EFFECTS OF ACL RECONSTRUCTION ON MUSCULAR STRENGTH, ENDURANCE AND MITOCHONDRIAL CAPACITY

by

SHELBY L. WALTERS

(Under the Direction of Kevin McCully)

ABSTRACT

PURPOSE: The purpose of this study was to measure quadriceps and hamstring mitochondrial capacity, endurance, and strength in female collegiate athletes that have undergone ACL reconstructive surgery following injury on a single leg. **METHODS**: Ten subjects were included in this study. Quadricep and hamstring muscular strength were measured using data collected from a Biodex Multi-Joint System isokinetic dynamometer. Muscle endurance was measured using data from accelerometers. Muscle mitochondrial capacity was measured using near-infrared spectroscopy (NIRS) Portamon devices. All testing was completed in a single session. **RESULTS**: A significant difference was observed in hamstring strength for the weaker injured leg in comparison to the non-injured leg. Muscular endurance and mitochondrial capacity were not different in quadriceps or hamstring for injured and non-injured legs. **CONCLUSIONS**: This study demonstrated a three-pronged approach to measuring an athlete's skeletal muscle after injury. No evidence was found that muscle endurance or mitochondrial capacity were impaired after injury.

INDEX WORDS: mitochondrial capacity, Near-infrared spectroscopy, endurance, strength, rehabilitation, knee injury

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DEDICATION

I would like to dedicate this research to all injured athletes. You are not alone.

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I would like to thank many individuals that have been there for me in my academic, athletic, and personal journey. First and foremost, I thank God for all the opportunities He has given me. Without Him, I wouldn’t be able to navigate this amazing life. I would also like to thank my family for their support and encouragement. Mom and Dad, thank you for all your sacrifices and unending love. You both mean the world to me.

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CHAPTER 1

INTRODUCTION

‘Knee injuries’ are amongst the most common of injuries that occur while playing sports (Ireland, 1999; Spindler & Wright, 2008). Knee injuries typically involve damage, tearing, or complete rupture of tendons and ligaments in the knee joint (Ireland, 1999; Spindler & Wright, 2008). The injury, or tear, in sports can occur from a variety of instances including contact with another player or sports-related movements (Spindler & Wright, 2008). The most commonly injuries ligament is the anterior cruciate ligament or ACL is housed within the knee joint (Spindler & Wright, 2008). The ACL is composed of dense connective tissue and their primary purpose is to connect the femur to the tibia (Duthon, 2006). The ACL is a crucial structure in the knee joint and provide resistance against rotational loads and anterior tibial translation (Duthon, 2006).

The ACL is most commonly injured ligament in the knee joint (Duthon, 2006). On average, 175,000 ACL reconstructions are performed every year, costing the U.S. more than $2 billion (Spindler & Wright, 2008). The number of ACL reconstructions has been increasing in recent years (Spindler & Wright, 2008). Isolated tears are seen less than 10% of the time while compounding meniscus injury occurs 60-75%, articular cartilage injuries 46%, subchondral bone injuries 80% and complete collateral tears 5-24% (Spindler & Wright, 2008). Most of these injuries are sustained in non-contact sporting events where the athlete is cutting or pivoting (Bing Yu, 2007; Boden, 2010). Women are at a significantly higher incidence rate of tearing than men (Spindler & Wright, 2008). It has been measured to be 2-6 times higher (Spindler & Wright, 2008). Even with advancements in technology, gear, and coaching, there has not been a significant decrease in the number of female athletes experiencing ACL tears (Ireland, 1999).

Rehabilitation of an ACL tear is a long process. ACL injury is often treated with surgery to repair the tear followed by rehabilitation protocols. The range for an entire rehabilitation protocol can range anywhere from 12 months to 16-20 weeks (Flagg et al., 2019; Wright et al., 2008). No matter the period of recovery, the criteria for returning to play relies on several topics including pain and swelling control, full knee extension, and quadriceps function/neuromuscular control (Flagg et al., 2019; Panariello et al., 2016; van Grinsven et al., 2010). The methods by which to achieve these goals are still debatable (Risberg, 2004). Exercises such as immediate weight bearing, closed kinetic chain and open kinetic chain modalities, and isometric strength are found to be essential to rehabilitation (Risberg, 2004). Exercises involving the overall endurance of the injured leg and corresponding muscle groups are not a priority in rehabilitation protocols, instead the focus relies mainly on return of strength and range of motion (Flagg et al., 2019; Panariello et al., 2016; van Grinsven et al., 2010). A previous study concluded that 2 years post-knee injury hamstring muscle endurance was reduced in the affected leg (Faxon et al., 2018).

A common occurrence seen in athletes with ACL tears even after completing rehabilitation is a re-tear. Re-tear or reinjury of the ACL occurs 6-13% of the time after surgical reconstruction (Myklebust et al., 2003; Salmon et al., 2005; Thomeé et al., 2011; Waldén et al., 2006). Re-tears are known to occur as well as the initial tear being a contributing factor to a tear on the contralateral leg (Salmon et al., 2005; Swärd et al., 2010; Thomeé et al., 2011; Wright et al., 2007). Certain movements like cutting, pivoting and jumping are associated with a higher incidence rate of re-injury (Salmon et al., 2005).

An important underlying factor that often goes unnoticed for rehabbing athletes is their mental wellbeing. Many athletes are not able to return to their sport because of fear of reinjury (C. L. Ardern et al., 2011; Thomeé et al., 2011). There are multiple examinations now that can assess how an athlete perceives their injury. These could prove to be vital in assessing when an athlete is ready to return to their sport. It is vital to understand more about the physiological factors that can contribute to an athlete with an ACL injury.

Statement of Problem

The purpose of this study is to evaluate a potential mechanism for re-injury after ACL reconstruction. While rehabilitation may result in return of muscle strength and range of motion in the injured leg, deficits in muscle endurance and mitochondrial capacity may leave the athlete at risk for future injury.

Specific Aims

Specific Aim 1: Measure hamstring and quadriceps mitochondrial capacity in college aged females who have had ACL surgery using near-infrared spectroscopy

Specific Aim 2: Measure hamstring and quadriceps endurance in in college aged females who have had ACL surgery using tri-axial accelerometry

Specific Aim 3: Measure hamstring and quadriceps maximum voluntary isometric strength in in college aged females who have had ACL surgery using isometric ergometry.

Hypotheses

I. Hamstring muscle on the injured leg will have reduced mitochondrial capacity relative to the non-injured side. Hamstring muscle on the injured leg will have reduced muscle endurance relative to the non-injured side.
II. The quadriceps muscles on the injured side will be different in muscle mitochondrial capacity or muscle endurance compared to the non-injured side
III. The injured leg will be different in muscle strength compared to the non-injured leg.

Significance of Study

The goal of ACL rehabilitation is to optimize recovery and allow the athlete to return to her sport. In addition, ACL rehabilitation is designed to reduce re-injury rates. However, current models of ACL rehabilitation do not focus on return or recovery of muscle mitochondrial capacity or muscle endurance. The demonstration of reduced muscle endurance, especially if future research can link re injury rates to lower muscle endurance, will lead to revisions of ACL rehabilitation therapies to include endurance training. Including endurance training in ACL rehabilitation has the potential to significantly reduce re-injury rates.

CHAPTER 2

REVIEW OF LITERATURE

Knee Injury in Athletic Populations

Knee related injuries constitute about one third of all outpatient injuries reported in athletes (Kujala, 2012). From a range of studies, on average, 27.04% of all sports related injuries involve the knee (Devereaux & Lachmann, 1983; Galasko et al., 1982; Kvist & Järvinen, 1980; Newman et al., 1969; Orava & Puranen, 1978). There are a large number of injuries that can occur to the knee. In Majewski et. al's (2006) study, minor knee distortions occur in about 33.9% of cases. 10.6% of cases were lesions of the knee (Majewski, 2006). Fractures and dislocations did not contribute a large majority to the number of injures (Majewski, 2006).

The largest majority of injuries are located in the internal knee at 44.8% of cases (Majewski, 2006). These can be broken down into the anterior cruciate ligament, medial meniscus, lateral meniscus, medial cruciate ligament, lateral cruciate ligament, and posterior cruciate ligament (Majewski, 2006). Among these structures, the anterior cruciate ligament (ACL) was damaged about 45.4% of the time (Majewski, 2006). The medial and lateral menisci follow, respectively, in order of next largest occurring sites of injury (Majewski, 2006).

