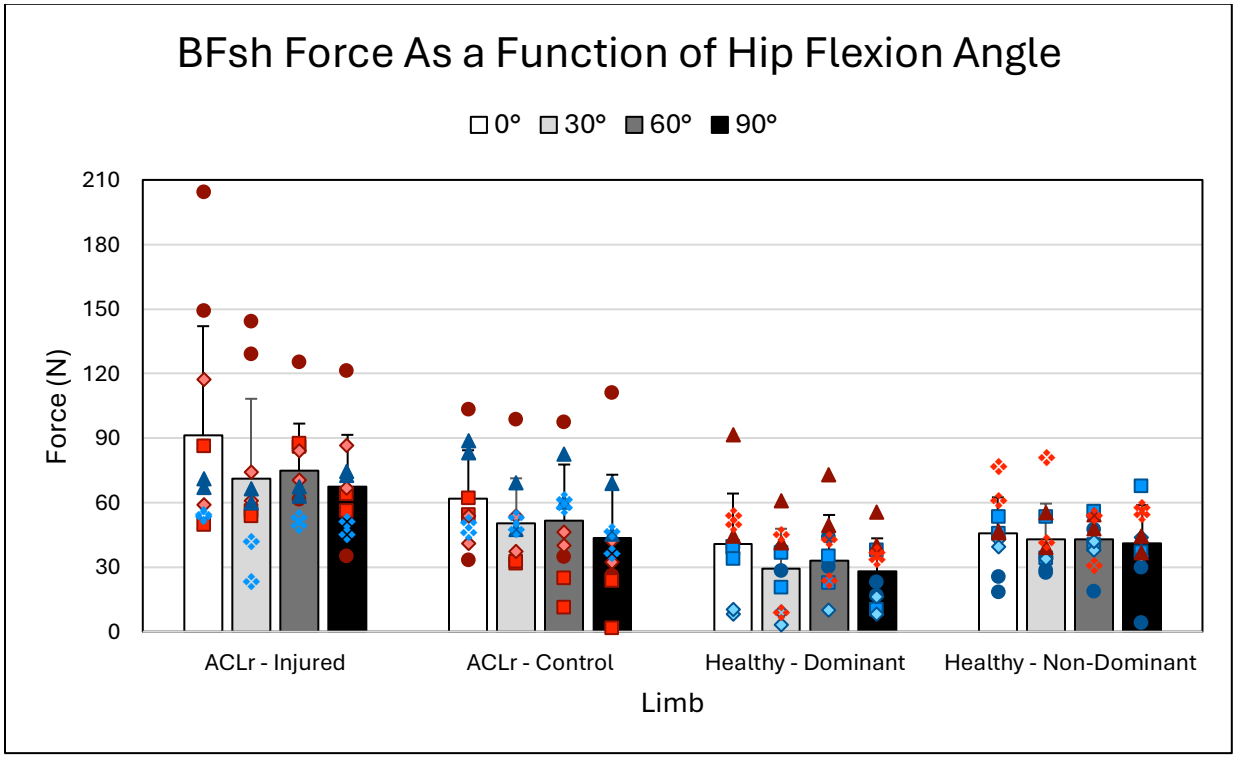
movements and less so on knee flexion or if any of the other hamstring muscles would have compensated for the ST during an isometric hip extension movement.

The additional BFsh force may lead to increased joint loading in the lateral knee joint compartment while reduced ST force would reduce medial knee joint loading. This altered knee joint loading might cause a breakdown of the cartilage due to disuse in the medial compartment and consequently medial tibiofemoral joint compartment OA development. Wellsandt et al. found a reduction of medial compartment loading prior to ACLr and in the months following to be a predictor of OA development[6]. A modeling study has suggested that increased joint contact forces from the SM are likely and would make up for reduced joint loading from the ST[7]. However, in this limited sample size study there was not an increase in SM forces. This means there was an overall reduction of medial knee joint loading. Overtime, this disuse might lead to a breakdown of the cartilage and OA development. The additional lateral knee joint compartment loading may also damage the knee joint cartilage, but this seems unlikely given the relatively small magnitude increases in lateral hamstring forces observed. Given that this study took place at a single timepoint, we cannot determine how knee joint loading has changed in the ACLr limb or if these changes will ultimately influence OA development.

