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Neuromuscular Strategies for Regulating Knee Joint Moments in Healthy and Injured Populations

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PhD Thesis

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General Abstract

Background: Joint stability has been experimentally and clinically linked to mechanisms of knee injury and joint degeneration. The only dynamic, and perhaps most important, regulators of knee joint stability are contributions from muscular contractions. In participants with unstable knees, such as anterior cruciate ligament (ACL) injured, a range of neuromuscular adaptations has been observed including quadriceps weakness and increased co-activation of adjacent musculature. This co-activation is seen as a compensation strategy to increase joint stability. In fact, despite increased co-activation, instability persists and it remains unknown whether observed adaptations are the result of injury induced quadriceps weakness or the mechanical instability itself. Furthermore, there exists conflicting evidence on how and which of the neuromuscular adaptations actually improve and/or reduce knee joint stability.

Purpose: The overall aim of this thesis is therefore to elucidate the role of injury and muscle weakness on muscular contributions to knee joint stability by addressing two main objectives: (1) to further our understanding of individual muscle contribution to internal knee joint moments; and (2) to investigate neuromuscular adaptations, and their effects on knee joint moments, caused by either ACL injury and experimental voluntary quadriceps inhibition (induced by pain).

Methods: The relationship between individual muscle activation and internal net joint moments was quantified using partial least squares regression models. To limit the biomechanical contributions to force production, surface electromyography (EMG) and kinetic data was elicited during a weight-bearing isometric force matching task.

A cross-sectional study design determined differences in individual EMG-moment relationships between ACL deficient and healthy controls (CON) groups. A crossover placebo controlled study design determined these differences in healthy participants with and without induced quadriceps muscle pain. Injections of hypertonic saline (5.8%) to the vastus medialis induced muscle pain. Isotonic saline (0.9%) acted as control. Effect of muscle pain on muscle synergies recruited for the force matching task, lunging and squatting tasks was also evaluated. Synergies were extracted using a concatenated non-negative matrix factorization framework.

Results/Discussion: In CON, significant relationships of the rectus femoris and tensor fascia latae to knee extension and hip flexion; hamstrings to hip extension and knee flexion; and gastrocnemius and hamstrings to knee rotation were identified. Vastii activation was independent of moment generation, suggesting mono-articular vastii activate to produce compressive forces, essentially bracing the knee, so that bi-articular muscles crossing the hip can generate moments for the purpose of sagittal plane movement. Hip ab/adductor muscles modulate frontal plane moments, while hamstrings and gastrocnemius support the knee against externally applied rotational moments.

Compared to CON, ACL had 1) stronger relationships between rectus femoris and knee extension, semitendinosus and knee flexion, and gastrocnemius and knee flexion moments; and 2) weaker relationships between biceps femoris and knee flexion, gastrocnemius and external knee rotation, and gluteus medius and hip abduction moments. Since the knee injury mechanism, is associated with shallow knee flexion angles, valgus alignment and rotation, adaptations after ACL injury are suggested to improve sagittal plane stability, but reduce frontal and rotational plane stability.

During muscle pain, EMG-moment relationships of 1) semitendinosus and knee flexor moments were stronger compared to no pain, while 2) rectus femoris and tensor fascia latae to knee extension moments and 3) semitendinosus and lateral gastrocnemius to knee internal rotation moments were reduced. Results support the theory that adaptations to quadriceps pain reduces knee extensor demand to protect the joint and prevent further pain; however, changes in non-painful muscles reduce rotational plane stability.

Individual muscle synergies were identified for each moment type: flexion and extension moments were respectively accompanied by dominant hamstring and quadriceps muscle synergies while co-activation was observed in muscle synergies associated with abduction and rotational moments. Effect of muscle pain was not evident on muscle synergies recruited for the force matching task. This may be due to low loading demands and/or a subject-specific redistribution of muscle activation. Similarly, muscle pain did not affect synergy composition in lunging and squatting tasks. Rather, activation of the extensor dominant muscle synergy and knee joint dynamics were reduced, supporting the notion that adaptive response to pain is to reduce the load and risk of further pain and/or injury.

Conclusion: This thesis evaluated the interrelationship between muscle activation and internal joint moments and the effect of ACL injury and muscle pain on this relationship. Findings indicate muscle activation is not always dependent on its anatomical orientation as previous works suggest, but rather on its role in maintaining knee joint stability especially in the frontal and transverse loading planes. In tasks that are dominated by sagittal plane loads, hamstring and quadriceps will differentially activate. However, when the knee is required to resist externally applied rotational and abduction loads, strategies of global co-activation were identified. Contributions from muscles crossing the knee for supporting against knee adduction loads were not apparent. Alternatively hip abductors were deemed more important regulators of knee abduction loads.

Both muscle pain and ACL groups demonstrated changes in muscle activation that reduced rotational stability. Since frontal plane EMG-moment changes were not present during muscle pain, reduced relationships between hip muscles and abduction moments may be chronic adaptions by ACL that facilitate instability. Findings provide valuable insight into the roles muscles play in maintaining knee joint stability. Rehabilitative/ preventative exercise interventions should focus on neuromuscular training during tasks that elicit rotational and frontal loads (i.e. side cuts, pivoting maneuvers) as well as maintaining hamstring balance, hip abductor and plantarflexor muscle strength in populations with knee pathologies and quadriceps muscle weakness.

List of Abbreviations and Units

°	degrees
%RC	percent relative change
α_s	strength ratio
β	beta coefficient
Φ_M	mean direction of muscle activation
Φ_T	mean direction of moment generation
ACL	anterior cruciate ligament
ADD	hip adductors
AMI	arthrogenic muscle inhibition
BASE	baseline
BF	biceps femoris
BM	body mass
C	synergy activation coefficient matrix/vector
CON	control
CNS	central nervous system
EMG	electromyography
$\text{EMG}/\text{EMG}_{\text{max}}$	experimental EMG value over max EMG value from MVIC trial
iEMG	integrated EMG
GM	gluteus medius
GMP	group mean peak
GRF	ground reaction force
HYP	hypertonic saline
ICC	intraclass correlation coefficient
INJ	with injection
ISO	isotonic saline
LG	lateral gastrocnemius
MAO	moment arm orientation
MG	medial gastrocnemius
Nm	newton metres
Nm/kg	newton meters per kilogram body mass
R^2	regression model goodness-of-fit
r	Pearson correlation coefficient
REC	recovery
RF	rectus femoris
S	synergy matrix/vector
SPM	statistical parametric mapping
ST	semitendinosus
TFL	tensor fascia latae
T_E	experimental torque
T_I	ideal torque
T_M	model torque
VAF	variance accounted for
VL	vastus lateralis
VM	vastus medialis
X_{EMG}	mean magnitude of activation

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Preface

Due to their anatomical orientation, the major muscles crossing the knee are typically categorized as knee flexors (hamstring, gastrocnemius) and extensors (quadriceps), and can aptly stabilize the knee against sagittal plane loads. However, physiological loading conditions are multifactorial and do not limit loads to a single axis of rotation. It remains unclear how the muscles crossing. It is unclear how these muscles contribute to knee stability during frontal and transverse plane loading.

The goal of this thesis was to determine which muscles or group of muscles are most important at modulating knee joint moments and subsequent knee joint stability. The two main objectives of this thesis were to (1) quantify the interrelationship of individual muscle contribution to internal knee joint moments and (2) to investigate the effects of anterior cruciate ligament injury and experimentally reduced quadriceps function on these relationships. To achieve these, five sub-objectives were proposed:

1-A to quantify the relationship between individual muscle activation patterns and internal net joint moments and determine if these relationships change with increasing effort;

1-B to identify muscle synergies important for stabilising the knee during direction specific loading conditions;

1-C to identify muscles synergies recruited during dynamic lunging and squatting tasks and determine if synergies are task specific or shared common characteristics;

2-A to elucidate muscle activation patterns that either increase or reduce joint moments related to knee instability;

2-B to determine the effect of experimentally induced quadriceps inhibition on individual activation and synergistic control of lower limb muscles.

This thesis is assembled in article format. Chapters 1, 2 and 3 consist of the general introduction, review of literature and general methods, respectively. Chapter 4 includes five independent studies whose references are standalone and may not be sourced in the general reference section at the end of this thesis. Study 1 has been accepted for publication at the journal of *Medicine & Science in Sports & Exercise*. Study 2 is under review at the *Journal of Orthopaedic Research*. Studies 3-5 are intended to be submitted for publication.

These sub-objectives however are not specific to one given study. Chapter 5 of this thesis provides a general discussion briefly restating the main findings of the five studies as it relates to the two main goals and corresponding sub-objectives. Chapter 5 also mentions any findings, limitations and suggestions for future research not mentioned in the discussion section of the articles.

CHAPTER 1: Introduction

The knee joint is exposed to high contact forces and moments during activities of daily living (Glitsch and Baumann, 1997; Kim et al., 2009). The ability to safely oppose forces and moments acting on the knee from the external environment is known as knee joint stability (Winter, 2009). Knee joint stability arises from the instantaneous integration of soft tissue restraints, articular geometry, and compressive forces of body weight and muscle action (Panjabi, 1992). The joint's soft tissue restraints can contribute to knee joint stability through their passive mechanical properties. However, during loading conditions that exceed tissue tolerance levels, the main contributor to knee joint stability and contact mechanics is compressive forces produced by muscle action. In fact, muscle forces can maintain stability even when passive tissue restraints have been removed in vitro (Hsieh and Walker, 1976; Yack et al., 1994). In such loading conditions, soft tissues are important in providing afferent feedback of force and motion through various mechanoreceptors located in ligaments, tendons, and muscle fibres (Panjabi, 1992).

Variations in neuromuscular function have been experimentally linked to reduced knee joint stability (MacWilliams et al., 1999) and are believed to be an underlying mechanism for traumatic knee joint injuries (DeMorat et al., 2004; Hewett et al., 2005; Hashemi et al., 2007) and joint degeneration (Herzog and Longino, 2007; Palmieri-Smith and Thomas, 2009). However, the direct contributions of muscle contractions to knee joint loads, stability and corresponding pathologies remain unknown.

Knee muscles are typically categorized as knee flexors (hamstring, gastrocnemius) and extensors (quadriceps) (Nordin and Frankel, 2001; Hamill and Knutzen, 2004; Agur and Dalley, 2005). This is primarily due to their anatomical orientation relative to the knee joint center resulting in large flexion and extension moment arms. Since knee joint range of motion is greatest in the sagittal plane, it is commonly accepted that the extensors will support the knee against an externally applied flexion moment while the flexors oppose extension moments. However, physiological loading conditions are multifactorial and do not limit loads to a single axis of rotation, so how does the knee remain stable when frontal, rotational and/or shear loads are applied?

Smaller muscles crossing the knee, such as gracilis, sartorius, and tensor fascia latae, possess relatively large varus and valgus moment arms; however, due to their small cross-sectional area they have low force generation capacity (Brand et al., 1986; Zhang and Wang, 2001; Zhang et al., 2001) and minimal contribution to supporting loads acting in the frontal plane (Lloyd and Buchanan, 1996; Buchanan and Lloyd, 1997). The only muscle crossing the knee joint whose moment arm orientation (MAO) dominates the transverse plane is popliteus. However, its role is maintaining rotational knee stability is debatable since its cross-sectional area is small (Brand et al., 1986), it can only internally rotate the tibia when the knee is flexed (Harner et al., 1998), a position not often associated with knee injury or instability (Boden et al., 2000), and no observable differences in activation between ACL injured and control exists (Weresh et al., 1997).

Rather than individual muscles opposing a given force, earlier studies began to acknowledge antagonist co-activation is an effective strategy to stabilize the knee. By contracting muscles spanning both sides of a joint, the femoral condyles are driven deeper into the tibial plateau, thus increasing joint stiffness under shear and torsional loading (Markolf et al., 1976). Since their moment arms also possess varus-valgus components, the knee joint flexors and extensors can support frontal plane loads by co-activating such that respective flexion and extension moments are neutralized and remaining frontal moments combine to generate a frontal plane moment (Goldfuss et al., 1973; Lloyd and Buchanan, 2001). Similarly, co-activation of the quadriceps and hamstring muscles has been shown to reduce tibial rotation (MacWilliams et al., 1999; Wojtys et al., 2003; Yoo et al., 2005).