The Anterior Cruciate Ligament (ACL)

The anterior cruciate ligament, or ACL, is one of the four major ligaments found in the knee joint and is mainly responsible for keeping the tibia from sliding forward towards the femur (Coffey, 2023; Cynthia R LaBella, 2014; Swain et al., 2014). It is also helpful in preventing hyperextension, valgus and varus movements as well as tibial rotation (Cynthia R LaBella, 2014). Its anatomy is vital in protecting the menisci from shearing forces involved in athletic movements (Cynthia R LaBella, 2014). It consists of two major fiber bundles known as the posterolateral and anteromedial bundles (Coffey, 2023). The structure of the ACL provides it a unique ability to be both flexible to withstand torque, but also strong to allow force exertion (Duthon, 2006).

The ACL is one of the most common knee injuries experienced by athletes (Duthon, 2006). They are experienced most heavily in men's football and women's gymnastics (Cynthia R LaBella, 2014). A large number of injuries are also reported in soccer and basketball (Bing Yu, 2007). ACL tears can have detrimental effects on the activity level of the athlete and overall quality of life (Bing Yu, 2007; Swain et al., 2014). After tearing one's ACL, other injuries and issues can arise in the knee. Studies show that these can include knee instability, menisci damage and osteoarthritis (Bing Yu, 2007; Finsterbush, 1990; GB Irvine, 1992; P. Kannus, 1990).

Most ACL injuries do occur through non-contact which can be defined as sudden deceleration or stopping, landing or pivoting actions that are repeatedly performed causing wear-and-tear on the ligament (Bing Yu, 2007; Boden, 2010). It can also be a singular incidence where great force is applied to the ligament causing it to rupture under that load (Bing Yu, 2007). Non-contact ACL tears make up about one third of total non-contact injuries (Boden, 2010).

ACL Injury Diagnostic Testing

Popping, swelling and instability are the first indicators that an ACL tear has occurred in an athlete (Swain et al., 2014). The most common diagnostic test used clinically is the Lachman Test (Coffey, 2023). It has been noted as the most sensitive and specific for ACL rupture testing (Coffey, 2023). Overall, the test assesses the ligament's ability to translate the tibia anteriorly

(Coffey, 2023). It can also directly test the relation of the two bundles' anatomical positioning (Coffey, 2023). The test itself does not require any sort of technical equipment (Coffey, 2023).

Before beginning the Lachman Test, proper evaluation and history should be administered and gathered from the patient. These include physical examinations of strength, range of motion, flexibility, stability, as well as special ACL specific tests (Coffey, 2023). Another important concept in testing is evaluating the un-injured knee for comparison (Coffey, 2023). The patient can exhibit a laxity in the testing that is not caused by injury (Coffey, 2023). Another note for the Lachman test is to evaluate as soon as possible from the initial occurrence of the injury to eliminate negative guarding and excessive swelling when administering the test (Coffey, 2023).

The test is administered with the patient in a supine position with the suspected injured knee at a 20-to-30-degree flexed position (Coffey, 2023). It is also externally rotated slightly (Coffey, 2023). The clinician stabilizes the distal femoral head with one hand while the other is located on the proximal tibia (Coffey, 2023). An anterior force is applied to the tibia while stabilizing the femur to evaluate if the tibia is moving forward (Coffey, 2023). If the tibia has a noticeable movement greater than the un-injured leg, as well as a lack of a firm endpoint, then the test is positive (Coffey, 2023). This test usually has a sensitivity rating of about 87% and specificity rating of 93% (Coffey, 2023). Magnetic resonance imaging (MRI) or a knee arthroscopy are definitive ways to check for ACL rupture but performing a Lachman Test or other forms of the test provide assuring ways of testing as well (Coffey, 2023; Solomon et al., 2001). Other forms include the pivot test and the anterior drawer test (Coffey, 2023; Jackson et al., 2003; Kaeding et al., 2017).

ACL Surgical Repair

The golden standard of ACL repair after rupture is surgery (Lyman et al., 2009; Mall et al., 2014; Nwachukwu et al., 2019; Sanders et al., 2016). There have been advancements of surgical techniques throughout the years though. Before arthroscopic procedures, ACLs were repaired using an open technique implementing arthrotomy (Nwachukwu et al., 2019). The results were “mediocre” and had high re-tear and and re-operation rates, as well as a higher percentage of surgical morbidity (Engebretsen et al., 1989; Feagin & Curl, 1976; Kaplan et al., 1990; Lysholm et al., 1982; Nwachukwu et al., 2019; Odensten et al., 1984; Sherman et al., 1991). There have been advancements in this type of ACL repair that have led to better results (Nwachukwu et al., 2019). Differing techniques for this method include primary suture repair, biologic scaffolding, microfracturing, and mechanical augmentation (Achtnich et al., 2016; Drogset et al., 2006; Heusdens et al., 2019; Hoffmann et al., 2017; Meunier et al., 2007; Mukhopadhyay et al., 2018; Murray et al., 2016; Nwachukwu et al., 2019; Smith et al., 2016; van der List & DiFelice, 2017). The most widely used non-arthroscopic ACL repair is called dynamic intraligamentary stabilization (DIS) (Evangelopoulos et al., 2017; Regli, 2016). Arthroscopic techniques have reduced occurrences of complications and post-operative failures tremendously with greater success in overall outcomes, time of return to play, patient satisfaction and graft survival (Leiter et al., 2014; Nwachukwu et al., 2019; Nwachukwu et al., 2017; Poehling-Monaghan et al., 2017). Overall, surgical intervention is the most successful method of repairing a torn ACL.

ACL Rehabilitative Measures

Over 300,000 ACL reconstructions are performed every year making rehabilitative measures essential to an athlete’s full recovery (Flagg et al., 2019). There has been much debate and research into the best practices for rehabilitation post ACL reconstruction. In general, the timeline for return to play ranges from 12 months to 16-20 weeks (Flagg et al., 2019; Wright et al., 2008). Physicians now are suggesting timelines based on the type of surgery. For example, bone-to-bone graft types are at least 6 months whereas hamstring grafts are approximated around 7 months (Flagg et al., 2019). Others suggest that no matter the graft the general range stands at 12 weeks to 12 months with the average being around 6 months (Barber-Westin & Noyes, 2011; Flagg et al., 2019).

No matter the period of recovery, the criteria for return to play relies on several topics including pain and swelling control, full knee extension, and quadriceps function/neuromuscular control (Flagg et al., 2019; Panariello et al., 2016; van Grinsven et al., 2010). Each timeline is relative to the patient and their goals. It is not suggested to base the progression on an end date, and it should instead be based on individual goals and have a steady system of objective measures (Flagg et al., 2019; van Melick et al., 2016).

The first phase of rehab begins immediately following surgery and usually spans 2-4 weeks (Flagg et al., 2019). This period focuses on strength and range of motion (ROM) as well as using ice therapy to mitigate swelling and pain (Flagg et al., 2019; van Grinsven et al., 2010). Crutches are often used as well to assist with gait control and to reduce quadriceps atrophy (Flagg et al., 2019; van Grinsven et al., 2010). Progression into new phases of rehabilitation depend on knee extension and at least 90-degree of flexion ROM as well as gait control (Flagg et al., 2019). Neuromuscular recruitment strengthening in the quadricep methods have been scrutinized over the years whether open-kinetic chain (OKC) versus closed-kinetic chain (CKC) is the better method, but recent research suggests that both methods have their own validity and effectiveness (Flagg et al., 2019; Kruse et al., 2012; Lobb et al., 2012; Wright et al., 2008).

Overall, the most important emphases within this first period of rehabilitation are proper mechanics and quality of movements (Flagg et al., 2019).

Although the goals of ACL recovery have been established, the methods by which to get there are not as clear (Barber-Westin & Noyes, 2011; Flagg et al., 2019; van Melick et al., 2016). In previous studies, only 13% of data showed measurable objectives for return to play including only one or two benchmarks (Barber-Westin & Noyes, 2011; Flagg et al., 2019). Blood flow restriction (BFR) has been a new development that has proven to be helpful in strengthening quadriceps and increasing limb size (Flagg et al., 2019).

Even with high quality and up-to-date rehabilitation, over 35% of athletes do not return to their sport within 2 years (C. L. Ardern et al., 2011; Clare L. Ardern et al., 2011; Flagg et al., 2019; Thomeé et al., 2011). A recent study concluded that although 80-90% of athletes appeared to have normal values to return to their sport, the full return rates were still low (C. L. Ardern et al., 2011; Thomeé et al., 2011). One area of rehabilitation that has been noted to need change is the lack of advanced strength and conditioning training (Flagg et al., 2019). Plyometrics and sports-specific training is often overlooked with an athlete returning (Flagg et al., 2019). Historically, return to play training has believed that sports-related movements should only be performed in the later stages of rehabilitation when there is minimal protection of the joint (Flagg et al., 2019). Others suggest that these athletic movements should be performed early on to promote further progression in the later stages of rehab (Panariello et al., 2016). A common criticism for ACL return to play protocol is that it is not sensitive or demanding enough to properly evaluate the knee function (Thomeé et al., 2011). The research behind this concept is not fully developed and still requires extensive evaluation (Murphy et al., 2003; Parkkari et al., 2001; van Melick et al., 2016).

