A Dissertation

entitled

Examining Neural Alterations as the Origins of Disability in Patients Following Anterior Cruciate Ligament Reconstruction

by

Adam S. Lepley

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Doctor of Philosophy Degree in Exercise Science

Phillip A. Gribble Ph.D., ATC, Committee Chair

Brian G. Pietrosimone, Ph.D., ATC, Committee Member

Abbey Thomas Ph.D., ATC, Committee Member

Michael A. Tevald, PT, Ph.D., Committee Member

David H. Sohn, MD, JD, Committee Member

Patricia R. Komuniecki, Ph.D., Dean
College of Graduate Studies

The University of Toledo
May 2014
An Abstract of

Examining Neural Alterations as the Origins of Disability in Patients Following Anterior Cruciate Ligament Reconstruction

by

Adam S. Lepley

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Doctor of Philosophy Degree in Exercise Science

The University of Toledo
May 2014

**Objective:** To examine quadriceps spinal-reflexive, corticospinal, and intracortical excitability before, at 2-weeks post and at 6-months post-anterior cruciate ligament reconstruction (ACLr) compared to healthy controls. A secondary aim of this study was to investigate the association between quadriceps neural excitability and neuromuscular, biomechanical and self-reported function at times of pre-surgery and 6-months post-surgery. **Patient and Other Participants:** Seventeen ACL injured patients scheduled to undergo surgical reconstruction (9 Female, 8 Male; age:21.0 ± 4.8 years; height:173.1±7.3cm; weight:77.7±11.2kg; 35.8±14.8 days-post-injury) volunteered and were compared to seventeen healthy controls (9 Female, 8 Male; age:22.1 ± 3.8 years; height:173.3±10.7 cm; weight:75.3±21.3 kg). **Methods:** This investigation utilized a case-control study design. For aim 1, quadriceps spinal-reflexive, corticospinal, and intracortical excitability were tested at pre-surgery, 2-weeks post-surgery (2-wks; average: 15.8 ± 2.5 days post-surgery) and 6-months post-surgery or when returned to participation (6-mo; average: 28.1 ± 2.8 wks post-surgery). For aim 2, quadriceps
strength, voluntary activation, knee joint biomechanics during stair walking and self-reported function were collected at the pre-surgery and 6-mo post time points. For aim 1, all measures were collected bilaterally in the ACLr group and in a dominance matched limb of the control group. For aim 2, outcome measures were collected in the injured limb of ACLr patients and a matched limb from the healthy control group for aim 2.

**Main Outcome Measures:** Quadriceps spinal-reflexive excitability was assessed using Hoffmann reflexes normalized to maximal muscle responses. Corticospinal excitability was evaluated with active motor thresholds (AMT) and motor evoked potentials at 120% of AMT. Intracortical excitability was assessed using short interval intracortical inhibition and intracortical facilitation. Quadriceps strength and voluntary activation were quantified using maximal voluntary isometric contractions and the central activation ratio, respectively. Peak knee joint angles and internal extension moments were calculated during the first 50% of stance phase during stair ascent and descent gait trials. The International Knee Documentation Committee questionnaire was used to evaluate self-reported function. **Statistical Analyses:** *Aim 1:* 3x3 (limb x time) repeated measures ANOVAs were performed with Tukey post-hoc tests where appropriate. *Aim 2:* Spearman Rho correlation matrices were performed at the pre-surgery and 6-mo post-surgery time points on the injured limb of the ACLr group and matched limb of the control group independently. **Results:** *Aim 1:* ACLr patients demonstrated lower spinal-reflexive excitability than controls at pre-surgery and 2-wks. At 6-mo post-surgery, spinal-reflexive excitability was not different between groups. Over-time, spinal-reflexive excitability in the ACLr group decreased from pre-surgery to 2-wks, and increased higher than pre-surgery at 6-mo, while controls did not change. Corticospinal excitability was
not different between groups at pre-surgery or 2-wks post-surgery, however ACLr patients had lower corticospinal excitability at 6-mo compared to controls. Corticospinal excitability in the ACLr group increased from pre-surgery to 2-wks, and at 6-mo was decreased compared to pre-surgery, while controls did not change. Intracortical excitability was not different between groups, nor did the values change over time in either group. **Aim 2:** At pre-surgery, corticospinal excitability was related to knee joint moments during stair ascent in ACLr patients, with higher corticospinal excitability relating to higher joint moments during gait. At 6-mo post-surgery, intracortical facilitation was inversely related to spinal-reflexive excitability. In addition, quadriceps strength had strong correlations with self-reported function and knee joint angle during stair ascent, indicating that ACLr patients with weaker quadriceps muscles reported higher levels of dysfunction and demonstrated straighter knee joint angles during stair ascent. **Conclusions:** Spinal-reflexive deficits are present before surgery and at 2-wks post-reconstruction, but not at a time when these individuals are cleared for full activity. In contrast, corticospinal deficits existed at 6-mo post-surgery, however not prior to or at 2-wks post-surgery. Deficits in neural excitability are present following ACL injury, potentially due to loss of ligament mechanoreceptors or joint effusion. Separate neuromuscular alterations occur at different stages of injury, with decreases in spinal-reflexive excitability early (pre-surgery, 2-weeks post) and deficits in corticospinal excitability observed at a time when individuals are returned to participation. Early rehabilitation strategies targeting spinal-reflexive excitability may help to improve post-operative outcomes, while later-stage rehabilitation may benefit from modalities aimed at improving corticospinal excitability. In addition, these neural alterations may have
negative effects on neuromuscular, biomechanical and self-reported function. Clinically, targeting these neural changes may benefit patients who are recovering from ACL injury and surgical reconstruction, and future research should examine the efficacy of the treatments proposed to target neural alterations.
Acknowledgements

Dr. Brian Pietrosimone: Your guidance, enthusiasm, knowledge and relentless dedication have immensely contributed to my professional development. I cannot thank you enough for the time and effort you have put forth throughout the past four years.

Drs. Gribble, Thomas, Tevald and Sohn: Thank you for being a part of my dissertation committee, and thank you for your continued support and contribution to this project. I could not have completed this task without your expertise and collaboration.

Hayley Ericksen, Amanda Murray, Masafumi Terada, Megan Quinlevan, Michelle McLeod, “Jimmy” Shinohara and many others: I have been fortunate to work with some of the greatest PhD students during the last four years. The opportunity to grow with, and share the experience of obtaining a doctoral degree with such accomplished individuals has been priceless.

My family: Your love and encouragement has shaped me into the person I am today, and I owe this accomplishment to you.

Lindsey (& Maggie): Thank you for keeping me grounded and for helping me to realize the important aspects of life. I am excited to begin the next chapter of our lives!
Table of Contents

Abstract...........................................................................................................................................iii

Acknowledgements.......................................................................................................................vii

Table of Contents.........................................................................................................................viii

List of Tables ................................................................................................................................xiii

List of Figures ...............................................................................................................................xiv

List of Abbreviations ....................................................................................................................xvi

1. Introduction................................................................................................................................1

   1.1 Background........................................................................................................................... 1

   1.2 Significance.......................................................................................................................... 3

   1.3 Specific Aims ......................................................................................................................... 4

2. Literature Review ....................................................................................................................... 7

   2.1 Introduction........................................................................................................................... 7

   2.2 Strength and Activation of Musculature Surrounding the Knee Joint ......................... 8

      2.2.1 Background ................................................................................................................... 8

      2.2.2 Muscle strength and activation following knee joint injury ................................. 10
2.2.3 Influence of strength and activation on function and disease progression ... 13

2.3 Spinal-Reflexive Excitability of Musculature Surrounding the Knee joint ....... 15
   2.3.1 Background ........................................................................................................ 15
   2.3.2 Alterations in spinal-reflexive excitability following knee joint injury ....... 20
   2.3.3 Effect of spinal-reflexive alterations on function and disease progression.... 23

2.4 Corticospinal Excitability of Musculature Surrounding the Knee Joint .......... 25
   2.4.1 Background ........................................................................................................ 25
   2.4.2 Alterations in corticospinal excitability following knee joint injury .......... 30
   2.4.3 Effect of corticospinal alterations on function and disease progression .... 32

2.5 Summary of ACL Rehabilitation........................................................................... 33

2.6 Conclusion of Literature Review.......................................................................... 36

3. Methodology ........................................................................................................ 37
   3.1 Research Design for Specific Aims 1 and 2 ...................................................... 37

   3.2 Overview of Methods ......................................................................................... 37
       3.2.1 Patients ........................................................................................................... 37
       3.2.2 Power analysis ............................................................................................... 38
       3.2.3 Patient timeline ............................................................................................. 38

   3.3 Outcome Measures for Specific Aim 1 ............................................................. 40
       3.3.1 Aim Overview ............................................................................................... 40
       3.3.2 Quadriceps spinal-reflexive excitability testing ........................................... 40
       3.3.3 Quadriceps corticospinal excitability testing ............................................... 42
3.3.4 Intracortical excitability testing ................................................................. 44

3.4 Outcome Measures for Specific Aim 2 ........................................................... 46

3.4.1 Quadriceps muscle strength ........................................................................... 46

3.4.2 Voluntary quadriceps muscle activation ....................................................... 46

3.4.3 Knee joint mechanics during stair ambulation .............................................. 48

3.4.4 Patient self-reported function ....................................................................... 52

3.5 Statistical Analyses ............................................................................................. 52

3.5.1 Demographics .................................................................................................. 52

3.5.2 Specific aim 1 ................................................................................................... 52

3.5.3 Specific aim 2 ................................................................................................... 52

4. Results .................................................................................................................. 54

4.1 Subject Demographics ....................................................................................... 54

4.2 Results - Specific Aim 1 .................................................................................... 55

4.2.1 Quadriceps spinal-reflexive excitability ......................................................... 55

4.2.2 Quadriceps corticospinal excitability – active motor threshold (AMT) ........ 57

4.2.3 Quadriceps corticospinal excitability – motor evoked potentials (MEP) at 120% of AMT .............................................................................................................. 59

4.2.4 Quadriceps intracortical excitability – short interval intracortical inhibition (SICI) .................................................................................................................. 61

4.2.5 Quadriceps intracortical excitability - intracortical facilitation (ICF) .......... 63

4.3 Results - Specific Aim 2 .................................................................................... 65

4.3.1 Descriptive statistics of outcome variables .................................................... 65
4.3.2 Correlations at pre-surgery ................................................................. 66

4.3.3 Correlations at 6-months post-surgery ........................................... 70

5. Discussion ............................................................................................... 73

5.1 Summary of Results ............................................................................... 73

5.2 Specific Aim 1 ..................................................................................... 74

5.2.1 Early alterations in quadriceps spinal-reflexive excitability ............. 74

5.2.2 Alterations in quadriceps corticospinal excitability are present at return to activity ................................................................. 77

5.2.3 No evidence of alterations in intracortical facilitation or inhibition .... 80

5.3 Specific Aim 2 ..................................................................................... 82

5.3.1 Correlations at pre-surgery ............................................................... 82

5.3.2 Correlations at 6-months post-surgery ........................................... 84

5.4 Clinical Relevance ............................................................................... 85

5.5 Limitations ........................................................................................... 87

5.6 Conclusion ............................................................................................ 87

References ..................................................................................................... 89

Appendix A ................................................................................................... 114

Adult Subject Consent Form ................................................................. 114

Appendix B ................................................................................................. 121

Minor Assent Form ................................................................................... 121

Appendix C ................................................................................................ 124

TMS/AMT Data Collection Sheet ............................................................ 124

xi
Appendix D ........................................................................................................................................... 126
CAR Testing Protocol ......................................................................................................................... 126
Appendix E .......................................................................................................................................... 127
Dimensions of Custom Built Staircase ............................................................................................... 127
Appendix F .......................................................................................................................................... 129
Post-processing Visual 3D Pipelines .................................................................................................. 129
Appendix G .......................................................................................................................................... 142
International Knee Documentation Committee Questionnaire (IKDC) ........................................... 142
Appendix H .......................................................................................................................................... 145
Normality Assessment for Variables in Aim 2 .................................................................................. 145
List of Tables

Table 2.1  Recent systematic reviews/meta-analyses reporting strength and activation deficits following knee joint injury. ..........................................................12

Table 2.2  Articles assessing corticospinal excitability following knee joint injury. ..31

Table 2.3  Summary of studies evaluating isokinetic knee extension strength (60°/sec) in ACLr patients at 6mo post-surgery .............................................34

Table 3.1  Summary of outcome measures and time of collection. .................................39

Table 4.1  Participant demographics. .............................................................................54

Table 4.2  Means ± standard deviations for outcome variables used in specific aim 2. ..............................................................................................................66

Table 4.3  Spearman correlation matrix analyzing associations among variables at pre-surgery within the injured limb of the ACLr group. .........................68

Table 4.4  Spearman correlation matrix analyzing associations among variables at pre-surgery within the matched injured limb of the healthy control group.69

Table 4.5  Spearman correlation matrix analyzing associations among variables at 6-months post-surgery within the injured limb of the ACLr group. ...............71

Table 4.6  Spearman correlation matrix analyzing associations among variables at 6-months post-surgery within the healthy control group. ..........................72
List of Figures

Figure 2-1  Quadriceps voluntary activation testing and calculation of the central.
activation ratio (CAR) ............................................................10
Figure 2-2  Hoffmann reflex and muscle response pathways. ..........................16
Figure 2-3  Recruitment curve of Hoffmann reflex and maximal muscle response. ...17
Figure 2-4  Representation of presynaptic inhibition. ....................................18
Figure 2-5  Representation of postsynaptic/recurrent inhibition. ........................19
Figure 2-6  Representation of corticospinal pathways of neural transmission. ....27
Figure 3-1  Testing position for spinal-reflexive excitability. ............................42
Figure 3-2  Testing for corticospinal and intracortical excitability. .....................44
Figure 3-3  Electrode placement for CAR testing. ............................................48
Figure 3-4  Retroreflective marker placement for 3D motion capture. ...............49
Figure 3-5  Participant performing stair ascent trial on custom built staircase. ....51
Figure 3-6  Screenshot of Cortex and Visual 3D software. .................................51
Figure 4-1  Spinal-reflexive excitability at each time point. ...............................56
Figure 4-2  Spinal-reflexive excitability changes over time. ..............................57
Figure 4-3  Corticospinal excitability, as measured by active motor threshold, at each
time point. ..............................................................................58
Figure 4-4  Changes in corticospinal excitability over time, as measured by active motor threshold. .......................................................... 59

Figure 4-5  Corticospinal excitability, as measured by motor evoked potentials at 120% of active motor threshold at each time point. ............................... 60

Figure 4-6  Changes in corticospinal excitability over time, as measured by motor evoked potentials at 120% of active motor threshold. ............................... 61

Figure 4-7  Short interval intracortical inhibition (SICI) at each time point. No differences were detected. .......................................................... 62

Figure 4-8  Changes in SICI overtime ............................................................... 63

Figure 4-9  Intracortical facilitation (ICF) at each time point. No differences were detected. .......................................................... 64

Figure 4-10  Changes in intracortical facilitation over time. .............................................. 65

Figure 5-1  Changes in spinal-reflexive and corticospinal excitability over time. ....... 80
List of Abbreviations

ACL .........................Anterior cruciate ligament
ACLr ..........................Anterior cruciate ligament reconstruction
AMT .........................Active motor threshold

CAR ..........................Central activation ratio

EMG ..........................Electromyography

H:M ..........................Hoffmann reflexes normalized to maximal muscle response
ICF ..........................Intracortical facilitation
IKDC .........................International knee documentation committee questionnaire

MEP ..........................Motor evoked potential
MEP:M .........................Motor evoked potential normalized to maximal muscle response
MVIC .........................Maximal voluntary isometric contraction

SIB ..........................Burst superimposition technique
SICI ..........................Short interval intracortical inhibition

TMS ..........................Transcranial magnetic stimulation

OA ..........................Osteoarthritis
Chapter 1

Introduction

1.1 Background

Anterior cruciate ligament rupture, and subsequent reconstruction (ACLr), is one of the most common sports related injuries, with an estimated 250,000 ACL ruptures occurring annually in the United States.\(^1\) Economically, ACLr and therapeutic rehabilitation results in approximately $3 billion annually in direct costs.\(^1\) - \(^3\) Although there have been advancements in surgical and rehabilitative techniques, quadriceps muscle strength deficits have been reported to exceed 20% at a time of return to activity, and can persist many years following ACL reconstruction (ACLr).\(^4\) - \(^5\) Persistent quadriceps weakness has been linked to decreases in physical performance,\(^6\) increased risk of re-injury,\(^7\) and has been hypothesized to contribute to the development of post-traumatic joint osteoarthritis.\(^8\) - \(^9\) Specifically, recent data\(^10\) suggests that deficits in quadriceps strength are associated with joint space narrowing at a time of four years post-surgery, highlighting the link between neuromuscular dysfunction and osteoarthritic joint changes that are frequent following ACLr. Further, quadriceps strength deficits have also been strongly associated with self-reported disability that is commonly reported in the
years following ACLr,\textsuperscript{11,12} highlighting the importance of restoring and maintaining optimal quadriceps function in patients following ACL injury.

The origins of quadriceps strength deficits remain unknown, however, recent literature suggests that the genesis of neuromuscular dysfunction occurs through neural inhibition of the muscle.\textsuperscript{4,9,13-16} These neural alterations are hypothesized to occur in fundamental neural excitability pathways that are responsible for generating movement, specifically at the spinal-reflexive and corticospinal levels. Previous experiments have utilized an experimental knee joint effusion model to demonstrate deficits in quadriceps spinal-reflexive excitability pathways,\textsuperscript{17-20} resulting in impaired physical function.\textsuperscript{14,21} Alterations in quadriceps spinal-reflexive excitability have also been observed following ACLr, with spinal-reflexive excitability increasing from pre-surgery to three months post reconstruction.\textsuperscript{22} Quadriceps corticospinal alterations have been discovered in ACL injured individuals,\textsuperscript{23} suggesting neural changes in central pathways are also present following injury. Although changes in neural pathways have been discovered following knee joint injury and simulated effusion models, no prospective data based on time of ACLr surgery exists, and no longitudinal investigation has been performed on these ACLr patients. It remains unclear how these neural pathways are affected following ACL injury, and how changes in these pathways progress from time of injury to return to participation. Further, it is unknown how these neural excitability pathways contribute to the persistent deficits in quadriceps strength observed following ACLr, or the association between neural changes and deficiencies in biomechanical and self-reported function.

Despite clinician’s best efforts, standard therapeutic rehabilitation following surgery has failed to restore knee function to pre-injury levels.\textsuperscript{5} Specifically, traditional
rehabilitation programs have not been able to restore quadriceps strength to healthy levels. Targeting neural impairment is often overlooked in traditional rehabilitation, and failure to directly treat the potential underlying influences of quadriceps weakness may result in unsuccessful restoration of quadriceps strength. In order for clinicians to develop new and innovative rehabilitative strategies that target these underlying mechanisms, it is imperative to understand how excitability of these influential neural mechanisms are altered following ACLr and how these alterations are associated with neuromuscular, biomechanical and self-reported function.

1.2 Significance

Neuromuscular deficits that are common and persistent following ACLr, specifically in the quadriceps muscle, are hypothesized to contribute to the development of post-traumatic OA and poor outcomes following ACLr. Recent literature suggests that the genesis of neuromuscular dysfunction occurs through neural inhibition of the muscle, yet it remains unknown how underlying neural pathways, specifically corticospinal and spinal-reflexive, contribute to neuromuscular deficits and function following ACLr. Identifying alterations in these neural pathways will allow future research to develop interventions that target neural inhibition and positively affect outcomes following not only ACLr, but also other knee joint injury populations that experience long-term neuromuscular deficits, such as OA, meniscal injury and anterior knee pain patients.

Traditional rehabilitation has not been able to eliminate persistent quadriceps weakness, resulting in individuals returning to activity with sustained neuromuscular
deficits. Recent evidence suggests that the inhibition of neural pathways, which influence quadriceps muscle function, lead to diminished quadriceps strength, which continues following ACLr. However, no evidence is available to evaluate how both spinal-reflexive and corticospinal pathways are affected post-injury and following ACLr, or the association these neural pathways have on neuromuscular, biomechanical and patient self-reported function following ACLr. This project is innovative because it is the first to prospectively evaluate spinal-reflexive and corticospinal excitability in patients post-ACL injury and reconstruction, and examine the effect of these alterations on neuromuscular, biomechanical and self-reported function. Identifying neural alterations at both the corticospinal and spinal-reflexive level is a novel approach in determining the genesis of neuromuscular and biomechanical dysfunction, as well as self-reported disability that is present and persistent following ACLr.