However, to what extent the strategy of increased antagonist co-activation is advantageous is debatable. Rudolph et al. (2007) believe that adapting a co-activation strategy to stabilise the knee to a certain level no longer stabilises the joint efficiently but instead introduces the joint to adverse loads, thus contributing to the development and progression of joint degeneration. Adverse loads can be even more detrimental if co-activation is unbalanced. In populations with unstable knees, significant reductions in medial muscle activations compared to lateral, increases abduction loads externally acting on the knee (Palmieri-Smith et al., 2009) – a major contributor to the ACL injury mechanism (Hewett et al., 2005). Furthermore, the role of co-activation in populations with knee pathologies have been limited to the quadriceps and hamstring muscles, even though the gastrocnemius and hip muscles have been shown to contribute to knee joint mechanics (Fleming et al., 2001b; Henriksen et al., 2009a; Sritharan et al., 2012; Morgan et al., 2014). There exist conflicting interpretations on how and which components of these neuromuscular variations actually improve and/or reduce knee joint stability (Berchuck et al., 1990; Sinkjaer and Arendt-Nielsen, 1991; Aune et al., 1997; Kvist and Gillquist, 2001; Rudolph et al., 2001; Chmielewski et al., 2005; Alkjær et al., 2012). Understanding role of individual muscles as it relates to knee joint loads, specifically in the frontal and transverse planes, and how the muscles activate collectively to stabilize the knee against a given loading condition is warranted.

1.1 Methodological Considerations

In an attempt to better understand muscular contribution to joint stability, researchers often assess functional tasks (e.g. walking, jumping, perturbations etc.). While these studies provide insight into how the system functions as a whole, identifying direct links between neuromuscular contributions to stabilising forces are confounded by the biomechanical factors during force production (Wilkie, 1950). Isometric exercise is a common approach to reduce the problem complexity; however, studies typically limit analysis to a single loading axis (flexion-extension). Appreciating that loads experienced during functional activities are multidirectional, Buchanan et al. (1997) implemented a force matching protocol to evaluate neuromuscular strategies used to support various direction dependant loads. Participants sat and transferred various combinations of flexion-extension adduction-abduction forces via the shank to a load cell while the foot remained free. Building from previous work (Andriacchi et al., 1984; Buchanan et al., 1986), a muscle's activation was concluded to be dependent on its anatomical orientation. For example, semitendinosus has a moment arm relative to the knee joint centre (tibial eminence) that is posterior and slightly medial. Thus, the greatest activation would occur when a flexion and slight adduction moment generation is required. When a required moment incorporates a component of the semitendinosus' MAO, activation still occurs but to a lesser degree.

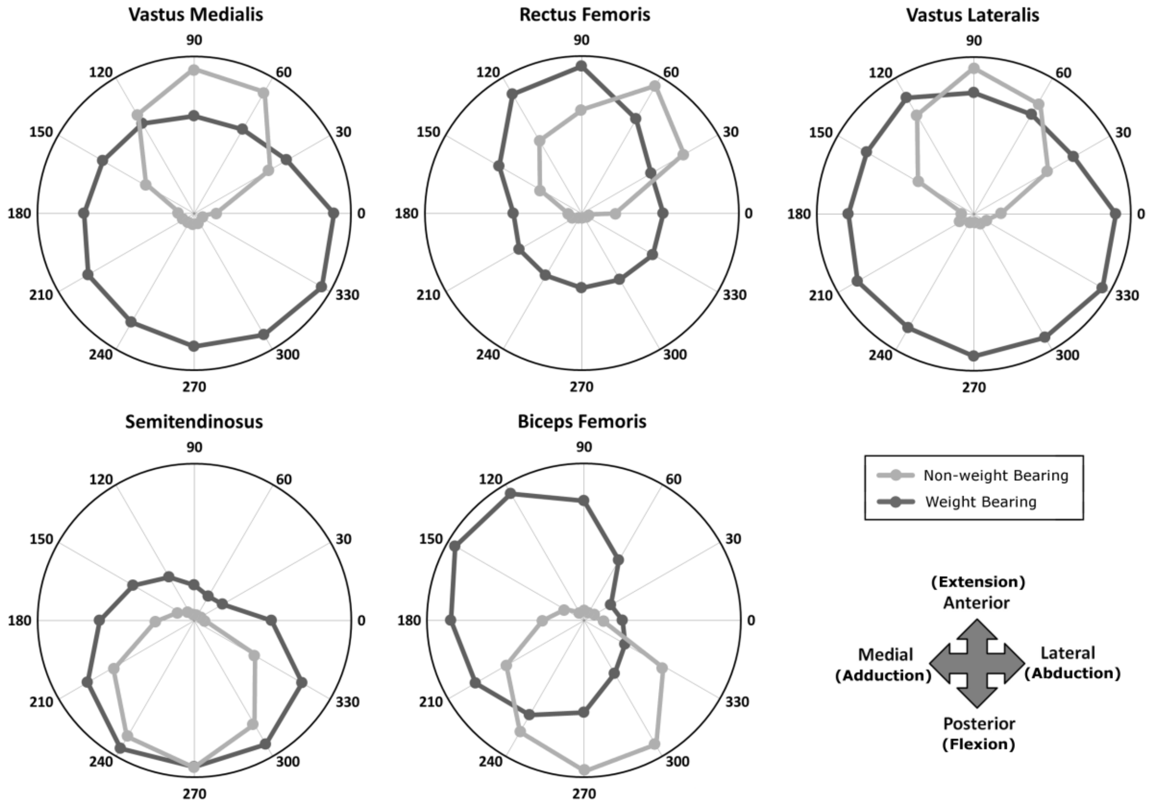

Figure 1: EMG polar plots of non-weight bearing (Krishnan et al., 2008) and weight-bearing (Flaxman et al., 2012) activation patterns of hamstring and quadriceps muscles in young healthy male adults. Both studies required participants to flex their knee to 30 degrees and generate 30% of maximal effort in each loading direction. Numbers along the circumference of the plots identify loading direction in degrees and where the pattern intersects the radii represents relative muscle activation magnitude at that loading direction. The units for the EMG magnitude are arbitrary, and the patterns are scaled to the maximum EMG value recorded for each muscle and loading condition.

Although this study sheds light on the roles of knee joint muscles in supporting direction-dependent loads, most functional activities are performed while weight bearing, consequently altering lower limb kinematics, joint loading conditions, and corresponding muscle activation characteristics compared to non-weight bearing tasks (Escamilla et al., 1998; Stensdotter et al., 2003; Shultz et al., 2009). As such, our group developed a force matching protocol that requires participants to stand, bear weight and modulate direction dependant horizontal ground reaction forces (GRFs) (Flaxman et al., 2012, 2013; Smith et al., 2012). Even though our protocol had similar magnitudes of effort and knee kinematics, our muscle activation patterns do not resemble those observed in non-weight bearing studies (Buchanan and Lloyd, 1997; Williams et al., 2003; Krishnan et al., 2008). While some muscles showed preferential activation corresponding to their MAO, other muscles showed preferential activation in loading directions opposite of their moment arm orientation (biceps femoris), or their activation that was not direction specific (vastii) (refer to Figure 1). In addition, greater antagonist activation was observed. As such, we re-defined muscle activation to include a stabilising role (Flaxman et al., 2012).

To date, individual muscle activation has been evaluated as a function of GRF direction and in turn, qualitatively related to knee joint loads. In this thesis, our analytical approach was extended to include the quantification of muscle activation as it relates to knee joint moments. With this, the roles of individual muscles can be more clearly associated with a measured load, thus providing a basis for understanding muscular contributions to knee joint stability.

Technically, knee joint motion only requires the contraction of a single muscle. For example, knee extension can be accomplished with only the contraction of the vastus lateralis. However, 12 different muscles cross the knee joint such that physiological motion exhibits mechanically redundant and complex co-contraction of agonist and antagonist muscle groups. Several studies support the notion that the central nervous system (CNS) can control numerous degrees of freedom of the body by a reduced set of activation signals called *muscle synergies* (Ting and Macpherson, 2005; Torres-Oviedo et al., 2006; Neptune et al., 2009; Ting et al., 2015). This type of structure not only simplifies muscle activation but may also be functionally related to biomechanical variables that are important for movement control (Ivanenko et al., 2004; Torres-Oviedo et al., 2006; Neptune et al., 2009; Chvatal et al., 2011; Walter et al., 2014).

Muscle synergy analysis is not only methodologically favorable because it simplifies muscle activation, but muscle synergies are suggested to represent basic neural mechanisms that are common across different dynamic conditions (Ivanenko et al., 2004; Hug et al., 2010; Torres-Oviedo and Ting, 2010; Chvatal et al., 2011; Chvatal and Ting, 2012). As aforementioned, the assessment of functional tasks (e.g. side-cuts, jumping, perturbations etc.) is preferable since it provides insight into how the system functions as a whole.

1.2 Clinical Considerations

Knee joint stability has been identified as a key contributor to joint health such that a lack of stability may result in traumatic knee joint injury (Hewett et al., 2005) or whole joint degeneration (Suter and Herzog, 2000; Fitzgerald et al., 2004; Roos, 2005). The first half of this thesis furthers our understanding of muscle contribution to healthy knee joint stability. The second half of this thesis investigates the muscular adaptations to knee joint injury and reduced muscle function and their effects on knee dynamics.

Rupture of the anterior cruciate ligament (ACL) causes loss of mechanical knee joint stability, sensory feedback and a voluntary inhibition of the quadriceps musculature, all of which dramatically affects the function of the knee (Urbach et al., 2001; Krogsgaard et al., 2011). Knee instability reduces mobility, increases risk of subsequent injuries, and mechanically drives the development of osteoarthritits (OA) (Daniel et al., 1994; Suter et al., 1998; Suter and Herzog, 2000; Palmieri-Smith and Thomas, 2009). Neuromuscular adaptations after injury have been commonly identified as increased knee flexion angles, decreased knee extensor moments, and increased antagonist activation of the hamstring and gastrocnemius muscles (Kvist and Gillquist, 2001; Rudolph et al., 2001; Williams et al., 2003; Chmielewski et al., 2005; Alkjaer et al., 2011; Shanbehzadeh et al., 2015). Although these differences are thought to decrease loads acting at the injured knee and increase joint stiffness (Hortobágyi and Devita, 2000; Rudolph et al., 2001), just 50-70% of ACL injured athletes regain pre-injury levels of physical activity within 5 years (Thomeé et al., 2007; Frobell et al., 2013), even when injury rehabilitation programs are implemented (Mikesky et al., 2006; Logerstedt et al., 2010; Frobell et al., 2013). Not surprisingly, information is lacking about how and which components of these neuromuscular changes are effective in modifying movement patterns or neuromuscular control during dynamic activities.

Almost instantaneously, ACL-injured individuals present deficits in voluntary muscle activation and force generation. This deficit has been identified almost universally in studies examining quadriceps activation in patients after ACL rupture (Chmielewski et al., 2004; Palmieri-Smith et al., 2008) as well as in populations with other knee pathologies causing joint pain (Powers et al., 1997; Slemenda et al., 1997). However, in a select number of cases, individuals with ACL deficient knees are able to "cope" and maintain pre-injury activity levels with no reports of "giving-way" episodes. The most distinguishable difference between ACL deficient copers and non-copers is quadriceps muscle strength and voluntary function (Rudolph et al., 2001; Alkjær et al., 2012; MacLeod et al., 2013b), which begs the question: *does instability result from compromising the mechanical integrity of the joint or the neuromuscular deficits after injury and the corresponding inability to adapt to external loads?* Thus, investigating the effect of instantaneous reductions in muscle function, before compensation strategies can be applied, may contribute to knowledge regarding the mechanisms of knee joint injury and its effects on stability.