The final call in if an athlete is ready to return to play is based entirely on the clinician (Flagg et al., 2019). Research suggests that there are certain criteria the athlete should possess before moving forward with sports-related movements such as minimal pain and swelling, full knee extension, at least 130 degrees of knee flexion, and normal gait (Flagg et al., 2019; van Grinsven et al., 2010). This transition usually occurs around the 9-to-16-week post-operative threshold (Flagg et al., 2019; van Grinsven et al., 2010). The athlete can then move into weight-bearing exercises as well as stabilized jumping and controlled running (Flagg et al., 2019; van Grinsven et al., 2010). At the week 16-22 marker, more advanced jumping, strengthening, agility and sports-related movements can be incorporated (Flagg et al., 2019; van Grinsven et al., 2010). Quadriceps and hamstring strength should be within >75% of the un-involved leg (Flagg et al., 2019; van Grinsven et al., 2010). There are many tests that have been used historically to definitively conclude that an athlete is ready to return to their sport. These include plyometric tests with >10% deficit which can be tested by single leg hoping (Flagg et al., 2019). The Tegner Scale has been used, predominately, to determine if the athlete has reached their desired level of activity (C. L. Ardern et al., 2011; Clare L. Ardern et al., 2011; Czuppon et al., 2014; Flagg et al., 2019; Kruse et al., 2012; Wright et al., 2008). As established in many papers, there are several rehabilitative protocols that can be followed that best fit to the athlete and their recovery.

ACL Reinjury

Reinjury of the ACL occurs in about 6-13% of reconstructed knees (Myklebust et al., 2003; Salmon et al., 2005; Thomeé et al., 2011; Waldén et al., 2006). There is an ACL injury on the contralateral side, 2-6% of the time following a previous rupture (Salmon et al., 2005; Swärd et al., 2010; Thomeé et al., 2011; Wright et al., 2007). Graft rupture has a 3-fold chance of occurring from the same contact mechanism that led to initial tear (Salmon et al., 2005). The chances of a retear are highest within the first year of reconstruction (Salmon et al., 2005). Within this time frame, there is not, however, a higher chance between a retear of the contralateral ACL or the original tear (Salmon et al., 2005). In a five-year period, retear rates are at about 12% (Salmon et al., 2005). There were certain risk factors that were identified including side-stepping, pivoting, jumping, and contact within sports (Salmon et al., 2005).

Athletes who suffer from an ACL tear are at a higher risk of either tearing their other ACL or re-tearing the original injury (Waldén et al., 2006). One area of focus looking into why retear rates are so prevalent is the type of grafts being used.

Bone-Patella Tendon-Bone (BPTB) autografts make up about 23% of ACL reconstructions (Middleton et al., 2014; Tibor et al., 2016; Widner et al., 2019). It offers the greatest biomechanical similarity to the original ACL (Widner et al., 2019). It also offers the greatest maximum-to-failure load, even though it does decrease with age (Widner et al., 2019; Woo et al., 1999; Woo et al., 1991). It is also easiest to harvest, requiring minimal dissection to gain access to the patellar tendon (Widner et al., 2019). The disadvantage of this graft type is donor site morbidity (Widner et al., 2019). This can lead to patella fracture, patellar tendon rupture, anterior knee pain, and other issues (Nawabi et al., 2017; Webster et al., 2016; Widner et al., 2019). While patellar fracture and tendon rupture occur in cases only about 0.4-1.3% and .18-.25%, respectively, anterior knee pain is evident in about 52% of cases and witnessed at 65% when the patient is attempting to kneel (Widner et al., 2019).

Hamstring autografts account for about 33-53% of all grafts (Middleton et al., 2014; Tibor et al., 2016; Widner et al., 2019). Similar to the BPTB grafts, hamstring tendon (HT) grafts also are noted to have donor site morbidities (Widner et al., 2019). These include anterior knee pain, and sensory and strength deficits (Widner et al., 2019). Sensory deficits occur in about 40-

88% of patients and can be explained by the disruption of the infrapatellar and sartorial branches of the saphenous nerve as a result of dissection (Hardy et al., 2017; Widner et al., 2019).

The quadriceps tendon (QT) has become less and less popular over the years since studies have shown its inferior ability in maximum load to failure in comparison to the native ACL (Noyes et al., 1984; Widner et al., 2019). Since that original study though, there have been contrasting evidence suggesting that the QT is more closely related to the native ACL than previously thought (Shani et al., 2016; Stäubli et al., 1999; Widner et al., 2019). Currently, it makes up about 11% of total grafts (Middleton et al., 2014; van Eck et al., 2010; Widner et al., 2019). Similarly, to the HT and BPTB there are donor site morbidities that include knee pain, numbness, and strength deficits (Widner et al., 2019).

Allografts, or grafts taken from a cadaver, make up a wide variety including hamstring, patellar, quadriceps, Achilles, and anterior/posterior tibialis tendons (Widner et al., 2019). The allograft is often considered inferior to an autograft but has been found to rival the autograft when taken from a younger donor (Bottoni et al., 2015; Widner et al., 2019). Allografts are commonly used in retear incidents where a patient has already undergone an autograft harvest (Widner et al., 2019).

In terms of which graft is best, it is up for debate. BPTB has been accredited the best in a recent meta-analysis that demonstrated a retear rate of only 2.8% when compared to HT retear rates at 2.84% (Samuelsen et al., 2017; Widner et al., 2019). In another study, all autograft types demonstrated the same retear rates (Mouarbes et al., 2019; Widner et al., 2019).

There are several factors that can attribute to retear rates. In one study, young age, higher MARX activity scores, and use of an allograft was associated with a 4.4% retear rate (Kaeding et al., 2015; Widner et al., 2019). Other factors like young age, male sex, tobacco abstinence and

HT graft use resulted in higher patient-reported scores in this same study (Kaeding et al., 2015; Widner et al., 2019).

Sex-Specificity in ACL Injury

Females, on average, are reported to sustain ACL tears at a rate three times higher than males (Giugliano & Solomon, 2007; Kramer et al., 2007; Prodromos et al., 2007). The highest ratio reported has been at over nine times higher for collegiate athletes (Prodromos et al., 2007). In high school sports, the rate is even higher at over five times more likely (Kramer et al., 2007). In the case of retears, females were not found to have a higher incidence rate than males (Salmon et al., 2005).

There are multiple factors that have been thought to contribute to this disparity including anatomical, biomechanical, neuromuscular recruitment and hormonal differences (Kramer et al., 2007). In a study investigating those with history of ACL injury versus those without there were significant differences in general laxity, tibial varum, hamstring flexibility, ITB flexibility, Q angle, and pelvic tilt (Kramer et al., 2007). Females have been known to demonstrate greater flexibility and joint laxity than males, but it is not certain whether that is a determining factor in one's injury (Kramer et al., 2007; Uhorchak et al., 2003).

A growing tibia and femur can cause an increase in torque and resulting instability of the knee (Giugliano & Solomon, 2007; Hewett et al., 2005). Men can compensate for this change by increasing strength and power (Giugliano & Solomon, 2007; Hewett et al., 2006; Hewett et al., 2005). Other anatomical factors include the Q angle or the quadriceps femoris angle. This describes the acute angle that connects the anterior superior iliac spine to the patella and the line connects the tibial tubercle to the same reference point of the patella (Giugliano & Solomon, 2007; Hungerford & Barry, 1979). Q angles have shown to be larger in women than in men, as well as being larger in those that suffered an ACL injury in those that have not (Giugliano & Solomon, 2007; Horton & Hall, 1989; Hvid et al., 1981; Ireland, 2002). A larger Q angle causes a higher degree of pull on the quadriceps muscle which adds medial strain to the knee thus increasing risk to the ACL (Giugliano & Solomon, 2007; Shambaugh et al., 1991). Q angle can be predictive for 32.4-46% of injuries (Buchanan, 2003; Giugliano & Solomon, 2007).