1.3 Specific Aims

The long-term goal of this research is to decrease disability following lower extremity joint injury. In order to advance towards this goal, the overall objective of this current work is to identify alterations in spinal-reflexive and corticospinal neural pathways in ACL patients at different time points post-injury and post-ACLr, and to investigate the association these alterations have on muscle function, performance and self-reported function. The central hypothesis that will be tested is that alterations in both corticospinal and spinal-reflexive neural pathways are present in the quadriceps following ACLr, and that these altered neural pathways are related to deficits in both physical and self-reported function. This hypothesis is formulated based on our labs preliminary data suggesting that neural alterations exist following acute knee injury.
The rationale motivating this investigation is that 1) neuromuscular dysfunction and weakness of the quadriceps is linked to disability in people with acute knee injuries, and 2) current rehabilitation protocols have not been able to restore pre-injury quadriceps strength, therefore a shift in ideas may be needed to target the origins of these neuromuscular problems. To test this central hypothesis, the following specific aims will be addressed:

1. **Determine differences in quadriceps neural excitability in an ACLr population at pre-surgery, 2-weeks post-surgery, and 6-months post-surgery.**
   It is hypothesized that ACLr individuals will experience bilateral alterations in spinal-reflexive excitability (Hoffmann reflexes normalized to muscle response ratio), corticospinal excitability (active motor threshold, motor evoked potentials) and intracortical excitability (intracortical inhibition and intracortical facilitation) compared to healthy individuals at all time points.

2. **Determine the association between quadriceps neural excitability, strength, voluntary activation, biomechanical function and patient self-reported function at pre-surgery and 6-months post-surgery in the ACLr injured limb.** It is hypothesized that a strong correlation will exist between quadriceps spinal-reflexive excitability, corticospinal excitability, intracortical excitability, strength (maximal voluntary isometric contractions), voluntary activation (central activation ratio), biomechanical function (knee joint kinematics and kinetics
during stair ambulation) and patient self-reported function (International Knee Documentation Committee questionnaire) at both time points.
Chapter 2

Literature Review

2.1 Introduction

Rupture of the anterior cruciate ligament (ACL) is one of the most common sports related injuries, with nearly 250,000 occurring annually in the United States.\(^1\) Surgical reconstruction of the ACL (ACLr) results in an annual cost of $3 billion,\(^29\) and an eight to ten fold increase in the development of osteoarthritis (OA) as soon as 12 years post-surgery.\(^30\) Comprehension of the neuromuscular and physiologic changes that occur following ACLr is necessary to enhance the understanding of the risk associated with development and progression of OA. Having a true appreciation of the neuromuscular deficiencies that occur following ACLr will aid in the development of future interventions that look to restore normal knee function and prolong knee joint health. Therefore, the purpose of this literature review is to develop a comprehensive examination of the evidence concerning neuromuscular and physiological outcomes following knee joint injury and ACLr, as well as their link to function and disease progression.
2.2  Strength and Activation of Musculature Surrounding the Knee Joint

2.2.1  Background

Neuromuscular function is a term that describes how the nervous and muscular systems are interacting and operating. As implied, there is a neural component and a muscular component, which work synergistically to initiate and control musculoskeletal movement. The initial section of this review will focus on muscular strength and volitional activation. Although there is an inherent neural component to muscle force generation, this section will focus mainly on the gross muscular strength components of muscle function and activation. Later sections, specifically sections 2.3 and 2.4, will address neural pathway’s contribution to neuromuscular function, including the influence of spinal-reflexive and corticospinal level neural pathways.

Muscle strength is a common outcome measure used to assess muscle function in both healthy and pathological populations. Lower extremity muscle strength has been shown to correlate with self-reported function, level of physical activity, lower extremity gait kinematics, balance, and physical function/performan. However, optimal muscle force generation relies on one’s ability to volitionally contract available motor units. Decrease in motor neuron recruitment or firing rate can cause deficits in both volitional activation and muscle strength. Recruitment of motor neurons can be inhibited at numerous points along neural pathways, however, as stated above, those specific inhibitory pathways will be discussed in sections 2.3 and 2.4.

Muscle strength has previously been used as a method to evaluate muscle activation, however, simply measuring force generation capabilities does not allow for
evaluation of the neural system’s integration. The central activation ratio (CAR) is currently one of the more common methods used to assess volitional activation.\textsuperscript{39-41} CAR incorporates both a maximal voluntary isometric contraction (MVIC) from the participant, as well as a supramaximal stimulus in conjunction with the MVIC. The stimulus is intended to stimulate motor neurons that may not be fully activated by the voluntary effort. The force generated during the stimulus represents an estimate of the combination of motor neuron recruitment and rate coding, and therefore the actual value of activation cannot be interpreted as a direct reflection of either. The force generated without the stimulus is divided by the total force generated with the stimulus, giving the following equation: \textsuperscript{42} $\text{CAR} = \frac{F_{\text{MVIC}}}{F_{\text{MVIC}} + F_{\text{SIB}}}$ (Figure 2-1). An increase in the CAR demonstrates either an increase in the number of motor neurons available to be recruited, or an increase in firing rate.\textsuperscript{41-43}
Muscle strength and activation following knee joint injury

Quadriceps muscle strength is an essential component needed for normal knee joint function. Following knee joint injury, however, individuals suffer from persistent quadriceps strength deficits that cause long lasting neuromuscular dysfunction.³⁹,⁴⁴

Figure 2-1: Quadriceps voluntary activation testing. The participant performs an isometric contraction of their quadriceps muscles until they reach their maximal voluntary isometric contraction (MVIC). One MVIC is reached; a supramaximal electrical stimulus is provided causing an increase in muscle force output by electrically activating motor neurons that cannot be voluntarily activated by the patient. Central activation ratio (CAR) is a calculation to estimate voluntary activation using the MVIC and SIB values. Specifically, this figure represents a depiction of the torque graph seen by the participant during MVIC and CAR testing. The dashed line represents the average MVIC produced during the practice trials. The top, solid black line represents 120% of the average MVIC. Participants were instructed to attempt to reach the solid black line to ensure maximal effort. An automated triggering system is armed once the torque output reaches the dotted line. Once the participant’s torque drops 1 NM below their peak torque, the automated stimulus is delivered to the quadriceps muscle. If the participant is unable to reach the dotted line during the trial, the stimulus will never be delivered and more rest is provided between trials. MVIC values and SIB values were used to calculate CAR as seen by the equation in the figure.
Deficits in quadriceps strength have been documented up to 15 years post ACLr, and may persist bilaterally.\textsuperscript{45,46} Bilateral quadriceps activation deficits have also been observed in ACLr and ACL deficient (ACLd) patients\textsuperscript{44} as well as those suffering from anterior knee pain (AKP) and diagnosed with knee joint OA.\textsuperscript{39} Recent systematic reviews of current literature have compiled data that assessed quadriceps strength and activation deficits among a variety of knee joint injuries. These investigations reveal an average quadriceps strength deficit of 18\% in the involved limb of ACLr individuals. Further, individuals suffering any knee joint injury have, on average, only 83\% quadriceps activation on their involved side compared to 95\% in healthy matched controls, a deficit of nearly 12\% (Table 2.1).

Deficits in the contralateral limb were also reported in these investigations, showing that the uninvolved quadriceps muscle may also be affected. In the uninjured limb, quadriceps activation levels ranged from 78.6\%\textsuperscript{44} in those with AKP, to 80.6\%\textsuperscript{39} and 87\%\textsuperscript{44} in those with OA and ACLr respectively (Table 2.1). Bilateral deficits in these populations make side-to-side comparisons difficult when assessing quadriceps strength and volitional activation, most likely underestimating the deficits observed. In addition, this does not allow for interpretation of these deficits as either a cause of, or result from the injury. Future investigations would benefit from the inclusion of healthy controls for comparison. It should also be noted that these investigations include a variety of populations, graft types, years post-injury etc. Therefore, these values represent a general analysis of strength and activation deficits following knee joint injury, and may vary depending on different injury characteristics.
Table 2.1: Recent systematic reviews/meta-analyses reporting strength and activation deficits following knee joint injury

<table>
<thead>
<tr>
<th>Author</th>
<th>Population</th>
<th>Manuscripts Included</th>
<th>Total Injured Subjects</th>
<th>Avg. Quadriceps Strength Deficits (% of Uninvolved)</th>
<th>Quadriceps Activation</th>
<th>Avg. Hamstring Strength Deficits (% of Uninvolved)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Injured</td>
<td>Contralateral</td>
</tr>
<tr>
<td>Hart et al.⁴⁴</td>
<td>ACLr</td>
<td>4</td>
<td>99</td>
<td>-</td>
<td>88%</td>
<td>87%</td>
</tr>
<tr>
<td>Ingersoll et al.⁴</td>
<td>ACLr</td>
<td>22</td>
<td>1155</td>
<td>18%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hart et al.⁴⁴</td>
<td>ACLd</td>
<td>10</td>
<td>372</td>
<td>-</td>
<td>81.4%</td>
<td>-</td>
</tr>
<tr>
<td>Hart et al.⁴⁴</td>
<td>AKP</td>
<td>3</td>
<td>38</td>
<td>-</td>
<td>79.6%</td>
<td>78.6%</td>
</tr>
<tr>
<td>Pietrosimone et al.³⁹</td>
<td>OA</td>
<td>14</td>
<td>NA</td>
<td>-</td>
<td>82.23%</td>
<td>80.6%</td>
</tr>
<tr>
<td>Total Average Deficits</td>
<td></td>
<td>53</td>
<td>&gt;1664</td>
<td>18%</td>
<td>82.8%</td>
<td>82.1%</td>
</tr>
</tbody>
</table>

Abbreviations: ACLr, anterior cruciate ligament reconstruction; ACLd, anterior cruciate ligament deficient; AKP, anterior knee pain; OA, osteoarthritis
2.2.3 Influence of strength and activation on function and disease progression

Decreases in quadriceps strength and volitional activation have been shown to alter lower extremity kinematics, leading to increased loading at the knee joint\textsuperscript{14} and negatively affecting self-reported measures of disability.\textsuperscript{13} Individuals with quadriceps strength in the injured limb of less than 80% of the contralateral side displayed lower knee flexion angles and reduced knee joint moments during the loading phase of both walking and jogging gait.\textsuperscript{47} Many researchers suggest that the reduced ability to generate quadriceps force in injured individuals does not allow the quadriceps to adequately contract eccentrically, therefore creating an inability to control joint loading during gait.\textsuperscript{14,44,48} This increase in force through the joint is hypothesized to weaken and deteriorate joint cartilage overtime, potentially leading to initiation and progression of joint disease and OA.\textsuperscript{4,13,14,21,44}

Quadriceps dysfunction is also a major factor in determining self-reported outcomes following injury. In an ACLr population, preoperative quadriceps strength significantly predicted 30% of knee joint function two-years following surgery, as scored by the Cincinnati knee scale.\textsuperscript{49} In addition, those who had preoperative quadriceps strength deficits of greater than 20%, had significantly larger strength deficits compared to their uninjured side at the two-year follow up.\textsuperscript{49} Quadriceps strength and activation failure have also been shown to predict 46% of a principle component score of function, which included outcomes of both self-reported and physical function.\textsuperscript{13} This particular investigation showed that the best predictive model of function following injury included measures of quadriceps strength and activation failure, coupled with gender. Individuals with deficits in both quadriceps strength and activation demonstrate the lowest levels of
overall function. However, function is improved in strong individuals with higher levels of quadriceps activation failure compared to strong individuals with low levels of activation failure, suggesting that quadriceps activation may moderate the relationship between strength and function. This is an interesting finding, considering that traditional rehabilitation programs currently focus on muscle strength and resistance training, with only moderate improvements observed in physical function. In addition, quadriceps weakness still persists long after injury and the incidence of knee OA following ACLr has not diminished with current rehabilitation programs. This data suggests that restoring quadriceps activation may need to be addressed prior to strengthening in order to ensure higher levels of function following knee joint injury. Pietrosimone et al. found that changes in quadriceps activation of OA patients significantly predicted changes observed in quadriceps strength following a four-week therapeutic rehabilitation program. In contrast, however, Scopaz et al. demonstrated that baseline activation values prior to rehabilitation in an OA population did not predict strength gains associated with a six-week rehabilitation program. Although these data are contradicting, and are found in an OA population instead of ACLr, it demonstrates the need for future research to determine what effect quadriceps activation has on quadriceps strength and function following joint injury. Given that muscle weakness and activation deficits persist long after injury, and the probability of developing early onset of OA remains high, it is important to understand the underlying cause to these deficits in order develop and implement optimal rehabilitation programs to help restore normal gait patterns and overall function.
2.3 Spinal-Reflexive Excitability of Musculature Surrounding the Knee joint

2.3.1 Background

Measuring spinal-reflexive excitability is of importance because it represents the number of alpha motor neurons (αMN) located in a selected muscle that are capable of responding to an excitatory stimulus from the nervous system.\textsuperscript{53-55} It has been reported to give a representation of the nervous system’s influence on motor output, specifically through spinal-reflexive neural pathways.\textsuperscript{55}

The Hoffmann reflex (H-reflex) is an electrically induced reflex equivalent to the spinal stretch reflex,\textsuperscript{53,55,56} and is commonly referred to as a method of measuring motor-neuron pool excitability (MNPE).\textsuperscript{17,55,57-60} This interpretation, however, could be seen as inaccurate given that the H-reflex cannot separate out inhibitory mechanisms, such as presynaptic or postsynaptic inhibition, which could occur between spinal nerves and the central nervous system (CNS).\textsuperscript{56,61} Evaluating the H-reflex as a representation of MN excitability is a common misconception in the literature.\textsuperscript{56} Therefore, instead of a direct measure of MNPE, it is often referred to as an estimate of MNPE,\textsuperscript{56} specifically when presynaptic inhibition\textsuperscript{61} and intrinsic excitability\textsuperscript{53} of the motoneurons is constant.\textsuperscript{56} More specifically, spinal-reflexive excitability may be a better term to describe what the H-reflex represents, incorporating both the afferent and efferent peripheral nerves as well as interneurons within the CNS.

As stated above, the H-reflex measurement includes both afferent and efferent nerve fibers, inherently measuring the efficacy of synaptic transmission as the stimulus travels through the spinal-reflexive loop (Figure 2-2).\textsuperscript{56}
Initial electrical stimulation of a mixed nerve excites the low threshold of the large diameter (Ia) afferent fibers. This excitation results in action potentials that travel along the afferent fibers, synapse at the CNS and interneurons, resulting in concurrent action potentials being generated by the αMNs. These efferent action potentials are propagated across the neuromuscular junction, causing an action potential in the associated muscle fibers, which can be visualized through the use of electromyography (EMG), and a twitch response. This muscle fiber action potential associated with the twitch is measured as the H-reflex. As intensity of the electrical stimulus increases, there is also a direct activation of the efferent fibers of the stimulated mixed nerve. Action potentials are then sent directly to the neuromuscular junction from the point of stimulation, producing another muscle twitch, or muscle response, known as the M-response. As intensity continues to increase, an antidromic response sending action
potentials toward the spinal cord causes complete cancelation of the H-reflex response (Figure 2-3).

![Figure 2-3: Recruitment curve of H-reflex and muscle response. Stimulus intensity starts low and gradually increases until maximal amplitude values are found. The H-reflex will reach a maximal peak-to-peak amplitude, and then decrease, whereas the muscle response will eventually plateau. This data is taken from the Joint Injury and Muscle Activation laboratory at the University of Toledo.]

Although the stimulation of the αMN results in a measurable response at the muscle, the H-reflex is debated as a direct way to measure MNPE. Other factors can influence αMN output, and therefore should be discussed when interpreting the H-reflex. Firstly, the action potentials traveling up the afferent nerve fibers must synapse at the spinal cord before being sent back down the efferent αMN fibers. In representing the H-reflex as a direct measure of MNPE, inhibition or facilitation that may occur at this synapse is not accounted for. This process is known as presynaptic inhibition. Presynaptic inhibition is generally caused by a reduction in the neurotransmitter release
from the presynaptic terminal, which results in a depression of the efferent output to the motor neuron pool (Figure 2-4).  

Once these afferent nerve fibers synapse at the spinal cord, they must be relayed by interneurons dispersed in the CNS. These interneurons act as relay stations, but can also inhibit antagonist muscle MNPE through muscle spindle or corticospinal involvement. Involvement of γ-aminobutyrate (GABA) interneurons and Ib inhibitory interneurons has also been documented to contribute to muscle inhibition by controlling presynaptic and postsynaptic mechanisms. Again, this inhibition cannot be isolated
when obtaining H-reflex measurements. Other factors not accounted for include Renshaw cell activity, which may also manipulate MNPE. Renshaw cells operate under control from the CNS, receiving information from supraspinal mechanisms and descending cortical pathways. Renshaw cells, located on the efferent loop of the αMN, can cause inhibition of the muscle by postsynaptic mechanisms. Renshaw cells have the ability to block transmission from the CNS to the αMN, resulting in recurrent inhibition of the affected MN pool (Figure 2-5).

Figure 2-5: Representation of postsynaptic/recurrent inhibition. Renshaw cells, located on the alpha motor neuron, can inhibit action potential transmission by postsynaptic means.
These naturally occurring inhibitory mechanisms cannot be separated during basic H-reflex testing, therefore it may not be appropriate to analyze this outcome as direct excitability of the MN pool. Instead, the H-reflex provides a representation of the efficacy of the entire spinal-reflexive loop. Spinal-reflexive excitability may be a more suitable term (as opposed to MNPE) to describe the outcomes of H-reflex testing. In addition, because the spinal-reflexive pathway ultimately affects αMN recruitment, alterations in this pathway may affect quadriceps activation as well as muscle strength.

As a measure of spinal-reflex excitability, the H-reflex has been commonly used in the athletic training literature to assess neuromuscular outcomes following musculoskeletal injuries as well as the effect of therapeutic modalities in manipulating this neural excitability. Specifically, many authors have used the H-reflex to evaluate persistent muscle weakness and inhibition that is present following joint injury or simulated joint effusions. Therefore, the next section of this review will focus on measures of spinal-reflex excitability and its association to the investigation of knee joint injury.

### 2.3.2 Alterations in spinal-reflexive excitability following knee joint injury

Persistent quadriceps weakness, atrophy, and an inability to fully contract the muscle are present following knee joint injury. Although the muscle itself is not damaged, these neuromuscular deficits significantly impede rehabilitative exercise goals, prohibiting reestablishment of muscle activation and strength gains. This inhibition of the muscles surrounding the knee joint is hypothesized to occur from altered excitability of the spinal-reflexive neural pathways, as described above. Arthrogenic muscle
inhibition (AMI) is a term often used to described the inability to volitionally contract the muscle following injury.\textsuperscript{24} AMI specifically refers to inhibition generated from the joint itself, however muscle inhibition may manifest from multiple sources in the nervous system, including through presynaptic and postsynaptic mechanisms along the spinal-reflexive pathway, as described in the preceding section. Therefore, for the purpose of this review, the term muscle inhibition will be used to account for neural inhibition occurring at all levels of the nervous system. Quadriceps inhibition has been shown to originate from presynaptic, postsynaptic, and gamma loop mechanisms, all effecting αMN output.\textsuperscript{9,71} It is most likely that quadriceps inhibition results from a combination of mechanisms along the spinal-reflexive loop, including mechanisms that may be currently unknown.\textsuperscript{9}

Muscle inhibition following knee joint injury is a natural response, hypothesized to be a short-term protective mechanism to limit excessive forces placed on the joint.\textsuperscript{24} However, these short-term protective mechanisms may interfere with normal muscle function long after injury, contributing to the persistent strength deficits and altered kinematics that remain following knee joint injury, specifically in the quadriceps muscle group after ACLr.\textsuperscript{4,24} Previous investigations have used experimentally induced knee joint effusions to show immediate alterations in both quadriceps\textsuperscript{14,17,19,20,64,72} and soleus\textsuperscript{57} spinal-reflex excitability. In addition, decreased muscle force output has been demonstrated following the same knee joint effusion model, suggesting that alterations in spinal-reflex excitability may manifest as decreased quadriceps function during dynamic activity.\textsuperscript{14,19} It is important to note that these models eliminate other factors associated with acute and chronic joint injury, such as pain and inflammation. On one account, this
type of research is beneficial, demonstrating that individuals who are pain free following joint injury may still present with dysfunctions in spinal-reflex excitability. In contrast, this method is still only a model, and does not fully correspond with actual joint damage or account for other concomitant factors.