1.3 Objectives

This thesis will try to answer the question: *which muscles or group of muscles are most important at regulating joint loads and subsequent knee joint stability?* To answer this question, two main objectives have been proposed: **(1)** to quantify the interrelationship between muscle activations (both individual and synergistic) and internal knee joint moments, thus specifying the functional contributions of muscles to maintaining knee joint stability; and **(2)** to determine how these relationships change in the presence of a traumatic knee joint injury or quadriceps muscle pain. Each main objective consisted of several sub-objectives which are described below:

Objective 1-A: quantify the relationship between individual muscle activation patterns and internal net joint moments and determine if these relationships change with increasing effort level.

Objective 1-B: to identify muscle synergies important for stabilising the knee during direction specific loading conditions (i.e. GRF directions) evoked from the force matching task.

Objective 1-C: to identify muscles synergies recruited during dynamic lunging and squatting tasks and determine if these synergies were task specific or shared common characteristics.

Objective 2-A: to identify muscle activation patterns that either increase or reduce joint moments related to knee instability in an ACL deficient populations.

Objective 2-B: of this thesis was to identify the effect of voluntary quadriceps inhibition (experimentally induced with muscle pain) on individual muscle activation and synergistic control**.**

1.4 Relevancy

Anterior cruciate ligament (ACL) injury is the most common debilitating knee injury in active adults with around 100,000 – 300,000 cases reported annually in the United States (Mall et al., 2014). At an estimated cost per case of approximately $17,000 USD, this injury places a large financial burden on our health care system (Lubowitz and Appleby, 2011). Rupture of the ACL causes loss of mechanical knee joint stability, sensory feedback and a voluntary inhibition of the quadriceps musculature, all of which dramatically affects the function of the knee (Urbach et al., 2001; Krogsgaard et al., 2011). Due to the functional impairment, ACL injured people are likely to suffer subsequent injuries, are 5 times more likely to develop OA, and perceived quality of life is reduced (Daniel et al., 1994; Lohmander et al., 2004; Frobell et al., 2013).

Muscular contractions are the only dynamic modulator of knee joint stability. Deficits in neuromuscular function has been experimentally (MacWilliams et al., 1999) and clinically (Slemenda et al., 1997; Rudolph et al., 2001) linked to reduced knee joint stability and is believed to be the underlying mechanism for traumatic knee joint injury (Hewett et al., 2005; Hortobagyi et al., 2005) and subsequent joint degeneration (Herzog and Longino, 2007; Palmieri-Smith and Thomas, 2009). Although several injury rehabilitation programs have been designed and shown to improve the stability and function of the knee (Mikesky et al., 2006; Frobell et al., 2010; Logerstedt et al., 2010), just 50-70% of ACL injured athletes regain pre-operative levels of physical activity within 5 years post-injury (Thomeé et al., 2007; Frobell et al., 2013). Information is lacking about how and which components of these training programs are effective in modifying movement patterns or neuromuscular control to increase stability during dynamic activities. By investigating the contributions of lower limb muscles to supporting the knee against external joint loads and how this differs in injured populations, we can identify activation patterns that may improve functional stability at the joint and contribute to rehabilitative or perhaps preventative interventions.

At the end of this thesis, we hope to describe the roles of muscles crossing the knee in maintaining joint stability, especially when the knee is exposed to frontal and rotational loading conditions. By evaluating the contributions of lower limb muscles to knee stability in both healthy and injured populations, we can identify activation strategies that may improve functional joint stability and contribute to the development of rehabilitative as well as preventative exercise programs.

CHAPTER 2: Literature Review

2.1 Muscular Contribution to Knee Joint Stability

The knee joint is exposed to high contact forces and moments during activities of daily living. Joint contact forces can range from 1.9-2.5x body weight while walking (Kim et al., 2009) and up to 14x while running (Glitsch and Baumann, 1997). For the purpose of this thesis, knee joint stability will be defined as the ability to safely oppose forces and moments acting on the knee from the external environment (Winter, 2009). An unstable joint is one that exposes soft tissues to forces/deformations exceeding the tissue's tolerance threshold or physiological limits causing micro- or macro-trauma. Knee joint stability arises from the integration of articular geometry, passive tissue restraints, and compressive forces from muscular contractions. Since the articulating surfaces of the femur and tibia are incongruent, and soft tissues only provide sufficient joint stability in low to moderate loading conditions, the compressive forces from muscular contractions are considered the most important contributors to knee stability (Hsieh and Walker, 1976). In fact, joint stability can be maintained by muscular contractions, even when the passive structures are removed (Hsieh and Walker, 1976; Yack et al., 1994). Rather, the importance of passive structures in regulating joint stability during high loading conditions is associated with their role in providing afferent force and motion feedback through various mechanoreceptors located in ligaments, tendons, and muscle fibres and ability to alter muscle activation strategies (Panjabi, 1992; Williams et al., 2001; Krogsgaard et al., 2011).

Due to their anatomical orientation, the major muscles crossing the knee are typically categorized as knee flexors (hamstring, gastrocnemius) and extensors (quadriceps) (Nordin and Frankel, 2001; Hamill and Knutzen, 2004; Agur and Dalley, 2005). This is because they possess large flexion and extension moment arms (the length between the knee joint centre and the line of force produced by these muscles). As such, it is commonly accepted that the extensors will support the knee against an externally applied flexion moment while the flexors oppose extension moments because knee joint range of motion is greatest along the sagittal plane. However, physiological loading conditions are multifactorial and do not limit loads to a single axis of rotation. It remains unclear how the muscles crossing the knee aide in knee joint stability when frontal, transverse and/or shear loads are applied.

Theoretically, each of the 12 muscles crossing the knee has a unique orientation so that when activated it can generate a moment along a specific line of action and oppose the given external force causing that moment (Brand et al., 1982). The gracilis, sartorius, and tensor fascia lata possess relatively large varus and valgus moment arms; however, due to their small cross-sectional area they have low force generation capacity (Brand et al., 1986; Zhang and Wang, 2001; Zhang et al., 2001) and minimal contribution to supporting loads acting in the frontal plane (Lloyd and Buchanan, 1996; Buchanan and Lloyd, 1997). Additionally, the medial and lateral hamstrings and gastrocnemius have been shown to contribute to external and internal rotational stability, respectively, however this is only significant when the knee is flexed (Yoo et al., 2005) – a posture that is not commonly associated with episodes of knee instability (Boden et al., 2000; Shimokochi and Shultz, 2008). Ultimately, there is very little literature to explain this stabilizing phenomenon that occurs during even the most basic functional tasks.

2.1.1 EMG-moment relationship

Evaluations of neuromuscular function with respect to knee joint stability typically involve dynamic assessments (i.e. cutting maneuver, perturbation etc.) such that results are functionally comparable to activities of daily living and sport. However, due to methodological limitations, stability during such tasks cannot be directly quantified. Inferences from in vitro and in silico simulations (Aune et al., 1997; MacWilliams et al., 1999; Sritharan et al., 2012; Morgan et al., 2014), or surrogate measures of stability during in vivo investigations (Sinkjaer and Arendt-Nielsen, 1991; Hortobágyi and Devita, 2000; Rudolph et al., 2001; Cashaback and Potvin, 2012) must be made instead. One surrogate measure is to evaluate the relationship between muscle activation and the loads acting on the knee. For example, Hortobagyi and Devita (2000) evaluated age-related differences in muscular contribution to a measure of joint stiffness (quantified as ratio of ground reaction force and angular displacement). Internal knee joint moments can also be used as a surrogate for understanding knee stability because in order to effectively oppose loads acting on the body from the external environment, the activity of all muscles that cross the joint must be integrated so that when summated, the muscular force products create a net joint moment-of-force that is opposite in direction to the external load.

Due to confounding biomechanical factors related to dynamic motion (i.e. joint velocity, position, and direction of movement) (Wilkie, 1950). The relationship between muscle activation and force generation built predominantly on experiments using isometric contractions. However, due to its primary plane of motion, studies typically limit analysis to the flexion-extension loading axis. Appreciating that loads experienced during functional activities are multidirectional, Buchanan et al. (1997) implemented a force matching protocol to evaluate neuromuscular strategies used to support various direction dependant loads. Participants sat and transferred various combinations of flexion-extension adduction-abduction forces via the shank to a load cell while the foot remained free. Findings suggest that muscle activation is dependent on a muscle's moment arm orientation (i.e. muscle's line of action relative to the joint's centre or rotation). This notion is supported by previous works (Andriacchi et al., 1984; Buchanan et al., 1986) and is commonly used in musculoskeletal modeling (Lloyd and Buchanan, 1996; Winby et al., 2009).

Although Buchanan et al. (1997) have shed light on the roles knee joint muscles play in supporting direction-dependent loads, the applicability of this relationship to functional tasks is limited for several reasons: (1) it is conducted in non-weight bearing conditions, where the joint is uncompressed from body weight (Kiefer et al., 1998; Fleming et al., 2001a; Shultz et al., 2009), (2) the majority of muscles crossing the knee are bi-articular so the contributions from the adjacent hip and ankle joints will alter the force-muscle activation relationship (Escamilla et al., 1998; Stensdotter et al., 2003; Shultz et al., 2009), and (3) rotational loads are not included which is a major component of knee injury mechanism (Li et al., 2007; Shimokochi and Shultz, 2008). A study that simulates physiological loads in a controlled yet more functionally relevant manner is critical to gain a better understanding of the neuromuscular contribution to force generation and joint stability.

As such, our group developed a force matching protocol that required participants to stand, bear weight and modulate direction dependant horizontal GRFs (Flaxman et al., 2012, 2013; Smith et al., 2012). Even though our protocol had similar magnitudes of effort and knee kinematics, our muscle activation patterns did not resemble those observed in the non-weight bearing force matching tasks (Figure 1, page 5). We attribute these differences to the added need to support the body, stabilise the knee against frontal and rotational loads, and the contributions of adjacent hip and ankle joints to bi-articular muscle action. Rather than a muscle's activation amplitude being dependent on moment arm orientation, a muscle's activation is also related to its role in maintaining knee joint stability. The roles of knee muscles were classified as (1) a *general joint stabilizer*: a muscle who's activation is independent of loading directions; (2) a *moment actuator*: a muscle that demonstrates preferential activation in a loading direction corresponding to its moment arm orientation; and (3) a *specific joint stabilizer*: a muscle that demonstrates preferential activation in a loading direction opposite of its moment arm orientation (Flaxman et al., 2012).

2.1.2 Mono- and bi-articular muscles

The angular displacement of the three major joints (hip, knee and ankle) determines the movement of the lower limb, while the direction of GRF is determined by the distribution of the net joint moments. The angular displacements and the net joint moments can vary independently, and even be opposite in direction. For example, during squat descent there is an increase in knee flexion angle accompanied by an increase in knee extension moments because the body must be supported. Since bi-articular muscles simultaneously influence two joints, the distribution of the net joint moments must be efficiently regulated; otherwise, unwanted co-contractions or eccentric actions would result if only mono-articular muscles were available. Therefore, the mono- and bi-articular muscles play different roles in multi-joint force control tasks: the mono-articular muscles provide positive work independently of the required net joint moments while the bi-articular muscles finely regulate the distribution of the net joint moments of the joints crossed (Jacobs and van Ingen Schenau, 1992; van Ingen Schenau et al., 1992; van Deursen et al., 1998).