ACLr – ST3 attempted to make up for his absence of a ST with additional SM force (534.0±84.5N on injured, 402.0±41.6N on control, p=.004, d=1.353) across the different muscle length and torque level conditions, but overall, SM force was consistent between the ACLr affected and contralateral limbs (345.6±142.3N on injured, 358.6±105.9N on control, p=.532, d=.100). BFlh force was also consistent between the limbs, with the injured limb producing 211.4±90.4N compared to the control limb's BFlh producing 247.2±128.7N for a small effect size of d=.301. This meant there was significantly and meaningfully less force produced on the

injured limb from the four muscles measured (575.0±174.7N on injured, 761.6±204.4N on control, $p<0.001$, $d=.961$), despite both limbs hitting the same target isometric torques. In the Healthy – ST group, the summed forces between the dominant and non-dominant limbs were not different from one another ($p=.559$, $d=.082$). This finding was similar to Messer et al. who found a significantly reduced ST T2 relaxation time in the ACLr limb but no significant differences between any of the other muscles compared to the contralateral limb following a Nordic hamstring curl protocol, suggesting there was reduced force production from the hamstring muscle group as a whole[24].

Figure 16. Mean BFsh muscle force by limb as a function of hip flexion angle. Error bars represent SD. Points represent individual datapoints with each colored symbol representing a participant. Both torque conditions are represented in the chart.



It is possible that other knee flexors such as the gastrocnemii and sartorius were producing greater levels of muscle force to achieve the target torque in the injured limb. Indeed, the gastrocnemii have been shown to produce the highest level of knee flexion torque when the

knee is extended or close to extended, as was the case in this study[90]. Another potential explanation is a change in the ST moment arm leading to greater joint torque contribution for a given force. Prior research has found the muscle-tendon attachment to move more proximally, medially, and superficially following ACLr[19,36]. This would potentially cause increased joint torque for a given muscle force. Research examining the impact of a severed extensor carpi ulnaris in the wrist on muscle force and moment found that while there were reductions in force, they were relatively small[91]. The increased moment arm was more than enough to overcome the reduction in muscle force, leading to higher muscle moments in the injured extensor carpi ulnaris than the healthy one. This thesis did not examine the ST moment arm so we cannot speculate how it may have been altered in the injured limb. However, based on prior research regarding changes in the ST muscle-tendon attachment point and our finding that summed hamstring muscle forces were lower in the affected limb for a given joint torque, a similar concept may be present as in the injured extensor carpi ulnaris. The injured ST may be producing a higher muscle moment per a given muscle force. Future research should explore ST moment arms, and the relationship between muscle force and joint torque, following ACLr with a hamstring graft.

Semitendinosus force production deficits were more pronounced at longer muscle lengths, but there were no differences based on torque level.

Sub-hypothesis 2 and SH3 hypothesized that deficits in ST force production would be most pronounced at shorter muscle lengths and at lower levels of torque production, respectively. For the ACLr – ST injured limbs, there was no meaningful difference in ST raw force or load share for any of the participants between the tested muscle lengths, with the force remaining consistent across the length spectrum tested. However in the contralateral, healthy limbs, there was a clear decrease in ST force as muscle length increased, leading to a significant decrease in

deficits between the injured and control limbs as muscle length increased. The 90° of flexion deficits were significantly and meaningfully less than the deficits at the other angles tested. This supported the hypothesis, but not in the expected way. It was anticipated that ST force on the injured limb would increase as the muscle length increased due to it being in a more optimal position; at shorter lengths, the long, compliant tendon would lead to short muscle fiber lengths at rest that would likely be reduced beyond their working range in an active state due to the increased tendon compliancy compared to the contralateral limb. Instead, the participants produced relatively constant levels of force across the force length spectrum on their injured limb, with very slight increases as 90° of hip flexion. This suggests that the ACLr affected ST may have limited force production capabilities across the force-length spectrum. It is possible that there may be a neuromuscular inhibitory mechanism that is preventing the ST from producing force above a certain threshold.

On the other hand, the results from the healthy limb suggest that the optimal muscle lengths for ST force production may be at shorter muscle lengths such as 0° and 30° of hip flexion. In the healthy group, the effect of hip angle on ST force was less clear. Still, as a group, the dominant limb produced significantly more ST force at shorter muscle lengths despite the lower peak torques at these angles, and consequently target torques, in these positions. This was in line with the findings from Kellis and Blazevich's narrative review exploring hamstring muscles' force-length relationships. They found that ST force was impacted less by changes in muscle length due to its long muscle fibers, but that there was slightly higher force production at shorter muscle lengths[65].