Condylar width is another suggested factor in ACL injury (Giugliano & Solomon, 2007). As height increases, in both men and women, condylar width increases (Giugliano & Solomon, 2007). However, men have a greater degree of growth compared to women (Giugliano & Solomon, 2007; Shelbourne et al., 1998). One study reported that women with narrow intercondylar notches had a 16.8% higher incidence rate of ACL injury than those that did not (Giugliano & Solomon, 2007; Uhorchak et al., 2003). Other studies have also shown that a narrow notch can increase the severity of the tear (Giugliano & Solomon, 2007; LaPrade & Burnett, 1994; Souryal & Freeman, 1993). An NWI, or notch width index, is the width of intercondylar notch divided by the width of distal femur and has been used to determine risk of ACL injury (Giugliano & Solomon, 2007).

ACL size has also been a studied factor. ACLs, when normalized for body size, are smaller in women than in men (Anderson et al., 2001; Giugliano & Solomon, 2007). There are many studies that demonstrate a positive correlation between smaller ACLs and injury rates (Giugliano & Solomon, 2007; Shelbourne et al., 1998; Uhorchak et al., 2003). A smaller ACL can encounter greater amounts of stress when compared to larger ACLs (Giugliano & Solomon, 2007). Another characteristic of ACLs that has been evaluated is the mechanical value. Female ACLs have been found to have a lower mechanical quality than males in terms of strain at failure, stress at failure and modulus of elasticity (Chandrashekar et al., 2006; Giugliano & Solomon, 2007).

Female ACLs are also known to have a greater degree of laxity, defined as the combination of joint hypermobility and musculotendinous flexibility, than males and may be a contributing factor to the higher incidence of tears in females (Giugliano & Solomon, 2007; Rozzi et al., 1999). After puberty, females, unlike males, gain a higher degree of laxity (Giugliano & Solomon, 2007; Hewett et al., 2006). Laxity may not seem like an important factor in ACL health, but muscle stiffness is essential in general knee health because it helps in knee stability and injury prevention (Giugliano & Solomon, 2007; Huston et al., 2000). The muscles surrounding the knee are able to contract under force and dissipate that strain away from the ACL (Giugliano & Solomon, 2007; Huston et al., 2000). General knee joint laxity can strain the ACL by increasing sagittal knee motion or hyperextension, coronal knee motion, and anterior tibial translation (Giugliano & Solomon, 2007; Hewett et al., 2006). Hamstring laxity has been shown to reduce the timing in co-contraction with the quadriceps and increase the risk of ACL injury (Ford, 1997; Giugliano & Solomon, 2007).

Hormonal factors, like an increased amount of estrogen in women, are thought to be an underlying cause of ACL injuries (Giugliano & Solomon, 2007; Gray et al., 1985; Zelisko et al., 1982). In the ACL fibroblasts there are estrogen receptors that produce the collagen necessary for load bearing (Giugliano & Solomon, 2007; Hewett et al., 2006; Huston et al., 2000). When estrogen is present in these fibroblasts the synthesis of collagen is reduced which can decrease strength of ACL increase risk of injury (Giugliano & Solomon, 2007; Hewett et al., 2006; Slauterbeck et al., 1997). High levels of estrogen have also been shown to decrease neuromuscular control of the knee and increase laxity (Deie et al., 2002; Giugliano & Solomon, 2007; Heitz et al., 1999; Hewett et al., 2006; Shultz et al., 2005). The overall effect of hormones and hormonal changes throughout puberty and with contraceptives is not fully clear and remains under investigation.

Neuromuscular factors have also been investigated as a possible factor. Unlike girls, boys have neuromuscular growth spurts that correspond with their growth spurts (Giugliano & Solomon, 2007; Hewett et al., 2004). This lack of coordination in girls at or around puberty, matched with a sudden weight and height gain, might contribute to a higher incidence rate (Giugliano & Solomon, 2007; Hewett et al., 2004). Females are also known to show leg dominance which is the imbalance of muscle strength, flexibility, and coordination between the lower extremities (Ardern et al., 2012; Giugliano & Solomon, 2007; Ly, 2006). Co-activation of the quadriceps and hamstring is important in protecting the knee against excessive anterior drawer, abduction, and lower extremity valgus (Giugliano & Solomon, 2007; Hewett et al., 2006). Women are found to be quadricep dominant which can increase anterior tibial displacement and increase risk of ACL injury (Giugliano & Solomon, 2007; Hewett et al., 2006). After ACL injury, women are also found to exhibit increased single-leg sway which suggests trauma to the proprioceptive system (Giugliano & Solomon, 2007; Haycock & Gillette, 1976).

There are even external suggestions for ACL injuries. One such suggestion includes the shoe and playing surface type that influences ACL tear rates in female sports (Giugliano & Solomon, 2007). Surfaces, like artificial grass or turf, can influence the friction between athlete and surface when the athlete is moving and could lead to more ACL tears (Giugliano & Solomon, 2007). Even drier surfaces have been shown to lead to more ACL injuries than wet surfaces (Giugliano & Solomon, 2007; Scranton Jr et al., 1997). There is not a full understanding of shoe types and how they can affect injury rates, but smaller shoes with more cleats were found to lead to a higher incidence rate (Giugliano & Solomon, 2007; Lambson et al., 1996; Torg & Quedenfeld, 1971).

Evaluating Muscle Strength

Muscular strength is one of the main indicators that is used to determine if a player is ready to return to their sport (van Melick et al., 2016). Quadriceps and hamstring musculature are the main areas of concern when testing for strength. The quadriceps muscles have been shown to have a 5-40% strength deficit following ACL injury and can last for 7 years following (de Jong et al., 2007; Hiemstra et al., 2007; Thomas et al., 2013; Yasuda et al., 1991). Hamstrings, on the other hand, report a 9-27% strength deficit and can last for 3 years following (de Jong et al., 2007; Hiemstra et al., 2007; Thomas et al., 2013; Yasuda et al., 1991).

The requirement for return to play is >90% of strength returned to the injured leg (Myer et al., 2006; O'Malley et al., 2018; Osterås et al., 1998; Sapega, 1990; van Melick et al., 2016). Isokinetic dynamometry tests are the most common form of muscular testing post ACL reconstruction (Barber et al., 1990; Eastlack et al., 1999; Fitzgerald et al., 2000; Keays et al., 2003; Liu-Ambrose et al., 2003; Noyes et al., 1991; Östenberg et al., 1998; Petschnig et al., 1998; Ross et al., 2002; Tegner et al., 1986; van Melick et al., 2016; Wilk et al., 1994). Its overall importance has been questioned in the past because of low correlations between the test results and functional performance (Finsterbush, 1990; Greenberger & Paterno, 1995; Östenberg et al., 1998; Pincivero et al., 1997a, 1997b; van Melick et al., 2016). There is also the discussion of open vs closed kinetic chain training in terms of strength rehabilitation and testing (Augustsson & Thomee, 2000; van Melick et al., 2016).

The Biodex Multi-Joint System isokinetic dynamometer is the most often used machinery to conduct these strength tests (de Vasconcelos et al., 2009; Dvir, 2002; Roberts et al., 2007).

This machine requires the patient to perform a series of maximal voluntary isokinetic contractions (MVIC) of knee extensions and flexions to determine the peak torque (Nm) that is created (de Araujo Ribeiro Alvares, 2015; de Carvalho Froufe et al., 2013; Roberts et al., 2007; Sung et al., 2019). The reference angle used in this type of testing has been widely at 60 degrees (Hannon et al., 2022; Šarabon et al., 2021; Whiteley et al., 2012; Zvijac et al., 2014). The measurements that are created are used to determine the musculature strength of the quadriceps and hamstrings (Roberts et al., 2007). This method of testing is considered the "gold standard" of strength testing following ACL reconstruction and is highly used to determine whether an athlete is ready to return to their sport (de Vasconcelos et al., 2009).

Evaluating Muscle Endurance

Muscle endurance has been described as the ability to sustain repeated muscle contractions (Hunter et al., 2005; Willingham & McCully, 2017). There are many experimental protocols that have been used to measure muscle endurance (Willingham & McCully, 2017). Historically, maximal voluntary activation or high frequency electrical stimulation has been used to induce muscle contractions in order to measure endurance (Jakobi et al., 2001; McDonnell et al., 1987; Willingham & McCully, 2017). These methods have a higher incidence of orthopedic injury, muscle damage, blood flow occlusion and lower tolerability from the patient (Bickel et al., 2004; Biering-Sørensen et al., 1990; Delitto et al., 1992; Gray & Staub, 1967; Willingham & McCully, 2017). Low frequency electrical stimulation produces lower forces of muscles contractions and may be more tolerable to the patient as well as reducing the risk of injury (Bickel et al., 2004; Willingham & McCully, 2017). Another advantage of low frequency stimulation is the ability to form cross-bridge turnovers which can increase force production (Willingham & McCully, 2017).