The most noteworthy finding from our previous work evaluating muscle activation patterns during multi-directional force control was that the mono-articular vastii activated to the same level indifferent of loading direction (Flaxman et al., 2012, 2013), contrasting previous non-weight bearing studies where vastii's activation was dependent on its extension moment arm (1997; Williams et al., 2003; Krishnan et al., 2008) (Figure 1, page 5). Instead of the mono-articular muscles only activating to produce positive work (as the above discussion suggests), the vastii muscles were classified as general joint stabilisers while weight-bearing, contracting independent of loading direction to brace the knee, while the bi-articular rectus femoris and hamstring muscles transfer work from the hip down to the foot-ground interface (Flaxman et al., 2012, 2013). The role of the mono-articular vastii as general joint stabilisers is supported by several works: Sasaki and Neptune (2010) demonstrated that the vastii are primary contributors to axial joint force at heel strike; Cashaback and Potvin (2012) determined that the vastii muscles, particularly the vastus lateralis, provide the greatest contribution to rotational stiffness in all flexion-extension, varus-valgus, and transverse loading planes; Hashemi et al. (2007) even observed a decrease in ACL strain when quadriceps force is increased during simulated landing. Although some light has been shed onto the individual muscular contributions to knee joint stability, it still remains unclear how the knee remains stable when frontal and transverse plane loads are applied and the different roles mono- and bi-articular muscles play in maintaining knee stability. Therefore, **Objective 1-A** of this thesis was *to further elucidate the role of lower limb muscles as it relates to joint stability by quantifying the relationship between individual muscle activation patterns and internal net joint moments in all* *three loading planes*. This is especially beneficial when trying to determine the function of bi-articular muscles spanning the hip and knee: which joint is the muscle more likely to act on?

Under isometric conditions, the relationship between muscle activation and force is frequently linear (Bigland-Ritchie, 1981). Yet again, this relationship has been established from non-weight bearing conditions and limit analysis to a single axis (Bigland-Ritchie, 1981; Buchanan and Lloyd, 1997; van Deursen et al., 1998; Alkjær et al., 2012). In addition, when comparing different populations, varied muscle activation characteristics are often accompanied with varied moment magnitudes (Krishnan and Williams, 2009; Alkjær et al., 2012). As such, **Objective 1-A** also sought *to determine if muscle activation patterns, and muscle roles as it relates to knee joint stability, will change with a change in force level*.

2.1.3 Co-activation

Technically, knee joint motion only requires the contraction of a single muscle. For example, knee extension can be accomplished with only the contraction of the vastus lateralis. However, 12 different muscles cross the knee and physiological motion exhibits mechanically redundant co-contraction of agonist and antagonist muscle groups. From a musculoskeletal modeling point of view, this is deemed inefficient and unnecessary to achieve a given functional goal. Since loads acting at the knee are not limited to the single axis of flexion-extension and are continually changing, sometimes too fast to elicit protective reflexes, it is speculated that co-activation helps to “prepare” the knee for unforeseen perturbations by increasing joint compression and stiffness (Hortobágyi and Devita, 2000).

Since their moment arms possess varus-valgus components, the knee joint flexors and extensors are suggested to support frontal plane loads by co-activating such that respective flexion and extension moments are neutralized and remaining components combine to generate a frontal plane moment (Goldfuss et al., 1973; Lloyd and Buchanan, 2001). Similarly, co-activation of the quadriceps and hamstring muscles has been shown to reduce tibial rotation (MacWilliams et al., 1999; Wojtys et al., 2003; Yoo et al., 2005). According to Markolf et al. (1976) joint stability can be increased from the contractions of the muscles spanning both sides of the joint by driving the femoral condyles deeper into the tibial plateau, thus increasing joint stiffness under shear and torsional loading.

However, it remains unclear to what extent co-activation is beneficial to maintaining knee joint stability. It is suggested that adapting a co-activation strategy to stabilise the knee to a certain level no longer stabilises the joint efficiently but instead introduces the joint to adverse loads, thus contributing to the development of joint degeneration (Rudolph et al., 2001) or increasing risk of injury (Palmieri-Smith et al., 2009). Co-activation can also be detrimental if unbalanced. In populations with unstable knees, significant reductions in medial muscle activations compared to lateral, increases risk valgus loading (Hubley-Kozey et al., 2009; Palmieri-Smith et al., 2009).

There is the basic assumption that knee joint extensors (quadriceps) and knee joint flexors (hamstrings and gastrocnemius) are antagonistic and co-activation of medial and lateral muscles will support the knee against externally applied lateral and medial loads, respectively (Lloyd and Buchanan, 2001; Zhang et al., 2001; Lewek et al., 2005). However, based on our previous work (Flaxman et al., 2012, 2013), muscle activation as it relates to knee stability is not dependant on its anatomical orientation. Also, the antagonistic relationship of hamstrings and gastrocnemius to quadriceps is often debated (Mills and Hull, 1991; Durselen et al., 1995;

Fleming et al., 2001b). Using traditional measures (i.e. co-activation indices) that assume certain groups of muscles are antagonistic may therefore not be appropriate since it remains unclear how muscles work collectively to oppose direction specific loads. A more comprehensive evaluation of neuromuscular control, such as muscle synergy analysis, may provide more insight.

2.1.4 Muscle Synergies

Execution of human movement requires the neuromuscular system to coordinate multi-articular limbs with many degrees of freedom while interacting with an unpredictable, ever changing environment. Although highly complex patterns of muscle activation appear necessary to preform functional tasks, a number of studies have shown that human motion exhibits reproducible patterns of coordinated muscle activity and that these patterns may be described by an underlying organization of co-active muscles or “muscle synergies” (Ivanenko et al., 2004; Ting and Macpherson, 2005; Torres-Oviedo et al., 2006; Neptune et al., 2009; Chvatal et al., 2011; Moghadam et al., 2013). The key hypothesis is that the CNS controls muscle activations using a set of basic control signals (i.e. synergies) and each synergy defines a group of muscles that co-activate to work as a single functional unit and achieve a given outcome.

Although many studies have attempted to identify muscle synergies, a common assumption has been that each muscle belonged to only one synergy, but these synergies failed to account for the high degree of complexity in human movement (Lee, 1984; Buchanan et al., 1986). Therefore, a new assumption was proposed: “each muscle can be simultaneously activated by multiple synergies, so that no two muscle activation patterns are the same, yet significant partial correlations may exist across many muscles" (Tresch et al., 1999). This lead to Ting and Macphersen's (2005) study that determined four synergies could account for 95% of the muscular responses during direction dependent perturbation tasks. Interestingly, each synergy was recruited for a specific perturbation direction (i.e. anterior, posterior, medial, and lateral). Based on their findings, Ting and Macpherson (2005) suggested that muscle synergies are recruited as a function of the desired biomechanical outcome.

Recent works have tried to interpret the inter-relationship between muscle synergies and biomechanical data such as center of mass deviation (Chvatal et al., 2011), lower limb kinematics (Ivanenko et al., 2004; Neptune et al., 2009), mechanical constraints during force control tasks (Hug et al., 2011), and concentric/eccentric phases of exercise (Kristiansen et al., 2013). The consideration of biomechanical variables while extracting muscle synergies leads to functionally relevant results. However, no study has quantified lower limb muscle synergies in humans as it relates to knee joint loads. Therefore, **Objective 1-B** of this thesis was to *determine whether knee muscle activity elicited during our force control task can be characterized by muscle synergies and related to knee joint moments important for stabilising the knee.* Our quasi static, standing GRF matching task was used to elicit various combinations of flexion-extension, adduction-abduction, and internal-external rotational knee joint moments. If muscular responses to multidirectional loading can be explained by the activation of a limited set of muscular synergies then the synergies associated with injury inducing loads can be focused on for rehabilitative or preventative interventions.

Muscle synergy analysis was extended to functional tasks to see if observable differences from the quasi-static task are reflected in dynamic motion. Studies of dynamic muscle coordination in the lower limb have been limited to postural balance tasks and gait (Torres-Oviedo et al., 2006; Torres-Oviedo and Ting, 2010; Chvatal et al., 2011; Chvatal and Ting, 2012). Although functionally important, such tasks have relatively low magnitudes of external force acting on the knee (Glitsch and Baumann, 1997). More insight into muscle synergies and how they relate to joint stability may be gained in more demanding tasks. The forward lunge and two-legged squat represent movements that are primarily controlled by the quadriceps, elicit relatively greater knee joint loads, and challenges knee joint stability, while having functional and clinical relevance (MacLean et al., 1999; Alkjaer et al., 2011). Furthermore, muscle synergies are suggested to represent basic neural mechanisms that are common across different dynamic conditions (Ivanenko et al., 2004; Hug et al., 2010; Torres-Oviedo and Ting, 2010; Chvatal et al., 2011; Chvatal and Ting, 2012). **Objective 1-C** of this thesis was *to extract muscle synergies used during dynamic squatting and lunging tasks and determine if extracted synergies share characteristics or are task specific.*

2.1.5 Hip contribution to knee loads

Studies investigating the muscular contribution to knee joint loads typically focus on muscles that cross the knee joint. Although several studies have associated a reduction in hip joint muscle function with knee pathologies such as patellofemoral pain syndrome (Ireland et al., 2003), ACL deficiency (Thomas et al., 2013) and OA (Hinman et al., 2010), only a few studies have investigated how hip muscles actually contribute to the knee joint forces. Using a model of experimental muscle pain, Henriksen et al. (2009a) induced a voluntary activation deficit up to 40% in the gluteus medius. As a result, hip abduction and knee adduction moments were reduced by 6.4% and 4.2%, respectively. Along the same lines, reduced hip muscle function induced by fatigue is associated with increased knee valgus angles and knee adduction moments during weight acceptance of jumps, cuts, or running tasks (Jacobs et al., 2007; Geiser et al., 2010). It is theorised that a reduction in hip function de-stabilises the femur, causing an increase in frontal plane lower extremity motion and potential increases the risk of a knee injury (Powers, 2010).

Contrasting reduced muscle function, interventions focusing on strengthening hip muscles have shown to reduce medial compartment knee loads and improve OA symptoms and/or varus alignment (Bennell et al., 2010). In addition, Sritharan et al. (2012) computed individual muscle contributions to frontal plane moments and joint reaction forces using a least squares approach and demonstrated that knee spanning muscles compress the whole joint (reflecting a stabilisation role), while hip abductors were the greatest contributors to knee adduction moments, thus compressing the medial compartment and unloading the lateral. Since hip muscles are large determinants of femoral orientation and knee joint loads, this thesis will not limit evaluation to muscles that cross the knee joint. Results will help in providing a more complete picture of how muscles of the lower limb can indirectly contribute to knee joint loads and stability.

2.2 Reduced Stability and ACL injury

The ACL is commonly known to resist anterior tibial translation (Kennedy et al., 1974). Knee extensor moments have been shown to cause anterior tibial translation and increase the strain placed on the ACL in vitro (Kennedy et al., 1974; Yoo et al., 2005). This is most prominent as the knee becomes more extended (Kennedy et al., 1974; Yoo et al., 2005). As such, hyperextension or shallow flexion angles are the most common characteristic observed in injury reports (Shimokochi and Shultz, 2008) and increased knee extensor moments have been considered a contributor to the ACL injury mechanism (Fleming et al., 2001b; DeMorat et al., 2004). However, in non-contact episodes, knee extension moments alone do not cause injury (Besier et al., 2001; McLean et al., 2004). Most ACL injuries occur during a weight-bearing, noncontact episode, typically during deceleration, lateral pivoting, or landing tasks (Boden et al., 2000; Shimokochi and Shultz, 2008).

The mechanism of ACL injury is multifactorial where knee extension combined with knee valgus and/or knee rotation moments will increase ACL strain to injury inducing levels (Markolf et al., 1995; Fleming et al., 2001a; Hame et al., 2002; Hewett et al., 2005; Shimokochi and Shultz, 2008). In many instances these loads can be opposed by efficiently coordinated kinematics and muscular forces; however, a small discrepancy in the tension generated by muscles can act to misalign the segments or ineffectively oppose external forces and create instability. As such, variations in muscle activation are thought to be a major contributor to the knee injury mechanism (Hewett, 2000; Hewett et al., 2005; Shimokochi and Shultz, 2008).