The implications of this for ST rehabilitation following ACLr with a ST graft are multi-fold. In a healthy population, working at shorter hamstring muscle lengths such as 0° of hip and

15° of knee flexion might preferentially activate the ST. Following ACLr the ST tendon is more compliant, so the same optimal fiber length is reached at a longer muscle length. This may necessitate the need to work at longer muscle lengths to aid in ST recovery. However, how this optimal working range changes throughout the donor site healing process is yet to be known. If an inhibitory mechanism is at play, rehabilitation methods should specifically work to minimize this effect through tools such as targeted exercises and verbal cues. This would potentially be an easier problem to address than a mechanical inability to activate due to a lack of significant tendon stiffness. However, future research is needed to determine why and how the ST is recruited following ACLr.

Sub-hypothesis 3 hypothesized that deficits in ST force production between the affected and healthy limb would be more pronounced at lower contraction levels since the other hamstring muscles would be able to compensate for the ST. This hypothesis was not supported. For both the ACLr – injured and ACLr – control limb, there was no effect of torque level on ST load share. The same occurred in the dominant and non-dominant limbs of the healthy group. This suggests that the tendon stiffness reductions in the ACLr – injured limbs did not ultimately impact how large the deficits would be, nor is ST load share impacted by torque level. As a greater muscle force must be met with a greater change in tendon length for a given tendon stiffness (Hooke's Law), it was also presumably possible that increased torque level would lead to greater shortening of the ST muscle outside of its working range. This would be the opposite of the presented hypothesis. Perhaps this theory worked in tandem with the presented hypothesis that the ST would need to contribute to torque production at higher torque levels, ultimately cancelling out the impact of torque level on ST load share.

Medial hamstring neuromuscular activity is reduced at longer muscle lengths, but there were no differences between ACL and contralateral limbs.

EMG data revealed no significant differences between the medial hamstring activity in the injured and contralateral limb in the ACLr – ST group. Looking at the EMG data by itself would thus suggest that there are no deficits in the ST following ACLr with a hamstring graft. The SWE data strongly rejected this. This disagreement might be due to the nature of EMG measurements. EMG measures the strength of the neuromuscular signal within the muscle, which is determined by the amount of active fibers[92]. The number of active fibers or the strength of the neuromuscular signal does not consider the force-length relationship nor is it always directly related to muscle force. Additionally, surface electrodes such as the ones used in this study are unable to differentiate between the superficial ST and the deep SM, meaning signal strength is impacted by the medial hamstring activity in its entirety as opposed to just the ST.

As there were not deficits between limbs, joint position nor torque level impacted the deficits. There was no support for SH2, SH3, and SH4 within the EMG data. However, there was a significant impact of position within the limb. As hip joint angle increased, increasing the length of the hamstring muscles, the medial hamstrings EMG signal was reduced. This finding was significant with a large effect size in the ACLr – Injured limb. There were moderate to large effect sizes due to position in the ACLr – Control, Healthy – Dominant, and Healthy – Non-Dominant limbs as well, with signal strength reducing as the muscle length increased. This was consistent with prior research that has found medial hamstring muscle EMG signals to be greater as shorter muscle lengths[56,61–65] as well as the SWE data in the ACLr – Control and Healthy dominant and non-dominant limbs which also found reduced ST force at longer muscle lengths. This may be related to the optimal length for the ST. As Kellis and Blazevich described, the

optimal length for the ST tends to be at shorter muscle lengths than where peak knee flexor torque occurs[65]. While the effect of position on EMG signal lines up with SWE data for the ACLr – Control and Healthy limbs, it does not align with the SWE findings for the ACLr – injured limb; consistent ST force was found across the muscle length spectrum tested. While ST force may have been consistent, the amount of motor units necessary to produce this force may have varied due to the fiber length being less optimal at shorter muscle lengths leading to increased neuromuscular signal. The greater reduction in ST EMG signal at the edges of the force-length curve following ACLr with a hamstring graft was also found during isokinetic contractions in another study[56]. However, this study found decreases at shortened muscle lengths with high knee flexion while this thesis found decreases at the other extreme of the muscle length curve during isometric contractions. Perhaps neuromuscular signal at the edges of the force length curve is reduced more in the medial hamstrings following ACLr with a ST graft than in unaffected limbs due to a shorter optimal operating range of the muscle.

Differences in quadriceps force sharing following ACLr with a BPTB or QT graft were not observed, but quality, active SWE images were difficult to capture.

There was complete or close to complete healing of the of the PT and QT following ACLr with BPTB and QT grafts in most individuals so large meaningful differences in force sharing and muscle recruitment patterns were not found, as expected. However, the quality of the active SWE data should be viewed with caution. Many ACLr and Healthy participants in the quadriceps groups had trouble holding the 15% and 30% isometric contractions steady, without their muscles shaking. Participants commonly remarked that they were not having difficulty holding the isometric contraction, but their muscles were still visibly shaking. This made it very

difficult for the SWE signal to propagate. These issues were most pronounced in the VM and the RF, and at higher knee flexion angles.

