

**The Role of Supraspinal Excitability in the Development of Healthy-Limb Deficits
Following Anterior Cruciate Ligament Injury**

By

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ABSTRACT

In the current study, we examined how supraspinal and spinal excitability were altered bilaterally after unilateral anterior cruciate ligament reconstruction (ACLR). 7 participants with ACLr and 7 healthy controls underwent transcranial magnetic stimulation (TMS) and electrical stimulation. To evaluate supraspinal excitability, resting motor thresholds (RMT) and motor evoked potential (MEP) stimulus response curves (SRC) were used. To measure spinal excitability, H-reflex SRC gain was assessed. Mixed factorial ANOVAs were used to compare measures between limbs and between groups. Cohen's d was used to assess effect sizes between groups. Data indicated no significant differences between subject groups or between limbs. However, large effect sizes were found between limbs for H-reflex gain and RMTs suggesting that ACLr can have an effect on some of the variables examined. This study identified decreases in strength in the injured limbs and that subjects with an ACL injury exhibited decreases in spinal and supraspinal excitability of the quadriceps compared to Healthy controls.

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List of Symbols, Nomenclature or Abbreviations

Anterior Cruciate ligament (ACL)
Anterior Cruciate Ligament Reconstruction (ACLR)
Anterior Knee Pain (AKP)
Central Nervous System (CNS)
Electromyography (EMG)
Healthy-Limb Deficit (HLD)
Maximum Stimulator Output (MSO)
Maximum Voluntary Contraction (MVC)
Motor Evoked Potential (MEP)
Quadriceps Activation (QA)
Resting Motor Threshold (RMT)
Stimulus Response Curve (SRC)
Total Knee Arthroplasty (TKA)
Transcranial Magnetic Stimulation (TMS)
Vastus Medialis (VM)

Introduction

The anterior cruciate ligament (ACL) is made up of two bundles of fibers with attachments at the lateral femoral condyle as well as at the top of the intercondylar notch of the tibia. The primary function of the ligament is to prevent anterior tibial translation under varying degrees of flexion, contributing up to 85% of the restraining force at 30 and 90 degrees of knee flexion (Butler et al., 1980; Sebastianelli, 2011). The ACL also helps to prevent knee hyperextension, limit tibial rotation and acts as a secondary restraint to varus and valgus stresses on the knee (Liu-Ambrose, 2003). Furthermore, the ACL contains sensory receptors such as mechanoreceptors which provide the central nervous system (CNS) with information regarding the tension being placed on the ligament (Krauspe et al., 1995). Injury to the ACL can occur via contact or noncontact mechanisms, with the majority of injuries resulting from non-contact mechanisms, that is, no physical contact was present on the knee during the time of injury (Lin et al., 2012). Rapid decelerations during running or excessive rotation of the tibia during landing are frequent causes of non-contact injuries (Sebastianelli, 2011). ACL injuries are one of the most common knee pathologies in sports today, increasing the importance of understanding the injury itself and its resulting long term neuromuscular and performance related effects.

An ACL injury presents immediate symptoms as well as long term chronic consequences, most commonly residual weakness of the quadriceps muscle (Rosenberg et al., 1992; Lewek et al., 2002; Williams et al., 2005; Heroux & Tremblay, 2006; Hodges et

al., 2009; Palmieri-Smith & Thomas, 2009; Rosenthal et al., 2009). This weakness is thought to be at least partially caused by a combination of muscle atrophy and ongoing neural inhibition preventing the quadriceps from fully activating (Rice and McNair, 2010). Quadriceps weakness after injury can vary in severity and may persist for months or even years following the initial injury, increasing susceptibility to additional functional limitations (Chaudhari et al., 2008; Hart et al., 2010). It is well documented that there are acute and chronic effects of ACL injury and that these effects can be observed in the joint, surrounding soft tissue and/or musculature of the injured limb. Only recently, has research started to focus on the effects of unilateral ACL injury on the contralateral, uninjured limb. A healthy-limb deficit (HLD) occurs when a unilateral injury, creates or results in a decrease in functioning of the contralateral, uninjured limb. Researchers have reported decreases in proprioceptive function (Roberts et al., 2000) gamma loop dysfunction (Konishi et al., 2007) as well as diminished muscular strength (Urbach et al., 1999; Hiemstra et al., 2007; Hart et al., 2010; Rice & McNair, 2010) in the uninjured limb after ACL injury. In addition, injury and damage to tissue other than the ACL have also been known to result in HLDs. Individuals with unilateral ankle sprain (Wikstrom et al., 2010), total knee arthroplasty (TKA) (Byrne et al., 2002; Milner, 2008), and individuals with anterior knee pain (AKP) (Suter et al., 1998; Hart et al., 2010; Ingram et al., 2015) have been reported to have functional alterations in the injured limb as well as the uninjured limb. Although these healthy-limbs exhibit similar deficits to those observed in the injured limb, the mechanisms responsible are more complex and less well

understood. Peripheral changes to gamma loop function and cortical alterations after injury are two hypothesized causes of HLDs.

The purpose of this thesis is to examine one of the hypothesized causes of HLD following ACL injury. Specifically it will examine whether factors contributing to these deficits are related to changes in excitability at either the cortical level or the spinal level. The thesis will add to current literature in an effort to further understand the cause of health-limb deficits following this injury. The following is a review of the literature, providing background on the anatomy and function of the ACL, potential mechanisms of ACL injury, injury treatment, acute and chronic effects of the injury, and the nature and potential mechanisms of HLDs.

ACL Anatomy & Function

The ACL is arguably the most important of four strong ligaments connecting and stabilizing the bones of the knee joint. The role of the ACL is to provide stability to the joint throughout its range of motion, in more than one degree of freedom. In addition, the ACL restrains excessive knee motion in response to externally applied forces. The ACL is comprised of two bundles, anteromedial and posterolateral, which mutually act to provide stability to the knee joint. These bundles are made up mostly of collagen, but also contain a small amount of elastin which is important for storing energy during activity and ensuring the ligament returns to its resting length (Sebastianelli, 2011). Furthermore, the ACL is able to provide the CNS with sensory feedback, relaying information regarding

tension, position, as well as velocity and acceleration of motion (Krauspe et al., 1995; Dyhre-Poulsen & Krogsgaard, 2000; Friemert et al., 2010).

Sensory Function

The ACL contains multiple sensory nerve endings, each providing the CNS with information regarding movement characteristics, ligament length and joint angle (Krogsgaard et al., 2011). The ACL ligament has three types of mechanoreceptors along with free nerve endings. The mechanoreceptors found in the ligament are:

- 1) Golgi-like tension receptors: Slow adapting mechanoreceptors that are thought to detect stimulus characteristics such as speed and acceleration. These receptors are located near the bony attachments of the ACL and underneath the synovial membrane (Schultz et al., 1984; Zimny et al., 1986).
- 2) Ruffini Receptors: Slow adapting mechanoreceptors that are well suited to measure speed and acceleration. These receptors are sensitive to stretching and are located at the surface of the ligament, mainly on the femoral portion where deformations are the greatest (Zimny et al., 1986).
- 3) Vater-Pacini Receptors: Fast adapting mechanoreceptors that measure change in the environment, such as motion. These receptors are predominately located at the femoral and tibial attachments of the ACL (Zimny et al., 1986; Krauspe et al., 1995).

In addition to projecting to alpha motor neurons, afferent information from the ACL is known to project to gamma (γ)-motor neurons (Sojka et al., 1991). Early research in this area was primarily done in cats. Sojka et al. (1991) recorded hamstring muscle spindle activity in lightly anaesthetized cats during increased tension on medial and lateral collateral ligaments. Increased tension was found to evoke changes in the primary spindle afferents leading to the conclusion that ligament receptor afferents contribute to the regulation of activity in the (γ)-muscle-spindle system. This system includes motor neurons, muscle spindles and afferent pathways which all work together to coordinate muscle contractions, and movement position and sense (Sjolander et al., 2002). Gamma motor neurons innervate muscle spindle fibers, which are highly sensitive to stretch and tension, allowing control of the spindle sensitivity, which is independent of the control of the motor neurons (Manuel & Zytnicki, 2011). Spindle receptors project these stretch and tension changes to afferent pathways, which relay the information back to alpha and gamma motor neurons, allowing a uniform contraction of the intrinsic and extrinsic muscle fibers (Sjolander et al., 1989; Zehr, 2002). As such it is likely that these mechanoreceptors, via their projections to the gamma motor system, contribute to the regulation of muscle stiffness surrounding the joint, and therefore the functional stability of the knee joint (Borsa et al., 1997; Krogsgaard et al., 2011). Work by Hagbarth et al. (1986) has also demonstrated that a properly functioning gamma motor system is required in order to for muscles to provide maximum voluntary contractions. Given the strong projection from ACL receptors to the gamma motor system, ACL injury therefore has the

potential to negatively impact both the gamma motor system and all of the muscle functions is helps to regulate.

Mechanoreceptors in the ACL also are stimulated by movement, tension and position change of the knee joint. These afferent pathways relaying information from the ACL are known to project to several supraspinal structures, and have been known to cause sensations of movements when stimulated (Sjolander et al., 2002). Alternatively, proprioceptive feedback may serve to update motor programs in the spinal cord, which control co-activation of muscles during motion. For example, dynamic contraction of the quadriceps can put strain exceeding failure on the ACL in the last 80 degrees of knee flexion before full extension is reached. Co-contraction of the hamstrings during this movement unloads the ACL and smooths' the motion (Krogsgaard et al., 2011). High performance athletes use an inhibited co-activation of the antagonist muscle to produce greater joint moments (Hagood et al., 1990). Through skill acquisition and proprioceptive feedback, trained athletes may reconfigure motor programs altering the co-contraction of the hamstrings to allow greater joint moments, even though it increases the risk of injury by placing greater loads on the ACL (Krogsgaard et al., 2011).

The ACL is not just a passive stabilizer, functioning to simply resist forces and prevent unwanted movement in the knee joint. The ACL also acts as a relay center, equipped with sensory mechanisms to provide the CNS with information regarding the speed, acceleration and change in motion of the knee joint. As a result, damage to the ligament can have an immense effect on the overall functioning of the knee joint. In addition to changes in joint mechanics, these effects can include alterations in knee

muscle function, which can subsequently lead to changes in the bone-on-bone forces the joint experiences. For these reasons, injury to the ACL can result in both short and long term deficits in muscle and joint function.

Acute & Chronic Effects of Injury

If the ACL is completely torn it is not capable of repairing itself. To address the resulting functional limitations and potential for relatively rapid onset of degenerative changes and development of osteoarthritis (Friel and Chu, 2013), surgical replacement of the ligament is a very common treatment option. As reviewed by Chambat et al. (2013) surgical intervention consists of replacing the torn ligament with a graft from the either the patellar or hamstring tendon or in some cases from a patient allograft. While the focus of this thesis will be on individuals who have undergone ACL reconstruction (ACLR), acute and chronic effects after both the initial injury and eventual surgical repair are similar and are described below.

ACL injuries are known to be accompanied by both acute and chronic symptoms. The duration and degree of symptoms is usually unique to each individual, mainly because of the variety of damage that may occur during the injury. Typical acute symptoms that follow an ACL injury include pain, swelling, bone marrow lesions, stiffness, a giving away feeling, and clicking or grinding of the knee during motion (Roos et al., 1998; Frobell et al., 2008). Swelling can be caused by bleeding or an increase in joint fluid and usually indicates a more severe tear. This swelling in turn creates stiffness of the joint restricting the range of motion. A giving away sensation is a common

complaint of individuals with ACL deficiency, and is in part, caused by lack of mechanical stability of the knee (Buss et al., 1995; Hasler et al., 1997; Roos et al., 1998; Lewek et al., 2002). Acute symptoms can also be experienced after ACLr. This surgery can lead to a temporary increase in joint fluid and bone marrow lesions, suggesting that an additional or longer rest period is needed after surgery compared to conservative non-operative rehabilitation (Chaudhari et al., 2008).