2.2.1 Neuromuscular Contributions to ACL injury

The most common symptom among populations with knee pathologies is reduced quadriceps strength (defined as the ability to produce a maximal voluntary knee extension torque) compared to the contralateral limb and controls (Powers et al., 1997; Slemenda et al., 1997; Rudolph et al., 2001; Chmielewski et al., 2004; Palmieri-Smith et al., 2008) and reduced strength, or weakness, of this muscle group is related to poor functional scores (Rudolph et al., 2001; Reinke et al., 2011). The effect of quadriceps weakness as it relates to the ACL injury mechanism is not well understood. Several studies have suggested that increased quadriceps force increases ACL strain in vitro (DeMorat et al., 2004) and in vivo (Fleming et al., 2001b). However, Hashemi et al., (2007) observed that during a simulated landing (with externally applied vertical GRF of 1120N, or about 2x body mass for a 60kg person) low quadriceps force levels (165N) were conducive to ACL rupture. Interestingly, greater quadriceps tension (650N) significantly reduced the ACL strain under identical loading conditions. Similarly, Aune et al. (1997) showed that increased quadriceps muscle force increases shear stiffness and reduced ACL strain. Furthermore, females have a greater incidence of ACL injury and reduced knee extensor strength (Hewett, 2000). While current clinical belief may state otherwise, a case can certainly be made that rather than the cause of injury, the quadriceps muscles may have a protecting effect on the ACL due to the compressive forces it exerts.

In addition to weak quadriceps, perhaps inappropriately timed hamstring muscle co-activation is a contributing cause to ACL injury. During weight-bearing, hamstring muscles resist anterior translation, tibial rotation, and subsequent loads on the ACL (MacWilliams et al., 1999). Delayed hamstring activation in ACL deficient participants compared to control has also reported in several studies (Wojtys and Huston, 1994; Steele and Brown, 1999) (Hewett 2005, Rudolph 2001) In support, Rudolph et al. (2001) showed that both ACL deficient non-copers (individuals who cannot stabilize their knees during moderate to high demand activities) and copers had high levels of quadriceps muscle activity. However, non-copers demonstrated a significantly delayed peak hamstring activity during weight acceptance while copers demonstrated similar activation timing to the healthy control group. Insufficient hamstring-quadriceps muscle co-activation may also be a causal factor in the injury mechanism, as demonstrated in female athletes, a population who is at an increased risk of ACL injury (Hewett et al., 2005), compared to their male counterparts (DeMont and Lephart, 2004; Sigward and Powers, 2006; Palmieri-Smith et al., 2009).

Also a knee flexor, the gastrocnemius plays a large role in knee joint loads. Due to its geometrical orientation, O'Conner (1993) proposed that gastrocnemius causes a relative anterior tibial translation throughout the entire flexion-extension range of motion. In fact, isolated gastrocnemius loads applied in vivo (Fleming et al., 2001b) causes respective increases in tibial translation and ACL strain. However, this relationship is contradicted in studies simulating weight-bearing tasks with physiological loading magnitudes (Durselen et al., 1995; Morgan et al., 2014). It is even suggested that elevated gastrocnemius activity is synergistic with the quadriceps to help compress the knee and lower ACL forces (Morgan et al., 2014). Despite contradictory evidence, reported differences in gastrocnemius activity exist in populations with knee instability (Rudolph et al., 2001; Benoit et al., 2003) and this changing activation will alter knee loads. Taken together, a consensus about mechanisms leading to joint injury is lacking. Since all muscles crossing the knee joint have been shown to affect ACL loads, it seems that a reasonable first step would be to investigate individual muscle contributions to loads acting on the knee and joint stability as this may shed light onto this matter.

2.2.2 Adaptations post-ACL injury

Rupture of the ACL causes loss of mechanical knee joint stability, sensory feedback and a voluntary inhibition of the quadriceps musculature, all of which dramatically affects the function of the knee (Rudolph et al., 2000; Urbach et al., 2001; Krogsgaard et al., 2011). Participants with ACL deficiency commonly display reductions in knee joint range of motion and knee extensor moments (Barrance et al., 2006; Alkjaer et al., 2011), earlier recruitment, and increased activation magnitudes of the antagonistic hamstrings and gastrocnemius during functional activities (Sinkjaer and Arendt-Nielsen, 1991; Kvist and Gillquist, 2001; Rudolph et al., 2001; Alkjær et al., 2002, 2012; Chmielewski et al., 2005). Increased co-activation is believed to be a neuromuscular compensation strategy to increase compressive loads and minimise the excessive intra-articular translations/rotations caused by an ACL rupture (MacWilliams et al., 1999; Yoo et al., 2005; Li et al., 2007), while the reduced knee flexion angles and moments is an adaptive "stiffening response" such that the external loads are minimised and joint compression is increased (Hortobágyi and Devita, 2000).

Even though in vitro evidence indicates co-activation is an effective stabilisation strategy (MacWilliams et al., 1999), there exists conflicting evidence whether such neuromuscular adaptations after injury help to protect the knee or are a causal effect for reduced knee joint function. (Berchuck et al., 1990; Sinkjaer and Arendt-Nielsen, 1991; Aune et al., 1997; Kvist and Gillquist, 2001; Rudolph et al., 2001; Williams et al., 2003; Chmielewski et al., 2005; Alkjær et al., 2012; Shanbehzadeh et al., 2015). For example, co-activation can introduce adverse loads if unbalanced (Hubley-Kozey et al., 2009; Palmieri-Smith et al., 2009). A new approach for understanding the relationship between neuromuscular control and knee joint stability in needed.

Therefore, **Objective 2-A** of this thesis was *to identify muscle activation patterns that either increase or reduce joint loads related to knee instability*. This was achieved by evaluating how differences in internal net joint moments between an ACL deficient and healthy control populations were interrelated with differences in individual muscle activations patterns. Muscle activation patterns were measured with surface electromyography (EMG) signals and described as temporal changes in amplitude. Internal net joint moments were used as a surrogate to strategies of modulating knee stability because in order to effectively oppose a load externally applied to body from the environment, the activity of all muscles that cross the knee must be integrated so that when summated, the muscular force products create a net joint moment-of-force that is opposite in direction to the external load. Determining how activation strategies differ across ACL deficient participants will improve our understanding of injury and knee joint stability and permit informed judgements to be made for rehabilitative interventions.

2.3 Quadriceps Muscle Weakness

ACL injured individuals commonly exhibit reduced knee extensor strength and voluntary quadriceps activation deficits up to 25% (Urbach et al., 2001; Chmielewski et al., 2004; Palmieri-Smith et al., 2008). Although reconstruction and aggressive rehabilitation can be successful, the restitution of muscle strength is rarely achieved and deficits can persist for years following injury and/or reconstruction (Palmieri-Smith et al., 2008), chronically affecting physical activity levels and knee function (Kostogiannis et al., 2007). The resulting quadriceps weakness post-ACL injury results in part from the inability to voluntarily activate the muscle group. This muscle inhibition, known as arthrogenic muscle inhibition (AMI), is considered to be a natural, centrally mediated neural response designed to protect the injured knee by diminishing the motor drive to muscles surrounding the injured joint, likely helping to prevent painful and potentially detrimental movements (Hopkins and Ingersoll, 2000). The reduction in extensor muscle force appears to be exclusively associated with the loss of the ACL, as sham surgeries in animal models do not result in decreased muscle force or EMG signals (Suter et al., 1998).

Using a forward dynamics framework, Goldberg and Neptune (2007) simulated quadriceps muscle weakness by decreasing the maximum isometric strength parameter to 50% in a human walking model. In order to maintain a normal walking pattern plantarflexor musculotendon work was increased as compensation. This coincides with works reporting increased gastrocnemius activation in ACL deficient participants (Rudolph et al., 2001; Benoit et al., 2003), and suggests the gastrocnemius to be synergistic to quadriceps muscle function and increased activation is a compensation strategy for quadriceps muscle weakness (Morgan et al., 2014).

Simulated quadriceps muscle weakness also resulted in increased activation of the weakened quadriceps muscles (Goldberg and Neptune, 2007; van der Krogt et al., 2012). This upregulation of the quadriceps muscles also is commonly observed in ACL deficient populations in various exercise conditions, despite deficits in knee extensor strength (Palmieri-Smith et al., 2008) (Aune et al., 1997; Rudolph et al., 2001; Williams et al., 2003; Reed-Jones and Vallis, 2008). In lieu of a musculoskeletal model or a method to quantify voluntary activation deficits, studies interpret this upregulation of a weakened muscle group's activation level as a compensatory strategy to increase motor drive and maintain a required force level (Rudolph et al., 2001; Reed-Jones and Vallis, 2008). However, van der Krogt et al. (2012) and Goldberg and Neptune (2007) highlighted that the increased activation of weakened quadriceps muscles still produced insufficient force and generated unbalanced joint moments. Therefore, the relationship between muscle activation (described as EMG amplitude) and force generation in injured and healthy populations may not be the same. To help interpret our findings from Objective 2-A, a secondary objective was to quantify knee extensor strength deficits in an ACL deficient population using measures of EMG (normalized to maximum voluntary isometric activation values) and maximum torque levels (Appendix D).

Interestingly, in a select number of cases, individuals with ACL deficient knees are able to “cope” and maintain pre-injury activity levels with no report of “giving-way” episodes. The most distinguishable difference between ACL deficient copers and non-copers is changes in quadriceps muscle strength, muscle morphology and voluntary muscle activation (Rudolph et al., 2001; Alkjær et al., 2012; MacLeod et al., 2013b). This discrepancy among copers and non-copers in addition to the evidence presented above causes one to question: *does instability result from compromising the mechanical integrity of the joint or from the neuromuscular deficits after injury and the corresponding inability to adapt?*

2.3.1 Experimental models of muscle weakness

Experimental models of injury have been used to evaluate the acute effect of injury on neuromuscular control. Palmieri-Smith et al. (2007) simulated an intra-articular derangement with experimental joint effusion. Here, both low and high effusion conditions reduced vastii activation up to 29.3% and 51.8%, respectively. These deficits can predict reductions in knee extensor muscle strength (i.e. maximal knee extensor torque generating ability) and knee extensor moments during gait (Pietrosimone et al., 2014). Similarly, Hodges et al. (2009) induced joint pain with hypertonic saline injections to the patellar fat pat which delayed onsets and decreased amplitudes of the vastii during stair ascent and descent tasks.

Experimental muscle pain has also been used to decrease voluntary quadriceps muscle activation and impair joint function comparable to clinical observations of patients with knee pathologies such as OA, ACL-D, and patellofemoral pain (Graven-Nielsen et al., 2002; Henriksen et al., 2007, 2010; Palmieri-Smith et al., 2008). Muscle pain is achieved with intra-muscular hypertonic saline injections, causing the perception of pain by stimulating nociceptors and centrally mediating a reduction in muscle activation (Graven-Nielsen et al., 2002; Arendt-Nielsen et al., 2008). Due to its less invasive method, experimental muscle pain is a preferred model to investigate muscular adaptations to injury and weakness.