While participants were not asked about knee pain, and healthy participants were to be free from any current or chronic knee injuries, simulated pain has been shown to affect isometric knee extensor force steadiness in healthy adults at contraction levels as low as 10% of MVIC[93]. This might have explained the issues with force steadiness in the ACLr knees, but the healthy group should have been free from any knee pain. Additionally, force steadiness has been shown to be reduced at 90° of knee flexion compared to 30°, which aligns with the angles at which issues were most often encountered[94]. While some individuals struggled during the 15% isometric contractions, difficulties were more pronounced during 30% efforts. To try to mediate these, the contraction level was often dropped to 22-26% of the peak torque. Additional rest times were also provided. This helped in some cases. It is unknown if differences in muscle force production between limbs may have become evident if there were less signal propagation issues that led to doubts in the data.

The EMG data revealed no differences between the ACLr affected and contralateral limbs for any of the muscles. This was consistent with Letter, who found no differences in neuromuscular activation or the contribution from different muscles between the affected and contralateral limb during MVICs following ACLr with a BPTB or QT graft[95]. This finding may have been different had we analyzed functional activities or components of EMG beyond signal strength such as electromechanical delay and signal frequency, which have frequently been shown to be altered following ACLr[96]. MVICs and sub-maximal isometric contractions are a more controlled activity than the functional activities in which ACL injury risk is higher.

Limitations and Clinical Significance

This thesis study explored donor site tendon recovery following ACLr with an autograft and subsequent changes in muscle group force sharing. The study results suggested that the PT and the QT have superior recovery to the ST tendon in terms of tendon stiffness and subsequent changes in muscle group sharing. The ST produced significantly less force and had a significantly lower contribution to total muscle force production on the ACLr limb. This may contribute to altered knee joint loading which could negatively influence cartilage health over time. However, the size of the deficit between the affected limb's ST tendon stiffness and the contralateral limb did not influence the degree of ST force production deficits following ACLr or deficits in the donor site's muscle group isokinetic or isometric strength. Knowing how the donor site tendon recovers following ACLr, and what other deficits this may cause can help influence graft choice based on the needs of the individual. It can also help physical therapists determine the best rehabilitation plan to give the ST MTU the strongest chance of healing and producing force voluntarily.

However, this study was not without limitations. The primary limitation was the sample size. There were only 5 ACLr – ST, 6 ACLr – PT, and 4 ACLr – QT participants in the study. Given this small sample size and the varying behaviors within each group, it was not possible to draw conclusions nor run full factorial ANOVA designs. The decision was made to analyze data between the injured and control limb in the ACLr groups and the dominant and non-dominant limbs in the healthy groups. This decision may have impacted some conclusions drawn, but the primary findings regarding donor site tendon properties, ST force production, and knee flexor strength appear to be largely unchanged regardless of analysis type. Additionally, active SWE images of the quadriceps muscles were not reliable due to inadequate muscle steadiness. Finally,

the muscle fascicle lengths used to estimate muscle PCSA were not measured but estimated from the literature. As muscle fascicle length is variable across individuals and has been shown to reduce in the ST following ACLr, this may have led to underestimations of ST PCSA and muscle forces, particularly in the injured limb.

This study does provide a good exploratory basis for future research and supports the idea that ST force production is reduced following ACLr with a semitendinosus graft due to incomplete recovery of tendon stiffness. This problem is not as prominent following ACLr with a BPTB or QT graft due to the surgical procedure utilizing a portion of the tendon.

Conclusion

Assessing donor site tendon recovery and subsequent muscle force sharing with SWE following ACLr with a ST tendon, BPTB, or QT autograft revealed that the ST tendon has increased compliancy which contributes to reduced ST force production on the affected limb. These deficits in donor site tendon stiffness were not as pronounced following ACLr with a BPTB or QT graft. Tendon recovery may be more likely in these procedures since only a central part of the tendon is sourced; the remaining tendon may act as a blueprint and scaffold for the tendon to recover along. Reduced ST force production was met with an increase in BFsh force production despite it being a small, uniarticular, lateral hamstring muscle compared to the biarticular, medial ST. There was significantly less summed hamstring muscle force in the injured limbs, suggesting that the joint torque produced per a given ST muscle force may be altered due to changes in the muscle-tendon attachment site or greater contributions from other knee flexors. Future research should study the impact rehabilitation methods such as electrical stimulation and exercises at longer muscle lengths may have on ST tendon recovery and

voluntary muscle recruitment as well as the relationship between ST muscle force and moment following ACLr.