Even if surgical repair of the torn ligament is successful, there is potential for negative, long lasting chronic effects. One commonly reported deficit is weakness of the quadriceps muscle (Rosenberg et al., 1992; Lewek et al., 2002; Williams et al., 2005; Heroux & Tremblay, 2006; Hodges et al., 2009; Palmieri-Smith & Thomas, 2009; Rosenthal et al., 2009). In concert with this weakness, abnormal gait is evident in patients after ACLr (Lewek et al., 2002). During early stance, when the injured leg is accepting the weight of the body, decreased knee flexion and reduced internal knee extensor moments are often observed, in what is suggested to be an effort to limit anterior tibial translation (Rudolph et al., 1998; Lewek et al., 2002). Quadriceps contraction near full extension may create unwarranted anterior tibial translation, producing a sense of giving away (Lewek et al., 2002). Furthermore, changes in movement patterns can result in variations to loading applied to cartilage as well as other ligaments, increasing the likelihood of developing osteoarthritis (Hasler et al., 1997; Chaudhari et al., 2008; Friel and Chu, 2013). The risk of developing osteoarthritis following ACL injury/repair is almost 4 times as high as it is in individuals with healthy knees (Friel and Chu, 2013).

This is problematic when you consider that most individuals who sustain this injury are under 30 and are therefore likely to develop OA at a relatively young age.

The main long term consequence of an ACL injury that will be focused on in this study is persistent weakness of the quadriceps muscle. Two ways to assess quadriceps weakness are through force production and voluntary activation measures (Chmielewski et al., 2004; Keays et al., 2007). Quadriceps activation (QA) failure is clinically defined as any voluntary activation value less than 95% (Chmielewski et al., 2004; Hart et al., 2010). The prevalence of QA failure among subjects with ACL injury is inconsistent. A recent review of 99 subjects from multiple studies found occurrence rates of QA failure in ACLr knees ranging from 0 – 71% (Hart et al., 2010). Possible causes of this variance could be the type of graft used, extent of rehabilitation, as well as differences in methods used to assess QA failure.

Weakness of the quadriceps muscle varies from individual to individual and as a function of time since injury (Urbach et al., 2001; Andrade et al., 2002; Keays et al., 2007; Hart et al., 2010). The majority of studies examine subjects within approximately the first two years after surgery. Weakness of the quadriceps appears to be greatest in the first few months after surgery. Some studies have found strength deficits as high as 25% and 50% in the first 3 months when comparing the injured leg to the contralateral uninjured or a healthy control leg (Yasuda et al., 1992; Williams et al., 2005). Andrade et al. (2002) examined patients up to 8 months post-surgery. They discovered that during months 4 through 8, quadriceps peak torque consistently increased but was always lower than the uninjured limb. At the 8 month mark, patients displayed on average a 33%

deficiency in peak torque in their injured leg compared to their uninjured leg (Andrade et al., 2002). Similarly, significant torque deficits have been found in the injured leg in patients 18 months after surgery (Mattacola et al., 2002). One 6 year follow-up study (Keays et al., 2007) reported satisfactory strength outcomes in both bone-patellar-tendon-bone and hamstring tendon grafts. For both graft groups there were no statistically significant difference in strength when compared to a control group. Contrary to these findings Hiemstra et al. (2007) reported large and statistically significant strength deficits in a group of individuals who were an average of 40 months post ACLr. In a similar study, Krishnan & Williams (2011) examined quadriceps strength deficit and its correlation to voluntary muscle activation, hamstring moment and peripheral changes in the muscle. Subjects were 2-15 years post-ACLR providing valuable long term data on the effects of ACLr on muscle function. Substantial quadriceps strength deficits were found in 30% of the ACL group with the remaining individuals showing minimal levels of weakness. One reason these results show less evidence of chronic quadriceps weakness may be the large range of time that had passed between individuals' surgery and their participation in the study. As discussed above, quadriceps strength appears to increase over time post-ACLR – having large variability in time post-surgery would likely decrease evidence of strength changes in the cohort studied (Andrade et al., 2002). Although strength is reported to increase over time after ACLr, quadriceps weakness is still observed in patients' years after injury (Hart et al., 2010), warranting further research into the possible mechanisms underlying quadriceps weakness and how these mechanisms change over time.

Healthy-Limb Deficits

Following ACL injury and repair it is obvious that there would be functional deficits in the injured limb. Less obvious, however, is the possibility of the injury impacting non-injured limb function. In a general sense the non-injured limb has not been subjected to the trauma, swelling, pain related muscle inhibition and potential disuse of the injured limb. As such it would be expected to continue to function normally. Research findings, however, suggest that an ACL injury in one limb does affect the contralateral healthy-limb's function. Urbach et al. (2001) and Chmielewski et al. (2004) have reported that individuals who undergo ACLr exhibit decreased voluntary activation of the quadriceps muscle in their healthy-limb when compared to healthy controls. Urbach et al. (2001) tested a group of ACL injured patients before surgery as well as 2 years after ACL graft reconstruction was performed. Before surgical repair patients were found to have significant deficits in voluntary activation in both injured and healthy-limbs when compared to control subjects. Two years later voluntary activation in the injured limb and healthy-limb had increased, although remained less than that of the controls (Urbach et al., 2001). In a recent review, Hart et al. (2010) reported that 34.2% of ACL deficient patients, across ten studies, were found to have QA failure in the uninjured limb. Roberts et al. (2000), examined patients who had undergone unilateral ACLr to measure proprioception in both the injured and uninjured limbs. Results showed that patients with a unilateral reconstruction of the ACL had trouble detecting passive range of motion in

both the injured and uninjured limbs when compared to healthy controls (Roberts et al., 2000).

We have coined the term healthy-limb deficit (HLD) to describe these negative effects of unilateral injury on function in the non-injured limb. As such we define HLDs as decreases in joint or muscle function in the contralateral limb after a person has sustained a unilateral, joint or muscle injury. Similar to the injured limb, the contralateral healthy-limb experiences some deficit in function (i.e. strength, muscle activation, proprioception) when compared to controls. In contrast to the injured limb, the healthy-limb has no damage to joint structures making it difficult to understand why these deficits occur.

Although HLDs are commonly reported after ACL injury (Urbach et al., 1999; Konishi et al., 2003; Hiemstra et al., 2007; Konishi et al., 2007; Hart et al., 2010; Rice & McNair, 2010), deficits have also been reported following unilateral ankle sprain (Wikstrom et al., 2010), total knee arthroplasty (TKA) (Byrne et al., 2002; Milner, 2008), and in individuals with anterior knee pain (AKP) (Suter et al., 1998; Hart et al., 2010; Ingram et al., 2015). In a review of quadriceps voluntary activation literature, Hart et al. (2010) found reports of HLDs in patients with unilateral ACL deficient and reconstructed knees. Patients with ACL deficient knees were more likely to have QA failure (i.e. Central Activation Ratio < 95%) than patients who had undergone subsequent ACLr. This indicates an improvement with reconstructive surgery, although deficits in both injured and healthy-limbs were still found to be commonly reported after ACLr (Hart et al., 2010). In addition to ACL injuries, a recent review on postural control has shown deficits

bilaterally in subjects who had sustained a unilateral ankle sprain when compared to controls. Changes in proximal muscle activation along with strength deficits were reported, hypothesizing spinal and or supraspinal alterations as possible explanations behind the deficits (Wikstrom et al., 2010). Moreover, Byrne et al. (2002) reported that during a step up task, subjects who had recently undergone TKA in one limb were found to have substantial alterations in knee function of both surgical and non-surgical knee compared to age-matched controls. It was believed that the similarity was in part caused by bilateral changes in knee performance. HLDs have also been experienced in patients with AKP. Bilateral deficits in QA have been observed in patients suffering AKP in one limb (Suter et al., 1998; Hart et al., 2010; Ingram et al., 2015). HLDs can result in increased joint loading leading to possible increased risk of injury and the development of degenerative disease and early onset of osteoarthritis (Palmieri-Smith & Thomas, 2009). As such, a comprehensive understanding of the mechanism underlying their occurrence is essential if effective treatments are to be developed.

Causes of HLDs

Despite the evidence indicating their existence, there is relatively little research that has examined the underlying cause of HLDs. Such research is important as development of effective interventions aimed at improving healthy-limb function require a good understanding of their cause. Despite the lack of research in this area there are several factors that have been hypothesized to be related to the development of HLDs. These include disuse (Hiemstra et al., 2007), changes in supraspinal functioning triggered

by altered sensory feedback from the ACL (Urbach & Awiszus, 2002; Heroux and Tremblay, 2006), changes in spinal level function driven by lack of sensory feedback from the ACL (Roberts et al., 2000; Hiemstra et al., 2007; Konishi, 2011), or changes in either supraspinal or spinal function that are created due to the altered mechanics of the joint. These will now be reviewed in detail below.

ACL injuries mostly happen to active people who are involved in sport or leisure activities (Daniel et al., 1994). After injury this active population undergoes a period of restricted activity, creating a situation that is similar to the training-detraining model (Hiemstra et al., 2007). Detraining or disuse is known to cause decreases in strength after a period of training (Hakkinen et al., 1985). Furthermore, it is generally accepted that reduced activity can lead to significant reductions in maximal voluntary contraction, while total inactivity, which may occur after ACL injury, leads to rapid loss in muscle strength. Atrophy, a main cause of strength decreases, occurs most quickly in the lower limb muscles and also in the first few weeks of inactivity (Bruton, 2002). Therefore after ACLr and prior to rehabilitation, these patients may already display bilateral strength loss when compared to their pre-injury status or healthy controls (Hiemstra et al., 2007) simply due to inactivity. Although it is recognized that these inactivity deficits do exist, it is not agreed upon to what extent disuse affects strength deficits. Research by Snyder-Mackler et al. (1994) concluded that disuse and atrophy were unable to account for the entire decrease in quadriceps strength following ACL injury. In addition, rate of force loss due to disuse decreases over time and while patients usually return to some level of activity, eliminating the disuse factor, weakness of the quadriceps muscle persists for

months and even years, long after the disuse has ceased (Bruton, 2002; Hart et al., 2010). An additional argument against disuse being a primary factor is that in some instances there is enhancement of function seen in certain healthy-limb joints. For example, following knee replacement (Byrne et al., 2002), while the healthy-limb exhibits deficits in knee strength and range of motion, the hip joint on the healthy-limb side is observed to produce increased moments during functional tasks. If disuse were a primary cause then certainly all joints of the limb would be expected to show deficits.

When the ACL is completely torn, and requires surgery, the removal of the native ACL causes sensory signals from the ligament to no longer be conveyed to the spinal cord and gamma motor neurons (Krogsgaard et al., 2011). It is possible that these changes in sensory function may contribute to the existence of HLDs following ACL injury/repair. As a result of the change in sensory function, inhibitory afferent signals could be sent, via interneurons in the spinal cord, to the quadriceps femoris of the contralateral leg (Konishi et al., 2003). Cross-over effects to the contralateral leg have been demonstrated with unilateral training regimes, reflex inhibition, and prolonged vibration (Hiemstra et al., 2007; Konishi, 2011). During unilateral strength training, increase in force has been shown in the contralateral limb, a process called cross-education. This process is thought to occur due to a “spill-over” of neural drive that induces adaptations in the control system for the opposite limb, confirming that sensory signals in one limb are able to influence the functioning of the opposite limb (Carroll et al., 2006). Moreover, in cats it has been proven that the primary afferent spindle response of one limb can be modified by afferent feedback from the other limb (Appelberg et al., 1986). Similarly, in humans it

has been shown that passive or active movement in one limb triggers inhibition of the H-reflex in the contralateral limb (Collins, McIlroy & Brooke, 1993). While the above studies present evidence to suggest the possibility of altered sensory function on one side effecting function on the other the exact mechanisms through which this happens are not well understood.