The use of accelerometers has been widely used in order to measure muscle endurance (Willingham & McCully, 2017). Accelerometer-based mechanomyography (aMMG) measures acceleration of changes in the muscle as a result of contraction (Barry et al., 1992; Orizio et al., 1999; Willingham & McCully, 2017). Previous studies of aMMG have demonstrated their signals indicate force measures and electromyography from muscle contractions as well as acceleration measures that can be used to assess muscle endurance (Akataki et al., 2001; Gobbo et al., 2006; Madeleine et al., 2001; Tarata, 2003; Willingham & McCully, 2017). aMMG has been used to measure muscle endurance during electrically stimulated muscle contractions (Bossie et al., 2017; Willingham & McCully, 2017). The device itself measures using a triaxial plane to distinguish movements of the muscle (Willingham & McCully, 2017). This method of measuring muscle endurance is considered superior because it is non-invasive, requires inexpensive equipment, and only takes as little as five minutes to test (Jones & McCully, 2020).

Evaluating Muscle Mitochondrial Capacity

The mitochondria of a muscle group are important in understanding the oxidative capacity and metabolic function during work (Hamaoka et al., 2011; Kent-Braun et al., 1995; Ryan et al., 2012). There are several non-invasive methods that have been developed in order to measure this including magnetic resonance spectroscopy (MRS) and the kinetics of phosphocreatine (PCr) resynthesis (McCully et al., 1993; Meyer, 1988; Ryan et al., 2012). These methods can be time consuming as well as very costly, therefore they do not provide the easiest way of collecting this data (Ryan et al., 2012).

Near-infrared spectroscopy (NIRS) provides a non-invasive, cost-effective, small and portable method for muscle oxygenation and mitochondrial measurements (Ferrari et al., 2004; Ferrari et al., 2011; Hamaoka et al., 2007; McCully & Hamaoka, 2000; Ryan et al., 2012). NIRS device provide information on oxygenated hemoglobin/myoglobin (O_2Hb), deoxygenated hemoglobin/myoglobin (HHb), and total hemoglobin (tHb) (Ryan et al., 2012).

NIRS measurements are performed with arterial occlusion to assess the muscle's rate of desaturation and assess the skeletal muscle oxygen consumption or mVO_2 (Pelka et al., 2023). A series of repeated arterial occlusions following muscle activation can be used to assess the mVO_2 change over time (Pelka et al., 2023). This data is plotted on a mono-exponential curve that provides the recovery kinetics or K-constant (Pelka et al., 2023).

Transcutaneous electrical stimulation has been used to evoke the metabolic changes needed to assess the mitochondrial capacity, and have been shown to be similar to voluntary muscle contractions (Pelka et al., 2023; Ryan et al., 2013). This is an easily accessible way to measure mitochondria. The downside to this method is that there could possibly be unaccounted variation in force production affecting the amount of type of fibers involved in the contractions (Pelka et al., 2023). In a recent study, researchers found that under similar work the electrically stimulated muscle groups accounted for a greater muscle oxygen demand when compared to voluntary movement (Pelka et al., 2023). However, the mitochondrial recovery between the two methods proved to be similar (Pelka et al., 2023).

Evaluating Psychological Readiness to Return to Sport

An important concept that has received more attention over the years, is an athlete's mental health and psychological well-being in general, as well as following injury (Czuppon et al., 2014; Flagg et al., 2019; van Melick et al., 2016; Wright et al., 2008). Fear of injury is one of the main reasons that athletes either do not return to their sport or choose to participate at a lower level of competition (C. L. Ardern et al., 2011; Thomeé et al., 2011). Athletes report anger, depression, lack of confidence, anxiety, and fear of sustaining an injury again which can lead to an impairment in their rehabilitative ventures (Ardern et al., 2016; Johnston & Carroll, 1998; Reese et al., 2012).

There are numerous methods that have been developed to assess an athlete's readiness to return to their sport. One such assessment is called the ACL-return to sports after injury or ACL-RSI (Kim et al., 2022). This scale was developed in 2008 by Webster et al. (Webster et al., 2008). It consists of 12 questions based on the 3 subcategories assessing the patient's emotions, confidence in their physical performance, and the level of perceived risk (Kim et al., 2022). Athletes have reported that their confidence in returning to sport largely relies on their beliefs in the rehabilitation completion, that they will not suffer another similar injury and that their performance is not hindered from the injury (Ardern et al., 2016; Podlog et al., 2015).

With low return to sport statistics and new research illustrating the importance of an athlete's mental capabilities, it could be hypothesized that this avenue could lead to better outcomes for athletes (Ardern et al., 2016). Studies have shown that there is an equal importance on both the physical and psychological rehabilitation of athletes (Ardern, 2015; Ardern et al., 2016; Ardern et al., 2013). Examples of such psychological rehabilitation methods include goal setting, education, modeling, rapport building, imagery and relation training (Ardern et al., 2016; Ardern et al., 2012; Covassin et al., 2015; Nyland & Brand, 2012; Podlog et al., 2015). Physical rehabilitation is vital to get an athlete well and back to their sport, but it is just as important, if not more, to also assess an athlete's ability to mentally recover from their injury.

CHAPTER 3

EFFECTS OF ACL RECONSTRUCTION ON MUSCULAR STRENGTH, ENDURANCE AND MITOCHONDRIAL CAPACITY[1]

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Abstract

PURPOSE: The purpose of this study was to measure quadriceps and hamstring strength, endurance, and mitochondrial capacity in female collegiate athletes that have undergone ACL reconstructive surgery on a single leg. **METHODS**: 10 subjects were included in this study. Quadricep and hamstring strength were measured using data collected from a Biodex Multi-Joint System isokinetic dynamometer. Muscle endurance was measured using data from accelerometers. Muscle mitochondrial capacity was measured using near-infrared spectroscopy (NIRS) Portamon devices. All testing was completed in single session. **RESULTS**: There was not a significant difference of strength for injury ($F = 3.65$, $p = 0.088$). Muscular endurance did not demonstrate a significant difference for injury ($F = 1.137$, $p = 0.314$). Mitochondrial capacity was not different in quadriceps or hamstring for injured and non-injured legs ($F = 4.169$, $p = 0.055$). **CONCLUSIONS**: This study did not demonstrate impairments in mitochondria or endurance. However, these measures remain important in rehabilitation from knee injury.

Introduction

Knee injuries are the most experienced disability among athletes (Hughston, 1962). The joint itself is in a vulnerable location and has functional limitations in its range of motion (Hughston, 1962). It is also comprised of a variety of ligamentous structures that provide mobility but also leave it susceptible to many forms of injury. It can also be noted that because of the structure of the joint, when one tissue is injured, there is a high possibility of another structure being injured as well (Fadale & Hulstyn, 1997). The most common injuries to the knee joint occur to the anterior cruciate ligament (ACL), posterior cruciate ligament (PCL), medial collateral ligament (MCL), lateral collateral ligament (LCL), meniscus, and the patellofemoral joint (Fadale & Hulstyn, 1997).

ACL injuries have grown with increasing frequency in the athletic community, making up about 50% of knee injuries (Fadale & Hulstyn, 1997; Flagg et al., 2019). Even greater ACL injuries are seen in female athletes (Giugliano & Solomon, 2007). Females are two to eight times more likely to tear their ACL in a sport-related event than men are (Giugliano & Solomon, 2007). There are several physiological reasons that have been placed for this disparity including bone length, neuro-musculature differences, hormonal factors, and skill/level of exposure (Giugliano & Solomon, 2007).

There are preventative strategies in training that have shown results in lowering ACL injury incidence rates by 3.7% (Giugliano & Solomon, 2007). A higher level of importance has been placed on female athletes with ACL injuries receiving the research and attention that they deserve. One emphasis has been placed on evaluating rehabilitative measures and the measures used to “return-to-play”. Up to 35% of athletes do not return to their sport within 2 years of their injury (Flagg et al., 2019). Re-injury rates are also quite common from initial ACL tears (Flagg et al., 2019). Our study investigates the measure of strength, endurance and mitochondrial capacity in an individual with an ACL tear, which has not been fully reported in previous literature.

The purpose of this study was broken into three specific aims of assessing quadriceps and hamstring muscular strength, muscular endurance, and muscular mitochondrial capacity on a singular leg in female athletes following ACL reconstructive surgery. We compared mitochondrial capacity, endurance index, and strength between both legs of the same participant. We hypothesized that the hamstring muscle would have a greater reduction in mitochondrial capacity than quadriceps, the hamstring muscle would have a greater reduction in endurance than quadriceps, and that the injured leg would be different in muscle strength compared to the non-injured leg.