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Appendix A: Informed Consent Document

Title of Study: Hamstrings and Quadriceps Muscle Recruitment Following ACL Reconstruction Based on Graft Type

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: Hamstrings and Quadriceps Muscle Recruitment Following ACL Reconstruction Based on Graft Type

Principal Investigator: Elizabeth Klemm
Institution, Department or Division: ECU, Department of Kinesiology
Address: 332A Ward Sports Medicine Building, Greenville, NC 27858
Telephone #: 732-927-0108

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 how ACL reconstruction with an autograft impacts thigh muscle use after rehabilitation. You are being invited to take part in this research because you had an ACL reconstruction with a hamstring tendon, quadriceps tendon, or patellar tendon graft from the injured side or you are a healthy volunteer. The decision to take part in this research is yours to make. By doing this research, we hope to learn how ACL reconstruction impacts hamstring and quadriceps force sharing and voluntary force production of the affected muscle(s).

If you volunteer to take part in this research, you will be one of about 30 people to do so.

Are there reasons I should not take part in this research?

I understand I should not volunteer for this study if I am under 18 years of age, have a history of chronic hamstring or quadriceps strains, am not medically cleared for full activity, have an implanted pacemaker, or have a BMI greater than 30. If I have not had an ACL reconstruction, I should not participate if I have been diagnosed with knee osteoarthritis.

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 at Ward Sports Medicine Building. You will need to come to the Biomechanics Laboratory in Room 332 two (2) times during the study. The second session will take place within 10 days of the first session. Each session will take 1.5 hours. One limb (i.e. right or left leg) will be tested per session. The total amount of time you will be asked to volunteer for this study is 3 hours.

What will I be asked to do?

You will be asked to do the following:

1. Refrain from strenuous lower body workouts within 48 hours before scheduled data collections.

2. Have your height and weight measured.
3. Fill out a brief demographics survey.
4. Complete strength testing of your hamstrings or quadriceps muscles in four different positions.
    a. If you had an ACL reconstruction with a hamstring tendon graft or are a healthy participant placed in the hamstring group, hamstring strength will be measured with a dynamometer while you are positioned in a custom-made apparatus that allows the researchers to alter your hip position. The hip angle will be adjusted to four different positions ranging from standing straight up to bent over with a flat back (90° hip hinge).
    b. If you had an ACL reconstruction with a quadriceps tendon or patellar tendon graft or are a healthy participant placed in the quadriceps group, quadriceps strength will be measured with a dynamometer. Joint angle will be adjusted to four different positions ranging from laying on your back to sitting upright (90° hip hinge) and a slightly bent knee (15° of flexion) to 90°.
5. Surface electrodes used to monitor muscle activity will be placed on your skin over the relevant muscles. The process is non-invasive but will require the skin to be cleaned and shaved where the electrodes will be placed. The electrodes used for data collection are used to measure the magnitude and timing of your muscle contractions only. There is no electrical current emitted from the electrodes into your body.
    a. If you had an ACL reconstruction with a hamstring tendon graft or are a healthy participant placed in the hamstring group, the electrodes will be placed over your hamstrings (back of thigh).
    b. If you had an ACL reconstruction with a quadriceps tendon or patellar tendon graft or are a healthy participant placed in the quadriceps group, the electrodes will be placed over your quadriceps (front of thigh).
6. Ultrasound gel and an ultrasound probe will be placed on you to take images of your muscles. We will measure resting muscle stiffness and muscle stiffness while you perform exercises at up to 40% of your peak strength in four different hip positions. We will also use the ultrasound to measure the size of your muscles and your tendon's stiffness. The ultrasound gel is water soluble and there is no radiation emitted. The positioning of the probe will be marked with permanent marker which will wash off within a couple of days.
    a. If you had an ACL reconstruction with a hamstring tendon graft or are a healthy participant placed in the hamstring group, the ultrasound probe will be placed on your hamstrings (back of thigh) while you complete leg curls.
    b. If you had an ACL reconstruction with a quadriceps tendon or patellar tendon graft or are a healthy participant placed in the quadriceps group, the ultrasound probe will be placed on your quadriceps (front of thigh) while you complete leg extensions.

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 be able to pay you for the time you volunteer while being in this study. You will receive a $40 Target gift card for participating in the study in its entirety. The award amount will be provided following the completion of the protocol, with $15 provided per session and a $10 bonus for completing both sessions. If you do not complete both sessions, the payment will be prorated accordingly.

Will it cost me to take part in this research?

It will not cost you any money to be part of the research.