One other possible mechanism that has received considerable attention as a possible factor underlying HLDs following ACL injury is the role of the gamma loop. In a recent study, Konishi et al. (2011) used prolonged patellar tendon vibration to show that bilateral dysfunction of the gamma loop existed in patients with unilateral ACLr. In this study, long duration vibration (20 minutes at 50 Hz) was applied to the patellar tendon of right and left limbs in those with unilateral ACLr. Normal response to long-term vibration is a reduction in force production post-vibration. In those with ACLr, Konishi et al. (2011) reported no drop in force, bilaterally, while healthy controls had bilateral force decrements. As this type of long-term vibration is thought to effect gamma motor neuron function, it was hypothesized by these authors that the lack of vibration effect indicates not only altered gamma loop function on the injured side but also on the healthy side. As the gamma loop is known to be integral to force production, this is one plausible explanation for HLDs following ACLr (Konishi et al., 2003). What is not known, however, are the pathways and mechanism through which the effect is passed to the contralateral limb.

Another possible mechanism that has been suggested is that functional changes in the healthy-limb may be centrally mediated by supraspinal systems such as the cortex

(Byrne et al., 2002; Konishi et al., 2003; Heroux & Tremblay, 2006). Somatosensory evoked potentials have been elicited from stimulation of an ACL graft, suggesting afferent information from ACL grafts are likely transmitted to supraspinal centers (Ochi et al., 2002). Research is limited regarding supraspinal influence on quadriceps function after injury, although studies have shown that pain and restrictions caused by bone and joint pathology can have large influences on supraspinal excitability (Zanette et al., 2004). Such research suggests that one possible mechanism underlying HLDs could be changes at the cortical level, likely triggered by the initial trauma of the injury or possibly residual sensory deficits. A recent study (Heroux & Tremblay, 2006), examined changes in spinal and supraspinal excitability associated with unilateral knee dysfunction secondary to ACL injury. Transcranial magnetic stimulation (TMS) was used to assess supraspinal excitability of the quadriceps. Asymmetries between limbs of the ACL participants were found for resting motor thresholds (RMT), which were significantly reduced in the injured limb. Since motor thresholds are relatively symmetrical between limbs in a healthy population, the lower RMTs found in the Heroux study reflect an increase in supraspinal excitability (lower RMTs mean the cells are more easily excited i.e. higher excitability). In addition to RMTs, motor evoked potential (MEP) stimulus response curves (SRC) were also used by Heroux and Tremblay (2006) as a measure of supraspinal excitability. A strong association was found between the steepness of the SRC and individual variations in strength in the injured limb. The steepness of the SRC indicates the strength of supraspinal projections, with greater slopes reflecting greater projections to a given motoneuron pool. The inconsistencies reported in the steepness of

the SRCs between limbs in the ACL subjects may resemble the variability also found in the prevalence and magnitude of QA failure commonly reported in the literature (Yasuda et al., 1992; Williams et al., 2005; Heroux & Tremblay, 2006; Hart et al., 2010). Heroux & Tremblay (2006) concluded that the decrease in RMT was likely occurring at the supraspinal level and that these changes were likely due to the alterations in sensory feedback, caused by the injury. Although these results suggest that sensory deficits are impacting supraspinal excitability and may lead to an explanation for HLDs following ACLr, there were several weaknesses in the study that make interpretation of the results challenging. These weaknesses include the following:

- 1) Limb dominance was not accounted for, therefore increasing potential variability within groups because motor performance has been shown to be different in dominant limbs compared to non-dominant limbs (Lanshammar & Ribom, 2011).
- 2) No measurement of maximal M-wave. In order to account for changes at the muscle level that may occur throughout the study, MEPs and H-reflex measures must be analyzed relative to a maximal M-wave (Zehr, 2002).
- 3) The use of two separate TMS coils – half the ACL group was stimulated with a circular coil while the rest were stimulated with a more efficient double cone coil. This decreased their statistical power by increasing the inter group variability of the TMS outcome measures.

Based on the literature review above, there is no clear understanding as to what causes HLDs. Answering this question is of important from both a basic and applied science perspective. For clinicians working with individuals post ACLr, an understanding

of the causes of HLDs is integral to developing effective and efficient interventions to optimize healthy-limb function. From a basic science perspective, improved understanding of the factors contributing to HLDs will further the understanding of the inter-relatedness of bilateral limb control. As such the present thesis will attempt to better understand this issue by examining the possible role of supraspinal and spinal excitability. This will be done by replicating the Heroux & Tremblay (2006) study described above and addressing the methodological issues identified in this study. As such the proposed research will ask the following research question:

Is supraspinal and spinal excitability altered bilaterally following unilateral ACL reconstruction?

Methods

Participants

Fourteen 14 participants were recruited from Memorial University and the surrounding community of St. John's, Newfoundland. Participants were recruited as part of either an ACLr group (7 participants, mean age: 29 +/- 8 years, 4 females) or a Healthy control group (7 participants, mean age: 29 +/- 8 years, 4 females). Participant age, weight, limb dominance, and date of surgery were recorded. Limb dominance was determined by asking the participant which foot they preferred to use to kick a soccer ball as hard as possible (Lagerquist et al., 2012). Members of the ACLr group were eligible for the study if they had undergone unilateral ACL reconstructive surgery at least one year prior to the testing date. Type of graft and method of surgery was not taken into account for this particular study. The Healthy control group was matched to the ACLr group for gender and age (+/- 5 yrs). Participants were excluded from the study entirely if they had any major non-ACL injuries to the lower limbs, bilateral ACL surgery or if the surgery to repair a unilateral ACL injury was within the 12 months prior to testing. In addition, all participants were clear of any known neurological or muscular disorders; were not taking medication that altered neurological functioning; had no history of brain/cranial surgery, migraines, seizures or concussions in the last year. Informed written consent was obtained from all participants before any testing was conducted. Full ethics

clearance for this study was approved by the Interdisciplinary Committee on Ethics in Human Research.

Summary of Experimental Protocol

All measurements collected during this study were done bilaterally. First, each participant's knee extensor strength was measured by having them perform maximum voluntary contractions (MVC). Following the strength assessment, two forms of stimulation were completed. TMS of the motor cortex was used to determine supraspinal excitability, and electrical stimulation of the femoral nerve was used to evaluate spinal excitability and maximal muscle responses. Participants in the study came to the lab on two separate days. Session one was a familiarization day that was used to introduce participants to the study protocol and testing procedures. TMS and femoral nerve stimulation were described and then a sample of each was performed on the participants. A range of intensities were used representing similar sensations that would be experienced during the second testing session. No data was collected during this session. During the second session, which lasted approximately 2 hours, all data collection took place. The details of this collection are described below.

Quadriceps Strength Assessment

Quadriceps strength was assessed by having participants perform MVCs. When performing MVCs participants were seated in a custom chair, with knees and hips flexed

at approximately 90 degrees. A load cell (Omega engineering Inc., LCCA 250, Don Mills, Ontario) was connected to the ankle using a padded ankle strap and a small chain. Force data were recorded for 7 -10 seconds and, with MVCs typically lasting 5 seconds, amplified (x1000) (CED 1902) and displayed on a computer screen. All force data was collected at 5kHz. The contralateral limb was at rest with a knee angle of 90 degrees during all strength testing. Harnesses over both the shoulders and around the waist were used to prevent unwanted body movement during MVCs (See Figure 1).

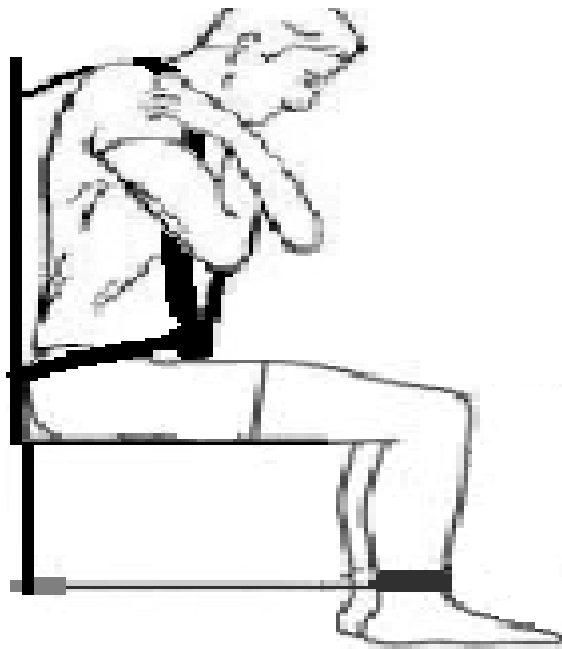


Figure 1: Position used for collection of knee extension MVCs. Subjects were secured to seat with a belt. A padded strap, attached to a load cell, was placed around the angle to record knee extensor force. See text for further details.

Quadriceps strength for all participants was measured bilaterally. Prior to performing MVCs, all participants warmed up on a stationary bike for 5 minutes at a speed between 55-65 RPM. Once seated in the custom recording chair, participants prepared for the MVC by completing 5 submaximal contractions at a self-perceived 50%

effort. Prior to each MVC, participants were instructed to extend their knee as hard and as fast as possible while having their arms crossed over chest and attempting to minimize the movement of the contralateral lower limb. Strong verbal encouragement was given during all MVC trials. The participants performed at least two MVCs with each limb and an MVC was considered maximal if the forces in two successive trials were within 5% of each other. If the difference between two successive MVCs was greater than five percent, additional MVCs were performed. To remove any effect of fatigue, at least a two minute rest was given between MVCs. In order to isolate the potential neurological effects of the MVC on subsequent measurements, participants rested for 15 minutes following their last MVC prior to proceeding with the remainder of the testing (Gandevia et al., 1999).

Stimulation Protocols

Following strength testing, supraspinal excitability and spinal excitability were assessed using TMS and H-reflex techniques, respectively. Supraspinal excitability was measured while participants were lying in a supine position on a table with bilateral shank and foot suspended off the end of the table (See Figure 2). In this position the knees were flexed to roughly 90 degrees and a pillow was placed under the thighs to reduce the amount of stress placed on the lower back. A padded strap was placed around the ankle, which was attached to a wall by a long chain. This setup allowed the leg to be restrained during stimulation. H-reflexes were assessed with participants in a fully supine position having their entire body on the bed (See Figure 3). In both these positions participants had a small pillow supporting their head and while testing occurred they were asked to have

arms folded comfortably across their chest and to close their eyes. Ideally the position used for the TMS and H-reflex testing should have been the same to reduce any effects of position on results. This was not possible however. The position used during TMS (Figure 2) was chosen so as to restrain the limb to limit jerking movements during stimulation. Secondly, while performing stimulation during a resting state was our main priority, at the outset of testing it was determined that a small active contraction would be used for individuals where MEPs could not be elicited at rest (NOTE: the option of active contractions during TMS did not end up being used for any subjects). To enable both