Few investigations have looked to assess spinal-reflex excitability following acute joint injury, particularly because it can be confounded by many factors such as pain, lack of baseline measurements and variability among subjects. Following an extensive literature search, only two articles were found in which spinal-reflex excitability outcomes associated with knee joint injury were examined. Engelhardt et al. examined quadriceps spinal-reflex excitability one-day prior to ACLr and again 6-8 weeks post ACLr finding no differences between the two time points. However, major limitations include that the participants were “injured” at both time points, and therefore dysfunction of the nervous system may have already occurred at injury and remained unchanged after surgery. In addition, there was no control group for comparative purposes. The other investigation by Hoffman and Koceja showed no significant differences in soleus spinal-reflex excitability between individuals 30-months post ACLr and healthy controls. Inhibition was also not detected in the contralateral muscle, which is in agreement with experimental effusion models.

Spinal-reflex dysfunction was not found in either investigation examining injured patients, whereas those that utilized effusion models unanimously showed inhibition following the effusion. The lack of baseline testing is likely a reason why spinal-reflex inhibition was not found in injured individuals, which makes it difficult to assess changes in the neural system. Variability also becomes an issue, as stated earlier.
Comparing group means may mask individual’s alterations in spinal-reflex excitability. Other methods of analysis, such as assessing change scores over multiple time periods, may be more beneficial. Nonetheless, it remains unclear why these two models are conflicting, however it stresses the need for further research on individuals who have actually suffered an acute or chronic joint injury to understand spinal-reflexive alterations in this population.

### 2.3.3 Effect of spinal-reflexive alterations on function and disease progression

It remains unclear how spinal-reflexive excitability is affected following joint injury, however effusion models, used to simulate joint injury, have been able to acutely induce quadriceps muscle inhibition and alterations in spinal-reflexive pathways.\textsuperscript{14,17,19,21,57,64,68,72,74} These investigations have suggested that these alterations may serve as the genesis of gross neuromuscular deficits that are present following joint injury.\textsuperscript{4,9,14,15} Conversely, neural inhibition following joint injury is not well understood, and although theoretically may be the possible origin of neuromuscular dysfunction, more research is needed on the actual population of interest.

The paucity of data on injured individuals makes it necessary to review evidence from effusion models in an attempt to understand how alterations in spinal-reflex excitability effect function and disease progression following joint injury. Following experimental effusions, subjects have demonstrated inhibition of the quadriceps muscle\textsuperscript{14,21} and facilitation of the hamstrings\textsuperscript{14,21} and soleus\textsuperscript{57} muscles during dynamic activity. Consequently, these individuals’ demonstrated increases in ground reaction
forces and decreases in net knee extension moments and peak knee flexion angle during a
dynamic jump-landing task.\textsuperscript{14,68} In contrast, Torry et al.\textsuperscript{21}, demonstrated greater knee
flexion angles during the stance phase of gait following joint effusion. The literature
suggests that inhibition of the quadriceps muscle can surprisingly result in either
increases or decreases in knee flexion angle during dynamic activity. The authors infer
that these alterations result from “quadriceps avoidance,” in which the individual has an
inability to fully contract that muscle during activity. “Avoidance” of the quadriceps
muscle during activity\textsuperscript{21} can either result in an inability of the quadriceps to extend the
knee joint and thereby increase knee flexion, or could cause a compensatory reliance on
hip musculature to keep the knee in a more extended position.\textsuperscript{14} This quadriceps
avoidance pattern has also been observed in patients with ACL injury.\textsuperscript{75}

Although it has been shown that quadriceps inhibition can cause either increases
or decreases in knee flexion angle, it appears that spinal-reflexive alterations results in
increased loading at the knee joint regardless of the increase or decrease in knee flexion
angle.\textsuperscript{9,14,21,68,74} This most likely occurs due to the failure of the quadriceps muscle to act
as a mechanical restraint during joint loading, causing increases in ground reaction forces
as well as the forces being transferred through the joint.\textsuperscript{14} This link between muscle
inhibition and improper force attenuation leads investigators to believe that muscle
inhibition and altered spinal-reflexive excitability may lead to the progression and onset
of knee joint OA. A possible theoretical model states that through modified afferent input
from joint damage, pain, etc., alterations in the spinal-reflexive loop are made to initiate
inhibition of the muscle, as well as dysfunction in muscle activation. Quadriceps
inhibition and activation failure leads to impairments in muscle strength, kinematics and
kinetics, resulting in altered knee joint loading. These alterations eventually would lead to abnormal wear patterns and cartilage degeneration.\textsuperscript{14}

It still remains unclear, however, how the spinal-reflexive pathway is affected, if at all, following ACLr. Because this has not been extensively researched in injured populations, there is no data to link spinal-reflexive excitability measures to disease progression or self-reported measures of disability. However, because of the unsatisfactory outcomes associated with conventional rehabilitation, some have suggested that targeting decreasing neural mechanisms may be needed to regain full muscle activation and force generating capabilities of the quadriceps muscles.\textsuperscript{5,6} But again, evidence has not shown that alterations in spinal-reflex excitability is linked to muscle strength and volitional activation, nor that restoring these levels will restore normal muscle function. In addition, spinal-reflexive mechanisms have only shown small contribution to knee extensor torque (~12%) in healthy individuals, suggesting involvement of other neural pathways, possibly supraspinal.\textsuperscript{76} Therefore, it is important to first understand the origins of neuromuscular dysfunction, and how the neural system is altered at different time points following joint injury, before treatment interventions are researched.

\subsection*{2.4 \textbf{Corticospinal Excitability of Musculature Surrounding the Knee Joint}}

\subsubsection*{2.4.1 \textbf{Background}}

Corticospinal pathways have been shown to play a major role in neuromuscular function and motor control.\textsuperscript{77,78} The motor cortex of the brain is an extensive area which
contains specific sections of corticospinal output that correspond to muscles and motoneuron pools around the body. It has been shown that the rapidly conducting axons in corticospinal pathways have a direct effect on discharging motor units and also will initiate motoneuron activity in voluntary muscle contraction. In addition, these pathways have the ability to respond to altered afferent inputs and adjust their excitability following muscle training and various pathologies. Therefore, this pathway can drive motor function by initiating movement, but also adapt to altered input allowing for modifications in the motor system.

As mentioned above, spinal-reflexive excitability is an estimate of muscle MNPE, specifically at the spinal level. However, when measuring MNPE in this manner, one cannot estimate the contribution of the neural signals being sent from the cortical level. Supraspinal mechanisms, which may manipulate the neuromuscular system, are therefore unable to be assessed by these methods. Corticospinal excitability incorporates both cortical and spinal-reflexive neural pathways when investigating neuromuscular control. These measures can be derived using transcranial magnetic stimulation (TMS), in which a specific area of the motor cortex is magnetically stimulated and a muscle twitch response is visualized using EMG in a specific muscle. Once the specific area of the motor cortex is stimulated, a simulation of motor control that is processed at the cortical level, an action potential is sent down descending efferent pathways of the spinal cord (Figure 2-6). Once at the spinal nerve associated with the muscle of interest, the action potential travels along the efferent limb of the spinal-reflexive loop to the muscle as described previously.
Corticospinal excitability has been previously investigated through the use of electroencephalography (EEG) and TMS, which is the direct magnetic stimulation of the motor cortex. It remains unclear whether the magnetic stimulus in TMS is directly activating corticospinal cells at the soma and axon, or indirectly by means of other cortical cells that synapse on the corticospinal tract. Regardless, most believe this to be irrelevant, as either response would be influenced by changes in the corticospinal pathway’s excitability, and therefore would result in the outcome of interest. Motor evoked potentials (MEP) induced by the magnetic stimulation are measured at the muscle through EMG, and provide beneficial information about the transmission in corticospinal pathways. However, some of the TMS literature is hesitant to make conclusions concerning corticospinal function, and leave interpretations to the transmission of the pathways only. Many researchers also recommend consistent TMS testing procedures.
such as coil size, stimulation type etc. to help validate conclusions on corticospinal excitability. Most agree that TMS is a powerful technique in the analysis of motor control, and that combining corticospinal excitability with spinal-reflexive measures allows for a more comprehensive evaluation of the neural system’s influence on neuromuscular function.

Two of the most common methods of quantifying corticospinal excitability via means of TMS are MEPs and motor thresholds (MT). Motor thresholds can be achieved during both active (AMT) and resting (RMT) states and is believed to be an estimate of pyramidal cell membrane excitability, as well as represent the integrated excitability of corticomotor projections. Although the physiological difference between AMT and RMT is debated, RMT is thought to only include small and slow-propagating pyramidal neurons, while AMT is thought to also include the contribution of fast-propagating pyramidal neurons. Therefore, increases in MT are interpreted as a decrease/decline in excitability, i.e., a higher stimulus is needed to excite the pyramidal cells. MEPs are used to ensure integrity of the corticospinal tract, as well as represent the excitability of the corticospinal system and its ability to send transmissions. MEPs are most commonly evaluated by peak-to-peak amplitude (magnitude of stimulus that is transmitted through the system), or latency (time it takes for stimulus to reach muscle of interest). Longer latency and smaller peak-to-peak amplitudes are interpreted as decreases in corticospinal function, i.e., it takes longer for the stimulus to reach the muscle, and less of the stimulus reaches the muscle of interest. These two measurements have been used extensively to evaluate the pathophysiology of psychiatric and neurological disorders and establish declines in corticospinal function in patients experiencing schizophrenia, mood disorders,
Tourette’s, stroke, epilepsy, traumatic brain injury, as well as other neurological and psychological disorders. Data examining corticospinal alterations in response to musculoskeletal injury are lacking, however, will be discussed in section 2.2.4 below.

In addition to measures of corticospinal function, TMS is often used to evaluate excitability of intracortical pathways, specifically though investigation or intracortical inhibition (SICI) and facilitation (ICF). A conditioning pulse is typically used in the measurement of intracortical excitability, which reduces the amplitude of synaptically evoked corticospinal volleys through GABA-ergic connections (gamma-aminobutyric acid), resulting in synaptic inhibition or faciliation of the corticospinal neurons that are targeted by a testing stimulus above the threshold level. SICI and ICF are thought to be mediated by GABA\(_A\) and NMDA (N-methyl-\(D\)-aspartate) receptors, respectively. Increases in SICI are interpreted as an increase in intracortical inhibition, mediated by GABA, and have been demonstrated in stroke patients as well as older individuals compared to younger. This increase in inhibition is thought to be a neuroplastic mechanism that contributes to deficits in neuromuscular function in these populations, however results are conflicting in other investigations. In contrast, increase in ICF is thought to represent an increase in facilitation in the intracortical network. Both increased ICF (more facilitation) and decreased SICI (less inhibition) represent a similar neurological outcome, these responses are suggested to be mediated by different mechanisms, as explained above. ICF has been previously examined following different interventions, to help understand if clinicians can increase facilitation of this network by pharmacological or therapeutic means. A few recent studies have shown that both pharmacological interventions (stroke, epilepsy) and therapeutic exercise (stroke,
healthy\textsuperscript{89,95} can increase intracortical facilitation, however as in SICI, results are conflicting.\textsuperscript{87} Unfortunately, no investigations have examined intracortical alterations following joint injury, and therefore is a novel approach in attempting to understand the origins of disability following lower extremity injury.

2.4.2 Alterations in corticospinal excitability following knee joint injury

Alterations in corticospinal pathways have been shown following ACL injury, further suggesting the contribution of these pathways to quadriceps dysfunction.\textsuperscript{23} However, there are very few investigations that have assessed corticospinal excitability following knee joint injury, which makes interpretation of these results difficult. The data does suggest that an increase in corticospinal excitability may be present following joint injury (Table 2.2).\textsuperscript{23,84} The authors propose that this increase in excitability is a response to altered input from the injured joint, and that corticospinal pathways are up-regulating in an attempt to maintain motor function.\textsuperscript{23,84}
Table 2.2: Articles assessing corticospinal excitability following knee joint injury.

<table>
<thead>
<tr>
<th>Author</th>
<th>Corticospinal Excitability Measurement</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baumeister et al.(^{84})</td>
<td>EEG</td>
<td>ACLr individuals demonstrated increases in EEG activity while quadriceps EMG activity remained similar to healthy controls during a knee extension task.</td>
</tr>
<tr>
<td>Engelhardt et al.(^{70})</td>
<td>TMS</td>
<td>No significant differences exist in MEP latency and amplitude values of the tibialis anterior prior to ACLr and post-ACLr.</td>
</tr>
<tr>
<td>Heroux et al.(^{23})</td>
<td>TMS</td>
<td>ACL injured participants demonstrated higher levels of corticospinal excitability than healthy controls. (Decrease in RMT, correlation between SR curve and quadriceps strength).</td>
</tr>
</tbody>
</table>

Abbreviations: EEG, electroencephalography; TMS, transcranial magnetic stimulation; ACLr, anterior cruciate ligament reconstruction; EMG, electromyography; MEP, motor evoked potential; RMT, resting motor threshold; SR, stimulus response.

It should also be noted that TMS has been used previously as an intervention, aimed at increasing voluntary activation and strength of muscles following joint injury.\(^{82}\) Urbach et al.,\(^{82}\) found that quadriceps voluntary activation can be immediately increased in arthroplasty patients when TMS is used simultaneously with MVICs. This enhancement in muscle activation even lasted up to 60 minutes post stimulation. The significance of this evidence is two-fold. First, this evidence suggests that corticospinal excitability may be altered following joint injury and may have an effect of muscle activation. Secondly, by stimulating the motor cortex and targeting corticospinal excitability, it may be possible to increase function following joint injury by targeting corticospinal neural levels. However, as described below, the overall effect that corticospinal pathways have on function following joint injury remains unknown.
2.4.3 Effect of corticospinal alterations on function and disease progression

Limited data show that alterations in corticospinal excitability are present following knee joint injury, however it is unknown how these alterations affect overall function. Baumeister et al.,84 showed that corticospinal excitability was up regulated following ACLr, with no change in force production or EMG activity when compared to healthy controls. The authors suggest that individuals may be able to up regulate corticospinal function in response to injury in order to maintain proper muscle function.

Other investigations have used fatiguing protocols to induce a decrease in corticospinal excitability and have observed an accompanying decrease in voluntary muscle activation, specifically in the elbow flexors.96 However, others refute this investigation’s conclusion, stating that decreases in these measures do not indicate a causal relationship.97-100 Other investigations have shown that muscle activation failure can occur following exercise and fatigue, but regardless of maintained or altered corticospinal excitability.99,100 Further, Kalmar et al.97 induced increases in quadriceps corticospinal excitability, through the use of caffeine, without accompanying enhancements in voluntary activation.

Unfortunately, there are limited investigations that look to determine how alterations in quadriceps corticospinal excitability affect kinematics, strength, self-reported function etc., specifically following joint injury. This leaves a significant knowledge gap in the understanding of how neural pathways affect disease progression and function.
2.5 Summary of ACL Rehabilitation

To successfully restore quadriceps strength and promote long-term knee joint health, several rehabilitation objectives have been identified following ACL reconstruction. Although the progression of each ACL rehabilitation protocol depends on surgical procedures, concomitant injuries and individual patient progression, clinicians can universally agree that the following items are essential components; control of post-operative pain and swelling, protection of healing graft, restoration of full range of motion, strengthening of lower extremity muscles that stabilize the knee, improve neuromuscular control and progression to functional activities.\textsuperscript{101-103}

Advances in ACL reconstruction and rehabilitation have allowed clinicians to achieve most of the above-mentioned goals, as most individuals have successfully controlled post-operative pain\textsuperscript{104,105} and swelling,\textsuperscript{105} restored knee joint range of motion\textsuperscript{106,107} and are capable of performing functional activities.\textsuperscript{101,108} Despite these successes, an optimal rehabilitation protocol that is capable of restoring pre-injury quadriceps strength has not been identified. A recent review\textsuperscript{5} demonstrated that ACLr individuals have a high magnitude of isokinetic quadriceps strength deficits at varying time points post-reconstruction. To highlight the effect that traditional rehabilitation has on restoring quadriceps strength, I have extracted the articles from this study\textsuperscript{5} that examined ACLr patients at the 6-month post-surgery time point. Quadriceps strength deficits exceed 24\% at 6-months post-surgery, which is a time when individuals are finishing traditional rehabilitation and cleared for full activity (Table 2.3). As quadriceps strength has been associated with joint space narrowing\textsuperscript{10} and self reported function\textsuperscript{11,12} following ACLr, it seems imperative that further advances in rehabilitation strategies are
needed in order to maximize quadriceps strength following ACL injury and reconstruction.

Table 2.3: Summary of articles evaluating isokinetic knee extension strength (60°/sec) in ACLr patients at 6mo post-surgery. Adapted from Palmieri-Smith et al., 2008.5

<table>
<thead>
<tr>
<th>Article</th>
<th>Quadriceps Strength Deficit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson et al.</td>
<td>28.7%</td>
</tr>
<tr>
<td>Aune et al.</td>
<td>25%</td>
</tr>
<tr>
<td>Beard et al.</td>
<td>29%</td>
</tr>
<tr>
<td>Cardone et al.</td>
<td>25%</td>
</tr>
<tr>
<td>Gobbi et al.</td>
<td>15.6%</td>
</tr>
<tr>
<td>Gokeler et al.</td>
<td>25.1%</td>
</tr>
<tr>
<td>Keays et al.</td>
<td>28.6%</td>
</tr>
<tr>
<td>Kaeys et al.</td>
<td>12%</td>
</tr>
<tr>
<td>Kaeys et al.</td>
<td>7.3%</td>
</tr>
<tr>
<td>Kobayashi et al.</td>
<td>36.8%</td>
</tr>
<tr>
<td>Lee et al.</td>
<td>28%</td>
</tr>
<tr>
<td>McHugh et al.</td>
<td>33%</td>
</tr>
<tr>
<td>Soon et al.</td>
<td>8.2%</td>
</tr>
<tr>
<td>Tashiro et al.</td>
<td>25%</td>
</tr>
<tr>
<td>Witvrouw et al.</td>
<td>40.5%</td>
</tr>
<tr>
<td>Wojtys et al.</td>
<td>24%</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>24.5%</strong></td>
</tr>
</tbody>
</table>

There are many variations in the approach researchers and clinicians take when developing rehabilitation protocols. Researchers have evaluated the effectiveness of accelerated versus traditional rehabilitation protocols, and typically agree that patients who participate in an accelerated program tend to demonstrate better quadriceps strength, knee joint motions and reduced anterior knee pain.125-127 Accelerated rehabilitation encourages immediate range of motion exercises, near-immediate weight bearing, close
kinetic chain exercises and early progression into strengthening and functional activities, in contrast to traditional approaches, which are typically more conservative in an attempt to protect the ligament. However, a recent randomized, double-blinded investigation demonstrated that at 2-years post-reconstruction, there were no clinically relevant differences in strength between individuals who participated in an accelerated versus a non-accelerated program. It has also been debated whether the typical concentric strength training used in traditional ACL rehabilitation protocols is the most efficient method to maximize strength gains. The application of early post-operative high-intensity eccentric resistance training has traditionally been contraindicated; however recent evidence suggests this mode of strength training is not only safe, but also more effective than concentric training.\textsuperscript{128} Greater gains in quadriceps strength have been discovered following both six\textsuperscript{129} and 12-week\textsuperscript{130} eccentric exercise interventions as opposed to traditional rehabilitation.

Another important component of rehabilitation, which is commonly overlooked, is the ability to remove inhibition of the surrounding musculature.\textsuperscript{15,16,24} As stated earlier in this review, it has been hypothesized that alterations in neural pathways may mediate strength deficits observed in this population. Although it has been theorized that supplementing traditional rehabilitation with modalities proposed to target neural pathways would be beneficial in the restoration of quadriceps strength, this topic remains understudied.
2.6 Conclusion of Literature Review

Neuromuscular dysfunction following knee joint injury is common, and often persists long after initial injury. Specifically, quadriceps dysfunction following ACLr can lead to alterations in lower extremity kinematics, possibly resulting in joint degeneration and the progression of OA. The origin of this neuromuscular dysfunction, however, remains unknown. Although it is hypothesized that neural pathways, such as the corticospinal and spinal-reflexive levels, play a role in altering muscle function, there is limited research to support the effects of neural pathways on neuromuscular function following joint injury. It remains imperative to understand the genesis of neuromuscular dysfunction, specifically the association between these measures and overall physical and self-reported function.
Chapter 3

Methodology

3.1 Research Design for Specific Aims 1 and 2

The research design used in this current research was a case-control study. The case group included patients who had a unilateral rupture of their anterior cruciate ligament (ACL) and were scheduled to undergo anterior cruciate ligament reconstructions (ACLr). The control group was comprised of healthy participants, with no history of lower extremity joint injury. The University of Toledo’s institutional review board approved this study (IRB #107707; Appendix A-B).