Methods

Study Participants

A total of 10 healthy female participants were recruited from the local athletic collegiate community. Recruitment was done using word of mouth and flyers posted around the campus. The inclusion criteria included: 1) Willingness to participate and provide informed consent, 2) female athlete between the ages of 18-25, 3) ACL injury must be confined to one limb, and 4) rehab protocol was completed 6 months to 5 years to the testing date. Participants were excluded if they had: 1) Untreated diabetes or other endocrinology disorders, 2) recent reconstruction surgery without therapy, 3) renal or peripheral venous disease, 4) uncontrolled hypertension, heart disease or lung disease, 5) prior musculoskeletal disease that would preclude participation, 6) psychosis or cognitive impairment such that compliance would be difficult, 7) sickle cell anemia, and 8) are or trying to become pregnant. This study was approved by the Institutional

Review Board of the University of Georgia and informed consent was obtained from all participants in this study (PROJECT00007003).

Study Design

This was a within-subjects cross-sectional design comparing injured to non-injured legs on the same individual. Participants were scheduled to come into the Non-Invasive Muscle Physiology Lab at the University of Georgia for one testing visit after a screening session to determine if they were eligible. The testing session lasted approximately two and half hours. Sessions began by collecting informed consent from the participants. Questionnaires collecting anthropometric data like age, gender, height, race as well as more information about their injury and experience were collected also.

The testing session contained three sets of measurements in the respective order: Muscular strength, muscular endurance, and muscular mitochondrial capacity. Participants were allowed to take breaks as needed throughout testing.

Experimental Procedures

Quadricep and Hamstring Muscular Strength Test

Muscular strength of both the hamstrings and quadriceps was adopted from a revised version of the Faxon et al. testing procedure (Faxon et al., 2018). Participants were instructed to complete 5 minutes of low-intensity warmup on a bike ergometer. After the 5 minutes, they were securely fastened into a Biodex System 4 Dynamometer. The testing protocol for this session included 3 rounds of 5 second contractions for flexion and extension at a 60-degree knee angle with 5 second breaks in-between. Participants would extend their leg, flexing their quadricep to measure its strength, and then have a 5 second break. They would then go straight into another 5 second contraction, only this time they would flex their leg in order to measure hamstring strength. They completed this series of flexion and extension 3 times. The peak torque was recorded for each series of movements.

Quadricep and Hamstring Muscular Endurance Test

The muscular endurance of both the hamstring and quadriceps was adopted from a revised version of the Faxon et al. testing procedure (Faxon et al., 2018). The endurance index relates to the percentage of muscle fibers that are fatigued throughout the course of the 5-minute exercise. The participant laid in a prone position for hamstring testing and supine position for quadricep testing on a padded table. Two 2” x 4” stimulation electrodes were placed proximally and distally to the muscle bellies of both the vastus lateralis (quadricep) and semitendinosus (hamstring) in separate testing. These were connected to a Theratouch EX4 electrical stimulator (Richmar, Middlebugh Heights, Ohio). In between these pads, a triaxial accelerometer (MetaMotionRL, Mbientlab Inc, San Francisco) was placed, and attached with double-sided tape. Appropriate electrical stimulation was achieved by determining the maximum level of intensity that could be tolerated while still seeing a visible contraction. The participant would then lay supine for 5 minutes while the quadricep was stimulated. After completion of this testing, the participant would then lay prone to stimulate the hamstring at the appropriate intensity level as described previously. The muscle acceleration was recorded using the triaxial accelerometer from both positions. Muscle acceleration was recorded at a frequency of 200 Hz which was used to derive muscle activity.

Quadricep and Hamstring Muscular Mitochondrial Capacity Test

The muscular mitochondrial capacity of both the hamstring and quadriceps was adopted from a revised version of the Ryan et al. testing procedure (Ryan et al., 2012). The mitochondrial capacity was determined for both the hamstrings and quadriceps in both legs of the participant. Testing was conducted on one leg at a time for the quadricep and hamstring musculature. Near-Infrared Spectroscopy (NIRS) devices were used to determine the rate of recovery of muscle oxygen consumption after arterial occlusions (Ryan et al., 2012; Sumner et al., 2020). The NIRS data for each participant was analyzed using the third channel on the device which was the deepest channel (40mm distance from the light source to the receiver). Arterial occlusion was done at ~270mmHg for all participants. Participants would lay supine for the entirety of the testing. A blood pressure cuff (Hokanson E20 cuff inflator; Bellevue, WA) was placed at the upper thigh above the NIRS devices. NIRS devices were placed both on the vastus lateralis (quadricep) and semitendinosus (hamstring) muscle bodies. Electrodes were placed proximally and distally to both the NIRS devices and connected to a Theratouch EX4 electrical stimulator (Richmar, Middleburgh Hts, Ohio). The NIRS devices were held to the muscle bodies with pre-wrap. The participant's leg was held in a bent position by cushioning, as to avoid contact with the devices and the platform. Stimulation intensity was adjusted to the highest tolerable level for each individual participant. The protocol began with a 2-minute rest period followed by a 30 second vascular occlusion from the cuff to establish the initial resting metabolism. After a minute of rest, the quadricep muscle was stimulated for 30 seconds, followed by another 30 seconds of cuffing. A rest period of 30 seconds followed to allow a return to baseline. The hamstring muscle was then stimulated for 30 seconds, followed by another 30 seconds of cuffing. A rest period of 30 seconds concluded the first stage of the NIRS procedure. The quadriceps and hamstring muscles were then stimulated simultaneously for 30 seconds and immediately followed by a series of short occlusions for 5 seconds on and 5 seconds off. This serial cuffing was repeated 6 times, which refers to one trial. The trial was then repeated 3 more times. At the conclusion of the trials, the participant rests for 5 minutes. Following the reset period, there is a final resting metabolic cuffing for 30 seconds. The return of metabolic rate to the original resting conditions was calculated using the rate of change in oxygenated to deoxygenated hemoglobin signals.

Self-Reporting Questionnaires

Before any testing, participants were asked to fill out a series of self-reported questionaries. Each form asked the participant a different series of questions but were all pertaining to their medical history and experience with their injury. The forms helped to gather information not only on the physicality of the athlete and their injury, but the underlying psychological hinderances that can be at play in their rehabilitation. A demographics form was included to obtain general information about the participants including age, gender, ethnicity, level of education, and sport. Other information in this demographics form included information about their knee injury like which leg was injured and graft type. The next form was TSK-11 (Tampa Scale 11) which assesses kinesiophobia, or fear of reinjury or movement (Hapidou et al., 2012). This scale assesses on a scale of 1 (strongly disagree) to 4 (strongly agree). Scores closer to 11 denotes negligible kinesiophobia while a score closer to 44 denotes severe fear. The next series of questions was the Tegner Scale which assesses general activity level pre- and post-injury. The Marx Activity Rating Scale (MARS) focuses primarily on confidence in four athletic movements like running, deceleration, cutting, and pivoting. A Knee Injury form gathered more in-depth information regarding the participant's injury. The ACLRSI or risk assessment for return to play looks primarily on an athlete's feelings on their overall readiness to play. A higher score (%) denotes a higher confidence index while a lower score denotes poor perceived confidence in ability.

Statistical Analysis

Descriptive data are presented as means and standard deviations. Statistical analysis was done using IBM SPSS Statistics 26 (IBM®, Armonk, New York). Differences between measurements were identified using two-way analysis of variance (ANOVA) comparing means of measures. This statistical test was used to measure values between the injured and non-injured legs, hamstrings and quadriceps, as well as any interactive effects. These were conducted for muscular strength, muscular endurance, and mitochondrial capacity. Significance was accepted at $p < 0.05$ for all comparisons. Effect size was calculated using Cohen's D.

Results

Subject Characteristics

Ten female athlete subjects were recruited for this study. All participants are active either in their sport or other quality activities. The physical characteristics of all participants are presented in Table 1. Responses to the self-reported questionnaires are reported in Table 2. These include the TSK-11, Marx, and ACLRSI questionnaires.

Hamstring and Quadricep Muscular Strength

Overall strength data are shown in Figure 4. There was no significant interaction between muscle and injury (F=0.707, $p = 0.422$). There was a significant main effect between hamstring and quadricep muscles using ANOVA such that the quadriceps were stronger than the hamstrings (F = 46.48, $p = 0.001$). There was no significant main effect of injury using ANOVA (F = 3.65, $p = 0.088$). The effect size calculation of Cohen's D for the significant main effect of muscle was large at 1.32.