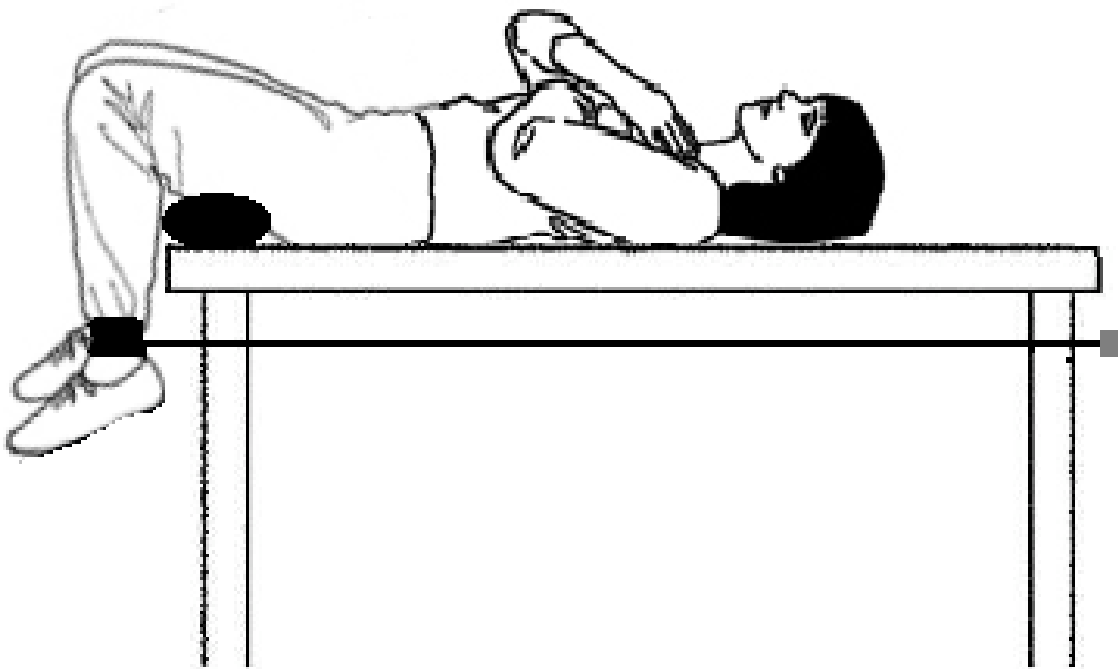


Figure 2: Positioning during TMS testing. Subject's testing limb was secured using padded ankle strap that was attached to a load cell to allow force measurements. The tested limb hung freely while the contralateral limb was supported on a low stool for comfort. See text for further details.

restraint and the measurement of an active contraction the knee had to be flexed over the edge of the bed and affixed to a strain gauge. Unfortunately pilot testing using the H-reflex protocol revealed that the optimal position to elicit H-reflexes was fully supine

(Figure 3). As such this position was used for H-reflex testing. These separate positions were viewed as suitable due to the structure of the data analysis (i.e. only comparing spinal measures to spinal measures and supraspinal measures to supraspinal measures).

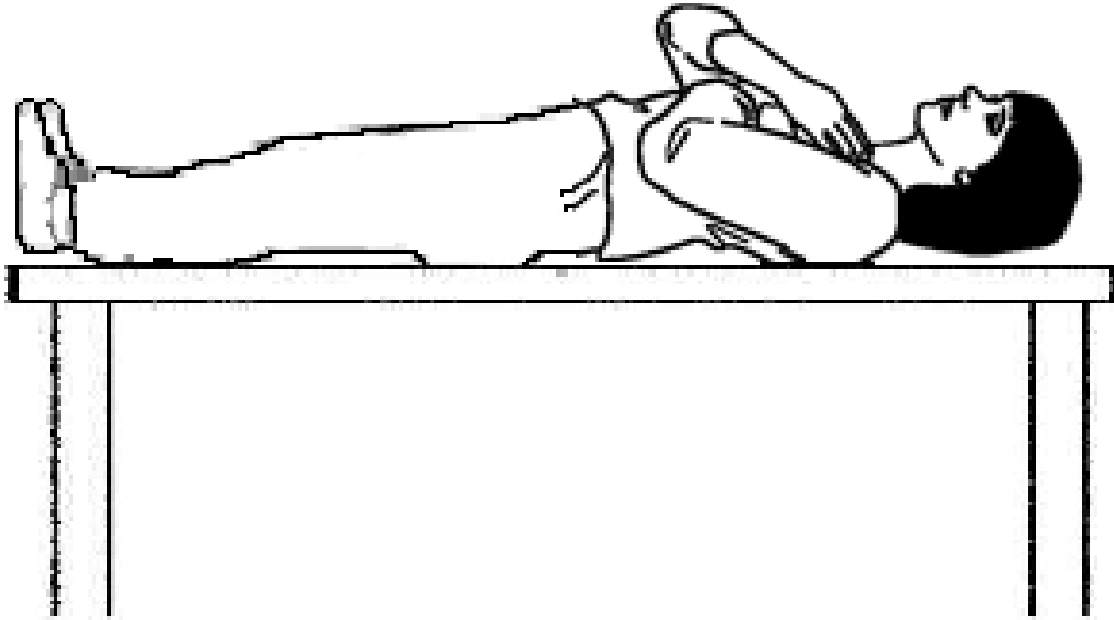


Figure 3: Illustration of position used to assess H-reflex amplitude. See text for further details.

The order in which the TMS and H-reflex testing occurred, along with limb order for each measurement was randomized for every participant. To evaluate both supraspinal and spinal excitability, electromyography (EMG) was recorded using surface electrodes (Ag/AgCl, Covidien, Kendall, Masfield, MA., USA) placed 1cm apart on the skin over the muscles. EMG was recorded bilaterally from vastus medialis (VM). Skin was prepped by shaving the area, abrading with sandpaper and then cleaning the skin with an alcohol swab. Electrodes for VM were placed on the muscle belly at a point 4cm above the superior-medial point of the patella with an approximate angle of 55 degrees to the longitudinal axis of the femur (Wong et al., 2006). In order to deliver stimulation to the

femoral nerve, two EMG electrodes (Ag/AgCl, Covidien, Kendall, electrodes, disc shaped and 10 mm in diameter, Masfield, MA., USA) were applied – one directly over the femoral nerve and one over the middle portion of the buttock on the ipsilateral side (Heroux & Tremblay, 2006). As stated above M-wave and H-reflexes were both recorded bilaterally. These were both acquired through stimulation of the femoral nerve. To locate the femoral nerve a researcher palpated along the inguinal crease until the pulse of the femoral artery was found. A non-permanent marker was used to mark the femoral nerve which was 1 -2 cm medial to the femoral artery (Heroux & Tremblay, 2006). Once the mark was placed, a stimulating wand was used to stimulate the area around the mark to find the optimal location (i.e. maximal amplitude M-wave) for producing H-reflexes. Once an optimal location was established an Ag/AgCl stimulating electrode was placed in the identified location. This electrode was used for the remaining femoral nerve stimulations delivered throughout the study. Once all electrodes were in place the simulation protocols were performed. All femoral nerve stimulations were delivered using a constant current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, UK). An interelectrode impedance of <5 kOhms was achieved prior to recording to ensure an acceptable signal-to-noise ratio. EMG signals were amplified ($\times 1000$) (CED 1902, Cambridge Electronic Design Ltd., Cambridge, UK)) and filtered using a 3-pole Butterworth filter with cut-off frequencies of 10–1000 Hz. All signals were analog-digitally converted at a sampling rate of 5 kHz using a CED 1401 (Cambridge Electronic Design Ltd., Cambridge, UK) interface.

M-wave Protocol

A square wave stimulus, with 0.2 ms pulse duration, was applied to the femoral nerve to elicit a maximal M-wave response in VM. The resulting signal, known as Mmax, was found by increasing stimulus intensity until no further increase in peak to peak amplitude could be produced. The average peak to peak value of three maximal muscle responses was recorded as Mmax and used for analysis. Mmax magnitudes are known to vary substantially between two positions or simply with time even in the absence of movement (Crone et al. 1999). Therefore Mmax measurements were elicited for each limb pre and post supraspinal and H-reflex testing for a total of 8 Mmax measurements recorded during the testing, 4 for each limb (Figure 4). As changes in both MEPs and or H-reflexes can be influenced by changes at the peripheral level (i.e., muscle) (Zehr, 2002) all MEP and H-reflex measurements were normalized to Mmax.

H-reflex Protocol

H-reflex responses evoked at rest were used as a way of assessing spinal level excitability. For this part of the testing participants lay fully supine as described above (Figure 3). Electrical stimulation, using a 1ms pulse duration, of the femoral nerve (see stimulations protocol for exact location) was used to elicit H-reflexes. To begin H-reflex testing, stimulation intensity was initially increased in 5mA intervals beginning at an intensity of 5mA until an H-reflex was visualized. Intensity was then decreased in 2mA intervals until the H-reflex disappeared. Starting from the point where the reflex disappeared, stimulus intensity was again increased in 1mA intervals until the H-reflex reappeared. The intensity at which the H-reflex re-appeared was defined as H-threshold.

Data to construct the H-reflex SRC were then collected by starting at a stimulation intensity that was 2mA below H-threshold. Intensity was then increased in 2mA intervals and concluded when the H-reflex peak to peak amplitude started to diminish with an increase in the M-wave being present or until the participant could no longer tolerate stimulations. Eight stimulations, at each intensity, were given at random, separated by at least 5ms in order to avoid post activation depression (Zehr, 2002). The majority of participants received 7 – 10 sets of 8 stimulations for each limb which were used to construct each SRC. H-reflex recordings from the 8 stimulations were averaged in real time to provide an average H-reflex response at each stimulation intensity.

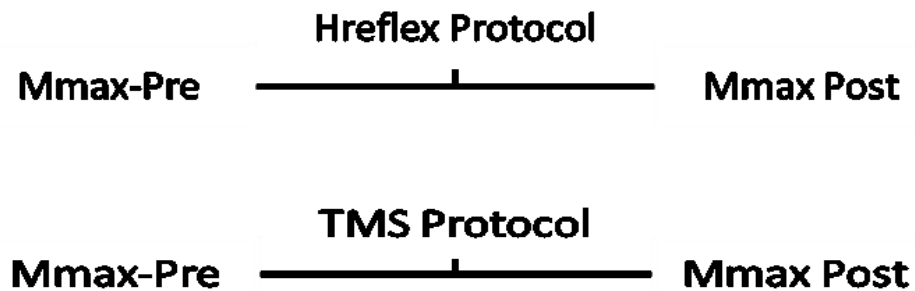


Figure 4: Example of stimulation protocol for one limb. Subjects were in different positions for H-reflex and TMS protocols.

Supraspinal Excitability

TMS was used to quantify supraspinal excitability. Two primary measures were used – RMT and MEP SRC. A double cone coil attached to a Magstim 200 stimulator (Magstim, Dyfed, UK) was used for all stimulations. As consistent coil position is

essential for accurate TMS results, participants were asked and consistently reminded to try and avoid any head movements during TMS testing to ensure coil placement remained the same. Furthermore, the same researcher held the coil for the entire study to increase consistency. As the noise the TMS coil creates can become quite loud and unpleasant due to proximity to the ears participants were given earplugs to wear during this portion of the testing to increase the comfort level.

RMT

To begin, the vertex of the participant was identified. This was done by locating two points: One at the midpoint of a line between the nasion and inion and another at the midpoint of a line between the left and right preauricular points. Small dots were placed at these locations on the skull with a marker. The vertex was defined as the intersection of these two points following their respected lines (Beam et al., 2009). Once the vertex was located and visibly marked on the skull, the researcher placed the coil over the marked area. Stimulation intensity was started at an intensity that was 50% of the maximal stimulator output (MSO). From this MSO, stimulation was increased by 10% MSO increments until visible MEPs could be evoked. Once visible MEPs were achieved, the center of the coil was moved in 1cm increments away from the vertex, in both the anterior-posterior or medial-lateral directions (Cai et al., 2012) in an effort to determine a location where the largest peak to peak amplitude MEP was produced. Once this location was found it was used for the remainder of the stimulations. The stimulation intensity was then lowered in 5% increments until no further MEP was produced. This was followed by

1% increases in stimulator intensity until the RMT was established. For the present study RMT was defined as the % MSO at which a MEP of greater than or equal to $50\mu\text{V}$, was produced in 4 out of 8 trials. The RMTs were manually recorded during testing for later analysis.