3.2 Overview of Methods

3.2.1 Patients

ACLr patients between the ages of 15-45 were recruited from two orthopedic surgeons at the University of Toledo medical center, Dr. Jason W. Levine MD, and Dr. David H. Sohn MD, JD, as well as from Dr. Joe Assenmacher of ProMedica Health
System, Toledo, OH. All patients invited to participate in the ACLr group had sustained a unilateral ACL rupture, which was confirmed by physician examination and magnetic resonance imaging. There were no previous orthopedic surgeries or ligamentous knee injury to either the uninvolved limb or other joints in the involved limb. Other exclusion criteria included a history of a lower extremity injury other than ACLr in the last 6 months, history of seizures, history of a concussion in the past 6 months and previous history of diagnosed cancer over magnetic stimulation points (brain) or electrical stimulation points (anterior thigh). Healthy matched control participants, with no previous history of lower extremity joint surgery or serious lower extremity musculoskeletal injury within the past 6 months, were recruited from the University of Toledo and local area high schools through the use of flyers and classroom announcements.

3.2.2 Power analysis

Prior to data collection, an initial power analysis was performed using means and standard deviations for active motor thresholds (AMT) from pilot data of 12 individuals (6 ACLr = 45.2 ± 13.4%tesla; 6 Healthy: 33.6 ± 9.6). Standardized effect sizes were calculated between groups, indicating that 12 participants per group would be needed to find statistical significance. This allows a 5% chance of committing a type I error and a 20% chance of committing a type II error.

3.2.3 Patient timeline

Outcomes were assessed at three different time points. Pre-surgery measurements
were evaluated after injury, but prior to surgery (average: 35.8 ± 14.8 days post-ACL injury). All outcome measures were also collected at 6-months post-surgery, or when patients were cleared for full activity (average: 28.1± 2.8 weeks post-surgery). Outcomes for specific aim one only were also collected at approximately 2-weeks post-surgery (average: 15.8 ± 2.5 days post-surgery). A summary of outcome variables and time points collected can be found in Table 3.1.

Table 3.1: Summary of outcome measures and time of collection.

<table>
<thead>
<tr>
<th>Pre Surgery</th>
<th>2-weeks post-surgery</th>
<th>6 months post-surgery (Return to activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinal-reflexive Excitability</td>
<td>Spinal-reflexive Excitability</td>
<td>Spinal-reflexive Excitability</td>
</tr>
<tr>
<td>- H:M</td>
<td>- H:M</td>
<td>- H:M</td>
</tr>
<tr>
<td>Corticospinal Excitability</td>
<td>Corticospinal Excitability</td>
<td>Corticospinal Excitability</td>
</tr>
<tr>
<td>- AMT</td>
<td>- AMT</td>
<td>- AMT</td>
</tr>
<tr>
<td>- MEP:M</td>
<td>- MEP:M</td>
<td>- MEP:M</td>
</tr>
<tr>
<td>Intracortical Excitability</td>
<td>Intracortical Excitability</td>
<td>Intracortical Excitability</td>
</tr>
<tr>
<td>- SICI</td>
<td>- SICI</td>
<td>- SICI</td>
</tr>
<tr>
<td>- ICF</td>
<td>- ICF</td>
<td>- ICF</td>
</tr>
<tr>
<td>Quadriceps Strength</td>
<td>Self-reported Function</td>
<td>Quadriceps Strength</td>
</tr>
<tr>
<td>- MVIC</td>
<td>- IKDC</td>
<td>- MVIC</td>
</tr>
<tr>
<td>Voluntary Quadriceps Activation</td>
<td></td>
<td>Voluntary Quadriceps Activation</td>
</tr>
<tr>
<td>- CAR</td>
<td></td>
<td>- CAR</td>
</tr>
<tr>
<td>Knee Joint Mechanics</td>
<td></td>
<td>Knee Joint Mechanics</td>
</tr>
<tr>
<td>- Peak knee flexion angle</td>
<td></td>
<td>- Peak knee flexion angle</td>
</tr>
<tr>
<td>- Peak internal knee extension moment</td>
<td></td>
<td>- Peak internal knee extension moment</td>
</tr>
<tr>
<td>Self-reported Function</td>
<td></td>
<td>Self-reported Function</td>
</tr>
<tr>
<td>- IKDC</td>
<td></td>
<td>- IKDC</td>
</tr>
</tbody>
</table>

Abbreviations: H:M, Hoffmann reflex normalized to maximal muscle response; AMT, active motor threshold; MEP:M, motor evoked potential normalized to maximal muscle response; SICI, short interval intracortical inhibition; ICF, intracortical facilitation; MVIC, maximal voluntary isometric contraction; CAR, central activation ratio; IKDC, International Knee Documentation Committee form.
3.3 Outcome Measures for Specific Aim 1

3.3.1 Aim Overview

All outcomes were collected bilaterally in the ACLr group, and in a dominance matched limb of the healthy control group. For example, if an ACL patient injured their dominant limb, the dominant limb of the healthy matched control participant would be the limb tested. Limb dominance was determined by asking the patient which limb they would use to kick a ball.

3.3.2 Quadriceps spinal-reflexive excitability testing

For electromyographic (EMG) assessment during spinal-reflexive excitability testing, two 10mm, pre-gelled Ag-AgCl (EL503, BIOPAC Systems Inc.) surface EMG electrodes were positioned over the distal vastus medialis muscle belly of both limbs with an inter-electrode distance of 1.75cm. When necessary, the hair over the collection sites were shaved and the skin over the recording electrode site was debrided and cleaned with alcohol.

Quadriceps Hoffmann reflexes normalized to maximal muscle responses (H:M) were used to assess quadriceps spinal-reflexive excitability. The stimulation used in this testing has been deemed safe for humans and has been used previously by members of this research team and other laboratories for assessing spinal reflexive-excitability. Participants were positioned supine on a padded treatment plinth with their arms comfortably placed at their side, their head in a neutral position, and their knee
slightly flexed and supported by a half bolster (Figure 3-1). EMG signals were band-pass filtered from 10 to 50 Hz and collected at 1024 Hz with a common-mode-rejection ratio of 110 dB using AcqKnowledge 4.0 software (Biopac Systems Inc., Goleta, CA). A 2mm shielded disc stimulating electrode (EL2524S, BIOPAC Systems Inc.) was positioned over the femoral nerve and secured with hypoallergenic tape. A 4x4cm self-adhesive electrode was placed over the ipsilateral hamstring muscle group to be used as a dispersive electrode. A 1ms square wave stimulus was produced with a BIOPAC stimulator module (STM100A, BIOPAC Systems, Inc.) and a 200 volt maximum stimulus adaptor (STMISOC, BIOPAC Systems Inc.) and delivered to the femoral nerve. Stimulus intensity was increased until a maximal Hoffmann reflex was found, in which an increase in the stimulus intensity resulted in a decrease of the peak-to-peak amplitude of the Hoffmann reflex. The stimulus intensity was then increased until a maximal muscle response was detected, in which an increase in the stimulus intensity resulted in no further increase in the peak-to-peak amplitude of the muscle response. The average peak-to-peak values of three maximal Hoffmann reflexes were normalized to the average peak-to-peak values of three maximal muscle responses to create the H:M outcome variable.
3.3.3 Quadriceps corticospinal excitability testing

Transcranial magnetic stimulation (TMS) was used to collect active motor threshold (AMT) and the amplitude of motor evoked potentials (MEP) elicited at 120% of AMT. The collection of MEPs has been safely collected and reported in variety of participant populations (ACL injuries, healthy participants, hemiparesis) and has been determined reliable. These methods have been previously used and presented by
members of the research team.\textsuperscript{27,28,132} EMG recording electrodes were positioned in the same manner as reported above during spinal-reflexive excitability testing.

During testing, participants were positioned seated in the Biodex System III Pro Dynamometer (Biodex Medical Systems, Shirley, NY), with their knees and hips at 90° of flexion and a lap belt placed over their lap. The tested limb was secured into the dynamometer arm using a Velcro strap. A lycra swim cap was placed on the participant’s head, and straight lines were drawn vertically in the sagittal (center of the occiput to the nose) and frontal planes (connecting each external auditory meatus).\textsuperscript{133} Intersection of these lines, at the vertex of the skull, allowed for identification of the motor cortex location. A double cone TMS coil (Magstim Company, Wales, UK) was positioned over the intersected lines, and a stimulus of 50% of the maximal stimulator output was used to locate the optimal stimulating point on the motor cortex. The coil was then moved in increments of 0.5 cm in an anterior-to-posterior direction over the vertex of the cranium until the optimal stimulating point was detected, which was defined as the optimal motor cortex location producing the greatest muscle response visualized through EMG.\textsuperscript{134} Once this area was detected and marked on the swim cap, the simulator was secured into that spot using a flexible camera mount (Manfrotto Company, Cassola, Italy) (Figure 3-2).

Once the stimulator was secured, AMT was located, which was defined as the lowest TMS intensity required to evoke a measureable (>100μV) MEP in five out of 10 trials (Appendix C).\textsuperscript{135} Once AMT was established, eight MEPs were evoked at 120% of AMT. The eight peak-to-peak MEP amplitude values were averaged and then normalized to the average of three maximal muscle responses, which were elicited during spinal-reflexive excitability testing.
In addition to AMT and MEP outcomes, measures of intracortical excitability (short interval intracortical inhibition and intracortical facilitation) were also collected using TMS. Intracortical excitability measures were collected in the same position and

3.3.4 Intracortical excitability testing

Figure 3-2: Testing setup for corticomotor excitability.
same manner as corticospinal excitability testing. A paired conditioning-test stimulus technique was used to assess both short interval intracortical inhibition (SICI) and intracortical facilitation (ICF).\textsuperscript{136,137} During testing, a conditioning pulse was set to 80% of active motor threshold in order to provide a subthreshold stimulus to produce a short period of suppression in EMG activity. Physiologically, the subthreshold conditioning pulse reduces the amplitude of synaptically evoked corticospinal volleys through GABAergic connections (gamma-aminobutyric acid), resulting in synaptic inhibition of the corticospinal neurons that are targeted by a testing stimulus above the threshold level.\textsuperscript{88,89} SICI and ICF are thought to be mediated by GABA\textsubscript{A} and NMDA (N-methyl-D-aspartate) receptors, respectively.\textsuperscript{90} Following the subthreshold stimulus, a subsequent testing stimulus of 120% of AMT was given at a time of 3ms during SICI testing and 15ms during ICF testing.\textsuperscript{136,137} The time interval between stimuli determines the effect the conditioning pulse has on the measured MEP.\textsuperscript{88} Eight SICI and eight ICF MEPs were evoked, normalized to raw MEP values at 120% AMT and averaged for analysis.\textsuperscript{88,136-140} It should be noted that a smaller SICI outcome value represents a greater level of inhibition in the intracortical neurons. Higher ICF value represents a greater level of facilitation.

During all corticospinal and intracortical excitability testing, each participant was directed to isometrically contract the limb at 5% of their MVIC,\textsuperscript{141} maintaining this contraction while the magnetic stimulus was given. Visual feedback of the torque generated from the contraction was provided in real time on a custom computer software program (Microsoft Visual Basic, Redmond, WA). Participants were given rest and instructed to stop contracting between stimuli.
3.4  **Outcome Measures for Specific Aim 2**

For specific aim 2, all outcomes were collected in the injured limb of the ACLr group, and in a dominance matched limb of the healthy control group.

**3.4.1 Quadriceps muscle strength**

Maximal voluntary isometric contractions (MVIC) of the quadriceps were used to assess quadriceps muscle strength. Participants were seated in the Biodex Systems III dynamometer in the same manner as during corticospinal excitability testing, with the knee and hip joints positioned in 90° of flexion. In addition, bilateral shoulder and lap straps were used to secure the participant into the dynamometer to limit any unwanted movement. To ensure maximal effort, both verbal and visual feedback was given. For the visual feedback, the torque generated from the contraction was provided in real time on a custom computer software program (Microsoft Visual Basic, Redmond, WA) and the participant was instructed to try and get the graph to go as high as they could. Participants were allowed warm up trials at their self-perceived effort of 25%, 50% and 75% of their maximal effort. Following the warm-up, participants performed MVIC trials until there was no more increase in torque. The last two trials were averaged and taken as the MVIC. At least one-minute rest was given between trials.

**3.4.2 Voluntary quadriceps muscle activation**

Voluntary quadriceps activation was assessed using the burst superimposition
technique (SIB) and quantified using the central activation ratio (CAR).\textsuperscript{39,51} An automated system was used to trigger the superimposed burst stimulation as it has been reported to improve reliability of this technique (Appendix D).\textsuperscript{143} Two 7x13cm self-adhesive stimulating electrodes were positioned on the proximal vastus lateralis and the distal vastus medialis (Figure 3-3). During activation testing, 125 volts (100ms train of 10 stimuli, at 100 pps, with pulse duration of 0.6 ms, and a 0.01 ms pulse delay) of electrical stimulation of the quadriceps was triggered through the use of a square wave stimulator (S88, GRASS telefactor, W. Warwick, RI) and a stimulation isolation unit (SIU8T, W. Warwick RI) while the participant performed an MVIC. A depiction of the calculation used to quantify voluntary activation can be found in an early figure of this manuscript (Figure 2-1, Appendix D).
3.4.3 Knee joint mechanics during stair ambulation

Knee joint mechanics (peak knee flexion angle, peak internal knee extension moments) were evaluated during stair ambulation using 12 Eagle motion capture cameras (Motion Analysis Corporation, Santa Rosa, CA) and an AMTI OR6-5 force plate (Advanced Motion Technology, Inc., Watertown, MA). Prior to collection, participants
were equipped with 35 retroreflective markers over various landmarks of the body including: bilateral acromioclavicular joints, anterior superior iliac spines, posterior superior iliac spines, iliac crests, greater trochanters, anterior thigh, medial and lateral femoral condyles, proximal, lateral and anterior shanks, medial and lateral malleoli, dorsum of foot, 2\textsuperscript{nd} and 5\textsuperscript{th} ray, calcanei and on cervical vertebra 7. (Figure 3-4).

Following application of the retroreflective markers, participants were instructed to stand in front of a custom built staircase with force plate integration (Dimensions of staircase can be found in Appendix E). Following an initial static trial, participants performed five ascending trials and five descending trials for each limb (total of 20 trials), in which the limb of interest would strike the force plate (Figure 3-5). A short rest was provided
between trials. Cortex 3.6.0.1312 motion capture/processing software (Motion Analysis Corporation, Santa Rosa, CA) was used for collection and tracking of all motion trials, while Visual 3D (Version 4; C-Motion, Inc., Germantown, MD) software was used for post-processing (Figure 3-6).

Lower limb joint rotations were defined based on the initial static trial of each participant and aligned with a three-dimensional lab coordinate system. A kinematic model consisting of seven skeletal segments (bilateral foot, shank and thigh segments, and pelvis) were created using the static trial. The three-dimensional marker trajectories of each stair walking trial were processed within the individual participant’s kinematic model. Joint rotations during the stair task were calculated using the Cardan rotation sequence and were expressed relative to each participant’s static trial. Kinematic data were sampled at 200Hz and synchronized ground reaction force data were sampled at 2000Hz and filtered using a fourth-order, zero-lag, low-pass Butterworth filter with a frequency cut-off of 6Hz. Filtered kinematic and ground reaction force data were entered into Visual 3D software using a standard inverse dynamics approach. Kinetic outputs were normalized to participant’s body height and mass (Nm/kg*m) and represented as internal moments. Biomechanical data were normalized to 100% of the stance phase, with gait markers of heel contact and toe-off equating to the time when vertical ground reaction force exceeded and fell below 10N. Peak knee joint angles and internal extension moments were evaluated in the sagittal plane over the first 50% of stance phase and averaged across the five trials for both ascending and descending tasks (Appendix F).
Figure 3-5: Participant walking up custom built staircase

Figure 3-6: Screenshot of Cortex software (A), used for capturing 3D motion data and Visual 3D software (B), used for post-processing and analysis of 3D motion data.
3.4.4 Patient self-reported function

The International Knee Documentation Committee (IKDC) questionnaire (Appendix G) was used to measure self-reported function. The IKDC has been proven reliable for self-assessing knee function.\textsuperscript{153,154}

3.5 Statistical Analyses

3.5.1 Demographics

Independent t-tests were performed on age, height and mass at baseline and mass at 6-months post-surgery to detect demographic differences between the ACLr and healthy control groups. Alpha level was set \textit{a priori} at $P \leq 0.05$.

3.5.2 Specific aim 1

Separate 3x3 (limb x time) analyses of variance (ANOVA) with repeated measures on time were used to assess differences between the ACL reconstructed limb, the uninjured ACL limb, and matched healthy limb in spinal-reflexive (H:M), corticospinal (AMT, MEP at 120\%AMT), and intracortical excitability (SICI, ICF) at the three separate times of pre-surgery, 2-weeks post and 6-months post-surgery. Tukey post-hoc comparisons were performed when appropriate. Alpha level was set \textit{a priori} at $P \leq 0.05$.

3.5.3 Specific aim 2

Prior to correlation analyses, normality was assessed using skewness (normal
distribution: < 0.5; > -0.5) and kurtosis (normal distribution: 3) statistics for each variable (Appendix H). Based on the non-normal distribution of outcome variables, separate Spearmen Rho correlation matrices were performed at the pre-surgery and 6-months post-surgery time points on the injured limb of the ACLr group and matched limb of the control group independently. Correlation analyses were performed to determine the association between quadriceps spinal-reflexive excitability (H:M), corticospinal excitability (AMT, MEP at 120 %AMT), intracortical excitability (SICI, ICF), strength (MVIC), voluntary activation (CAR), knee joint biomechanics (peak knee angles and moments during stair ambulation) and self-reported function (IKDC). Alpha level was set 

\textit{a priori} at P \leq 0.05.
Chapter 4

Results

4.1 Subject Demographics

A total of 34 individuals volunteered to participate (17 ACLr, 17 controls).

Complete demographic data can be found in Table 4.1. No demographic differences were observed between groups (P > 0.05).

Table 4.1: Patient demographics (Means ± SD)

<table>
<thead>
<tr>
<th></th>
<th>ACLr group</th>
<th>Healthy Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>17 (9 female, 8 male)</td>
<td>17 (9 female, 8 male)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.0 ± 4.8</td>
<td>22.1 ± 3.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.1 ± 7.3</td>
<td>173.3 ± 10.7</td>
</tr>
<tr>
<td>Mass at baseline (kg)</td>
<td>77.7 ± 11.2</td>
<td>75.3 ± 21.3</td>
</tr>
<tr>
<td>Mass at 6-month follow up (kg)</td>
<td>77.5 ± 11.7</td>
<td>75.7 ± 20.9</td>
</tr>
<tr>
<td>Days post-injury (Time point 1)</td>
<td>35.8 ± 14.8</td>
<td>N/A</td>
</tr>
<tr>
<td>Days post-surgery (Time point 2)</td>
<td>15.8 ± 2.5</td>
<td>15.1 ± 1.9 (between sessions)</td>
</tr>
<tr>
<td>Weeks post-surgery (Time point 3)</td>
<td>28.1 ± 2.8</td>
<td>27.5 ± 1.4 (between sessions)</td>
</tr>
<tr>
<td>Graft type</td>
<td>10 PT, 7 HS</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Abbreviations: ACLr, anterior cruciate ligament reconstruction; PT, Bone-pattellar tendon-bone autograft; HS, hamstring tendon autograft.
4.2 Results - Specific Aim 1

4.2.1 Quadriceps spinal-reflexive excitability

A significant time by group interaction was discovered for spinal-reflexive excitability ($F_{4.96} = 10.25$, $P < 0.001$). Significant main effects for time ($F_{2.96} = 16.08$, $P < 0.001$) and group ($F_{2.48} = 7.19$, $P = 0.002$) were also detected. ACLr patients demonstrated lower spinal-reflexive excitability in their injured limb compared to healthy controls at pre-surgery; however, no differences between limbs in the ACLr group were detected. At 2-weeks post-surgery, spinal-reflexive excitability was lower in both limbs of the ACLr patients compared to the controls. No spinal-reflexive excitability differences were detected when ACLr patients were cleared for full activity (Figure 4-1). Over time, spinal-reflexive excitability in the ACLr injured limb decreased from pre-surgery to 2-weeks post-surgery, and increased higher than pre-surgery values at 6-months post-surgery. The uninjured limb of the ACLr group showed a decrease in spinal-reflexive excitability from pre-surgery to 2-weeks post, which returned back to pre-surgery levels at 6-months. The control group did not change over time (Figure 4-2).

A secondary analysis was performed to determine the reliability of collecting H:M measures in the matched limb of our healthy group. A two-way mixed model intraclass correlation coefficient analysis was performed with absolute agreement to determine reliability over the three data collection time points in the healthy group only. Good reliability was discovered for H:M outcomes in the healthy matched limb (ICC = 0.868; 95% CI: 0.709, 0.948)
Figure 4-1: Spinal-reflexive excitability in the injured limb and uninjured limb of the ACLr group compared to a matched limb of the healthy control group at each time point.