Title of Study: Hamstrings and Quadriceps Muscle Recruitment Following ACL Reconstruction Based on Graft Type

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?
The investigators will keep your personal data in strict confidence by having your data coded. Instead of your name, you will be identified in the data records with an identity number. You name and code number will not be identified in any subsequent report or publication. The main investigator and the research students will be the only persons who know the code associated with your name and this code as well as your data will be kept in strict confidence. The computer file that matches your name with the ID number will be encrypted and the main investigators will be the only staff that knows the password to this file. Your data will be used for research purposes only. Data files will be kept for 6 years after the study is completed, after which time all records indicating who the subjects are and all identifiers will be destroyed.

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 732-927-0108 (work days, between 9am and 5pm).

If you have questions about your rights as someone taking part in research, you may call the ECU University and Medical Center Institutional Review Board (UMCIRB) at phone number 252-744-2914 (days). If you would like to report a complaint or concern about this research study, you may call the Director for Human Research Protections, at 252-744-2914.

Is there anything else I should know?
Identifiers might be removed from the identifiable private information or identifiable biospecimens and, after such removal, the information or biospecimens could be used for future research studies or distributed to another investigator for future research studies without additional informed consent from you or your Legally Authorized Representative (LAR). However, there still may be a chance that someone could figure out the information is about you.

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.

Title of Study: Hamstrings and Quadriceps Muscle Recruitment Following ACL Reconstruction Based on Graft Type

_____ _____

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**

Appendix B: Institutional Review Board Approval

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board
Willis Building · Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office **252-744-2914** · Fax **252-744-2284** ·
rede.ecu.edu/umcirb/

Notification of Amendment Approval

From: Biomedical IRB
To: Elizabeth Klemm
CC: Zachary Domire
Steven Philpott
Date: 3/3/2025
Re: Ame3_UMCIRB 24-001544
UMCIRB 24-001544
Hamstrings and Quadriceps Muscle Recruitment Following ACL Reconstruction With an Autograft

Your Amendment to update this study according to the revised human research regulations (as of January 21, 2019) has been reviewed and approved using expedited review on 2/28/2025. It was the determination of the UMCIRB Chairperson (or designee) that this amendment does not impact the overall risk/benefit ratio of the study and is appropriate for the population and procedures proposed.

Please note that any further 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 Final Report application to the UMCIRB prior to the Expected End Date provided in the IRB application. If the study is not completed by this date, an Amendment will need to be submitted to extend the Expected End Date. 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:

Document	Description
ConsentDocv4.docx(0.06)	Consent Forms
ThesisProposal_UpdatedMethodsforIRB.docx(0.08)	Study Protocol or Grant Application

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

Appendix C: Semitendinosus Muscle Force-Length Curve and Parameters

The percentage of its maximal force a muscle can produce is based on the length of the muscle fibers. At a muscle's optimal fiber length, the actin and myosin fibrils within the sarcomeres have an ideal amount of overlap. In this configuration, upon muscle contraction, the maximal number of cross bridges can be created allowing for maximal force production. If a muscle is outside of its optimal working length, force production is reduced. When the muscle fibers are too short or too long, it is difficult to impossible for cross bridges and the subsequent power stroke to be created as there is complete overlap or no overlap, respectively, of the actin and myosin fibrils. Joint angle, tendon length, and tendon stiffness impact muscle fiber length and as such the force-length profile.

Impact of Joint Angle

The ST is a biarticular muscle crossing both the hip and knee joints. It originates at the ischial tuberosity and inserts to the medial surface of tibia[97]. Alterations in both the knee and hip joint impact the resting length of the MTU, with increased hip flexion increasing the length and increased knee flexion decreasing muscle length. These changes in resting muscle length are made up by changes in the muscle fiber length as resting tendon length remains constant. The muscle fiber length is the key determinant of the amount of force a muscle can produce relative to its maximal capacities.

Technical limitations make it difficult to determine the optimal fiber length and joint angles for individual hamstring muscles. However, modeling has suggested that the optimal range of joint angles for ST knee flexion torque production ranges from approximately 45° of hip flexion with 45° of knee flexion at the short end and 45° of hip flexion and 0° of knee flexion at the long end[65]. It should be noted that torque is based on the product of muscle force and the

moment arm, which is typically shown to increase relative to the knee joint as the knee flexion angle increases. In a study done on frogs, peak ST force was obtained from a hip flexion angle of approximately 55° and knee flexion angle of 90° to a hip flexion angle of 80° and knee flexion angle of 135°[98].