Stimulus Response Curves

Data required to create the SRC curve was collected once the RMT was established. In order to generate a SRC it was essential that stimulation begin at an intensity that was below the RMT to ensure the MEP at the RMT was included in the curve. To ensure this happened stimulus intensity was initially set to a value that equalled $(\text{RMT} - 0.05*\text{RMT})$. Intensity was then increased in intervals equal to 5% of RMT until no further augmentation was seen in MEP amplitude. For example if RMT was at 80% MSO, then the SRC data collection began at 76% MSO and increased by 4% each interval. Participants received 5 stimulations at each intensity, separated by 5.6 – 8.6 seconds. The time delay was programed to vary randomly within 20% of 7 seconds to decrease predictability for the subjects. Results from the 5 stimulations were averaged in real-time by the Signal 4 software.

Data Analysis

Force Measurements

The force data from the final 2 MVC trials from each limb was examined to determine the highest force attained. This force was evaluated for quadriceps strength differences between limbs and between ACL and healthy subjects. A Strength Index was

used to compare strength between limbs of each individual subject. This index used the following formula: (Injured limb force (kg) / Uninjured limb force (kg)) *100 (As per Fitzgerald et al., 2000).

H-reflex

To assess spinal excitability, SRC slopes were determined for the right and left limbs of each participant. H-reflex measurements were manually extracted from Signal into Excel 2010 (Microsoft Corporation, Redmond, WA, USA). In Excel, the SRCs were created by plotting normalized H-reflex amplitudes against the current used to evoke the response. Slopes were calculated from the ascending portion of each curve using a linear

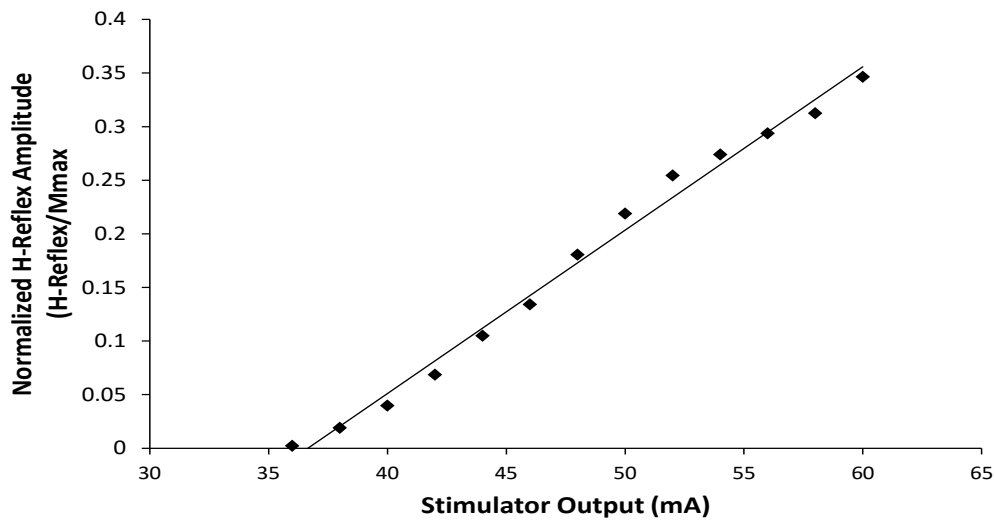


Figure 5: Example of the linear trend line used to determine the slope of the SRC for H-reflex data points. H-reflex magnitude, normalized to Mmax is plotted as a function of stimulation intensity. Data is from a healthy participant. See methods for more information regarding SRC protocol.

trend line (Figure 5), which was fitted to the curve in between points at H-min and H-max (as per Piscione et al., 2012). All trend-lines and slopes were recorded and entered manually in excel for graphing and comparison purposes.

TMS

To assess possible differences in the supraspinal excitability of VM between limbs and between subjects, both VM RMT and MEP SRC slopes were determined bilaterally for all participants. The RMT measures, presented as a percentage of MSO were compared between limbs and between subject groups. The MEP peak to peak amplitudes at each stimulation intensity were extracted manually into Excel after testing was

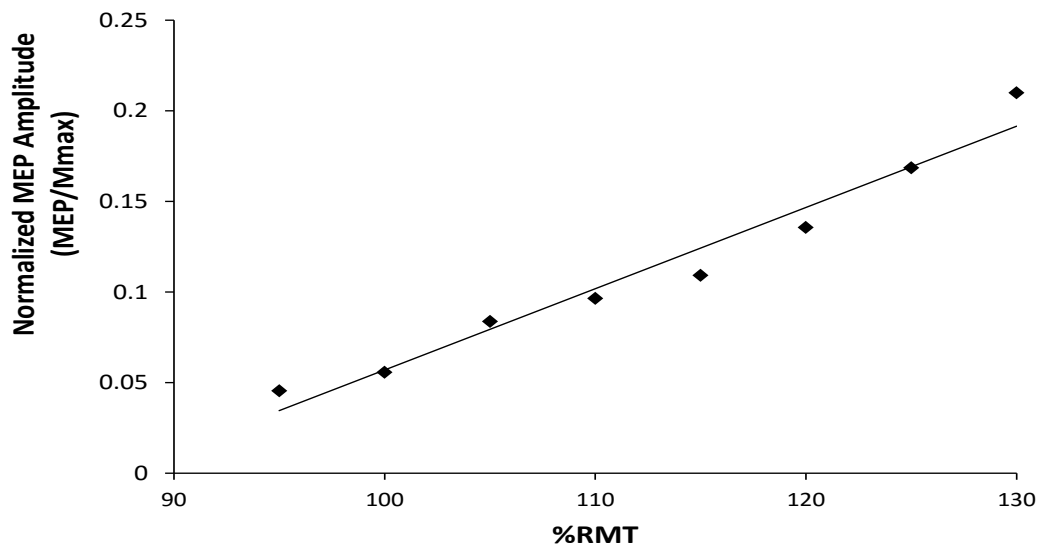


Figure 6: Example of linear line of fit used to determine the slope of the SRC, based on MEP data points on the ascending portion of curve. Stimulation magnitude, reported on the horizontal axis, was delivered at a magnitude that was a percentage of RMT. Data is from a healthy participant. See methods for further details regarding SRC.

completed. The SRCs were then created offline in Excel by plotting the normalized MEP amplitudes against the stimulus intensity used to elicit the response. The slope of the ascending limb of the SRC was found using a linear trend line that was fitted using points on the curve from 95% through to 130% of RMT (Figure 6) (as per Rosenkratz et al. 2014).

Statistical Analysis

Statistical analyses were completed using SPSS software (SPSS 18.0, IBM Corporation, Armonk, New York, USA). Assumptions of homogeneity (Box's test) and sphericity (Mauchly test) were tested and met for all dependent variables. A mixed factorial ANOVA was performed on all dependent variables to examine both within group and between group differences. The Cohen's d effect sizes (Cohen, 1988), were also calculated to compare differences in MVC, RMT averages, MEP SRC, and H-reflex SRC between limb groups. Figures and tables include absolute values and are presented as mean +/- standard error.

As a key purpose of the present research was to determine whether HLD deficits existed in the ACLr group it was necessary to compare both limbs of the ACLr group to the limbs of control participants. As such the following groups were defined. The limbs of members of the ACLr group were classified as being either injured (ACLi) or control (ACLc). The ACLi limbs were those that had undergone ACLr while the ACLc limbs were the non-surgical (or healthy) limbs of the ACLr participants. Members of the Healthy control group were matched to ACLr participants on the basis of gender and age.

Prior to data analysis a further matching was done on the basis of limb dominance. To facilitate this matching the limb dominance of the ACLi limbs was determined. This number was then used to create two limb groups in the healthy group – a Healthy injured group (Healthyi) and a Healthy control group (Healthyc). The Healthyi group had the same limb dominance characteristics as the ACLi group. Similarly the Healthyc group had the same limb dominance characteristics as the ACLc group. For example, if an ACL subject had ACLr on their dominant limb, then the healthy subject who was matched to that ACL subject by age and gender would have their dominant limb entered in the Healthyi group and their non-dominant limb classified as Healthyc. Similarly if an ACLr

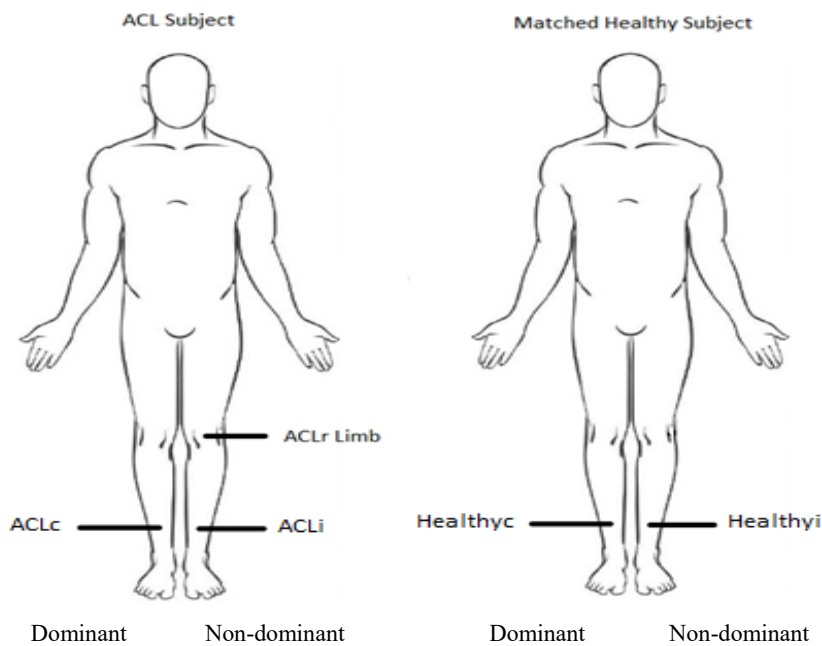


Figure 7: Figure illustrating group nomenclature used for the study. In the case shown above ACLi was the non-dominant limb of the ACL subject so the non-dominant limb of the age and gender matched Healthy participant was assigned to the Healthyi group. Similarly, because the dominant limb of the ACLr participants was ACLc the dominant limb of the age and gender matched control was assigned to the Healthyc group.

participant had ACLr on their non-dominant limb, then the health subject who matched that ACL subject would have their non-dominant limb classified as Healthy_i and their dominant limb would be considered Healthy_c. Matching the groups in this manner ensured limb dominance did not affect outcomes for spinal and supraspinal measures. A figure illustrating how participant grouping was done is provided in Figure 7.

Results

Demographics

Group demographics can be seen in Table 1. ACL subjects, on average weighed more than the Healthy control group. All subjects were right limb dominant, with 5 subjects having ACL reconstruction performed on their dominant limb and 2 on their non-dominant limb. The average time since surgery was 93.9 months, with a range of 23 to 276 months.

Table 1: Demographic data taken for ACL and healthy groups. Strength index is also included in this table. See statistical analysis for calculation details. Age, Weight, Time Since Injury and Strength Index are indicated as mean (+/- SD).