* Denotes lower spinal-reflexive excitability compared to Healthy matched limb at $P \leq 0.05$
4.2.2 Quadriceps corticospinal excitability – active motor threshold (AMT)

There was a significant time by group interaction effect for AMT ($F_{4,96} = 10.52$, $P < 0.001$). There was also a significant main effect for time ($F_{2,96} = 47.52$, $P < 0.001$), and a non-significant group main effect ($F_{2,48} = 1.77$, $P = 0.181$). First, it is important to note that an increase in AMT is interpreted as a decrease in corticospinal excitability. AMT was not different between the ACLr limbs and control limb at pre-surgery or 2-
weeks post-surgery as measured by AMT. Both limbs of ACLr patients had higher AMTs (lower corticospinal excitability) at 6-months post-surgery compared to controls (Figure 4-3). Both limbs in the ACLr group responded similarly over time, with AMT decreasing (corticospinal excitability increasing) from pre-surgery to 2-weeks post, and increasing higher than (decreasing corticospinal excitability) pre-surgery values at 6-months post-surgery. The control group did not change overtime (Figure 4-4).

Figure 4-3: Corticospinal excitability, as measured by active motor threshold, in the injured limb and uninjured limb of the ACLr group compared to a matched limb of the healthy control group at each time point. It is important to note that an increase in AMT is interpreted as a decrease in corticospinal excitability.

* Denotes lower corticospinal excitability compared to Healthy matched limb at P ≤ 0.05
4.2.3 Quadriceps corticospinal excitability – motor evoked potentials (MEP) at 120% of AMT

There was a non-significant time by group interaction effect for MEPs ($F_{4,96} = 0.94$, $P = 0.45$) and a non-significant group main effect ($F_{2,48} = 0.50$, $P = 0.61$). However a significant main effect for time was detected ($F_{2,96} = 3.96$, $P = 0.03$). No differences in MEPs were detected between either limb in the ACLr group or healthy matched control limb at any time point (Figure 4-5). The only significant change over time occurred in the

* Significant increase in corticospinal excitability from pre-surgery to 2-weeks post-surgery in both the ACLr injured and uninjured limbs at $P \leq 0.05$.
† Significant decrease in corticospinal excitability from 2-weeks post surgery to 6-months post surgery in both the ACLr injured and uninjured limbs at $P \leq 0.05$.
‡ Significant decrease in corticospinal excitability from pre-surgery to 6-months post-surgery in both the ACLr injured and uninjured limbs at $P \leq 0.05$. 
ACL uninjured limb, in which MEPs decreased from pre-surgery to 6-months post-surgery (Figure 4-6).

Figure 4-5: Corticospinal excitability, as measured by motor evoked potentials at 120% of active motor threshold, in the injured limb and uninjured limb of the ACLr group compared to a matched limb of the healthy control group at each time point. No differences were detected.
4.2.4 Quadriceps intracortical excitability – short interval intracortical inhibition (SICI)

No significant group by time interaction effect ($F_{4,96} = 0.82, P = 0.52$), group main effect ($F_{2,48} = 0.02, P = 0.98$), or time main effect were discovered ($F_{2,96} = 0.45, P = 0.64$). No differences in intracortical inhibition were observed at any time point (Figure 4-7). No change over time was detected for any limb (Figure 4-8).
Figure 4-7: Intracortical inhibition, as measured by short interval intracortical inhibition (SICI), in the injured limb and uninjured limb of the ACLr group compared to a matched limb of the healthy control group at each time point. No differences were detected.
Figure 4-8: Changes in intracortical inhibition over time, as measured by short interval intracortical inhibition (SICI), in the injured limb and uninjured limb of the ACLr group, and a matched limb of the healthy control group. No change over time was detected.

4.2.5 Quadriceps intracortical excitability - intracortical facilitation (ICF)

No significant group by time interaction effect ($F_{4,96} = 0.88, P = 0.48$), group main effect ($F_{2,48} = 0.24, P = 0.79$), or time main effect were discovered ($F_{2,96} = 0.20, P = 0.82$). No differences in intracortical facilitation were observed at any time point (Figure 4-9). No change over time was detected for any limb (Figure 4-10).
Figure 4-9: Intracortical facilitation, as measured by intracortical facilitation (ICF), in the injured limb and uninjured limb of the ACLr group compared to a matched limb of the healthy control group at each time point. No differences were detected.
4.3 Results - Specific Aim 2

4.3.1 Descriptive statistics of outcome variables

Means and standard deviations for all outcome variables in specific aim 2 can be found in Table 4.2. Normality statistics are reported in Appendix H.

Figure 4-10: Changes in intracortical facilitation over time, as measured by intracortical facilitation (ICF), in the injured limb and uninjured limb of the ACLr group, and a matched limb of the healthy control group. No change over time was detected.
<table>
<thead>
<tr>
<th></th>
<th>ACLr Injured Limb</th>
<th>Healthy Matched Limb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-surgery</td>
<td>6-months post</td>
</tr>
<tr>
<td>H:M</td>
<td>0.193 ± 0.126</td>
<td>0.250 ± 0.097</td>
</tr>
<tr>
<td>AMT (%Tesla)</td>
<td>39.5 ± 8.8</td>
<td>44.7 ± 8.6</td>
</tr>
<tr>
<td>MEP 120</td>
<td>0.020 ± 0.020</td>
<td>0.019 ± 0.017</td>
</tr>
<tr>
<td>SICI</td>
<td>0.510 ± 0.457</td>
<td>0.498 ± 0.390</td>
</tr>
<tr>
<td>ICF</td>
<td>0.709 ± 0.487</td>
<td>0.704 ± 0.824</td>
</tr>
<tr>
<td>AMT (%Tesla)</td>
<td>2.02 ± 0.56</td>
<td>2.47 ± 0.62</td>
</tr>
<tr>
<td>CAR (%)</td>
<td>82.6 ± 8.6</td>
<td>90.9 ± 6.7</td>
</tr>
<tr>
<td>Knee angle – Ascent (°)</td>
<td>62.3 ± 12.1</td>
<td>62.6 ± 8.1</td>
</tr>
<tr>
<td>Knee moment – Ascent (Nm/kg*m)</td>
<td>0.55 ± 0.25</td>
<td>0.53 ± 0.31</td>
</tr>
<tr>
<td>Knee angle – Descent (°)</td>
<td>28.6 ± 14.9</td>
<td>25.6 ± 10.9</td>
</tr>
<tr>
<td>Knee moment – Descent (Nm/kg*m)</td>
<td>0.31 ± 0.19</td>
<td>0.30 ± 0.22</td>
</tr>
<tr>
<td>IKDC</td>
<td>45.9 ± 5.4</td>
<td>74.5 ± 18.1</td>
</tr>
</tbody>
</table>

Abbreviations: H:M, Hoffmann reflex normalized to maximal muscle response; AMT, active motor threshold; MEP120, motor evoked potentials elicited at 120% of AMT; SICI, short interval intracortical inhibition; ICF, intracortical facilitation; MVIC, maximal voluntary isometric contraction; CAR, central activation ratio; IKDC, International Knee Documentation Committee score.

### 4.3.2 Correlations at pre-surgery

Within the ACLr group, short interval intracortical inhibition (SICI) had a strong positive association with intracortical facilitation (ICF; \( \rho = 0.60, P = 0.01 \)) and strong negative association with MEPs at 120% of AMT (\( \rho = -0.61, P = 0.01 \)). Peak knee extension moments during stair ascent had a moderate and positive correlation with MEPs (\( \rho = 0.50, P = 0.03 \)) and moderate negative association with knee angle during ascent (\( \rho = -0.49, P = 0.04 \)). Internal knee extension moments during stair descent had a moderate and negative association with knee angle during descent (\( \rho = -0.58, P = 0.01 \)).
as well as a moderate and positive association with quadriceps strength ($\rho = 0.53$, $P = 0.02$). (Table 4.3).

When analyzing the healthy control group, intracortical facilitation had a strong, positive association with intracortical inhibition ($\rho = 0.82$, $P < 0.001$), which was also observed in the ACLr group. Knee extension moments during stair descent had moderate to strong correlations with intracortical facilitation ($\rho = -0.60$, $P = 0.02$), knee angle during descent ($\rho = -0.55$, $P = 0.02$), and knee moments during ascent ($\rho = 0.62$, $P = 0.01$). Lastly, a moderate and negative correlation was found between knee angles during ascent and descent ($\rho = -0.69$, $P = 0.002$). (Table 4.4).
Table 4.3: Spearman correlation matrix analyzing associations among variables at pre-surgery within the injured limb of the ACLr group.

<table>
<thead>
<tr>
<th></th>
<th>H:M</th>
<th>AMT</th>
<th>MEP 120</th>
<th>SICI</th>
<th>ICF</th>
<th>MVIC</th>
<th>CAR</th>
<th>Knee angle – Ascent</th>
<th>Knee moment – Ascent</th>
<th>Knee angle – Descent</th>
<th>Knee moment - Descent</th>
<th>IKDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>H:M</td>
<td>ρ = -0.04</td>
<td>ρ = 0.23</td>
<td>ρ = -0.19</td>
<td>ρ = -0.40</td>
<td>ρ = 0.38</td>
<td>ρ = 0.10</td>
<td>ρ = -0.42</td>
<td>ρ = 0.17</td>
<td>ρ = 0.26</td>
<td>ρ = -0.07</td>
<td>ρ = 0.03</td>
<td></td>
</tr>
<tr>
<td>AMT</td>
<td>ρ = 0.01</td>
<td>ρ = -0.11</td>
<td>ρ = -0.20</td>
<td>ρ = -0.21</td>
<td>ρ = -0.37</td>
<td>ρ = -0.07</td>
<td>ρ = 0.06</td>
<td>ρ = 0.37</td>
<td>ρ = -0.38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEP120</td>
<td>ρ = -0.61*</td>
<td>ρ = -0.21</td>
<td>ρ = 0.39</td>
<td>ρ = 0.00</td>
<td>ρ = 0.04</td>
<td>ρ = 0.50*</td>
<td>ρ = 0.07</td>
<td>ρ = 0.34</td>
<td>ρ = -0.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SICI</td>
<td>ρ = 0.60*</td>
<td>ρ = -0.19</td>
<td>ρ = -0.16</td>
<td>ρ = 0.29</td>
<td>ρ = -0.43</td>
<td>ρ = -0.17</td>
<td>ρ = -0.07</td>
<td>ρ = 0.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICF</td>
<td>ρ = -0.32</td>
<td>ρ = 0.02</td>
<td>ρ = 0.19</td>
<td>ρ = 0.06</td>
<td>ρ = -0.10</td>
<td>ρ = -0.21</td>
<td>ρ = -0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVIC</td>
<td>ρ = 0.17</td>
<td>ρ = -0.16</td>
<td>ρ = 0.25</td>
<td>ρ = -0.39</td>
<td>ρ = 0.53*</td>
<td>ρ = 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAR</td>
<td>ρ = 0.11</td>
<td>ρ = -0.15</td>
<td>ρ = 0.07</td>
<td>ρ = -0.28</td>
<td>ρ = 0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Knee angle – Ascent</td>
<td>ρ = -0.49*</td>
<td>ρ = 0.23</td>
<td>ρ = -0.17</td>
<td>ρ = 0.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Knee moment - Ascent</td>
<td>ρ = -0.02</td>
<td>ρ = 0.19</td>
<td>ρ = -0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Knee angle - Descent</td>
<td>ρ = -0.58*</td>
<td>ρ = -0.21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Knee moment - Descent</td>
<td>ρ = -0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: H:M, Hoffmann reflex normalized to maximal muscle response; AMT, active motor threshold; MEP120, motor evoked potentials elicited at 120% of AMT; SICI, short interval intracortical inhibition; ICF, intracortical facilitation; MVIC, maximal voluntary isometric contraction; CAR, central activation ratio; IKDC, International Knee Documentation Committee score.

* denotes significant correlation at P ≤ 0.05.
Table 4.4: Spearman correlation matrix analyzing associations among variables at pre-surgery within the matched injured limb of the healthy control group.

<table>
<thead>
<tr>
<th></th>
<th>H:M</th>
<th>AMT</th>
<th>MEP 120</th>
<th>SICI</th>
<th>ICF</th>
<th>MVIC</th>
<th>CAR</th>
<th>Knee angle – Ascent</th>
<th>Knee moment – Ascent</th>
<th>Knee Angle – Descent</th>
<th>Knee moment – Descent</th>
<th>IKDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>H:M</td>
<td>ρ = 0.08</td>
<td>ρ = -0.21</td>
<td>ρ = -0.15</td>
<td>ρ = 0.06</td>
<td>ρ = 0.13</td>
<td>ρ = 0.27</td>
<td>ρ = -0.05</td>
<td>ρ = 0.29</td>
<td>ρ = 0.02</td>
<td>ρ = 0.04</td>
<td>ρ = -0.41</td>
<td></td>
</tr>
<tr>
<td>AMT</td>
<td>ρ = 0.05</td>
<td>ρ = -0.28</td>
<td>ρ = -0.28</td>
<td>ρ = 0.12</td>
<td>ρ = 0.18</td>
<td>ρ = -0.19</td>
<td>ρ = 0.04</td>
<td>ρ = -0.02</td>
<td>ρ = 0.24</td>
<td>ρ = -0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEP120</td>
<td>ρ = -0.14</td>
<td>ρ = -0.43</td>
<td>ρ = -0.30</td>
<td>ρ = 0.06</td>
<td>ρ = 0.22</td>
<td>ρ = 0.06</td>
<td>ρ = 0.06</td>
<td>ρ = 0.23</td>
<td>ρ = 0.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SICI</td>
<td>ρ = 0.82*</td>
<td>ρ = -0.07</td>
<td>ρ = -0.26</td>
<td>ρ = 0.02</td>
<td>ρ = -0.04</td>
<td>ρ = -0.19</td>
<td>ρ = -0.21</td>
<td>ρ = -0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICF</td>
<td>ρ = -0.04</td>
<td>ρ = -0.06</td>
<td>ρ = -0.09</td>
<td>ρ = -0.23</td>
<td>ρ = -0.08</td>
<td>ρ = -0.56*</td>
<td>ρ = 0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVIC</td>
<td>ρ = -0.20</td>
<td>ρ = -0.05</td>
<td>ρ = -0.09</td>
<td>ρ = 0.16</td>
<td>ρ = 0.05</td>
<td>ρ = 0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAR</td>
<td>ρ = 0.09</td>
<td>ρ = -0.36</td>
<td>ρ = 0.14</td>
<td>ρ = -0.40</td>
<td>ρ = 0.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee angle – Ascent</td>
<td>ρ = 0.00</td>
<td>ρ = 0.69*</td>
<td>ρ = -0.19</td>
<td>ρ = 0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee moment - Ascent</td>
<td>ρ = -0.21</td>
<td>ρ = 0.62*</td>
<td>ρ = -0.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee angle - Descent</td>
<td>ρ = -0.55*</td>
<td>ρ = 0.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee moment - Descent</td>
<td>ρ = -0.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: H:M, Hoffmann reflex normalized to maximal muscle response; AMT, active motor threshold; MEP120, motor evoked potentials elicited at 120% of AMT; SICI, short interval intracortical inhibition; ICF, intracortical facilitation; MVIC, maximal voluntary isometric contraction; CAR, central activation ratio; IKDC, International Knee Documentation Committee score.

* denotes significant correlation at P ≤ 0.05.
4.3.3 Correlations at 6-months post-surgery

Within the ACLr group, intracortical facilitation (ICF) had a moderate and negative association with spinal-reflexive excitability ($\rho = -0.51$, $P = 0.04$) at 6-months post-surgery. MEPs at 120% of AMT had a moderate and negative correlation to intracortical inhibition ($\rho = -0.61$, $P = 0.01$) and a strong positive association with peak knee extension moments during stair descent ($\rho = 0.55$, $P = 0.02$). Lastly, quadriceps strength had a moderate and negative association with knee joint angle during stair ascent ($\rho = -0.57$, $P = 0.01$) and a strong, positive correlation with self-reported function ($\rho = 0.73$, $P < 0.001$). (Table 4.5).

At the 6-months post-surgery time point for the healthy control group, intracortical facilitation had a strong and negative association with MEPs at 120% of AMT ($\rho = -0.76$, $P < 0.001$). Knee flexion angle during descent had a moderate, positive association with knee joint angles ($\rho = 0.52$, $P = 0.03$) and negative association with knee joint moments ($\rho = -0.51$, $P = 0.03$) during stair ascent. Lastly, knee joint moments during stair ascent moderately and positively correlated with knee joint moments during descent ($\rho = 0.59$, $P = 0.01$). (Table 4.6).
Table 4.5: Spearman correlation matrix analyzing associations among variables at 6-months post-surgery within the injured limb of the ACLr group.

<table>
<thead>
<tr>
<th></th>
<th>H:M</th>
<th>AMT</th>
<th>MEP 120</th>
<th>SICI</th>
<th>ICF</th>
<th>MVIC</th>
<th>CAR</th>
<th>Knee angle – Ascent</th>
<th>Knee moment – Ascent</th>
<th>Knee angle – Descent</th>
<th>Knee moment – Descent</th>
<th>IKDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>H:M</td>
<td>(\rho = -0.17)</td>
<td>(\rho = 0.46)</td>
<td>(\rho = -0.09)</td>
<td>(\rho = -0.51^*)</td>
<td>(\rho = -0.19)</td>
<td>(\rho = 0.14)</td>
<td>(\rho = -0.26)</td>
<td>(\rho = 0.35)</td>
<td>(\rho = -0.08)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMT</td>
<td>(\rho = -0.17)</td>
<td>(\rho = 0.30)</td>
<td>(\rho = 0.32)</td>
<td>(\rho = 0.04)</td>
<td>(\rho = 0.14)</td>
<td>(\rho = 0.13)</td>
<td>(\rho = 0.36)</td>
<td>(\rho = 0.05)</td>
<td>(\rho = 0.13)</td>
<td>(\rho = 0.28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEP120</td>
<td>(\rho = -0.61^*)</td>
<td>(\rho = -0.35)</td>
<td>(\rho = 0.28)</td>
<td>(\rho = -0.39)</td>
<td>(\rho = -0.01)</td>
<td>(\rho = 0.15)</td>
<td>(\rho = -0.40)</td>
<td>(\rho = 0.55^*)</td>
<td>(\rho = 0.11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SICI</td>
<td>(\rho = 0.19)</td>
<td>(\rho = -0.21)</td>
<td>(\rho = -0.06)</td>
<td>(\rho = 0.06)</td>
<td>(\rho = -0.03)</td>
<td>(\rho = 0.00)</td>
<td>(\rho = -0.35)</td>
<td>(\rho = 0.23)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICF</td>
<td>(\rho = 0.10)</td>
<td>(\rho = 0.33)</td>
<td>(\rho = -0.01)</td>
<td>(\rho = 0.37)</td>
<td>(\rho = 0.31)</td>
<td>(\rho = -0.26)</td>
<td>(\rho = 0.25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVIC</td>
<td>(\rho = 0.39)</td>
<td>(\rho = -0.57^*)</td>
<td>(\rho = 0.21)</td>
<td>(\rho = -0.16)</td>
<td>(\rho = 0.17)</td>
<td>(\rho = 0.73^*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAR</td>
<td>(\rho = 0.28)</td>
<td>(\rho = 0.23)</td>
<td>(\rho = 0.25)</td>
<td>(\rho = -0.08)</td>
<td>(\rho = 0.20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee angle – Ascent</td>
<td>(\rho = 0.21)</td>
<td>(\rho = 0.19)</td>
<td>(\rho = -0.21)</td>
<td>(\rho = -0.27)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee moment – Ascent</td>
<td>(\rho = 0.36)</td>
<td>(\rho = 0.33)</td>
<td>(\rho = 0.06)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee angle – Descent</td>
<td>(\rho = -0.21)</td>
<td>(\rho = -0.27)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee moment – Descent</td>
<td>(\rho = 0.24)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: H:M, Hoffmann reflex normalized to maximal muscle response; AMT, active motor threshold; MEP120, motor evoked potentials elicited at 120% of AMT; SICI, short interval intracortical inhibition; ICF, intracortical facilitation; MVIC, maximal voluntary isometric contraction; CAR, central activation ratio; IKDC, International Knee Documentation Committee score.

* denotes significant correlation at \(P \leq 0.05\).
Table 4.6: Spearman correlation matrix analyzing associations among variables at 6-months post-surgery within the matched injured limb of the healthy control group.