2.4.1 Compensation Strategies

Experimental muscle pain to the quadriceps consistently inhibits voluntary activation of the target muscle, thus decreasing activation levels and reducing knee extensor strength comparable to levels observed clinically (Graven-Nielsen et al., 2002; Palmieri-Smith et al., 2008; Henriksen et al., 2010). However, reported differences in non-painful muscle activation and movement strategies are highly variable. As Bank et al. (2013) highlight, some studies report upregulation, down-regulation, no change, or inconsistent changes in activation of non-painful muscles. Consequently, such studies do not align with simple theories of motor adaptation to pain (Roland, 1986; Lund et al., 1991). Rather than uniform inhibition or excitation of non-painful muscles, Hodges and Tucker (2011) proposed a new theory of motor adaptation to pain that involves the redistribution of activity within and between muscles. A more comprehensive analysis of the redistribution of muscle activity with pain may therefore be warranted and could be achieved through synergy analysis

Since the quadriceps is essential to maintaining knee joint stability, other muscles of the lower limb must adapt accordingly should its activation be inhibited. Unfortunately, the acute effects of muscle weakness on knee joint dynamics is not well studied due to other confounding factors such as joint pain, swelling, patient accessibility etc. A model of quadriceps muscle pain can act as a surrogate to AMI and provide insight into acute neuromuscular adaptions to injury, such as the AMI, and how this relates to knee joint stability. Therefore, **Objective 2-B** of this thesis was *to quantify changes in muscle activation and its effect on knee joint dynamics during experimental quadriceps (vastus medialis) muscle pain.* This was achieved by evaluating which internal net joint moments were interrelated with changes in individual muscle activations in those with and without muscle pain. The results allow us to evaluate the effects of pain and reduced function of a single muscle on stabilization strategies under our well- controlled conditions. Since the effect of pain on individual muscle activation may not be obvious, the change in muscle synergies with and without pain was also evaluated. Our findings will help identify key muscles important for regulating knee joint stability and contribute to evidence-based rehabilitation programs such that subsequent injury or joint degeneration is minimized.

CHAPTER 3: General Methodology

3.1 Study Design

There were two data collections in this thesis. The first data collection (**DC1**) was a cross-sectional experimental study designed to evaluate between group differences in muscle activation of healthy control (CON) and ACL-deficient (ACLD) participants.

The second data collection (**DC2**) evaluated the effect of experimental muscle pain on muscle activation strategies in healthy young adults. **DC2** was a crossover study with a placebo-controlled design. Each subject was tested on 2 days separated by 1 week. During a test day, intra-muscular saline injections to the vastus medialis were given. The saline was either a hypertonic (pain inducing) or isotonic (sham) solution. Only one type of solution was injected on a given test day whose order will be randomized (Refer to Figure 4, page 41).

3.2 Participants

Sample size

Three groups of participants were recruited for this thesis. The first two groups of participants took part in **DC1**: investigating the differences in neuromuscular activation between ACLD and CON populations. The third set of participants took part in **DC2**, where a test-retest approach investigated the effect of experimentally induced quadriceps muscle pain on muscle activation strategies in a young healthy adult population. A power analysis (G*power v 3.1, on our primary outcome data (muscle activation characteristics defined on page 44) was conducted after 10 individuals from each group were collected. A minimum sample size of 18 participants in each group was determined to be necessary to effectively test our research hypotheses at the alpha = 0.05 and a power of 80%. To effectively match CON and ACL groups for **DC1**, 38 and 34 participants were recruited and completed the study. For this thesis, 24 participants for each CON and ACL group were included in the analysis. To account for a 20% attrition rate for a test-retest study design 22 participants were recruited for **DC2**. All 22 completed **DC2** and were included in the analyses.

Recruitment/Eligibility

CON participants for **DC1** and **DC2** were recruited from the University of Copenhagen and the surrounding community. Potential CON participants were included if they were young (18-35 years old), physically active (defined by participation in exercise or sport at least twice a week), and reported no history of (1) traumatic injury of the lower limb (i.e. meniscal tear, ligament rupture), (2) lower limb sprain, muscle tendon injury, or fracture within six months, (3) lower extremity motor nerve lesion, presence of knee joint effusion, muscle atrophy or any other observed physical impairment that may affect the results of this study.

ACLD participants for **DC1** were recruited from the Bispebjerg University Hospital (Copenhagen, Denmark) by Dr. Michael Krogsgaard. Inclusion criteria for ACLD were ACL rupture confirmed by magnetic resonance imaging (MRI) or arthroscopy and intention to be surgically reconstructed. Participation was not limited to injured leg dominance. However, previous work demonstrate an effect of leg dominance on muscle activation (MacLeod et al., 2013a), therefore only participants who injured their dominant leg were included in this thesis.

3.3 Equipment and Data Collection

Kinematics and Ground Reaction Forces

Retro-reflective markers were placed on various anatomical landmarks on the subject's body according to the HMBL Cluster Marker set (Mantovani and Lamontagne, 2016) (Appendix A). Trajectories were recorded using a ten-camera motion analysis system (6 MXF-40s and 4 T40-series cameras, Oxford Metrics, Oxford, UK) sampling at 100 Hz with supporting Nexus software (version 1.8, Oxford Metrics, Oxford, UK). GRFs recorded from two force platforms (AMTI-OR6, AMTI, Watertown, MA) were also collected in Nexus at 1000Hz and amplified with an internal gain of 1000 (MSA-6, AMTI, Watertown, MA). The force platform for the leg of interest had a water ski boot (HO Skis, Snoqualmie WA, USA) fixed to its surface in order to maintain a solid base for force transfer from the foot to the force platform but still allowing mobility of the ankle joint.

Muscle Activations

Activations of 10 muscles of the lower leg were recorded using surface electromyography (EMG) signals. Electrodes (Blue-Sensor N-00-S, AMBU, Ballerup, Denmark) were placed in a bipolar configuration, with a 2cm inter-electrode distance, over the bellies of the rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), biceps femoris (BF), semitendinosis (ST), tensor fascia latae (TFL), lateral gastrocnemius (LG), medial gastrocnemius (MG), gluteus medius (GM), and adductor muscle group (ADD) following SENIAM (Hermens et al., 2000) guidelines and recommendations from DeLuca (1997).

EMG was detected with a 10 channel wireless device (MQAir, MARQ Medical, Farum, Denmark). All EMG signals with be sampled at 1000Hz with a 20-500Hz bandwidth and a 6dB/octave filter slope before A/D conversion. EMG signal were recorded with the MQ Fireworks supporting software. Signal quality and cross-talk was visual inspected during isolated exercise with manual resistance provided by the researcher. Hip flexion and seated knee extension evaluated respective RF and vastii, knee flexion evaluated BF and ST, seated plantarflexion evaluated MG and LG signals, hip abduction evaluated GM and TFL, and hip adductor evaluated ADD signal quality.

3.4 General Protocol

All experiments will be carried out at Dr. Alkjær and Dr. Simonsen's gait laboratory at the Department of Neuroscience and Pharmacology, University of Copenhagen, Denmark.

Data Collection 1 (DC1)

Informed consent, questionnaires, practice

All participants read and signed an informed consent form approved by local ethics committee for the Capital Region of Denmark (De Videnskabsetiske Komiteer for Region Hovedstaden, H-3-2013-126) and the University of Ottawa Research Ethics Board (H06-14-27). Participants also completed a series of subjective functional questionnaires including the Knee injury and Osteoarthritis Outcome Score (KOOS) (Roos and Lohmander, 2003), International Knee Documentation Committee (IKDC) subjective knee form (Irrgang et al., 2001), the Knee Numeric-Entity Evaluation Score (Comins et al., 2013), Lysholm scale (Lysholm and Gillquist, 1982), and Tegner physical activity score (Tegner and Lysholm, 1985).

Tasks involving force control at the foot-ground interface has been shown to have learning effect (van Deursen et al., 1998). Following the questionnaires, participants were introduced to the protocol with a demonstration by the researcher and completed 12 force-matching trails according to the description below before participant set-up. Subject anthropometrics (weight, height, thigh and tibial lengths, thing and tibial circumferences, knee and ankle widths) were then measured, and EMG electrodes and retro-reflective markers were placed.

Maximal voluntary contraction trials

Manual maximum voluntary isometric contractions (MVICs) were performed to normalize our experimental EMG values. Hip flexion, hip extension, hip abduction, and hip adduction exercises were performed while standing in neutral position (no hip or knee flexion) and effort was exerted against a strap placed above the ankle. For plantarflexion, participants stood and raised to their toes while resisting upward motion using wall mounted bars. Knee extension and flexion exercise was performed with manual resistance from the researcher while participants sat with hip flexed to 90° and knee to 30°. Participants were instructed to scale their perceived force from a zero level to their maximal and hold it for approximately three seconds. Ten seconds was allotted for each trial while researchers provided verbal encouragement.

The maximal isometric knee extension and flexion torque was also recorded using an isokinetic dynamometer (KinCom, Chattex, Chattanooga, TN). The same conditions as the manual MVIC trials were provided in addition to onscreen visual feedback of force amplitude. An external trigger synchronized recorded joint torque and EMG signals. Due to technical issues, only a subset of 14 ACLD and 17 CON participants completed these tasks and had their torque data recorded (reported in Appendix D).

The force matching protocol (described below) required participants to stand with their leg of interest in a boot attached to a force platform with approximate body positions of 30° hip flexion, 30° knee flexion, and 10° ankle plantar flexion angles (Figure 2). In order to normalise the effort required to successfully complete the force matching tasks, both maximal effort and relaxed standing GRFs were recorded while the participant maintained equal body weight on each leg. Maximal effort standing GRFs were recorded in the anterior, posterior, medial and lateral anatomical loading directions corresponding to the respective -Fy, +Fy, -Fx, +Fx force plate channels. Similar to MVIC trails, participants were instructed to scale their perceived force from a zero level to their maximal and hold it for approximately three seconds. Ten seconds was allotted for each trial while researchers provided verbal encouragement. Relaxed standing forces recorded in the Fx and Fy channels were subtracted from subsequent GRF readings, acting as a “zeroing” method.

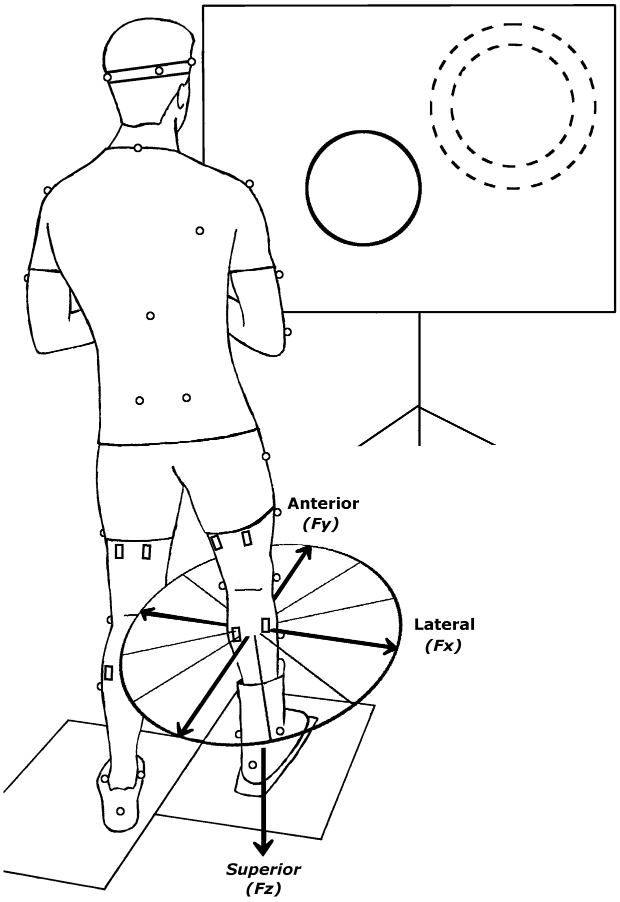

Figure 2: Laboratory setup: the subject stands with their dominant foot in a boot fixed to a force platform and the opposite foot located posteriorly and adjacently. A projector displays biofeedback of the GRF generated by the subject as a cursor (solid circle). Participants must position the cursor between the target’s two rings (dashed lines) by modulating the GRF in the horizontal plane (*Fx* and *Fy*). Cursor diameter increases or decreases with more or less body weight loading (*Fz*), respectively (Adapted from Flaxman et al. (2012)).

Target Match

While maintaining the same bodily configuration, participants modulated the GRFs of their fixed leg in order to move a projected cursor. The cursor moved with three degrees-of-freedom: 1) anterior/posterior loads (force along the ±y axis) moved the cursor upward/downward, 2) medial/lateral loads (force along the ±x axis) moved the cursor to the left/right, and 3) inferior/superior loads (force along the ±z axis) made the cursor smaller/larger (controlling the amount of applied body weight). Participants were required to match the cursor over a projected target, which was representative of a direction dependent ground reaction force magnitude (Figure 2).