Group	ACLr (n=7)	Healthy controls (n=7)
Age (yr)	30.0 (+/- 8.6)	29.86 (+/- 7.4)
Weight (kg)	81.676 (+/- 10.49)	67.857 (+/- 10.45)
Limb Dominance	7 Right	7 Right
Gender	4 Females, 3 Males	4 Females, 3 Males
Injured Limb	5 Non-Dominant, 2 Dominant	5 Non-Dominant, 2 Dominant
Time Since Surgery (mo)	93.9 (+/- 86.0)	N/A
Strength Index (%)	82.8 (+/- 12.2)	99.7 (+/- 17.4)

Quadriceps Strength Measures

Strength outcomes from the MVC trials are represented in Figure 8. There was a significant main effect of limb ($F(1,12) = 0.426, p < .05$) with MVCs in the uninjured limbs ($M = 60.06, SD = 19.95$) being greater than injured limbs ($M = 55.32, SD = 21.40$) irrespective of group. In addition, there was also a significant interaction effect between limbs and group ($F(1,12) = 0.476, p < .01$). Post-hoc testing revealed that this interaction effect was driven by the fact that the healthy-limbs of the ACL participants were significantly stronger than their injured limbs. Post-hoc testing of this interaction revealed

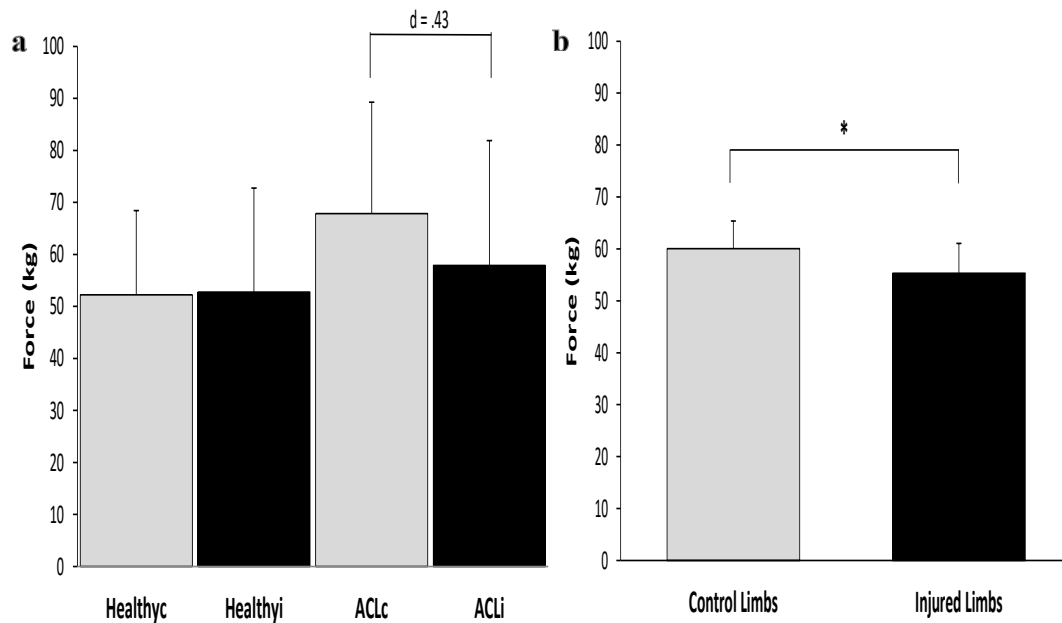


Figure 8: a) Average MVCs in kilograms, grouped by limb. See text for description of limb groupings. b) Knee extensor force collapsed across groups and indicating that uninjured limbs were, on average, stronger than injured limbs. * indicates a significant main effect of limb. Cohen's $d=0.43$ is also shown for the comparison of control and injured limbs in the ACL group.

no other significant differences (Figure 8a). Furthermore, Cohens d was used to interpret the magnitude of the effects in addition to the ANOVA outcomes. The Cohens d for the comparison of ACLi limbs to ACLc limbs was 0.43, indicating a moderate effect of the ACL repair on knee extensor strength. Irrespective of subject group, when comparing

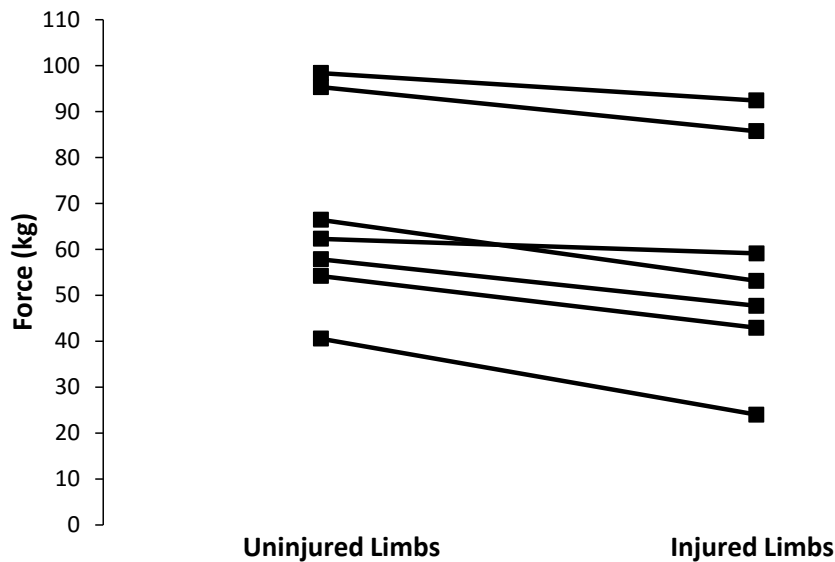


Figure 9: MVC outcomes from all ACL subjects. This figure provides a visualization of the strength index for each ACL subject. Each point represents the MVC for either the uninjured or injured limb.

strength in injured versus control limbs, Cohens effect size ($d=.03$) reflected a small effect size between the two groups (Cohen, 1988). Strength index calculations (Table 1) revealed that ACL subjects had, on average, greater asymmetry in strength measures between limbs than matched Healthy subjects.

RMT

RMTs were obtained in 7 healthy and 6 ACLr participants. In one ACLr subject, measureable MEPs under 100% MSO were unattainable. RMT averages are displayed in Figure 10. Visually, the RMT averages from both limbs of the Healthy group were similar, while the ACL subjects seemed to have slightly higher thresholds in the injured limb than in the uninjured limb. In addition, RMTs from injured and uninjured limbs resulted in no statistically significant differences found for either the main effect of limb ($F_{(1,11)} = .196, P = .667$), or the interaction effect ($F_{(1,11)} = 2.825, P = .121$). Despite this lack of statistical significance, Cohens d effect sizes showed large effects between ACLi and Healthyi limbs ($d = 1.12$) as well as between ACLc and Healthyc limbs ($d = .62$).

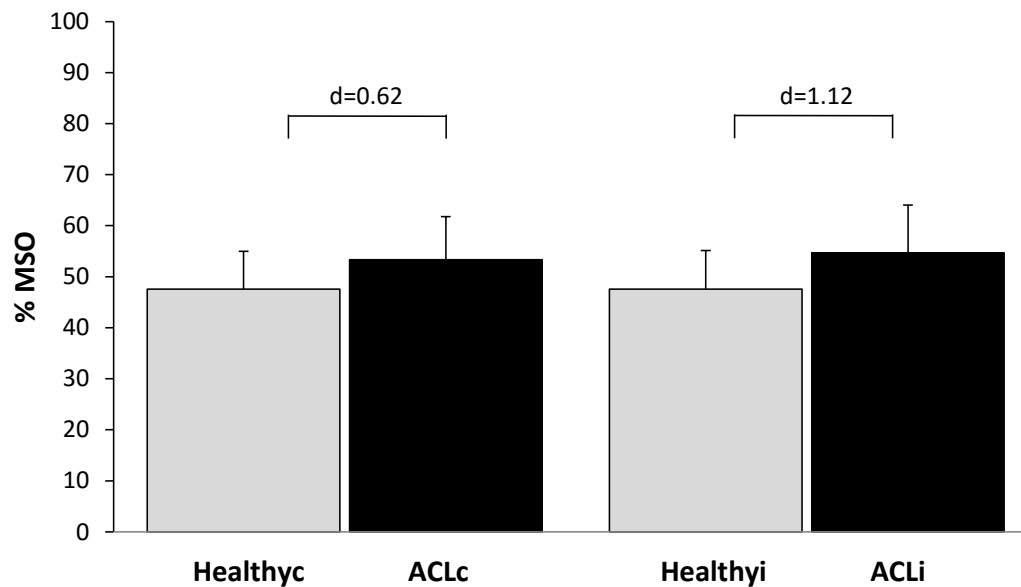


Figure 10: The RMT average magnitudes as a percentage of MSO. No statistically significant differences were found however large effect sizes were present and are indicated using the Cohen's d values indicated in the figure.

These results suggest that although the parametric analysis did not find a significant difference between groups, there appears to be a large effect of ACL reconstruction on RMT in the participants examined for this study.

MEP Slopes

A sample MEP profile from a typical participant is provided in Figure 11.

Recordable MEPs and therefore SRCs were able to be produced for 7 healthy subjects and 6 ACL subjects. As was the case with the RMT measures, there was no main effect of limb ($F_{(1,11)} = 1.242$, $P = .289$) and no interaction effects ($F_{(1,11)} = .094$, $P = .765$) for the MEP slope measures. Cohen's d analysis of this measure revealed only small effect sizes, also supporting the notion of similarities in MEP slope between the two subject groups. Slope averages for all participants are displayed in Figure 12.

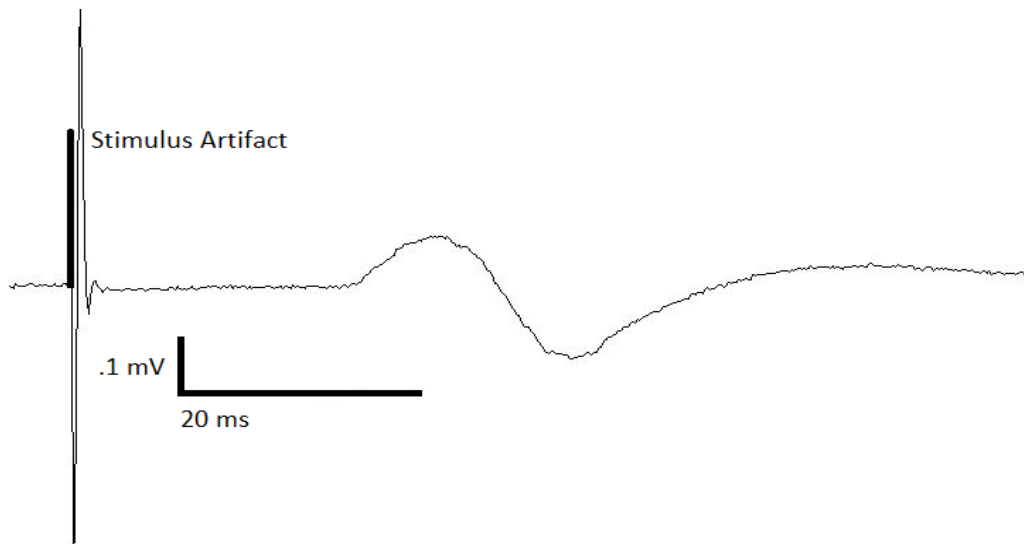


Figure 11: Sample raw MEP response recorded from the quadriceps muscle (VM) of one healthy subject during TMS trials. The stimulus artifact is indicated in the figure followed by a typical MEP.