<table>
<thead>
<tr>
<th></th>
<th>H:M</th>
<th>AMT</th>
<th>MEP 120</th>
<th>SICI</th>
<th>ICF</th>
<th>MVIC</th>
<th>CAR</th>
<th>Knee angle – Ascent</th>
<th>Knee moment – Ascent</th>
<th>Knee Angle – Descent</th>
<th>Knee moment – Descent</th>
<th>IKDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>H:M</td>
<td>ρ = -0.01</td>
<td>ρ = -0.02</td>
<td>ρ = -0.20</td>
<td>ρ = -0.21</td>
<td>ρ = 0.16</td>
<td>ρ = -0.05</td>
<td>ρ = -0.16</td>
<td>ρ = 0.30</td>
<td>ρ = -0.21</td>
<td>ρ = 0.12</td>
<td>ρ = -0.41</td>
<td></td>
</tr>
<tr>
<td>AMT</td>
<td>ρ = 0.38</td>
<td>ρ = 0.18</td>
<td>ρ = -0.37</td>
<td>ρ = 0.14</td>
<td>ρ = 0.40</td>
<td>ρ = 0.00</td>
<td>ρ = 0.12</td>
<td>ρ = 0.02</td>
<td>ρ = 0.26</td>
<td>ρ = -0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEP120</td>
<td>ρ = -0.30</td>
<td>ρ = -0.76*</td>
<td>ρ = -0.16</td>
<td>ρ = 0.08</td>
<td>ρ = 0.05</td>
<td>ρ = 0.18</td>
<td>ρ = -0.10</td>
<td>ρ = 0.30</td>
<td>ρ = 0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SICI</td>
<td>ρ = 0.37</td>
<td>ρ = -0.01</td>
<td>ρ = 0.34</td>
<td>ρ = 0.15</td>
<td>ρ = -0.01</td>
<td>ρ = -0.19</td>
<td>ρ = 0.17</td>
<td>ρ = 0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICF</td>
<td>ρ = -0.28</td>
<td>ρ = -0.20</td>
<td>ρ = -0.04</td>
<td>ρ = -0.08</td>
<td>ρ = 0.11</td>
<td>ρ = -0.20</td>
<td>ρ = 0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVIC</td>
<td>ρ = 0.10</td>
<td>ρ = -0.03</td>
<td>ρ = 0.07</td>
<td>ρ = 0.10</td>
<td>ρ = -0.25</td>
<td>ρ = 0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAR</td>
<td>ρ = 0.01</td>
<td>ρ = -0.10</td>
<td>ρ = 0.15</td>
<td>ρ = 0.28</td>
<td>ρ = 0.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee angle – Ascent</td>
<td>ρ = -0.48</td>
<td>ρ = 0.52*</td>
<td>ρ = -0.21</td>
<td>ρ = -0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee moment – Ascent</td>
<td>ρ = -0.51*</td>
<td>ρ = 0.59*</td>
<td>ρ = -0.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee angle – Descent</td>
<td>ρ = -0.26</td>
<td>ρ = 0.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee moment – Descent</td>
<td>ρ = -0.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: H:M, Hoffmann reflex normalized to maximal muscle response; AMT, active motor threshold; MEP120, motor evoked potentials elicited at 120% of AMT; SICI, short interval intracortical inhibition; ICF, intracortical facilitation; MVIC, maximal voluntary isometric contraction; CAR, central activation ratio; IKDC, International Knee Documentation Committee score. * denotes significant correlation at P ≤ 0.05.
Chapter 5

Discussion

5.1 Summary of Results

The purpose of this investigation was to determine if neural alterations in the quadriceps muscle were present in those who sustained an ACL injury, and to understand how these alterations evolved from time of initial injury to return to participation following surgical reconstruction. This investigation discovered alterations in spinal-reflexive excitability prior to, and at 2-weeks post-surgery, however no significant differences were detected when patients returned to activity. Alterations in corticospinal excitability were discovered when patients were returned to participation, however corticospinal excitability in the ACLr group did not differ from healthy controls prior to, or at 2-weeks post-surgery. No significant differences were found in intracortical excitability measures.

The secondary purpose of this investigation was to evaluate the association between quadriceps neural excitability and biomechanical, neuromuscular and self-reported function at times of pre-surgery and at 6-months post-surgery (return to participation) in the injured limb of ACLr patients. At pre-surgery, corticospinal
excitability was related to knee joint moments during stair ascent in ACLr patients, with higher corticospinal excitability relating to higher joint moments during gait. At 6-months post-surgery, intracortical facilitation was inversely related to spinal-reflexive excitability. In addition, quadriceps strength had strong correlations with self-reported function and knee joint angle during stair ascent, indicating that ACLr patients with weaker quadriceps muscles reported higher levels of dysfunction and demonstrated less flexed knee joint angles during stair ascent.

5.2 Specific Aim 1

5.2.1 Early alterations in quadriceps spinal-reflexive excitability

The ACLr patients used in this investigation demonstrated lower spinal-reflexive excitability at times of pre-surgery and 2-weeks post-surgery when compared to healthy matched controls. The spinal-reflexive deficits found in this study are consistent with previous investigations that found immediate reductions in spinal-reflexive excitability following acute experimental joint effusion. Others have also found a trend for spinal-reflexive excitability of the quadriceps to be lower in the injured limb of ACL deficient patients. To our knowledge, this is the first investigation comparing spinal-reflexive excitability in acutely injured ACL patients to healthy controls prior to and immediately after surgery. Our data suggest that during early stages of injury and reconstruction, deficits in spinal-reflexive excitability are present.

Interestingly enough, spinal-reflexive deficits were not present at a time when patients are returned to normal activity. These early deficits could be attributed to swelling and pain that are present early in the rehabilitation process. Our measurements
were taken on average 35.8 (± 14.8) days from injury, and again at 15.8 (± 2.5) days post-surgery. Although no measures of swelling were taken from these patients, it is possible they were experiencing some level of effusion and thus distension of the joint capsule. Data generated from previous experimental effusion models have suggested that effusion may cause altered afferent signaling from the joint, resulting in inhibition of the surrounding musculature and manifests as decreased spinal-reflexive excitability and deficits in neuromuscular function. Our ACLr individuals were also reporting high levels of self-reported dysfunction at pre-surgery (IKDC: 45.9 ± 5.4) and at 2-weeks post-surgery (IKDC: 27.1 ± 4.9). Level of pain has previously been associated with quadriceps dysfunction, however, pain as a mechanism of spinal-reflexive alterations has not been studied. Further supporting this rationale, Hopkins et al. demonstrated that following an experimental knee effusion; spinal-reflexive excitability could be increased with the application of cryotherapy and transcutaneous electrical nerve stimulation (TENS), two modalities that are thought to influence perception and level of pain. More research is needed to understand why deficits in spinal-reflexive excitability are present early in the injury process.

At pre-surgery, spinal-reflexive excitability was altered only in the injured limb of our ACLr patients compared to the healthy control limb, indicating a unilateral response. Palmieri et al. also showed a unilateral response to acute injury using an experimental effusion model, with contralateral H:M measures remaining constant in the uneffused limb. Our data, however, suggest that a bilateral response occurs sometime between 30-days post-injury to 2-weeks post-reconstruction. Inherent damage to the joint capsule and surrounding structures are evident following surgical intervention, and may cause a
greater magnitude of altered afferent information being sent from the joint. Unlike the investigation performed by Palmieri et al., patients in the current study sustained an acute joint injury, and also experienced additional physiological trauma to the joint through surgical reconstruction. Following this intensive procedure, it is possible that a systematic and bilateral neuromuscular response occurs in an attempt to limit any further injury, which has been previously hypothesized as a protective mechanism termed arthrogenic muscle inhibition. Further, these individuals are approximately two months from initial injury at the 2-week time point, causing uncertainty whether this bilateral deficit is a response to an acute injury, or an adaptation from chronically dealing with this injury over time. It is possible that ACLr individuals may develop bilateral alterations as a compensatory mechanism or adaptation to injury by the 2-week post-surgery time point, while the initial decrease in the involved limb may be due to acutely injured structures or joint effusion, as previously established by experimental effusion investigations. 

As stated above, both limbs of the ACLr groups demonstrated decreases in spinal-reflexive excitability from pre-surgery to 2-weeks post, which is in agreement with previous experimental knee effusion studies that demonstrated decreases in H:M outcomes following an experimentally induced injury. Spinal-reflexive excitability increased in both limbs of our ACLr group from 2-weeks to 6-months post-surgery. This result corresponds with previous data by Rosenthal et al., which showed increases in spinal-reflexive excitability from 1-month to 3-months post ACLr. Based on both the findings of the current investigation, and the conclusions from Rosenthal et al., it appears that spinal-reflexive excitability is decreased early in the injury process, however
increases sometime between 2-weeks post-surgery and 3-months post-surgery. This is a time when patients are typically undergoing an aggressive neuromuscular rehabilitation program, which potentially has the effect of increasing afferent information or producing excitatory signals around the joint. In addition, pain and swelling typically decrease during this time frame. Therefore, as proposed above, if pain and swelling are both modulators of spinal-reflexive alterations, the decrease in pain and swelling may explain the increase in spinal-reflexive excitability and the absence of differences observed at return to participation. More information is needed to understand exactly where in this time frame these spinal-reflexive alterations are changing. Capturing these spinal-reflexive measures at a variety of time points during this process may help to clarify how these neural alterations are progressing through the different phases of injury and rehabilitation.

5.2.2 Alterations in quadriceps corticospinal excitability are present at return to activity

Corticospinal excitability in the ACLr group was not different from our healthy controls at pre-surgery or 2-weeks post surgery. However, when these individuals were returned to participation, both limbs of the ACLr group demonstrated deficits in quadriceps corticospinal excitability (denoted by higher AMT compared to controls). Therefore, when these patients were fully cleared, their corticospinal system needed a higher input to allow the same amount of information to be sent to the muscle compared to the control group. Although the threshold to produce the response was different, the amplitude of the responses was not different from controls, as denoted by no differences in MEP values at any time point.
We had hypothesized that ACLr individuals would experience alterations in corticospinal excitability at pre-surgery and 2-weeks post, however this was not confirmed. Heroux and Trenblay\textsuperscript{23} discovered alterations in corticospinal excitability in ACL injured individuals, denoted by altered resting thresholds and stimulus response curves. It is difficult to compare the results of our current study to that of Heroux and Trenblay\textsuperscript{23} because it is inferred in their paper that they used an ACL deficient population. However, their patients presented with corticospinal alterations on an average of 22-months post-injury, or almost 2-years removed. Additionally, On et al.,\textsuperscript{156} evaluated corticospinal excitability in patients with chronic anterior knee pain and found increases in MEP amplitudes. This finding seems to correspond with the results of this current study, showing that corticospinal alterations are present in a chronic state of injury as opposed to acutely injured individuals. Providing further support, a recently accepted article performed by our lab group showed no changes in corticospinal excitability following an experimental knee joint effusion.\textsuperscript{157} It appears that alterations in corticospinal excitability may not occur in an acutely injured population, and rather have the potential to develop as an adaptation to the chronic nature of joint injury.

Even though corticospinal alterations were not found compared to healthy controls at pre-surgery and 2-weeks post, changes in AMT were found over time in both limbs of the ACLr group. This means that following surgery, there appears to be a bilateral up-regulation (decrease in AMT) in corticospinal excitability. Interestingly, this up-regulation in corticospinal excitability corresponded with the decrease in spinal reflexive excitability observed during the same time span. To put this in context, corticospinal excitability was not different from controls at pre-surgery or 2-weeks post,
however this was a time when spinal-reflexive alterations were present. Similarly, no spinal-reflexive alterations existed when patients were returned to participation, however corticospinal alterations were discovered at this time. It seems that these two pathways are potentially interacting over time, and inversely changing from 2-weeks post-surgery to 6-months post surgery (Figure 5-1). Previous work presented from our laboratory has shown an inverse association between these two neural pathways in an ACLr population,\textsuperscript{25} suggesting an interaction between these pathways in maintaining quadriceps function. It remains unclear what is occurring during the time frame from 2-weeks post-surgery to 6-months post-surgery that would cause these pathways to interact in this manner. Future research should aim to collect data between these time points to understand what is causing the alterations in corticospinal excitability, and ultimately how these two neural pathways are interacting. It is also important to note that MEP amplitudes in the ACLr group were not different from control participants. It is unclear why motor thresholds were different between groups, but not the amplitude of MEPs. More research is needed to understand both the physiological and clinical meaningfulness of this result.
5.2.3 No evidence of alterations in intracortical facilitation or inhibition

No differences were detected in quadriceps intracortical excitability, as measured through short interval intracortical inhibition and intracortical facilitation, at any time point following injury or reconstruction. Further, no changes in intracortical excitability were detected over time. A limited number of investigations have assessed intracortical
excitability in muscles of the lower extremity, as most investigations have focused on evaluating upper extremity musculature.\cite{93,158-160} Deficits in neuromuscular function, through age, fatigue, injury, inactivity, etc., have been hypothesized to result from changes in intracortical excitability, which may cause an inhibition of the musculature surrounding a joint.\cite{93,140,158} However, conflicting data exist on the effect that age,\cite{92,93,160} sex,\cite{161} and physical activity\cite{162,163} have on intracortical excitability.

The ACLr group in this investigation experienced deficits in corticopial excitability at 6-months post-surgery. It would have been interesting had this group also demonstrated lower SICI MEP values (greater level of inhibition), potentially indicating that this reduction in corticospinal excitability resulted from inhibitory mechanisms mediated by GABA\textsubscript{A} receptors. Recent advances in the understanding of intractorical mechanisms have led to improvements in pharmacological interventions aimed at benefitting motor intracortical excitability.\cite{90} The majority of this pharmacologic research has been aimed at targeting diseases such as Alzheimer’s\cite{164} and Parkinson’s,\cite{165} however if warranted, future research could examine its influence on musculoskeletal injury.\cite{90} Furthermore, recent evidence suggests that lower extremity muscle strength gains occurred in conjunction with a reduction in intracortical inhibition (as measured by SICI), potentially indicating that intracortical plasticity occurs as a result of strength training interventions.\cite{89} However, because no alterations in intracortical excitability were observed in our ACLr group, it is unclear if targeting intracortical excitability through either pharmacological or strength training interventions would be indicated.
It is important to note that this is the first investigation to examine intracortical excitability in lower extremity musculature following a lower extremity musculoskeletal injury, and more specifically after ACL injury. More research is needed to confirm or refute the results of this current study, as well as provide more evidence regarding changes in intracortical excitability in response to a musculoskeletal injury.

5.3 Specific Aim 2

5.3.1 Correlations at pre-surgery

The purpose of specific aim 2 was to evaluate the association between quadriceps neural excitability and biomechanical, neuromuscular and self-reported function at times of pre-surgery and at return to participation. The intracortical excitability measures of SICI and ICF shared a strong, positive association in both groups. As stated in the methods section (3.3.4), smaller SICI values represent a greater level of inhibition in the intracortical neurons, and higher ICF values represent a greater level of facilitation. Therefore, those with higher levels of intracortical inhibition demonstrated less facilitation in this network, and vice versa. In theory, either an increase in SICI (more inhibition) or a decrease in ICF (less facilitation) would result in decreased corticospinal excitability. The significant association discovered between these two outcomes may help to explain the lack of corticospinal deficits in the ACLr population found at pre-surgery, as these two variables appear to be working together at this time point as a potential mechanism to maintain corticospinal function. Further supporting this rationale, no significant association was found between our intracortical excitability variables at 6-months post-surgery, which was a time point where deficits in corticospinal excitability were discovered. However, no changes in SICI or ICF were observed over time, and no
deficits in these values were discovered at 6-months post surgery. Again, as this is the first investigation to examine measures of intracortical excitability in an ACLr population, more research is needed to truly understand the association between measures of intracortical excitability and neuromuscular, biomechanical and self-reported function.

At pre-surgery, corticospinal excitability (as measured by MEPs) was related to knee joint moments during stair ascent in ACLr patients, with higher corticospinal excitability relating to higher joint moments during gait. This association was not found in the healthy group. Limited data are available determining how alterations in quadriceps corticospinal excitability affect neuromuscular, biomechanical and self-reported function. Baumeister et al.,\textsuperscript{84} showed that corticospinal excitability was up regulated (increases in electroencephalographic activity) following ACLr, with no change in force production or electromyographic activity when compared to healthy controls. The authors\textsuperscript{84} suggest that individuals may be able to up-regulate corticospinal function in response to injury in order to maintain proper muscle function. This was not supported by our findings in aim 1, as no differences in corticospinal excitability were observed at pre-surgery. However, it is plausible that an undetectable up-regulation in quadriceps corticospinal excitability was present at pre-surgery in an attempt to maintain quadriceps strength levels, thereby affecting forces around the knee joint. Two results from the current study help to support this rationale. First, quadriceps strength was related to knee joint moments during stair descent in our ACLr population at pre-surgery, which was not found in healthy controls. This means that the ability to produce force at the quadriceps had an effect on the forces at the knee joint during stair walking. Second, there was an increase in corticospinal excitability over time from pre-surgery to 2-weeks post-surgery.
(Figure 4-4), indicating an up-regulation during this time frame. Although corticospinal levels were not different from controls at early time points, AMT in the ACLr group was changing over time, potentially effecting neuromuscular and biomechanical function. In contrast, however, individual correlations between quadriceps strength and corticospinal excitability were non-significant. Clearly, more research is needed to understand the full meaning of this association.

### 5.3.2 Correlations at 6-months post-surgery

At 6-months post surgery, higher corticospinal excitability was still associated with higher knee joint moments, however, at this time point during stair descent. Interestingly, intracortical facilitation was inversely related to spinal-reflexive excitability in ACLr patients. As discovered during aim 1, corticospinal and spinal-reflexive excitability seemed to be inversely related as patients progress through the injury process. These two pathways potentially interact as a mechanism to maintain quadriceps function. Based on the association found, it is plausible that corticospinal and spinal-reflexive alterations may originate from, or be mediated by, alterations in intracortical facilitation. As no other significant associations were discovered, and no differences were found in intracortical excitability between groups, this interpretation remains inconclusive while warranting further investigation.

Also in the ACLr population, quadriceps strength was related to knee joint angle during stair ascent. Those who had weaker quadriceps muscles had reduced peak knee flexion during activity, exhibiting a more extended joint during gait. Conversely, stronger ACLr patients were able to walk with a more flexed knee. More extended knee joints during stair ambulation have been reported in both ACL deficient\textsuperscript{166} and ACLr
patients. Additionally, quadriceps strength has previously been associated with knee joint angle and moments during gait in ACLr patients, supporting the findings of the current study. The result of poor quadriceps strength having a negative impact on joint kinematics and kinetics has been previously hypothesized as a mechanism of post-traumatic osteoarthritis in this population, emphasizing the need to maximize quadriceps strength following ACLr.

Another finding of this study that seems to support previous research, is the strong correlation discovered between quadriceps strength and self-reported function in the ACLr group at a time when individuals are returned to participation. Pietrosimone et al. demonstrated that quadriceps strength and corticospinal excitability were able to explain variations in self-reported function in an ACLr population. Similarly, Logerstedt et al. showed that pre-operative quadriceps strength was able to predict self-reported function at 6-months post-surgery. Therefore, results from this study and others indicate that greater quadriceps strength is associated with greater levels of self-reported function, further highlighting the importance of maximizing quadriceps strength in ACLr patients.

5.4 **Clinical Relevance**

Deficits in quadriceps muscle strength are common and persistent following ACLr, and has been linked to decreases in physical performance, self-reported function, increased risk of re-injury, and has been thought to contribute to the development of post-traumatic joint osteoarthritis. It has previously been hypothesized that alterations in motor generating neural pathways contribute to these lingering neuromuscular deficits, however until this time, no prospective, longitudinal...
investigation had been performed in an attempt to understand neural alterations following ACLr. Specifically, alterations in corticospinal excitability and spinal-reflexive excitability have been proposed as mechanisms of quadriceps weakness following ACLr.

Despite clinicians’ best efforts, standard therapeutic rehabilitation following surgery has failed to restore knee function to pre-injury levels. Targeting neural impairment is often overlooked in traditional rehabilitation, and failure to directly treat the potential underlying influences of quadriceps weakness may result in unsuccessful restoration of quadriceps strength. Results of the current study suggest that alterations in these neural pathways exist in ACLr patients, and that these alterations have the potential to negatively affect function. Further, our results reinforce previous recommendations that rehabilitation strategies used to target neural alterations may be beneficial for the restoration of muscle strength, and could help combat the persistent nature of quadriceps weakness. These results endorse further investigation into the modalities proposed to target these neural pathways, such as transcutaneous electrical nerve stimulation (TENS), neuromuscular electrical stimulation or electromyographic biofeedback. More specifically, our results suggest that spinal-reflexive alterations are present early in the rehabilitation process, and therefore the utilization of TENS for the purpose of increasing spinal-reflexive excitability may be beneficial to patients at this time. In addition, corticospinal deficits seem to present when patients return to activity and may benefit from modalities aimed at increasing corticospinal excitability, such as electromyographic biofeedback. Future research could look to supplement traditional rehabilitation with modalities directed at improving neural influences, and determine if
changes in these pathways overtime lead to improvements in muscle strength, biomechanics and self-reported function.