Hamstring and Quadricep Muscular Endurance Index (EI)

Endurance values are shown in Figure 5. There was no significant interaction between muscle and injury (F=0.032, p = 0.863). There was no significant main effect between the comparison of hamstring and quadricep endurance using ANOVA (F = 2.356, p = 0.141). There was no significant main effect of injury using ANOVA (F = 1.137, p = 0.314). In comparison to Faxon et al.'s paper, this data provides a differing outlook on endurance in these muscle groups. This could be contributed to the difference of the testing population.

Hamstring and Quadricep Muscular Mitochondrial Capacity

Mitochondrial values are shown in Figure 6. There was no significant interaction between muscle and injury (F=0.048, p = 0.831). There was no significant main effect between the comparison of hamstring and quadricep mitochondrial capacity using ANOVA (F = 4.169, p = 0.055). There was no significant main effect of injury using ANOVA (F = 0.00, p = 0.985).

Discussion

The first aim of the study was to determine the relationship of the injured leg and non-injured in terms of muscular mitochondrial capacity. We were unable to accept our hypotheses that the hamstring and quadriceps mitochondrial capacity differed from the injured to non-injured side. There was not a significant difference observed between the injury groups. This method of measuring injured and non-injured mitochondrial capacity is novel. Previous studies, like Hanna et al's (2021), measured healthy quadricep and hamstring. They found that the mitochondrial procedure was feasible for measuring mitochondrial capacity in the hamstring and quadriceps (Hanna et al., 2021). Growing research into this method of measurement is vital to understanding more about the rehabilitated athlete.

The second aim of this study was to evaluate the relationship between the injured and non-injured legs' quadriceps muscle endurance. We were not able to accept the conclusion that the muscle endurance of the quadriceps of the injured and non-injured was different, as well as that the hamstring endurance varied based on the injured to non-injured leg. The data showed no significant difference. This varies based on Faxon et al.'s (2018) paper as there was a significant difference found between the hamstring and quadricep musculature.

The last aim of this study was to investigate the relationship of muscular strength in injured and non-injured legs. We hypothesized that the strength would be different in the injured leg in comparison to the non-injured leg. We were not able to accept our hypothesis because there was not significant difference found between the injured and non-injured legs. There was however a significant relationship between the hamstrings and quadriceps strength. This coincides with modern ACL injury rehabilitation protocol that focuses mainly on quadriceps strength while excluding hamstring strength. Myer et al's study (2009) found that hamstring strength was also diminished in female athletes after knee injury, but quadriceps strength remained the same. This study coincides with the findings of our study.

This study did not find statistical differences for muscle endurance or mitochondrial capacity, in comparison to Faxon et al.'s that found an endurance difference. Other studies have shown that female athletes that have suffered ACL injuries demonstrate less endurance than their uninjured counterparts (Beniot). Our study population represents collegiate athletes that have thoroughly rehabbed with professionals for a consecutive time period. This could explain why endurance and mitochondrial values do not demonstrate significance. If testing was conducted on a differing athletic population, the data could illustrate a differing result. Further investigation into the relationship of ACL injuries and endurance of female athletes should be conducted.

This study only investigated the relationship of female athletes and knee injury, and not men. Numerous studies have been conducted on male athletes and even more comparing the differences between the two sexes. In this study, we wanted to focus solely on female athletes since they have a higher probability of knee injury in their sport.

Another component of this study was to test athletes after college athletic rehabilitation. Originally, recruitment was conducted through the athletic department of the University of Georgia. We were able to recruit a total of four current collegiate athletes. The remaining six were healthy and active college students. An issue for this study could be the consistency and success of treatment for each individual. Rehabilitation protocols are largely similar, though there can be some individual differences. We sought to understand this as well by including the self-reported questionnaires. Some reported that they were fully rehabbed while some reported some hesitation. This also highlights an issue for future studies.

This study was largely an explorative investigation into the possible components of ACL injuries and how to test for return to play. Although there is extensive literature on ACL injuries, there are diverse results from studies looking into the women’s physiology and other factors that might lead to the high proportion of ACL injuries being in female athletes. This largely results from Q angle, hormonal factors, and reduced muscular strength.

There are some limitations to our study. The study was underpowered, which might have had some influence on our results. Secondly, there were difficulties testing the muscle mitochondrial capacity and achieving a high enough STEM index to cause a metabolic change in the muscle, and properly mimic exercise. This could alter those results. It was also difficult in measuring both the quadriceps and hamstring simultaneously. Future studies might test them separately or develop another approach to measuring these muscle groups.

Conclusions

This study found no evidence that hamstring muscle mitochondrial capacity or muscle specific endurance were reduced following rehabilitation from knee injury/surgery. The study population did have reduced muscle strength in the injured leg, suggesting prolonged changes despite rehabilitation. This study provides a novel, noninvasive method of measuring muscular strength, endurance and mitochondrial capacity in athletic populations using twitch stimulations and helps to further add to the literature on ACL injuries. Future studies should investigate the reproducibility of these results, and further investigation into multiple surgeries, endurance of athletes or effects of other muscle groups is important to the evolution of this study. Also, future studies among varying athletic populations will be beneficial to the overall literature of ACL injuries and reduction of future cases.

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Table 1. Physical characteristics of participants

Participant	**Age** *(yr)*	**Education** *(College)*	**Sport**	**Injured Leg** *(L/R)*	**Type of Graft Used**	**Months Since Surgery**
1	18	Freshman	Basketball	L	Hamstring	6
2	21	Senior	Softball	L	Patellar	57
3	22	Senior	Softball	L	Quadriceps	80
4	19	Sophomore	Softball	R	None	37
5	21	Senior	Soccer	L	None	27
6	21	Junior	Lacrosse	R	Patellar	32
7	22	Senior	Swimming	L	Hamstring	12
8	20	Junior	Soccer	R	Hamstring	42
9	19	Freshman	Soccer	L	Patellar	29
10	20	Freshman	Lacrosse	R	Quadriceps	29

Table 2. Questionnaire Results. The TSK11 has a range of 1-4 (strongly disagree – strongly agree). Significant impairment for this test is a 24 or above. The Marx test has a range of 4-20 (less than once a month – 4 or more times a week). The ACLRSI has a range of 0-100 (not confident – fully confident). Significant impairment for this test is <56.

Participant	TSK11 Score	Marx Score	ACLRSI Score
1	16	20	60%
2	13	20	98%
3	13	20	90%
4	17	20	53%
5	18	16	59%
6	19	15	83%
7	18	7	36%
8	13	13	81%
9	22	20	65%
10	16	14	87%

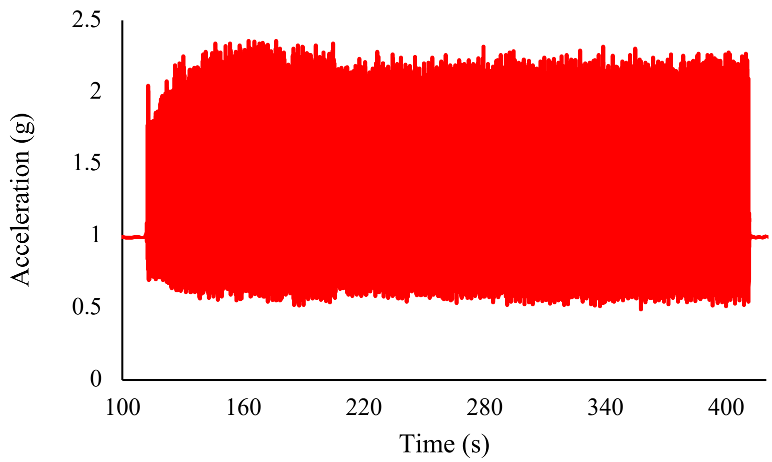

Figure 1. A representative example of the 5-minute Endurance Protocol from healthy quad. The acceleration values were calculated as the resultant vector from a tri-axial accelerometer. The stimulation was at 5 Hz.