Impact of Tendon Properties

Tendons are elastic and elongate while under stress. For simplicity, this means that during an isometric contraction, the overall muscle length remains constant as the tendon elongates and the muscle fibers contract. The amount of tendon elongation or strain is based on the tendon stiffness, as well as the amount of force produced. The change in length of the tendon can be determined through Hooke's Law, $F_m = k\Delta L_t$, where F_m is equal to muscle force, k is the stiffness of the tendon, and L_t is the change in tendon length from rest to contraction. As mentioned above, maximal isometric muscle force is based on muscle fiber length. Additionally, tendon stiffness k is assumed to be constant. This means that tendon elongation can be calculated for a given muscle force. Any tendon elongation during an isometric contraction must be met by an equal contraction of the muscle fibers, due to the constant length of the MTU.

To predict muscle fiber length during a maximal isometric contraction, a forward modeling simulation can be run. The resting muscle fiber length is predicted as a function of joint angle. The optimal muscle fiber length is set within the model and is used to predict percent of maximal force production as a function of fiber length. This fiber length for the given joint angle or MTU length is a function of tendon strain, which is a function of muscle force production. This leads to a series of iterations necessary to predict muscle fiber length, tendon strain, and muscle force production for a given joint angle.

Consequences Following ACLr with ST or STG graft

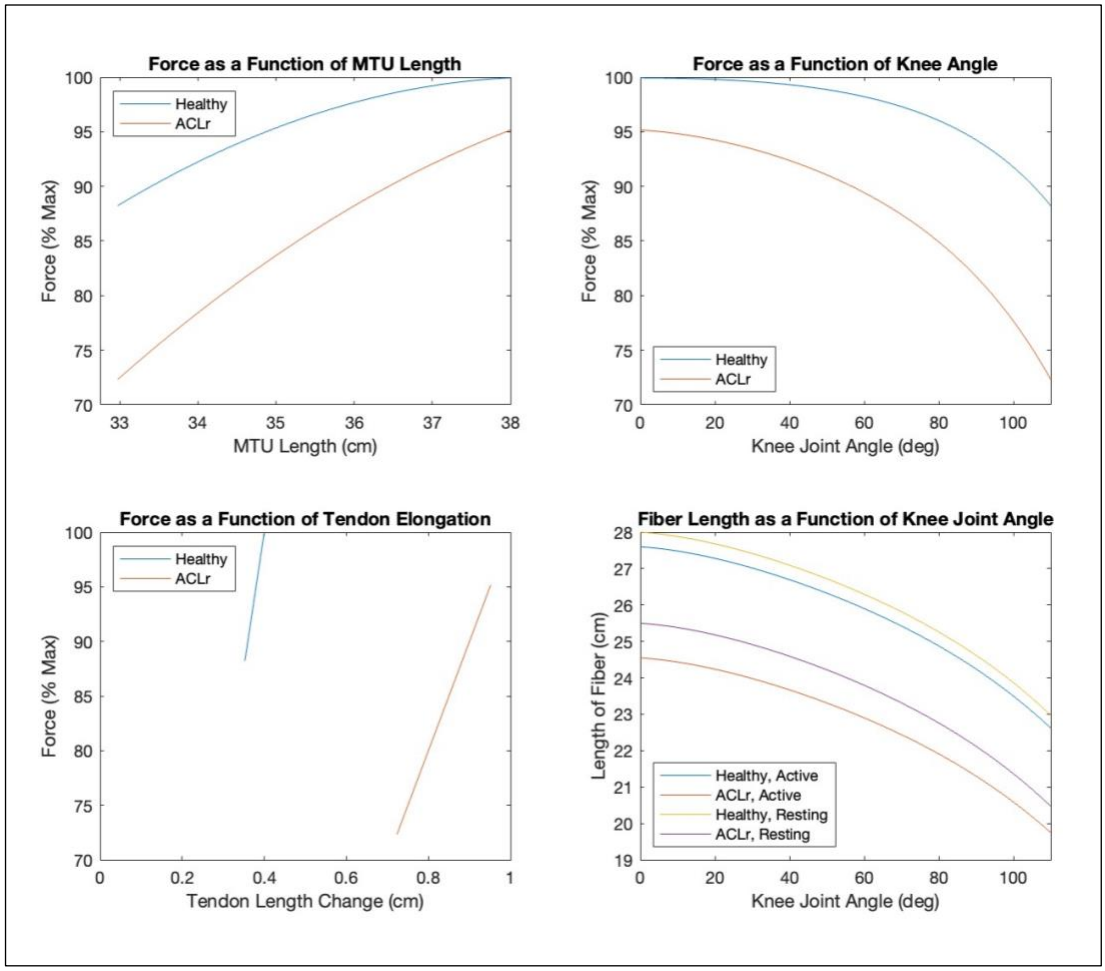
Following ACLr with a ST or STG graft, the ST tendon is generally less stiff[22,26] and often longer[18,19]. However, the MTU length for a given joint angle remains constant, assuming no change in tendon attachment site. This means that for a given joint angle, there are shorter fiber lengths and subsequently a shift in the force-length curve towards longer muscle lengths. The optimal fiber length occurs at a longer muscle length due to the increased percentage of tendon within the MTU. The decreased stiffness also leads to increased tendon elongation, and increased muscle fiber contraction, for a given amount of muscle force. This leads to an even larger shift in the force-length curve towards longer muscle lengths. To achieve optimal fiber length under contraction to produce maximal force, the resting muscle length needs to be in a longer position than in a healthy ST.