5.5 Limitations

This investigation was not without limitation. First, we included patients who underwent reconstruction from a total of three surgeons, included two different graft types, as well as patients with concomitant meniscal injuries. This does not allow for inferences about a single surgical technique, or the effect that different graft types have on neural excitability, however our data provides an overall representation of ACLr patients as a whole. Another limitation is that our study was not a true prospective investigation. We cannot conclude if neural alterations were present prior to injury, or if they were the result of injury. The lack of additional follow-up sessions between the 2-week and 6-month post-surgery time point may also be viewed as a limitation, however these sessions were chosen to give a representation of acute changes following surgery (2-week post) and at return to participation (6-month post). Based on the findings of this study, future research should examine ACLr patients at a variety of time points during this time frame. Lastly, we did not control for type of rehabilitation performed. Similar to above, this does not allow investigation into types of rehabilitation, which should be taken into consideration during future investigations.

5.6 Conclusion

The intention of this research was to examine alterations in neural level pathways in an ACLr population. Our results indicate that alterations are present in spinal-reflexive
and corticospinal neural pathways, and may contribute to the lingering neuromuscular deficits in these patients. ACL rupture remains one of the most common traumatic knee injuries, with upwards of 250,000 ACL injuries occurring in the United States each year. Quadriceps muscle strength deficits are common and persistent in these patients, and potentially have negative effects on long term outcomes following surgery. Alterations in neural pathways have previously been hypothesized to contribute to the persistent neuromuscular dysfunction observed in this population, however, no prospective and longitudinal investigation has been performed to examine these alterations until now. The data generated from this investigation provides clinicians and researchers with valuable information that is needed to understand the origins of neuromuscular dysfunction. Clinically, targeting these neural changes may benefit patients who are recovering from ACL injury and surgical reconstruction, and future research should examine the efficacy of the treatments proposed to target neural alterations.
References


27. Lepley AS, Murray AM, Bahhur NO, Gribble PA, Pietrosimone BG. Relationship between corticospinal excitability and muscle activation of the quadriceps following an experimental knee joint effusion. National Athletic Trainers' Association; 2012; St. Louis, MO.


101. Myer GD, Paterno MV, Ford KR, Quatman CE, Hewett TE. Rehabilitation after anterior cruciate ligament reconstruction: criteria-based progression through the


108. Keays SL, Bullock-Saxton JE, Keays AC, Newcombe PA, Bullock MI. A 6-year follow-up of the effect of graft site on strength, stability, range of motion, function, and joint degeneration after anterior cruciate ligament reconstruction:


150. Willson JD, Davis IS. Lower extremity mechanics of females with and without patellofemoral pain across activities with progressively greater task demands. *Clinical biomechanics*. Feb 2008;23(2):203-211.


157. Lepley AS, Bahur NO, Murray AM, Pietrosimone BG. Quadriceps corticomotor excitability following an experimental knee joint effusion. Knee Surgery, Sports Traumatology and Arthroscopy.; Accepted December 2013 [In Press].


Appendix A

Adult Subject Consent Form
ADULT RESEARCH SUBJECT INFORMATION AND CONSENT FORM

COMPREHENSIVE NEUROMUSCULAR/MUSCULOSKELETAL OUTCOMES FOLLOWING UNILATERAL KNEE JOINT SURGERY

Principal Investigator: Brian Pietrosimone PhD ATC

Other Staff (identified by role) Adam Lepley MA ATC (Co-Investigator)
Brittney Luc ATC (Co-Investigator)
Amy Clements ATC (Co-Investigator)
Jason Levine MD (Co-Investigator)

Contact Phone number(s): (419) 530-4467

What you should know about this research study:

- We give you this consent/authorization form so that you may read about the purpose, risks, and benefits of this research study. All information in this form will be communicated to you verbally by the research staff as well.
- Routine clinical care is based upon the best-known treatment and is provided with the main goal of helping the individual patient. The main goal of research studies is to gain knowledge that may help future patients.
- We cannot promise that this research will benefit you. Just like routine care, this research can have side effects that can be serious or minor.
- You have the right to refuse to take part in this research, or agree to take part now and change your mind later.
- If you decide to take part in this research or not, or if you decide to take part now but change your mind later, your decision will not affect your routine care.
- Please review this form carefully. Ask any questions before you make a decision about whether or not you want to take part in this research. If you decide to take part in this research, you may ask any additional questions at any time.
- Your participation in this research is voluntary.

PURPOSE (WHY THIS RESEARCH IS BEING DONE)
You are being asked to take part in a research study that looks at different outcomes following a knee joint surgery. The purpose of the study is to examine a multitude of outcomes pertaining to how the muscles around your knee work, how you move, and how you feel your knee is performing during different tasks. You were selected as someone who may want to take part in this study.
because you have recently suffered an injury to one of your knees which requires surgery. There will be approximately 300 people participating in this study at the University of Toledo.

DESCRIPTION OF THE RESEARCH PROCEDURES AND DURATION OF YOUR INVOLVEMENT
If you decide to take part in this study, you will be asked to report to the Joint Injury and Muscle Activation (JIMA) Laboratory in the Health Science and Human Services building (Room 1408), which is located on the Main Campus of the University of Toledo. You will be asked to come in a total of 5 times which will correspond with your physician appointments. The time points are as follows: time point 1: baseline/prior to surgery, time point 2: post-surgery physician appointment (5-14 days post-surgery), time point 3: 6-month follow-up, time point 4: 12-month follow-up, time point 5: 2 year-follow-up. We will work with you and the physician's office to schedule these appointments when you schedule your follow-ups to meet with Dr. Levine. Please remember that you can stop participating at any time. If you enroll now and choose not to participate in any of the follow-up appointments, it will not affect your medical care from the physician.

During the sessions, you will be asked to fill out knee injury questionnaires about how your knee feels during different activities. We will also test the neural function of both legs using 3 different methods. These methods include muscle activation testing, reflex testing and motor cortex testing. After this neural testing, your knee joint motion will then be recorded during a simple stair-walking task and jump-landing task. Each session will last approximately 2 hours in length.

Knee Injury Questionnaires – Time Points 1-5
You will be asked to provide us information regarding your previous history of any joint injury, current and past level of activity and how your joint injury currently affects you during different activities.

Muscle Activation Testing – Time Points 1, 3-5
You will be asked to sit near the testing chair and two electrodes treated with some gel will be placed on your thigh. One of the electrodes will be placed above your knee and the other will be given to you to place below your hips so that it lies flat when you are sitting. The electrodes will be held in place with an elastic bandage. These electrodes will be used to deliver a brief, mild electrical stimulus to your thigh muscles. The electricity will be approximately a half a second in duration and will contract your thigh muscle for that half second and relax.

You will be asked to sit in a chair that resembles a car seat. You will have a seat belt applied so that you do not move as you are contracting your leg muscles as hard as you can. You will then be asked to extend your leg as hard as you can and hold it for five seconds. While you are extending out, the electrical stimulus will be delivered to your thigh. This stimulus feels similar to a static electric shock that you could get from walking across a carpet in a dry room and then touching a doorknob, although the voltage is lower. You will be asked to perform this at least four times for each leg throughout each session. You will be allowed up to 1 minute of rest between each repetition.

Reflex Testing – Time Points 1-5
This testing provides an estimate of how well nerves in the lower leg are functioning. You will be instructed to lie on a table. You will have sticky electrodes placed on your thigh. These electrodes are called EMG electrodes which stand for Electromyography which is a recording of
the electrical (reflex) activity in skeletal muscle. The site of the EMG electrodes will be shaved and cleaned with alcohol. An electrode that provides a stimulus will be taped in the front of your hip. Several reflex measurements will be taken while you are lying down.

- These measurements include a 1-millisecond electrical stimulus.
- The intensity of this stimulus will vary depending on the reflex being elicited.
- The stimuli in this study feel similar to static electricity felt as you touch a doorknob after walking across a carpet.
- A series of measurements will be taken on your leg.

**Motor Cortex Testing – Time Points 1-5**

This testing provides us important information regarding how your brain is sending messages to muscles in your legs. You will be asked to sit in the same chair as above with your arms crossed at your chest. We will position a coil over your head and adjust the position of the coil until it is in the correct spot. We will ask you to wear a bathing cap and ear plugs. A brief magnetic stimulus will then be produced which will sound like a “click.” You will not have any associated pain or discomfort in your head, but rather may feel a brief muscle contraction in the muscles of your leg or thigh. You will be asked to flex certain leg muscles at a small to moderate intensity while we provide a series of brief magnetic stimuli to your head.

**Motion Analysis Testing**

*Star Walking: Time Points 1, 3-5*

This testing provides us information on how your joints are moving in relationship to the rest of your body. You will have small, round reflective markers placed on different landmarks of your leg. You will be asked to walk up and down a small set of stairs for a total of 6 times up and 6 times down while motion cameras record your movement.

*Jump-Landing: Time Points 3-5*

Along with the above testing, this provides us information on how your joints are moving in relationship to the rest of your body but during a more dynamic task. You will have small, round reflective markers placed on different landmarks of your leg. You will then be asked to jump off a box, and then jump as high as you can for a total of 3 times while motion cameras record your movement.

**Risks and Discomforts You May Experience if You Take Part in This Research**

**Likely Risks**

- Mild discomfort for a very brief period during the electrical stimulation.
- Mild transient muscle soreness from muscle activation testing.

**Less Likely Risks**

- Mild, transient skin irritation from the sticky electrodes.

**Very Unlikely Risks**

- Mild, transient headache following magnetic stimulation
• In people with a history of seizures there is a slight possibility of causing a seizure with the magnetic stimulation; therefore you must tell us prior to testing if you have ever had a seizure so we can exclude you from the study.
• Re-injury to the surgically repaired knee joint

RISKS TO UNBORN CHILDREN
It is unknown how the electrical stimulation used in this study would affect an unborn fetus; therefore, if you are pregnant you will not be allowed to participate in this study.

POSSIBLE BENEFIT TO YOU IF YOU DECIDE TO TAKE PART IN THIS RESEARCH
We cannot and do not guarantee or promise that you will receive any benefits from this research.

COST TO YOU FOR TAKING PART IN THIS STUDY
You are not directly responsible for making any type of payment to take part in this study. However, you are responsible for providing the means of transportation to the Joint Injury and Muscle Activation Laboratory. You will not be compensated for gas for travel or any other expenses to participate in this study.

ALTERNATIVE(S) TO TAKING PART IN THIS RESEARCH
The only alternative is not to participate in this study.

CONFIDENTIALITY - USE AND DISCLOSURE OF YOUR PROTECTED HEALTH INFORMATION
By agreeing to take part in this research study, you give to The University of Toledo (UT), the Principal Investigator and all personnel associated with this research study your permission to use or disclose health information that can be identified with you that we obtain in connection with this study. We will use this information for the purpose of conducting the research study as described in the research consent/authorization form.

The information that we will use or disclose includes history of knee joint injury, activity level, and strength or muscle activation measurements. We may use this information ourselves, or disclose this information as part of a research study. Under some circumstances, the Institutional Review Board and Research and Sponsored Programs of the University of Toledo may review your information for compliance audits. We may also disclose your protected health information when required by law, such as in response to judicial orders.

The University of Toledo is required by law to protect the privacy of your health information, and to use or disclose the information we obtain about you in connection with this research study only as authorized by you in this form. There is a possibility that the information we disclose may be re-disclosed by the persons we give it to, and no longer protected. However, we will encourage any person who receives your information from us to continue to protect and not re-disclose the information.

Your permission for us to use or disclose your protected health information as described in this section is voluntary. However, you will not be allowed to participate in the research study unless you give us your permission to use or disclose your protected health information by signing this document.

You have the right to revoke (cancel) the permission you have given us to use or disclose your protected health information at any time by giving written notice to Dr. Brian Pietrosimone, MS119 2601 W. Bancroft St. Toledo, OH 43606. However, a cancellation will not apply if we have acted with your permission, for example, information that already has been used or disclosed prior to the cancellation.
Also, a cancellation will not prevent us from continuing to use and disclose information that was obtained prior to the cancellation as necessary to maintain the integrity of the research study.

Except as noted in the above paragraph, your permission for us to use and disclose your protected health information will stop at the end of the research study. A more complete statement of University of Toledo’s Privacy Practices is set forth in its Joint Notice of Privacy Practices. If you have not already received this Notice, a member of the research team will provide this to you. If you have any further questions concerning privacy, you may contact the University of Toledo’s Privacy Officer at 419-383-3413.

IN THE EVENT OF A RESEARCH-RELATED INJURY
In the event of injury resulting from you taking part in this study, treatment can be obtained at a healthcare facility of your choice. You should understand that the costs of such treatment will be your responsibility. Financial compensation is not available through The University of Toledo or The University of Toledo Medical Center. By signing this form, you are not giving up any of your legal rights as a research subject. In the event of an injury, contact Brian Pietrosimone, PhD, ATC (419) 530-4467.

VOLUNTARY PARTICIPATION
Taking part in this study is voluntary. You may refuse to participate or discontinue participation at any time without penalty or a loss of benefits to which you are otherwise entitled. If you decide not to participate or to discontinue participation, your decision will not affect your future relations with the University of Toledo or The University of Toledo Medical Center.

NEW FINDINGS
You will be notified of new information that might change your decision to be in this study if any becomes available.

OFFER TO ANSWER QUESTIONS
Before you sign this form, please ask any questions on any aspect of the study that is unclear to you. You may take as much time as necessary to think it over. If you have questions regarding the research at any time before, during or after the study, you may contact: Dr. Brian Pietrosimone- (419) 530-4467

If you have questions beyond those answered by the research team or your rights as a research subject or research-related injuries, please feel free to contact the Chairperson of the University of Toledo Biomedical Institutional Review Board at 419-383-6796.
**SIGNATURE SECTION (Please read carefully)**

YOU ARE MAKING A DECISION WHETHER OR NOT TO PARTICIPATE IN THIS RESEARCH STUDY. YOUR SIGNATURE INDICATES THAT YOU HAVE READ THE INFORMATION PROVIDED ABOVE, YOU HAVE HAD ALL YOUR QUESTIONS ANSWERED, AND YOU HAVE DECIDED TO TAKE PART IN THIS RESEARCH.

BY SIGNING THIS DOCUMENT YOU AUTHORIZE US TO USE OR DISCLOSE YOUR PROTECTED HEALTH INFORMATION AS DESCRIBED IN THIS FORM.

The date you sign this document to enroll in this study, that is, today’s date, MUST fall between the dates indicated on the approval stamp affixed to the bottom of each page. These dates indicate that this form is valid when you enroll in the study but do not reflect how long you may participate in the study. Each page of this Consent/Authorization Form is stamped to indicate the form’s validity as approved by the UT Biomedical Institutional Review Board (IRB).

<table>
<thead>
<tr>
<th>Name of Subject (please print)</th>
<th>Signature of Subject or Person Authorized to Consent</th>
<th>Date a.m.</th>
<th>Date p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relationship to the Subject (Healthcare Power of Attorney, authority or Legal Guardian)</td>
<td>Signature of Person Obtaining Consent</td>
<td>Date</td>
<td></td>
</tr>
<tr>
<td>Name of Person Obtaining Consent (please print)</td>
<td>Signature of Witness to Consent Process (when required by ICH Guidelines)</td>
<td>Date</td>
<td></td>
</tr>
</tbody>
</table>

YOU WILL BE GIVEN A SIGNED COPY OF THIS FORM TO KEEP.
Appendix B

Minor Assent Form
CHILD RESEARCH SUBJECT ASSENT FORM
COMPREHENSIVE NEUROMUSCULAR/MUSCULOSKELETAL OUTCOMES FOLLOWING UNILATERAL KNEE JOINT SURGERY

Principal Investigator: Brian Patrousos, PhD, ATC (419-530-4467)
Other Investigators: Adam Leniey, MA ATC (Co-Investigator)
Brittney Luc ATC (Co-Investigator)
Amy Clemens ATC (Co-Investigator)
Jason Levine MD (Co-Investigator)

• You are being asked to be in a study to help understand people better.
• You should ask any questions you have before making up your mind. You can think about it and discuss it with your family or friends before you decide.
• It is okay to say “No” if you don’t want to be in the study. If you say “Yes” you can change your mind and then quit the study at any time without any problems.

We are doing a research study about how the muscles around your knee work, how you move, and how you feel your knee is performing during different tasks. A research study is a way to learn more about people. If you decide that you want to be part of this study, you will be asked to (asked to report to the Joint Injury and Muscle Activation [JIMAS] Laboratory in the Health Science and Human Services building [Room 1409], which is located on the Main Campus of the University of Toledo. You will be asked to come in a total of 6 times which will correspond with your physician appointments. The time points are as follows: time point 1: baseline pre-surgery, time point 2: post-surgery physician appointment (5-14 days post-surgery), time point 3: 6-month follow-up, time point 4: 12-month follow-up, time point 5: 2 year follow-up). We will work with you and your physician’s office to schedule these appointments when you schedule your follow-ups to meet with Dr. Levine. Please remember that you can stop participating at any time. If you enroll now and choose not to participate in any of the follow-up appointments, it will not affect your medical care from the physician.

During the sessions, you will be asked to fill out knee injury questionnaires about how your knee feels during different activities. We will also test the strength of both legs. At this strength testing, your knee joint motion will then be recorded during a simple stair-walking task and jump-landing task. Each session will last approximately 2 hours in length.

Knee Injury Questionnaires – Time Points 1-5
You will be asked to provide us information regarding your previous history of any joint injury, current and past level of activity and how your joint injury currently affects you during different activities.

Muscle Strength Testing – Time Points 1-5
You will be asked to sit in a chair that resembles a car seat. You will have a seat belt applied so that you do not move as you are contracting your leg muscles as hard as you can. You will then be asked to extend your leg as hard as you can and hold it for five seconds. You will be asked to perform this at least four times for each leg throughout each session. You will be allowed up to 1 minute of rest between each repetition.
Motion Analysis Testing
Start Walking; Time Points 1, 3, 5
This testing provides us information on how your joints are moving in relationship to the rest of your body. You will have small, round reflective markers placed on different landmarks of your leg. You will be asked to walk up and down a small set of stairs for a total of 6 times up and 6 times down while motion cameras record your movement.

Jump-Landing; Time Points 3-5
Along with the above testing, this provides us information on how your joints are moving in relationship to the rest of your body but during a more dynamic task. You will have small, round reflective markers placed on different landmarks of your leg. You will then be asked to jump off a box, and then jump as high as you can for a total of 3 times while motion cameras record your movement.

There are minimal risks for participating in this study, including possible muscle soreness from muscle strength testing and a very unlikely risk of re-injury to the surgically repaired knee joint during motion analysis testing.

Not everyone who takes part in this study will benefit. A benefit means that something good happens to you. We cannot and do not guarantee or promise that you will receive any benefits from this research.

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

If you have any questions about the study, you can ask Dr. Brian Bielmaier or one of the other investigators. You can call the investigator(s) listed at the top of this page if you have a question later.

If you are female, you cannot participate if you are pregnant. You should not become pregnant while you are in this study because we do not know if the study could hurt the baby.

You do not have to be in this study if you do not want to. You can decide later if you want to think about it for awhile. If you decide to be in this study, please print and sign your name below.

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

(Sign your Name) ________________________________ Date: ____________

Name of Person Explaining Assent (Print) ________________________________

Signature of Person Explaining Assent ________________________________ Date: ____________

I attest that I or my representative discussed this study with the above-named participant.