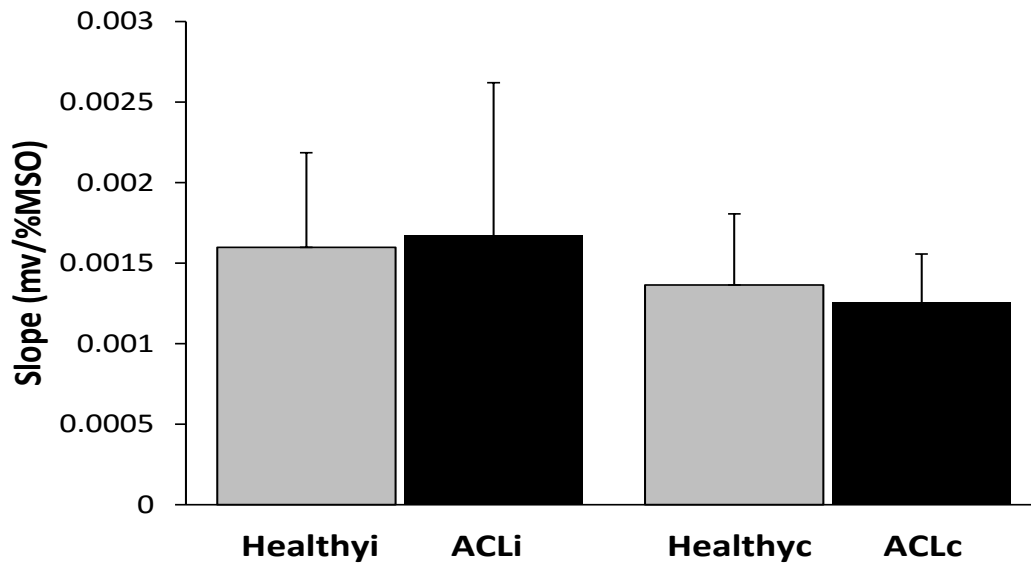


Figure 12: Average MEP slopes for all groups examined. The MEP slope is normalized to %MSO. There were no statistically significant differences between the groups. Effect sizes were also small.

H-Reflex Slopes

A sample H-reflex curve from a typical subject is provided in Figure 13, Measureable H-reflexes were only able to be elicited in 5 healthy and 6 ACLr participants. Distribution of H-reflex slopes can be found in Figure 14. There were no significant main effect of limb ($F_{(1,9)} = 2.519, P = .147$) and no interaction effect between groups or limbs ($F_{(1,9)} = 4.417, P = .065$). Examination of the effect sizes for these results showed small ($d=0.33$) and large ($d=1.16$) effect sizes for comparisons of ACLi vs Healthyi limbs and ACLc vs Healthyc limbs, respectively. This shows us that a possible effect of limb and group exists on H-reflex slope outcomes, despite the fact that the parametric analysis did not reach significance.

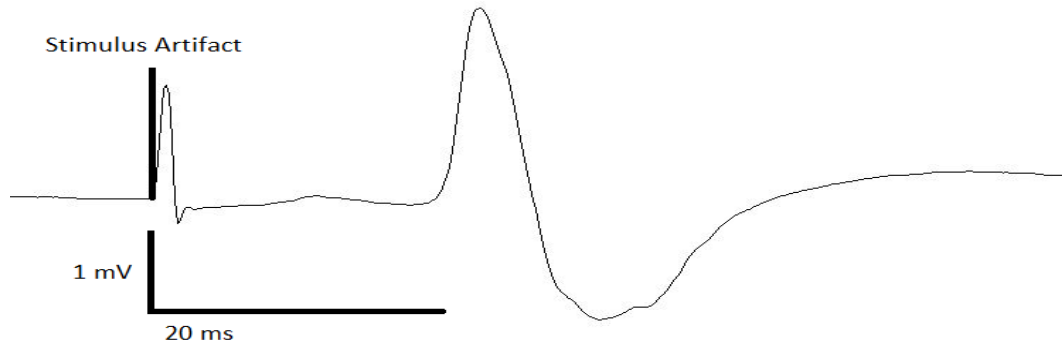


Figure 13: Sample raw H-reflex data taken from one healthy subject during testing. Stimulus intensity was low enough to not produce an accompanying M-wave. The stimulus artifact is indicated in the figure, followed by the H-reflex. Latency of reflex was consistently between 16-20ms.

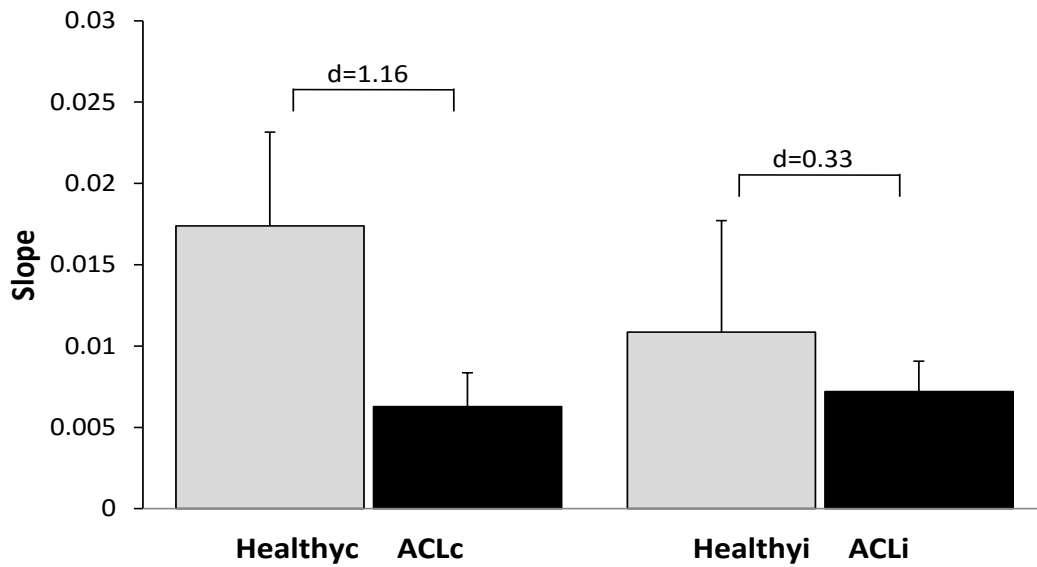


Figure 14: H-reflex SRC slopes displayed as group averages. There were no significant differences between the groups. Effect sizes, as indicated by Cohen's d values are also illustrated on the figure.

Discussion

This study was designed to examine spinal and supraspinal mechanisms underlying HLD which have been shown to occur, post unilateral ACL injury (Konishi et al., 2007; Hart et al., 2010; Rice & McNair, 2010). Despite examining a group of patients known to exhibit these deficits, strength measures indicated no HLDs. Given the lack of HLD found in our patient population, the original research questions were modified to enable us to more broadly explore the data and examine more general effects of ACLr on spinal and supraspinal excitability bilaterally. Data indicated that ACLr did not affect MEP gain, H-reflex gain and RMTs. This was likely due to small sample sizes ($n \leq 7$). However, large effect sizes found between limbs for H-reflex gain and RMTs suggest that ACLr did have an effect on neurological function in the participants examined.

In this study, injured limbs showed lower knee extensor strength compared to uninjured limbs (Figure 8b). These strength measures were important as they helped to more clearly describe the participant characteristics, although the inclusion of TMS (RMT and MEP Slopes) and H-reflex measures is what allowed us to further analyze and possibly isolate changes occurring at both supraspinal and spinal levels, respectively. The RMT measurements indicated that ACL subjects had lower levels of supraspinal excitability bilaterally compared to Healthy controls, while MEP slopes showed no differences between groups or limbs (Figure 10 and 12, respectively). H-reflex responses showed a tendency towards lower levels of spinal excitability, although this effect was not confirmed bilaterally and was only present in the uninjured limbs of the ACL group

(Figure 14). The discussion that follows will examine in detail these changes at the spinal and supraspinal levels in an effort to better understand the effects of ACLr.

Supraspinal Excitability

One focus of the present study was to explore the idea that changes in excitability at the supraspinal level occur following ACL reconstruction and that these excitability changes may be a possible mechanism contributing to strength deficits after ACLr. To examine supraspinal excitability, RMT and MEP SRCs were assessed. The following sections aim to interpret the RMT and MEP SRC outcomes as a means to identify possible supraspinal adaptations in relation to the strength deficits observed in this population.

RMT

Statistical analysis of the RMT averages (Figure 10) showed no significant differences across groups and limbs, although further inspection of the effect sizes suggested a large effect between limbs. Effect sizes were large and were found bilaterally when comparing ACL subject limbs to matched healthy subject limbs. This indicates the possibility that unilateral ACLr had a large effect on the RMT of the VM muscle bilaterally. RMTs were higher in the ACLr group than in the Healthy group, reflecting lower levels of supraspinal excitability due to the fact that higher stimulator outputs were required to achieve the same level or amplitude of response. Since both ACLi and ACLc

groups had higher RMT averages and large effect sizes were found, it is possible the ACL injury created a bilateral decrease in the level of supraspinal excitability for VM.

There is limited research examining supraspinal excitability measures after unilateral ACL injury (Heroux & Tremblay, 2006; Pietrosimone et al., 2013; Kuenze et al., 2015, Pietrosimone et al., 2015). Heroux and Tremblay (2006) are the only authors, to our knowledge, that have used RMT as a means to examine the effects of an ACL injury on supraspinal excitability. In contrast to the present study, however, they examined individuals who had suffered an ACL injury but had not undergone an ACLr. They demonstrated that unilateral ACL injury resulted in a lower RMT and therefore increased excitability in the injured limb. They suggested that increased excitability of the injured limb could be a coping mechanism that would enable individuals to have greater control over the musculature surrounding the injured knee joint (Heroux & Tremblay, 2006). As a knee joint with a torn ACL will behave differently both from a kinematic and kinetic perspective from one that has undergone surgical reconstruction (Urbach et al., 2001), it is very difficult to draw direct comparisons between the work of Heroux and Tremblay (2006) and the present research.

More recent studies have examined supraspinal excitability in individual's post-ACLR (Kuenze et al., 2015; Pietrosimone et al., 2015). Both groups of researchers measured supraspinal excitability bilaterally during active contraction (i.e. quantified active motor thresholds (AMT)) in subjects after unilateral ACL reconstruction. Results of the two studies differed slightly however. Kuenze et al. (2015) found significantly greater injured vs. uninjured limb AMT asymmetry compared to healthy controls. In

addition the AMT in injured limbs was greater than the AMT in the uninjured limbs but not different from healthy control limbs. The uninjured limbs AMT was also significantly less than the AMT in the healthy control limbs. This means that individuals in the ACLr group had lower supraspinal excitability for their injured limb and higher excitability on the uninjured side. In contrast to these findings Pietrosimone et al. (2015) reported that AMT was lower (i.e. excitability was higher) for the injured limb, while there were no supraspinal excitability differences noted on the uninjured side. The present study adds yet another outcome related to supraspinal excitability post ACLr. Although the differences were not statistically significant, the large effect sizes indicate that ACLr likely resulted in comparatively large changes in RMT in this group. Clearly there is much variability in results related to supraspinal excitability in those who have undergone ACLr. Some reasons for this variability are obvious. In addition to differing testing protocols and patient population, Heroux & Tremblay (2006) used two separate coils during the testing, while neither gender, age or limb dominance were accounted for when comparing to healthy control subjects. Kuenze et al. (2015) and Pietrosimone et al. (2015), examined supraspinal excitability during an active contraction as opposed to at rest. In addition Kuenze et al. (2015) did not account for limb dominance when comparing to healthy control subjects. Similarly, differences in time since injury / surgical repair could account for some of the differences (Lepley et al., 2015). Time since injury varied substantially between and within studies; (Heroux and Tremblay: Median: 22 (4-108) mo; Kuenze et al: Mean: 31 (23.5) mo; Pietrosimone et al: Mean: 48.1 (36.2)

mo; Current study: Mean: 93.9 (86) mo). These differences between studies could explain the variation in findings found across studies.