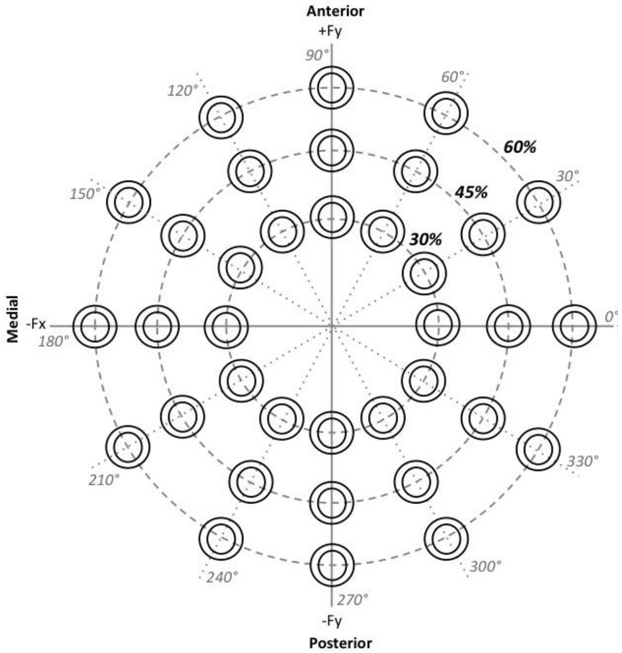

Figure 3: Force target locations representative of normalized horizontal GRF loading directions at three different force levels. Location along the radius represents normalised effort (30%, 45%, and 60%). Target directions are separated by 30° about a circular trajectory corresponding to the horizontal loading plane.

The successful match of the cursor over the target for 0.5s triggered the simultaneous recording of retro-reflective marker trajectories, GRFs, and EMG. Targets randomly appeared in 12 different directions evenly spaced by 30° about a circular trajectory (representative of various horizontal loading directions: 0°= lateral, 90°= anterior; 180°= medial; 270°= posterior).

Each direction had 3 different force targets appear along its radii corresponding to 3 different force levels (30%, 45% and 60% max effort) (Figure 3). This set of 36 targets was matched twice for 72 targets total.

The normalized force required to reach each target (F_{target}) was computed based on Equation 1 presented in (Krishnan et al., 2008). This equation has been adapted to include the removal of the relaxed horizontal GRFs:

$$\boldsymbol{F_{target}} = \sqrt{\left[\left(\mathbf{cos}\,\boldsymbol{\theta} \times (\boldsymbol{F_{x_p}} - \boldsymbol{F_{x_r}}) \times \ \%\boldsymbol{maxF}\right)^{\mathbf{2}} + \left(\mathbf{cos\,90} - \boldsymbol{\theta} \times (\boldsymbol{F_{y_p}} - \boldsymbol{F_{y_r}}) \times \ \%\boldsymbol{maxF}\right)^{\mathbf{2}}\right]}$$

where θ is the angle between the target and the + x axis, F_{x_p} and F_{x_r} are the respective peak and relaxed GRF produced along the ± X axis (medial/lateral), F_{y_p} and F_{y_r} are the respective peak and relaxed GRF produced along the ±Y axis (anterior/posterior) and $\%maxF$ is percent effort level.

After completion of the force target matching protocol, participants completed a set of dynamic functional tasks (two-legged squats, one-legged squats, lunges, forward hops, side-cutting maneuvers, and counter movement jumps); however, the data collected from these trials were not analysed in this thesis.

Data Collection 2 (DC2)

The testing procedure for **DC 2** similarly followed the methods outlined above for **DC 1**: However the number of force targets to match differed and the dynamic tasks performed were limited to squatting and lunging exercise.

During a test day, 4 sets of force targets and dynamic tasks were completed. Although the effects of the hypertonic saline on muscle function are present up to 25 minutes post injection, the perceived presence of pain only lasts 5 minutes (Henriksen et al., 2007, 2009a). Set one acted as a baseline measure and intramuscular saline injections were given immediately before sets 2 and 3. Rest times between sets 1-3 were 5 minutes. After set 3 a 20-minute break was held to ensure that any perception of pain was gone before completing set 4 (Figure 4). The perception of pain was monitored after the first injection using a verbal scale of 0-10 where 0 indicated no pain and 10 indicated worst imaginable pain. Sets 2-4 consisted of 12 targets at 60% effort level, 10 squats, and 10 lunges. Set 1 involved two repetitions of 12 targets, 10 squats and 10 lunges.

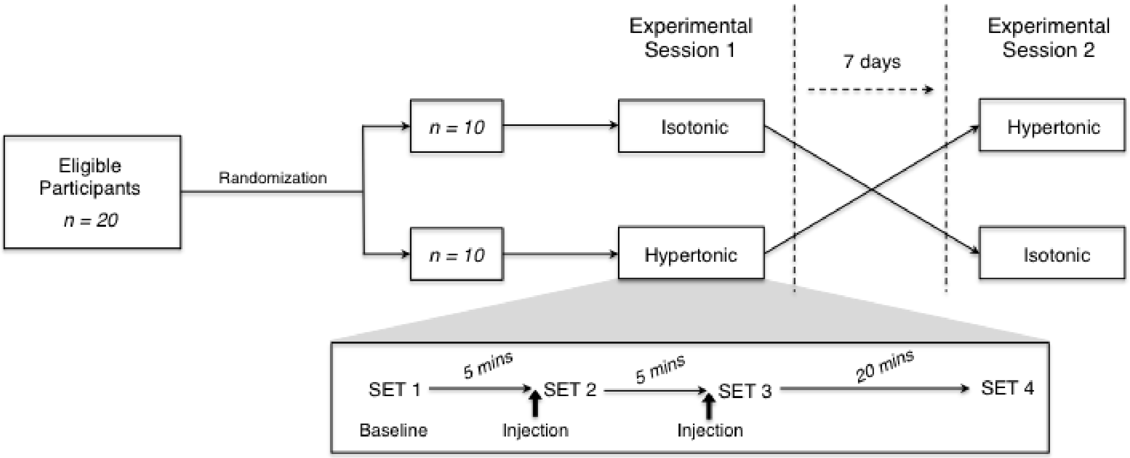

Figure 4: Crossover study design for experimental muscle pain injections (DC2). Eligible participants will be randomized into two groups – one group will receive only the isotonic injections in the first session and the other group will receive only the hypertonic injections. Participants will return one week later to repeat the experimental protocol but receive the opposite injection.

A metronome was used to help limit each squatting and lunging cycle to 4 seconds: 1s for descent, 1s for transition, 1s for ascent, and 1sfor a rest. Squatting tasks started with participants standing straight, feet hip width apart, and arms stretched out in front, parallel to the floor. Participants were instructed to squat until thighs are parallel to the floor while keeping weight on the heels and chest facing forward (perpendicular to the floor). Lunging tasks required participants to stand hip width apart, hands on the back of the head, and lunge forward with their test leg until their front knee is bent to 90°. Participants ascended by pushing off with their test leg until they reached starting position, all while keeping their chest up and facing forward. Participants returned one week later to repeat the same protocol except the opposite injection was used.

Intramuscular injection

Sterile hypertonic saline (5.8%) induced muscle pain while isotonic saline (0.9%) acted as a control. Intramuscular bolus injections of 1ml were placed 5cm proximal and 5cm medial to the medial corner of the patellar base. Manual injections were accomplished with a 5-ml plastic syringe with a disposable needle (27 gauge, 40 mm) at a depth of 3.5 cm.

3.5 Data Processing

Using Vicon Nexus software, the raw marker trajectories were reconstructed, labelled, gap filled and filtered with a 2nd order dual pass 15Hz low pass filter. Based on the recommendations of Bisseling and Hof (2006) and Kristianslund et al. (2012), raw GRF data was also filtered in the same manner. Conditioned marker trajectories and GRF data were exported to OpenSim (v 3.2)(Delp, 2007) to compute local coordinate systems of the lower limb and subsequent joint angles and internal net joint moments using inverse kinematics and dynamics.

All EMG signals were high-pass filtered at 20Hz with a 2nd order dual-pass Butterworth filter, full wave rectified, and low-pass filtered at 10Hz a with 2nd order dual-pass Butterworth filter. The filter order and cut-off frequency options that best represented muscle activity onset and offset during side-cutting maneuvers without causing negative values was opted for based on visual inspection. Although side-cuts were not analysed in this thesis, we chose this maneuver to decide our filter options because it was the most demanding task and comparisons across tasks could be made in future works.

Experimental EMG was normalized to maximal amplitude recorded from maximum voluntary isometric contractions (EMG_{max}). EMG_{max} was computed as a 50ms mean about the maximum value in the conditioned EMG signal of the MVIC trials across all exercises.

Squatting data were time normalized to 100% where the first 1-50% of the cycle was normalized to the descent phase and 51-100% represented the ascent phase. Each squat cycle was cropped to 90% of mean knee ROM. Lunging data were time normalised to 100% time spent on the force plate plus 20% percent of this time before heel strike (HS). Linear interpolation was used for time normalization. Individual participant data were ensemble averaged across repetitions for each condition.

For target match data, normalized EMG, kinematic and kinetic data were time averaged over the 0.5s of successful force match, ensemble averaged across repetitions, and plotted in polar coordinates to visually display muscle activation patterns. To better represent the change in GRF effort on EMG and joint moments, relaxed EMG and joint moments recorded during the system's GRF "zeroing" step was also subtracted from corresponding trial data. In other words, the joint loads and EMG from the resting standing position were treated as a baseline and were not included in our graphs or analyses.

Objective 1-A of this thesis was sub-divided into two aims: (1) to quantify the relationship between individual muscle activation patterns and internal net joint moments and (2) to determine if these relationships change with increasing effort level. To satisfy aim 2 of

Objective 1-A, muscle activation patterns were quantitatively described in terms of: (1) pattern symmetry, (2) mean direction of muscle activity, (3) mean magnitude of muscle activity, and (4) specificity index. These four characteristics are computed in Study 1 (page 55) but are not described in full. As such, the equations are provided below. Data analysis and statistical procedures for all other objectives are provided in methods section of respective journal articles.