Signature of Principal Investigator or Sub-Investigator ________________________________ Date: ____________
Appendix C

TMS/AMT Data Collection Sheet
<table>
<thead>
<tr>
<th>ST</th>
<th>Trial Number</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
</tbody>
</table>
Appendix D

CAR Testing Protocol

\[
CAR = \left( \frac{MVIC}{MVIC + SIB} \right) \times 100
\]
Appendix E

Dimensions of Custom Built Staircase
Appendix F

Post-processing Visual 3D Pipelines

File_New;

Set_Pipeline_Parameter_To_Folder_Path
(PARAMETER_NAME= FOLDER
(PARAMETER_VALUE=VISUAL3D_DEFAULT_DATA_FOLDER
(PARAMETER_VALUE_SEARCH_FOR=
(PARAMETER_VALUE_REPLACE_WITH=
(PARAMETER_VALUE_APPEND=
;

File_Open
(FILE_NAME= FOLDER
;

Assign_Tags_To_Files
(!MOTION_FILE_NAMES=
(TAGS= Right
;

Assign_Tags_To_Files
(!MOTION_FILE_NAMES=
(TAGS= Left
;

Create_Hybrid_Model
!CALIBRATION_FILE=
!SUFFIX=
!RANGE=ALL_FRAMES
;

Apply_Model_Template
/MODEL_TEMPLATE=
!CALIBRATION_FILE=
;
Set_Subject_Height
!CALIBRATION_FILE=
!HEIGHT=
;

Set_Subject_Weight
!CALIBRATION_FILE=
!WEIGHT=
;

Assign_Model_File
!CALIBRATION_FILE=
/MOTION_FILE_NAMES=ALL_FILES
!REMOVE_EXISTING_ASSIGNMENTS=FALSE
;

Build_Model
!CALIBRATION_FILE=
/REBUILD_ALL_MODELS=TRUE
!DISPLAY_RESULTS=TRUE
;

Multiply_Signals
/SIGNAL_TYPES=METRIC+METRIC
/SIGNAL_NAMES=MASS+HEIGHT
/SIGNAL_FOLDER=PROCESSED+PROCESSED
/RESULT_NAME=WEIGHTxHEIGHT
!RESULT_FOLDER=PROCESSED
;

!Start PROCESSING ****************************
Set_Use_Processed_Analog
/USE_PROCESSED=TRUE
;

Set_Use_Processed_Targets
/USE_PROCESSED=TRUE
;

Set_Force_Platform_Threshold
/THRESHOLD=10
/SET_AS_DEFAULT=TRUE
;

Select_Active_File
/FILE_NAME=ALL_FILES
;

Lowpass_Filter
/SIGNAL_TYPES=TARGET+ANALOG
! /SIGNAL_NAMES=
! /SIGNAL_FOLDER=ORIGINAL+ORIGINAL
! /RESULT_SUFFIX=
! /RESULT_FOLDER=PROCESSED
! /FILTER_CLASS=BUTTERWORTH
/FREQUENCY_CUTOFF=6
! /NUM_REFLECTED=6
! /TOTAL_BUFFER_SIZE=6
/NUM_BIDIRECTIONAL_PASSES=1
;

Modify_Force_Platform_Parameters
/FP_USED=2
/FP_TYPE=4+4
/FP_CHANNEL=1+2+3+4+5+6+0+0+0+0+0+0
/FP_ORIGIN=0+-0.04+-44+0.6+0.6+-38.9
/FP_CALMATRIX=1.505+0+0+0+0+0+1.516+0+0+0+0+0+0+5.906+0+0+0+0+0+0+592+0+0+0+0+0+0+0+0+0+0+0+0+305+3.054+0+0+0+0+0+0+3.077+0+0+0+0+0+0+11.686+0+0+0+0+0+0+0+0+1627+0+0+0+0+0+1606+0+0+0+0+0+0+0+0+1+777
/STORE_CALMATRIX=BYCOLUMN
/FP_ZERO=1+10
/FP_ZEROS=1+10+1+10
Modify_Force_Structure_Parameters
/USED=1
/TYP=4
/NUM_FP_IN=1
/FP_INDEX=1
/NUM_SURFACES_OUT=1
/SPEED_VALUES=0
!/SPEED_CHANNELS=
!/SPEED_SCALES=
/CORNER1=508+460.6+350
/CORNER2=508+30+350
/CORNER3=0+30+350
/CORNER4=0+460.6+350
/USE_FORCES_FOR_KINETICS=TRUE
!/COMBINE_INPUT_FORCES=FALSE
!/UPDATE_C3D_FILE=FALSE
;

!----------------------------------------------
! Rotations and Moments
!----------------------------------------------

Compute_Model_Based_Data
/RESULT_NAME=Right Hip Rotations
/FUNCTION=JOINT_ANGLE
/SEGMENT=RTH
/REFERENCE_SEGMENT=RPV_2
/RESOLUTIONCOORDINATE_SYSTEM=
!/USE_CARDAN_SEQUENCE=FALSE
!/NORMALIZATION=FALSE
! /NORMALIZATION_METHOD=
! /NORMALIZATION_METRIC=
! /NEGATEX=FALSE
! /NEGATEY=FALSE
! /NEGATEZ=FALSE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;
Compute_Model_Based_Data
/RESULT_NAME=Right Knee Rotations
/FUNCTION=JOINT_ANGLE
/SEGMENT=RSK
/REFERENCE_SEGMENT=RTH
/RESOLUTION_COORDINATE_SYSTEM=
! /USE_CARDANSEQUENCE=FALSE
! /NORMALIZATION=FALSE
! /NORMALIZATION_METHOD=
! /NORMALIZATION_METRIC=
! /NEGATEX=FALSE
! /NEGATEY=FALSE
! /NEGATEZ=FALSE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;
Compute_Model_Based_Data
/RESULT_NAME=Right Ankle Rotations
/FUNCTION=JOINT_ANGLE
/SEGMENT=RFT
/REFERENCE_SEGMENT=RSK
/RESOLUTION_COORDINATE_SYSTEM=
! /USE_CARDANSEQUENCE=FALSE
! /NORMALIZATION=FALSE
! /NORMALIZATION_METHOD=
! /NORMALIZATION_METRIC=
! /NEGATEX=FALSE
! /NEGATEY=FALSE
! /NEGATEZ=FALSE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;

Compute_Model_Based_Data
/RESULT_NAME=Left Hip Rotations
/FUNCTION=JOINT_ANGLE
/SEGMENT=LTH
/REFERENCE_SEGMENT=RPV_2
/RESOLUTIONCOORDINATE_SYSTEM=
! /USE_CARDAN_SEQUENCE=FALSE
! /NORMALIZATION=FALSE
! /NORMALIZATION_METHOD=
! /NORMALIZATION_METRIC=
! /NEGATEX=FALSE
/NEGATEY=TRUE
/NEGATEZ=TRUE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;

Compute_Model_Based_Data
/RESULT_NAME=Left Knee Rotations
/FUNCTION=JOINT_ANGLE
/SEGMENT=LSK
/REFERENCE_SEGMENT=LTH
/RESOLUTIONCOORDINATE_SYSTEM=
! /USE_CARDAN_SEQUENCE=FALSE
! /NORMALIZATION=FALSE
! /NORMALIZATION_METHOD=
! /NORMALIZATION_METRIC=
! /NEGATEX=FALSE
/NEGATEY=TRUE
/NEGATEZ=TRUE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;

Compute_Model_Based_Data
/RESULT_NAME=Left Ankle Rotations
/FUNCTION=JOINT_ANGLE
/SEGMENT=LFT
/REFERENCE_SEGMENT=LSK
/RESOLUTIONCOORDINATESYSTEM=
! /USE_CARDAN_SEQUENCE=FALSE
! /NORMALIZATION=FALSE
! /NORMALIZATION_METHOD=
! /NORMALIZATION_METRIC=
! /NEGATEXT=FALSE
/NEGATET=TRUE
/NEGATEZ=TRUE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;

Compute_Model_Based_Data
/RESULT_NAME=Right Hip Moments
/FUNCTION=JOINT_MOMENT
/SEGMENT=RTH
/REFERENCE_SEGMENT=
/RESOLUTIONCOORDINATESYSTEM=RPV_2
/USE_CARDAN_SEQUENCE=TRUE
/NORMALIZATION=TRUE
/NORMALIZATION_METHOD=NORMALIZE_TO_LOCAL_METRIC
/NORMALIZATION_METRIC=WEIGHTXHEIGHT
! /NEGATET=FALSE
! /NEGATET=FALSE
! /NEGATEZ=FALSE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;

Compute_Model_Based_Data
/RESULT_NAME=Right Knee Moments
/FUNCTION=JOINT_MOMENT
/SEGMENT=RSK
/REFERENCE_SEGMENT=
/RESOLUTIONCOORDINATESYSTEM=RTH
//USE_CARDAN_SEQUENCE=TRUE
//NORMALIZATION=TRUE
//NORMALIZATION_METHOD=normalize_to_local_metric
//NORMALIZATION_METRIC=weightxheight
! /NEGATEX=FALSE
! /NEGATEY=FALSE
! /NEGATEZ=FALSE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;

Compute_Model_Based_Data
/RESULT_NAME=Right Ankle Moments
/FUNCTION=JOINT_MOMENT
/SEGMENT=RFT
/REFERENCE_SEGMENT=
/RESOLUTION_COORDINATE_SYSTEM=RSK
/USE_CARDAN_SEQUENCE=TRUE
/NORMALIZATION=TRUE
/NORMALIZATION_METHOD=normalize_to_local_metric
/NORMALIZATION_METRIC=weightxheight
! /NEGATEX=FALSE
! /NEGATEY=FALSE
! /NEGATEZ=FALSE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;

Compute_Model_Based_Data
/RESULT_NAME=Left Hip Moments
/FUNCTION=JOINT_MOMENT
/SEGMENT=LTH
/REFERENCE_SEGMENT=
/RESOLUTION_COORDINATE_SYSTEM=RPV_2
/USE_CARDAN_SEQUENCE=TRUE
/NORMALIZATION=TRUE
/NORMALIZATION_METHOD=normalize_to_local_metric
/NORMALIZATION_METRIC=weightxheight
! /NEGATEX=FALSE
/NEGATEY=TRUE
/NEGATEZ=TRUE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;

Compute_Model_Based_Data
/RESULT_NAME=Left Knee Moments
/FUNCTION=JOINT_MOMENT
/SEGMENT=LSK
/REFERENCE_SEGMENT=
/RESOLUTION_COORDINATE_SYSTEM=LTH
/USE_CARDAN_SEQUENCE=TRUE
/NORMALIZATION=TRUE
/NORMALIZATION_METHOD=NORMALIZE_TO_LOCAL_METRIC
/NORMALIZATION_METRIC=WEIGHTXHEIGHT
! /NEGATEx=FALSE
/NEGATEY=TRUE
/NEGATEZ=TRUE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;

Compute_Model_Based_Data
/RESULT_NAME=Left Ankle Moments
/FUNCTION=JOINT_MOMENT
/SEGMENT=LFT
/REFERENCE_SEGMENT=
/RESOLUTION_COORDINATE_SYSTEM=LSK
/USE_CARDAN_SEQUENCE=TRUE
/NORMALIZATION=TRUE
/NORMALIZATION_METHOD=NORMALIZE_TO_LOCAL_METRIC
/NORMALIZATION_METRIC=WEIGHTXHEIGHT
! /NEGATEx=FALSE
/NEGATEY=TRUE
/NEGATEZ=TRUE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
Compute_Model_Based_Data
/RESULT_NAME=Right Knee Force
/FUNCTION=JOINT_FORCE
/SEGMENT=RSK
/REFERENCE_SEGMENT=
/RESOLUTION_COORDINATE_SYSTEM=RTH
! /USE_CARDAN_SEQUENCE=FALSE
! /NORMALIZATION=FALSE
! /NORMALIZATION_METHOD=
! /NORMALIZATION_METRIC=
! /NEGATEX=FALSE
! /NEGATEY=FALSE
! /NEGATEZ=FALSE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;

Compute_Model_Based_Data
/RESULT_NAME=Left Knee Force
/FUNCTION=JOINT_FORCE
/SEGMENT=LSK
/REFERENCE_SEGMENT=
/RESOLUTION_COORDINATE_SYSTEM=LTH
! /USE_CARDAN_SEQUENCE=FALSE
! /NORMALIZATION=FALSE
! /NORMALIZATION_METHOD=
! /NORMALIZATION_METRIC=
/NEGATEX=TRUE
! /NEGATEY=FALSE
! /NEGATEZ=FALSE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;

Compute_Model_Based_Data
/RESULT_NAME=Right GRF
/FUNCTION=GRF_DATA
/SEGMENT=RFT
/REFERENCE_SEGMENT=
! /RESOLUTIONCOORDINATE_SYSTEM=LAB
! /USERCARDAN_SEQUENCE=FALSE
/NORMALIZATION=TRUE
/NORMALIZATION_METHOD=DEFAULT_NORMALIZATION
! /NORMALIZATION_METRIC=
! /NEGATEX=FALSE
! /NEGATEY=FALSE
! /NEGATEZ=FALSE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;

Compute_Model_Based_Data
/RESULT_NAME=Left GRF
/FUNCTION=GRF_DATA
/SEGMENT=LFT
/REFERENCE_SEGMENT=
! /RESOLUTIONCOORDINATE_SYSTEM=LAB
! /USERCARDAN_SEQUENCE=FALSE
/NORMALIZATION=TRUE
/NORMALIZATION_METHOD=DEFAULT_NORMALIZATION
! /NORMALIZATION_METRIC=
! /NEGATEX=FALSE
! /NEGATEY=FALSE
! /NEGATEZ=FALSE
! /AXIS1=X
! /AXIS2=Y
! /AXIS3=Z
;

Recalc
;

Automatic_Gait_Events
! /SELECT_X=FALSE
! /SELECT_Y=FALSE
! /SELECT_Z=TRUE
! /FRAME_WINDOW=8
! /USE_TPR=TRUE
;

Highlight_Event_Label
/EVENT_LABEL= RHS + RTO + LHS + LTO
;

Switch_to_Event_Processing_Mode
;
Interactive_Graph_Signals
/SIGNAL_TYPES= FORCE
/SIGNAL_NAMES= FP1
!/SIGNAL_FOLDER=ORIGINAL
!/SIGNAL_COMPONENTS= Z
!/GRAPH_INDEX=  
!/GRAPH_SUBINDEX=  
!/REPLACE_CURRENT=FALSE
;

Interactive_Graph_Signals
/SIGNAL_TYPES= LINK_MODEL_BASED
/SIGNAL_NAMES= Right Knee Rotations
!/SIGNAL_FOLDER=ORIGINAL
!/SIGNAL_COMPONENTS= X
!/GRAPH_INDEX=  
!/GRAPH_SUBINDEX=  
!/REPLACE_CURRENT=FALSE
;
Interactive_Graph_Signals
/SIGNAL_TYPES= LINK_MODEL_BASED
/SIGNAL_NAMES= Right Knee Moments
/SIGNAL_FOLDER=ORIGINAL
/SIGNAL_COMPONENTS= X
! /GRAPH_INDEX=
! /GRAPH_SUBINDEX=
! /REPLACE_CURRENT=FALSE
;

File_Save_As
! /FILE_NAME=
;
Appendix G

International Knee Documentation Committee Questionnaire (IKDC)
2000 IKDC SUBJECTIVE KNEE EVALUATION FORM

Your Full Name: ________________________________

Today's Date: ____/____/____ Date of Injury: ____/____/____

Day Month Year Day Month Year

SYMPTOMS*:
*Grade symptoms at the highest activity level at which you think you could function without significant symptoms, even if you are not actually performing activities at this level.

1. What is the highest level of activity that you can perform without significant knee pain?
   - Very strenuous activities like jumping or pivoting as in basketball or soccer
   - Strenuous activities like heavy physical work, skiing or tennis
   - Moderate activities like moderate physical work, running or jogging
   - Light activities like walking, housework or yard work
   - Unable to perform any of the above activities due to knee pain

2. During the past 4 weeks, or since your injury, how often have you had pain?

   0 1 2 3 4 5 6 7 8 9 10 Constant

   Never

3. If you have pain, how severe is it?

   0 1 2 3 4 5 6 7 8 9 10 Worst pain imaginable

   No pain

4. During the past 4 weeks, or since your injury, how stiff or swollen was your knee?

   - Not at all
   - Mildly
   - Moderately
   - Very
   - Extremely

5. What is the highest level of activity you can perform without significant swelling in your knee?

   - Very strenuous activities like jumping or pivoting as in basketball or soccer
   - Strenuous activities like heavy physical work, skiing or tennis
   - Moderate activities like moderate physical work, running or jogging
   - Light activities like walking, housework, or yard work
   - Unable to perform any of the above activities due to knee swelling

6. During the past 4 weeks, or since your injury, did your knee lock or catch?

   - Yes
   - No

7. What is the highest level of activity you can perform without significant giving way in your knee?

   - Very strenuous activities like jumping or pivoting as in basketball or soccer
   - Strenuous activities like heavy physical work, skiing or tennis
   - Moderate activities like moderate physical work, running or jogging
   - Light activities like walking, housework or yard work
   - Unable to perform any of the above activities due to giving way of the knee
SPORTS ACTIVITIES:

8. What is the highest level of activity you can participate in on a regular basis?
   - Very strenuous activities like jumping or pivoting as in basketball or soccer
   - Strenuous activities like heavy physical work, skiing or tennis
   - Moderate activities like moderate physical work, running or jogging
   - Light activities like walking, housework or yard work
   - Unable to perform any of the above activities due to knee

9. How does your knee affect your ability to:

<table>
<thead>
<tr>
<th></th>
<th>Not difficult at all</th>
<th>Minimally difficult</th>
<th>Moderately Difficult</th>
<th>Extremely difficult</th>
<th>Unable to do</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Go up stairs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Go down stairs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Kneel on the front of your knee</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Squat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. Sit with your knee bent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f. Rise from a chair</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g. Run straight ahead</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>h. Jump and land on your involved leg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Stop and start quickly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FUNCTION:

10. How would you rate the function of your knee on a scale of 0 to 10 with 10 being normal, excellent function and 0 being the inability to perform any of your usual daily activities which may include sports?

FUNCTION PRIOR TO YOUR KNEE INJURY:

<table>
<thead>
<tr>
<th>Cannot perform daily activities</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>No limitation in daily activities</th>
</tr>
</thead>
</table>

CURRENT FUNCTION OF YOUR KNEE:

<table>
<thead>
<tr>
<th>Cannot perform daily activities</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>No limitation in daily activities</th>
</tr>
</thead>
</table>
Appendix H

Normality Assessment for Variables in Aim 2
### Variables at Pre-surgery

<table>
<thead>
<tr>
<th>Outcome Variable</th>
<th>Skewness</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACLr Group</td>
<td>Healthy Group</td>
</tr>
<tr>
<td>H: M</td>
<td>0.94</td>
<td>0.38</td>
</tr>
<tr>
<td>AMT</td>
<td>1.84</td>
<td>0.44</td>
</tr>
<tr>
<td>MEP120</td>
<td>2.99</td>
<td>1.50</td>
</tr>
<tr>
<td>SICi</td>
<td>2.00</td>
<td>1.00</td>
</tr>
<tr>
<td>ICF</td>
<td>1.47</td>
<td>0.99</td>
</tr>
<tr>
<td>MVIC</td>
<td>0.01</td>
<td>0.69</td>
</tr>
<tr>
<td>CAR</td>
<td>-0.75</td>
<td>-1.84</td>
</tr>
<tr>
<td>Knee angle – Ascent</td>
<td>1.09</td>
<td>0.26</td>
</tr>
<tr>
<td>Knee moment – Ascent</td>
<td>0.13</td>
<td>0.06</td>
</tr>
<tr>
<td>Knee angle – Descent</td>
<td>-2.85</td>
<td>-0.24</td>
</tr>
<tr>
<td>Knee moment - Descent</td>
<td>1.06</td>
<td>0.98</td>
</tr>
<tr>
<td>IKDC</td>
<td>0.10</td>
<td>-4.10</td>
</tr>
</tbody>
</table>

### Variables at 6-months Post-surgery

<table>
<thead>
<tr>
<th>Outcome Variable</th>
<th>Skewness</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACLr Group</td>
<td>Healthy Group</td>
</tr>
<tr>
<td>H: M</td>
<td>0.67</td>
<td>-0.06</td>
</tr>
<tr>
<td>AMT</td>
<td>1.20</td>
<td>0.46</td>
</tr>
<tr>
<td>MEP120</td>
<td>2.50</td>
<td>1.50</td>
</tr>
<tr>
<td>SICi</td>
<td>2.50</td>
<td>0.76</td>
</tr>
<tr>
<td>ICF</td>
<td>3.60</td>
<td>0.30</td>
</tr>
<tr>
<td>MVIC</td>
<td>-0.16</td>
<td>0.78</td>
</tr>
<tr>
<td>CAR</td>
<td>-0.67</td>
<td>-2.40</td>
</tr>
<tr>
<td>Knee angle – Ascent</td>
<td>-1.10</td>
<td>-0.34</td>
</tr>
<tr>
<td>Knee moment – Ascent</td>
<td>1.10</td>
<td>1.30</td>
</tr>
<tr>
<td>Knee angle – Descent</td>
<td>-0.40</td>
<td>-0.26</td>
</tr>
<tr>
<td>Knee moment - Descent</td>
<td>1.60</td>
<td>1.50</td>
</tr>
<tr>
<td>IKDC</td>
<td>-0.63</td>
<td>-4.10</td>
</tr>
</tbody>
</table>