To demonstrate the potential effect of an ACLr with ST tendon on the muscle's force-length profile, two simple muscle models were created. The first altered knee flexion angle and analyzed ST muscle force production abilities. The second altered hip flexion angles and analyzed the hamstring muscle group force production. In both models, the ACLr ST or summed hamstring tendon was 25% longer and had 40% of the stiffness of the healthy tendon. This was based on prior research which has found the regenerated ST tendon to be 9-50% longer[19,26] and to recover approximately 40% of healthy ST stiffness[22,26]. It was assumed that at a given joint angle, the MTU length was the same, implying that there was no change in tendon attachment site. It was also assumed that the maximal force the muscle can produce did not alter between the healthy and ACLr ST.

For the first model, Herzog's regression equation for ST distal moment arm as a function of knee joint angle[97] was used in conjunction with the relationship between moment arm and change in MTU length as a function of joint angle. At rest, the change in MTU length is the first

integral of moment arm with respect to joint angle[99]. The same idea was utilized for the second model looking at the hamstring muscle group force production as a function of hip flexion angle, and used Domire's regression equation[99]. Based on optimal fiber length, the force for a given fiber length can be calculated. However, the fiber length at a given joint angle under maximal isometric contraction is determined by tendon length which is impacted by tendon strain. The elastic tendon's elongation is also based on the force production. This leads to a circular problem, but through multiple iterations, it is possible to predict tendon strain, fiber length, and force production at a given joint angle based on tendon and muscle parameters.

Figure 17. Results of the ST muscle model as a function of knee flexion angle.



The results of the first and second models can be seen in **Figure 17** and **Figure 18**, respectively. Analyzing the top left graph in **Figure 17**, we see that the healthy ST has a greater percentage of maximal force produced for a given MTU length. This is because the healthy ST fibers are longer and at more optimal force producing lengths at these MTU lengths. The ACLr ST muscle fibers make up a smaller percentage of the MTU and are below optimal force producing levels. Moving to the top right graph, this relationship is further visualized – the healthy ST can produce a greater level of force at greater knee flexion angles as the muscle fibers are still long enough to produce high levels of force. As the knee flexes, an ACLr affected ST likely has too short of muscle fibers to produce optimal levels of force. These fibers are short due to not only the longer resting tendon, but also the increased tendon strain. As the bottom left graph shows, the ACLr ST tendon has significantly higher elongation despite the muscle producing less force. The bottom right graph emphasizes that the healthy fibers are consistently longer, and contract less while active, than the ACLr ST muscle fibers. As the other three plots explained, this is due to the longer and less stiff ST tendon leading to greater muscular contraction despite reduced force production.

The second model shows the effect of changing hip angle, from 20° of hip extension to 60° of hip flexion. As the hip flexes, the resting hamstring muscle fibers become longer due to reduced sarcomere overlap. This model predicts that the healthy limb produces peak isometric force at 8° of extension, before the fibers become too long to produce optimal levels of force due to decreased actin and myosin overlap. In high hip flexion, there is a significant drop in isometric force production in the healthy limb due to this. On the contrary, the ACLr limb's muscle fibers are too short to produce optimal levels of force in hip extension and do not reach peak isometric force levels until 31° of hip flexion. Until about 20° of hip flexion, which represents most

functional positions, the ACLr limb cannot produce as high a level of isometric force due to its increased tendon elongation leading to too great of a reduction in muscle fiber length. The muscles fibers are shorter and contract more for a given joint position. While the angles of optimal fiber length and force production might differ in practice, the patterns likely remain due to the altered tendon properties. The decreased tendon stiffness, coupled with the increased tendon length, pushes the regenerated ST to longer hamstring muscle positions for optimal muscle fiber length and force production.

Figure 18. Results of the hamstring muscle model as a function of hip flexion angle.