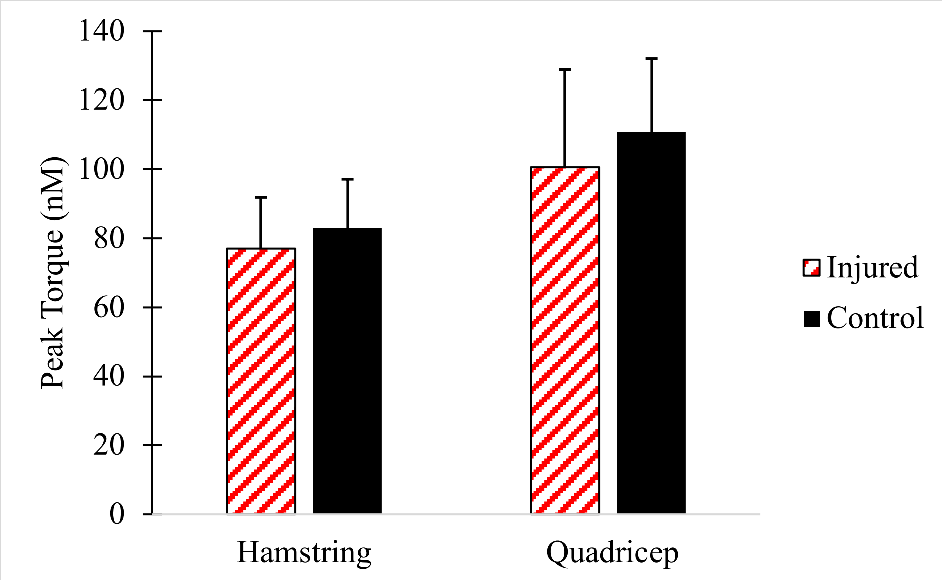

Figure 2. Hamstring and Quadricep Strength Measurements. Values are means and SD. Significant differences were seen for the main effects between hamstring and quadriceps strength.

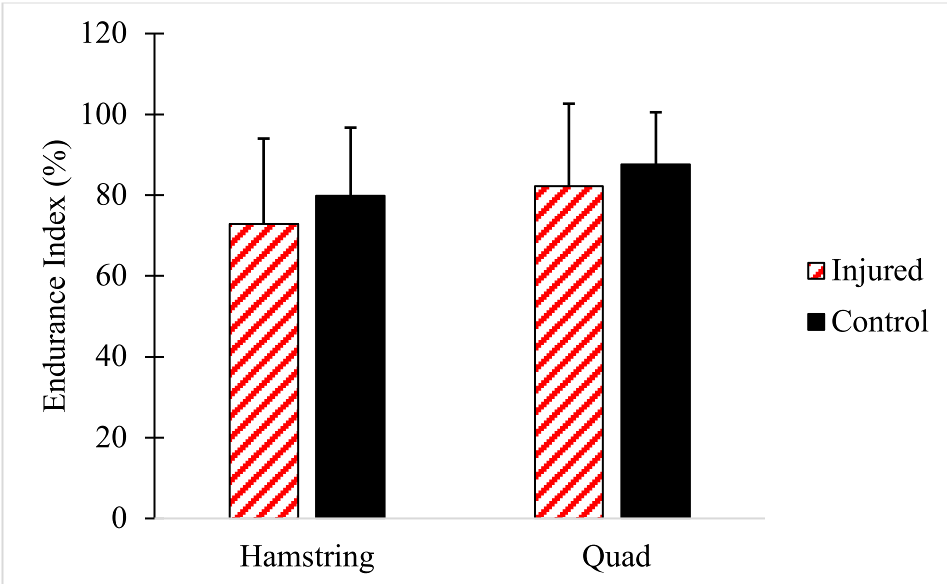

Figure 3. Hamstring and Quadriceps Endurance Measurements. Values are means and SD.

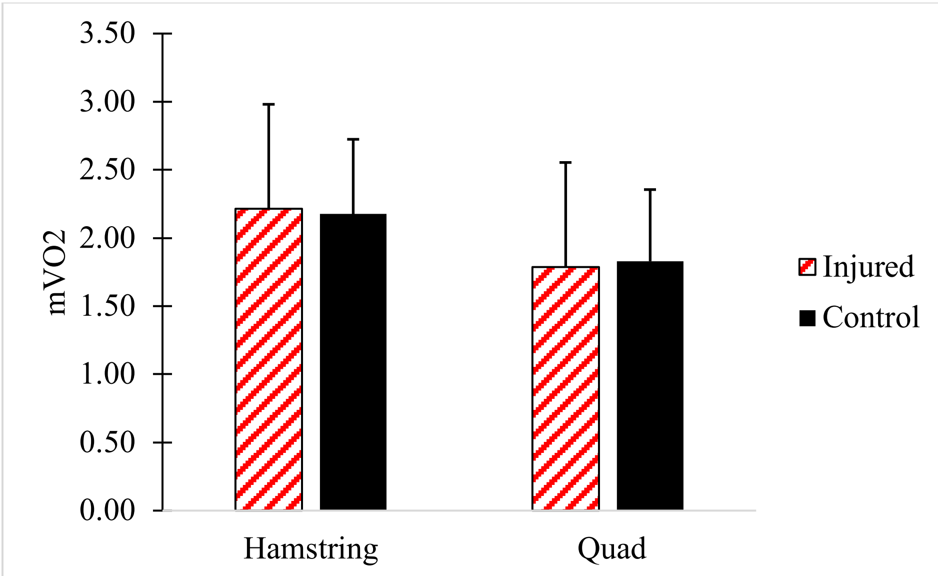

Figure 4. Hamstring and Quadriceps Mitochondrial Capacity Measurements. Values are means and SD.

CHAPTER 4

SUMMARY AND CONCLUSION

ACL injuries occur in female athletic populations at an alarming rate. There is little research into the testing protocols used for return to play in an athlete's recovery following reconstructive surgery. Previous studies have highlighted measurements of strength only and have excluded measurements of endurance or mitochondrial capacity (de Jong et al., 2007; Hiemstra et al., 2007; Osterås et al., 1998; Thomas et al., 2013). These measurements could provide more information about rehabilitative recovery. Without them there are limitations to how to properly measure when an athlete is fully rehabbed. Therefore, this study sought to measure muscle strength, endurance, and mitochondrial capacity in order to demonstrate a more well-rounded approach to understanding a fully rehabbed athlete. This study was a follow up to a previous study that measured strength and endurance after knee injury. In addition to repeating the previous study in a new sample of subjects, this study added muscle mitochondrial capacity.

Quadriceps and hamstring muscle strength, endurance and mitochondrial capacity were tested in ten female athletes with an ACL injury. The primary finding of this study was the relationship of muscular endurance and mitochondrial capacity. Though the statistical analysis did not yield significant results, there are confounding factors that could have affected them. The testing protocol as well as the testing population could all have effects on the results. Other studies have demonstrated that female athletes with past ACL injuries demonstrate a reduced level of endurance in comparison to their un-injured counterparts (Benoit et al., 2024). This area of rehabilitation could offer a solution to retear rates in female athletes. Further testing is necessary.

Strength measures demonstrated a significant difference in the hamstring of the injured leg versus the un-injured. Proper rehabilitation methods should be placed on this muscle group as well as the quadriceps. Current rehabilitation focuses on quadriceps but incorporating hamstrings gives a more well-rounded method of helping athletes.

A possible limitation to this study was the stimulation intensity not being high enough in the mitochondrial capacity testing. This could alter the metabolic change of the musculature and skew the results. The stimulation intensity is determined based on the participants comfortability with the stimulation, but if it is not high enough then there will not be a proper metabolic change. Future studies should seek to determine a more consistent method of testing for mitochondrial capacity and determining a proper stimulation intensity. Because of this limitation there is a question to the overall validity of the mitochondrial measures The upper portion of the leg is a difficult area to test when compared to the lower leg because the musculature does not cross a joint and it in a heavily dense area. The protocol for this testing attempted the best possible approach to gathering data. Several analyses were conducted over this data and the best possible data was presented.

Another limitation was the study being under powered. Despite that, we were able to demonstrate viable results regarding strength and endurance. Future studies should include a larger sample size of female athletes. This study initially partnered with the athletic department at the University of Georgia. However, recruitment levels were low for university athletes and other participants needed to be recruited. It is important to study on athletic populations, but it is often difficult to gather enough participants. Instead of recruiting from a college atmosphere like this study, one could recruit from an orthopedic clinic. This could be beneficial to the study because you can gather enough participants to make the study powerful. There are also many patients there with knee injuries that would fit the criteria. This could aid the progression of this research.

In conclusion, this study did not find deficits in the injury leg for hamstring muscle mitochondrial capacity or muscle specific endurance. However, this study demonstrated a three-pronged approach, including strength, endurance, and mitochondrial capacity, to measuring ACL injury recovery is a viable option in rehabilitating athletes. This method could prove useful in athletic clinical populations for all levels of sports and for all levels of athletes, because it's non-invasive and very informative with little equipment needed. From the data gathered, there are no considerations to be given regarding the current rehabilitation process. This does not however conclude that further research into this topic is not important for future athletes and their health.

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