The study that most closely resembled the current research was that of Pietrosimone et al. (2015). Although they used AMT as opposed to RMT, they matched participants for limb dominance, an important consideration as dominance has been shown to effect motor control (Lanshammar & Ribom, 2011). Decreases in supraspinal excitability of a given muscle have been thought to be a possible mechanism involved with persistent quadriceps weakness and strength deficits following ACL injury (Urbach et al., 2001; Heroux & Tremblay, 2006). It is possible that the present study along with Pietrosimone et al. (2015) and Kuenze et al. (2015) identified similar, but separate reorganization patterns, all with the ultimate effect of reducing the contraction of the quadriceps to avoid excessive forces in the injured limb. We showed that that members of the ACLr group had a tendency to exhibit lower supraspinal excitability levels in both limbs, possibly indicating a reorganization pattern in which bilateral reduction in supraspinal excitability occurred in order to reduce quadriceps strength, and thus possibly reduce knee joint loading. Similar to our findings, Pietrosimone et al. (2015) found lower levels of supraspinal excitability in ACL patients. Although the authors showed this was only the case in the injured limbs, these results still resemble a reorganization pattern seemingly aimed to reduce forces on the knee joint in the injured limb. Moreover, Kuenze et al. (2015) suggested that ACL patients exhibited “favoritism” of the uninjured limb based on the increased supraspinal excitability they found in this limb. This created significant asymmetry between limbs in the ACL patients which could represent yet

another reorganization pattern, purported to favor the uninjured limb as a means to avoid excessive contraction of the quadriceps muscle in the injured limb (Kuenze et al., 2015).

It should be noted that strength measures in the present study did not correspond with the bilateral changes observed in RMTs, as strength deficits were only found in the injured limbs of members of the ACL group. In a recent study looking at strength and supraspinal excitability as ways to predict disability in patients after ACLr, the two measures were found to have little association with each other (Pietrosimone et al., 2013). These findings suggest the possibility that muscle strength following knee injury is not directly related to level of supraspinal excitability. Moreover, bilateral decreases in supraspinal excitability (RMT) have also been found in patients with unilateral chronic ankle instability (Pietrosimone et al., 2012). Although it is difficult to make direct comparisons between their study and the present one due to the different joints examined, the findings of Pietrosimone et al. (2012) show further evidence of bilateral decreases in supraspinal excitability after a unilateral injury in the lower limbs. In interpreting their results Pietrosimone et al. (2012) suggested that changes in RMT may have a greater effect on more complicated tasks such as postural control and gait as opposed to ankle strength. This suggests that examining more complex functional tasks, which extend beyond strength, may be better suited to identifying the potential function related effects of changes in supraspinal excitability observed post-ACLR. Future studies in this area should consider including gait and balance related measures in addition to basic strength measures.

MEP Slope

There were no statistically significant differences in the slopes of the MEP SRCs found in the present studies (Figure 12). The small effect sizes further confirmed that the MEP slopes were likely similar between groups. The slope, hereafter referred to as gain, of a MEP SRC is thought to reflect the strength of supraspinal projections to a given motoneuron pool (Devanne et al, 1997; Boroojerdi et al., 2001). It can be recognised then, that all subjects in the present study displayed comparable strength of projections from the motor cortex to VM. This similarity is slightly different from what was seen during analysis of RMTs, with RMT averages lower bilaterally in limbs of ACL subjects. Although both techniques are able to provide insight regarding the projections onto the corticospinal tract, the gain of a MEP SRC and RMTs offer different information regarding supraspinal excitability. Gain is thought to provide information about the extent of a motor representation (i.e. the size of the area of the motor homunculus that projects to any given muscle). Thus, a higher gain would indicate a greater representation. On the other hand, Heroux & Tremblay (2006) have suggested, based on previous research (Abbruzzese & Trompetto, 2002; Chen, 2000), that RMT provides details about the most excitable elements under the coil, or the central core region of the motor representation. With RMT results showing potential decreases in supraspinal excitability and MEP gain similar between groups, it suggests a decrease in excitability of the central core region rather than a decrease in the size of the motor representation of VM.

To the authors' knowledge, no other study has looked at MEP SRC gain after ACLr. While Heroux & Tremblay (2006) did examine MEP SRC gain, they did so in

individuals with ACL injury who had not yet had surgical reconstruction. They reported no systematic differences between ACL patients and healthy subjects and suggested that this lack of difference was likely due to the high variability of their MEP measures. The authors identified three groups of ACL patients based on MEP data; patients who displayed greater MEP gain in the injured limb (n=4), those who showed greater MEP gain in the uninjured limb (n=3), and those who showed similar responses in both limbs (n=3). In the present study, we found similar variability and were able to group according to the same pattern; greater MEP gain in the injured limb (n=3), greater MEP gain in the uninjured limb (n=2) and those who showed similar responses in both limbs (n=1). Based on the findings of Heroux & Tremblay (2006) and those in the present study, MEP gain and thus alterations in the projections to the corticospinal tract are variable. It would seem logical that these variations in supraspinal excitability would be according to the extent of the deficits experienced, although it has been hard for studies to make predictive connections between alterations occurring at the supraspinal level and those that occur at a functional level (Pietrosimone et al., 2013; Lepley et al., 2014). While the role of which changes in supraspinal excitability may play in altering knee function is still unclear (Pietrosimone et al., 2013; Lepley et al., 2015), studies are beginning to bridge the gap between deficits shown on a functional level with changes that occur at a supraspinal level (Heroux & Tremblay, 2006; Pietrosimone et al., 2013; Kuenze et al., 2015; Pietrosimone et al., 2015). While this research represents a good start much more research is needed to better understand, what if any effect changes at the supraspinal level have on knee function.

While TMS is an excellent technique to assess supraspinal excitability, in order to differentiate between changes occurring at the spinal level and those occurring at the supraspinal level, H-reflex measures were required to account for spinal properties. It was also necessary to include this H-reflex measurement to assess spinal mechanisms due to literature acknowledging loss of sensory feedback and impaired gamma loop functioning after ACLr and its effects on spinal motoneurons.

Spinal Excitability

Reductions in spinal excitability have been regarded as a possible mechanism contributing to deficits in quadriceps strength after ACLr. This hypothesis stems from the loss of sensory feedback after ACLr as mechanoreceptors, which are removed with the ACL during surgery, are an integral part of a normal functioning motor system (Hoffman et al., 2000; Konishi et al., 2007; Krogsgaard et al., 2011). In the present study, there was no significant difference in the gain of the H-reflex SRCs between ACL and healthy subjects. Similar to RMT though, there were large effect sizes suggesting that an effect existed between limbs. Interestingly, analyses of the effect sizes showed a large effect (Figure 14) when control limbs (ACLc) of the ACL subjects and matched control limbs (Healthyc) of healthy subjects were compared. Comparison of ACL injured limbs (ACLi) to the matched limbs (Healthyi) of the healthy subjects resulted in only small effects sizes.

Most of the previous research examining H-reflex data in this population has examined the H:M ratio (Hoffman et al., 2000; Heroux & Tremblay, 2006; Kuenze et al.,

2015; Pietrosimone et al., 2015). To our knowledge, the present research represents the first time gain of the H-reflex SRC has been examined in this ACL patient population. Heroux and Tremblay (2006) and Kuenze et al. (2015) found no differences in H-reflexes between subject groups, despite both reporting significant differences in supraspinal measures. In contrast, Lepley et al. (2014) reported significant differences in spinal excitability measures after ACLr. These authors split the ACL subjects into low and high strength subsets and found that the high strength group also had significantly higher H:M ratio compared to the low strength subjects. Higher H:M ratios represented greater levels of spinal excitability, suggesting that in the lower strength group, decreases in strength may have been attributed to lower spinal excitability. In addition Lepley et al. (2014) reported that spinal, supraspinal and voluntary activation measurements significantly predicted variations in strength in the ACL subjects. The authors thus suggested that the strength changes observed in their participants were likely caused by neural deficits at the spinal level and not the supraspinal level. It should be mentioned that the regression model used by Lepley et al. (2014) was unable to significantly predict strength outcomes in the healthy population, raising questions about the magnitude of contributions of spinal and supraspinal measures to strength.

The findings of the present study, along with previous research demonstrate that ACL injuries have an effect on spinal excitability. There are considerable discrepancies between studies and much more research is needed to fully understand the extent of an ACL injury on spinal excitability and the associated mechanisms and consequences on more functional measures. Moreover, while some subjects seem to show strength deficits

that appear to be related to changes in spinal excitability, others are showing similar strength deficits that may be attributed to changes in supraspinal excitability. Even with the variability between studies, there is a foundation of research providing evidence of possible alterations at both the spinal and supraspinal levels and that these changes likely contribute to strength deficits.

Quadriceps Strength

This discussion would not be complete without spending some time examining the strength measures found in this study. As previously stated, the strength results for the present study were unexpected. The expectation was that the individuals in the ACLr group would exhibit bilateral strength deficits as voluntary activation and strength deficits have been reported by several authors (Urbach et al., 2001; Chmielewski et al., 2004; Hart et al., 2010; Rice & McNair, 2010). Surprisingly these bilateral deficits were not found. Further examination of individual participant data confirmed that every member of our ACLr group produced lower force in the injured limb compared to uninjured limb, While, on average, strength of ACLc limbs were greater than the strength observed in Healthy limbs. We are unsure as to why these results did not confirm our original hypothesis. There are a few possibilities that may have contributed to these findings. First, we did not account for activity level. This may have led to having ACL subjects that were more active than the Healthy control subjects, possibly contributing to greater knee extensor strength among the ACL participants. In addition, because we did not account for activity levels, we were unable to identify subjects as trained or untrained. A subject

who participates in resistance training on a regular basis would be likely to have higher strength. Therefore the ACL subjects could have been more trained, contributing to higher MVC forces in ACL subjects compared to healthy subjects. Even though HLDs were not observed in the ACL subjects, MVC results provided additional information to further understand how spinal and supraspinal changes may influence quadriceps strength outputs.

Methodological Considerations

Due to the nature of the recruiting and exclusion criteria of the study the sample size was small. This may have been a catalyst for not finding significant differences even though some large effect sizes were found. Another limitation of this study is that ACL patients varied largely in time since surgery, increasing the possible variance as some of the factors analyzed in this study are known to adapt over time. A final limitation in this study was that the type of replacement graft was not accounted for. The type of replacement graft has been shown to have an effect over time on quadriceps strength, with those receiving patella tendon grafts experiencing greater strength deficits compared to hamstring tendon grafts.

Although H-reflex as a research tool has been used for quite some time it is accompanied by a number of extraneous factors that create limitations when discussing its merit (Zehr, 2002). In the present study, we controlled for as many factors (See methods) as possible that fit within the purpose of the study to report H-reflex responses with greater validity and accuracy.

Conclusion

Although the original hypothesis that patients would experience HLDs was not confirmed, the results of this study offer supplementary data to the existing literature, advancing our understanding about the consequential effects of ACL injury and reconstruction. Most importantly, we were able to identify a patient population with strength deficits in the injured limb and that these patients were experiencing differences in spinal and supraspinal excitability compared to Healthy subjects. This additional evidence only puts more emphasis on the need for further understanding of the underlying mechanisms contributing to strength deficits after ACL injury.

Future Work

It is becoming more evident that changes in spinal and supraspinal excitability exist in this population. In order to improve rehabilitation techniques, it is important to better understand the relationship between these measures and the strength and functional deficits often experienced by this patient population. Further research in this area is needed to fully understand the relationship between supraspinal and spinal excitability and its role in strength deficits in injured and uninjured limbs. In the existing literature, there seems to be some disconnect between neural changes and the resulting functional deficits. This is even seen in healthy populations, where supraspinal excitability does not correlate to strength outcomes on a consistent basis (Pietrosimone et al., 2013). It is

important to fill this gap and gain a detailed understanding of how changes at the spinal and supraspinal level effect changes on a functional level. Only then will we be able to create sound rehabilitation programs targeting essential mechanisms to reduce the short and long term consequences of this injury. How these neural adaptations progress over time is also an important piece of the puzzle, due to the long-term nature of functional deficits in addition to secondary consequences such as osteoarthritis. As we gain further understanding of these mechanisms involved, we can decrease the severity of short and long term consequences as well as increase the likelihood of patients returning to prior activity level.

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