Quantitative description of target match muscle activation patterns

Our previous works describe muscle roles based on the relationship between the muscle’s activation pattern and reported moment arm orientation (MAO): a muscle with a symmetrical activation pattern (equal activation in all loading directions) was termed a *general joint stabiliser*; a muscle with an asymmetrical activation pattern concentrated about their theoretical MAO were deemed to be *moment actuators*; and specific joint stabilisers were muscles that displayed asymmetrical activation patterns opposite of their reported MAO. In order to classify muscle roles, target EMG were plotted in polar coordinates to visually represent muscle activation patterns (Dewald et al., 1995). To quantitatively describe these patterns, asymmetry about the polar plot origin was first tested (Curray, 1956):

$$\boldsymbol{p = e^{-L^2 n}10^{-4}}$$

where *p* if the probability of observing non-random asymmetry, *e* is the base of natural logarithm, *L* is the mean vector magnitude, and *n* is the number of observations. Asymmetry is observed if $p<0.05$, indicating preferential activation in a general loading direction. If asymmetrical, the “mean directions of muscle activity” (Φ) was computed by taking the arctan of the vectors’ summed Cartesian coordinates (x_i and y_i):

$$\boldsymbol{\Phi = tan^{-1}\left(\frac{\sum y_i}{\sum x_i}\right)}$$

The variance of muscle activation about the Φ was quantified as a “specificity index” (*SI*) described at the ratio of the muscles’ actual resultant vector to its absolute resultant vector:

$$\boldsymbol{SI = \frac{\left[\left(\frac{\sum x_i}{n}\right)^2 + \left(\frac{\sum y_i}{n}\right)^2\right]^{-1/2}}{\left[\left(\frac{\sum |x_i|}{n}\right)^2 + \left(\frac{\sum |y_i|}{n}\right)^2\right]^{-1/2}}}$$

Where *n*=12, representing the number of target locations. The SI ranges between 0 and 1, 0 indicating equal activity in all target directions (non-specific) whereas 1 indicates activity only at one target location (completely specific). Finally, a ‘‘mean magnitude of muscle activity’’ (X_{EMG}) was computed by averaging the normalised EMG at every target location:

$$\boldsymbol{X_{EMG} = \frac{\sum EMG_i}{n}}$$

CHAPTER 4: Journal Articles

Study 1: Predicting the Functional Roles of Knee Joint Muscles from Internal Joint Moments

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4.1.1 ABSTRACT

Introduction: Knee muscles are commonly labelled as flexors or extensors and aptly stabilize the knee against sagittal plane loads. However, how these muscles stabilize the knee against adduction-abduction and rotational loads remains unclear. Our study sought to: (1) classify muscle roles as they relate to joint stability by quantifying the relationship between individual muscle activation patterns and internal net joint moments in all three loading planes, and (2) determine if these roles change with increasing force levels. **Methods:** A standing isometric force matching protocol required participants to modulate ground reaction forces to elicit various combinations and magnitudes of sagittal, frontal and transverse internal joint moments. Surface electromyography measured activities of 10 lower limb muscles. Partial least squares regressions determined which internal moment(s) were significantly related to the activation of individual muscles. **Results:** Rectus femoris and tensor fascia latae were classified as moment actuators for knee extension and hip flexion. Hamstrings were classified as moment actuators for hip extension and knee flexion. Gastrocnemius and hamstring muscles were classified as specific joint stabilisers for knee rotation. Vastii were classified as general joint stabilisers because activation was independent of moment generation. Muscle roles did not change with increasing effort levels. **Conclusions:** Our findings indicate muscle activation is not dependent on anatomical orientation but perhaps on its role in maintaining knee joint stability in the frontal and transverse loading planes. This is useful for delineating the roles of bi-articular knee joint muscles and could have implications in robotics, musculoskeletal modeling, sports sciences and rehabilitation.

4.1.2 INTRODUCTION

Due to their anatomical orientation, the major muscles crossing the knee are traditionally classified as knee joint flexors (hamstring, gastrocnemius) and extensors (quadriceps) and their corresponding activation is typically classified as either agonistic or antagonistic to sagittal plane motion. However, functional loads are not limited to a single axis. Theoretically, each muscle crossing the knee has a unique orientation so that when activated it can generate a moment along a specific line of action and oppose the given external force causing that moment (Andriacchi et al., 1984; Buchanan and Lloyd, 1997). However, due to their small cross-sectional area, muscles with large frontal plane moment arms, such as the gracilis, sartorius, and tensor fascia latae, have low force generation capacity and minimal contribution to opposing frontal plane external loads (Brand et al., 1986; Buchanan and Lloyd, 1997). It remains unclear how the muscles crossing the knee effectively stabilize the joint when frontal and rotational loads are applied.

In vivo evaluations of neuromuscular function with respect to knee joint stability typically involve a dynamic assessment (e.g. cutting maneuver, perturbation etc.) such that results are functionally comparable to activities of sport and daily living. Yet the presence of biomechanical factors such as joint velocity, position, and direction of movement confounds the muscular contribution to force generation (Wilkie, 1950). To better elucidate this relationship, isometric exercises are commonly used. Previously, studies have limited such evaluations to a single axis (i.e. flexion-extension) and/or to a non-weight bearing condition (Buchanan and Lloyd, 1997; Van Deursen et al., 1998; Alkjær et al., 2012). Since non-contact knee joint injuries occur when the foot is in contact with the ground, and the individual bears weight, a study that simulates physiological loads in a controlled yet more functionally relevant manner is critical to gain a better understanding of the neuromuscular contribution to force generation and joint stability.

We have developed a task that requires participants to stand and modulate ground reaction forces (GRFs) while maintaining a static position (Flaxman et al., 2012; Smith et al., 2012). Our results indicate that when standing and bearing body weight, a muscle's activation is not dependent on moment arm orientation. Rather, we propose that a muscle's activation is also related to its role in maintaining knee joint stability. We have classified the roles of knee muscles as 1) a general joint stabilizer: a muscle that demonstrates activation independent of loading directions; 2) a moment actuator: a muscle that demonstrates preferential activation in a loading direction corresponding to its moment arm orientation; and 3) a specific joint stabilizer: a muscle that demonstrates preferential activation in a loading direction opposite of its moment arm orientation (MAO) (e.g. the biceps femoris activation pattern reported in (Flaxman et al., 2012) was specific about a medial loading direction which is opposite of its posterio-lateral MAO).

The purpose of this study was to further classify the roles of lower limb muscle as it relates to joint stability by quantifying the relationship between individual muscle activation patterns and internal net joint moments in all three loading planes (Aim 1). This is especially beneficial when trying to determine the function of bi-articular muscles spanning the hip and knee: which joint is the muscle more likely to act on? Based on our previous work (Flaxman et al., 2012) and that of van Ingen Schenau et al (1992), who suggested mono-articular muscles provide a general gross force while bi-articular muscles “fine tune” the distribution of the net joint moments across adjacent joints, it is hypothesized that 1) the internal net joint moments will be independent of changes in activation of the vastii muscles and 2) the activation of the bi-articular knee/hip muscles will be load dependent (significantly correlated by a general moment at each articulating joint), corresponding to their role as moment actuators.

Under isometric conditions, the relationship between muscle activation and force is frequently linear (Bigland-Ritchie, 1981). Yet again, this relationship has been established from non-weight bearing conditions and is limited to an analysis of a single axis (Buchanan and Lloyd, 1997; Van Deursen et al., 1998; Alkjær et al., 2012). When comparing different populations, varied muscle activation characteristics are often accompanied with varied moment magnitudes (Krishnan and Williams, 2009; Alkjær et al., 2012). As such, we also sought to determine if muscle activation patterns, and the roles of muscles as they relate to knee joint stability, will change with an increase in ground reaction force level (Aim 2). Based on works by Buchanan et al. (1986) and Levin et al. (2003), it is hypothesized that there will be a progressive increase in EMG amplitude with increasing force demand but a muscle's functional role will not change. With this, we can determine if observed between group differences indicate fundamental changes in neuromuscular control or simply a function of loading level.

4.1.3 METHODS

Participants and Equipment

Twenty-five healthy active adults [12 males (height=182.9±4.9cm; mass=81.2±10.1kg; age=29.2±6.7yrs) and 13 females (height=169.7.9±5.0cm; mass=63.1±9.0kg; age=25.6±7.3yrs)] with no previous reports of significant lower limb injury were recruited from the University of Copenhagen and the surrounding community. Experiments were carried out at the University of Copenhagen and all participants read and signed an informed consent form approved by the local ethics committee for the Capital Region of Denmark (De Videnskabsetiske Komiteer for Region Hovedstaden, H-3-2013-126) and the University of Ottawa Research Ethics Board (H06-14-27).

Retro-reflective markers were placed on various anatomical landmarks on the subject's body according to the HMBL Cluster Marker set (Mantovani and Lamontagne, 2016) (Figure 5A) (Appendix A). Trajectories were recorded using a ten-camera motion analysis system (6 MXF-40s and 4 T40-series cameras, Oxford Metrics, Oxford, UK) sampling at 100 Hz with supporting

Nexus software (version 1.8, Oxford Metrics, Oxford, UK). GRFs recorded from a force platform (AMTI-OR6, AMTI, Watertown, MA) were also collected in Nexus at 1000Hz and amplified with an internal gain of 1000.

Surface electrodes placed in a bipolar configuration collected electromyography signals (EMG) of rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), semitendinosus (ST), biceps femoris (BF), medial gastrocnemius (MG), lateral gastrocnemius (LG), tensor fascia latae (TFL), gluteus medius (GM), and adductor (ADD) muscle group of the dominant leg (defined as leg used to kick a soccer ball as far as possible). EMG was sampled at 1000Hz with a 20-500Hz bandwidth and a 6dB/octave filter slope recorded using a wireless EMG system (MQ air, Marq Medical, Farum Denmark).

Maximum voluntary isometric contractions (MVICs) were performed before the experimental protocol. Hip flexion, hip extension, hip abduction, and hip adduction exercises were performed while standing in neutral position (no hip or knee flexion) and effort was exerted against a strap placed above the ankle. For plantarflexion, participants stood and raised to their toes while resisting upward motion using wall mounted bars. Knee extension and flexion exercise was performed with manual resistance from the researcher while participants sat with hip flexed to 90° and knee to 30°. Visual feedback of force trajectories and verbal encouragement were provided. EMG_{max} for each muscle was computed as a 50ms mean about the maximum value in the conditioned EMG signal across all exercises.

Experimental Protocol

A force matching protocol assessed muscle activation patterns of participants while they modulated GRFs with their dominant limb (Flaxman et al. 2012; Smith et al., 2012). Participants maintained a staggered standing position during testing such that their feet were spaced hip width apart and their test leg had approximate joint angles of 30° hip flexion, 30° knee flexion, and 10° ankle plantar flexion (Figure 5A). A projected image of a cursor and a force target was placed in front of the subject, providing visual feedback of the direction and magnitude of the subject’s force applied to the force platform from the test leg. The cursor moved with three degrees-of-freedom: 1) anterior/posterior loads (force along the ±y axis) moved the cursor upward/downward, 2) medial/lateral loads (force along the ±x axis) moved the cursor left/right, and 3) inferior/superior loads (force along the ±z axis) decreased/increased the cursor’s size.

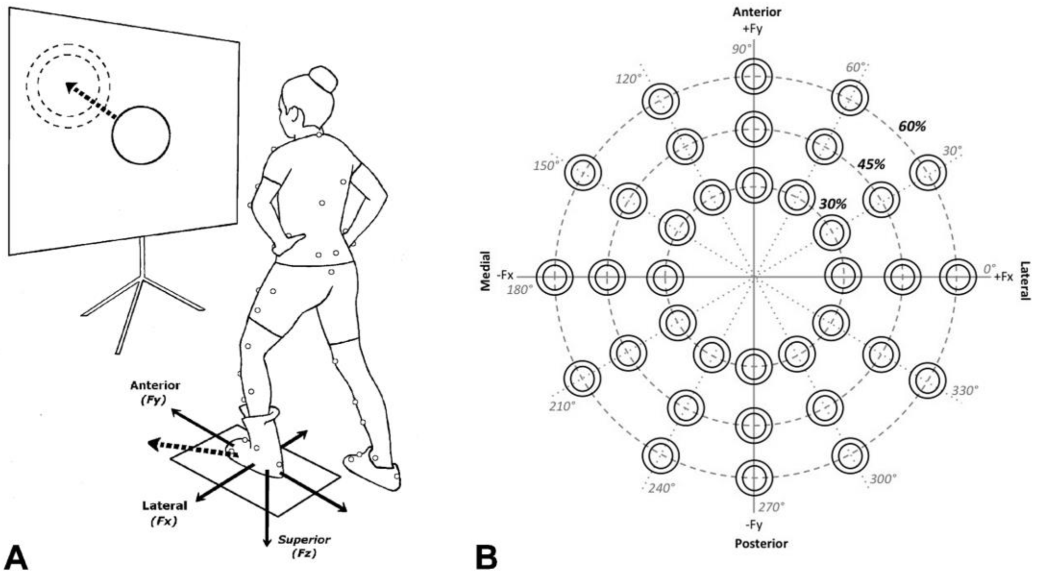

Figure 5: A) Subject stands with their dominant foot in a boot fixed to a force platform and the opposite foot located posteriorly and adjacently. A projector displays biofeedback of the applied forces as a cursor (solid circle). Participants must position the cursor between the target’s two rings (dashed circles) by modulating the force in the horizontal plane (***Fx*** and ***Fy***). Cursor diameter increases or decreases with more or less body weight loading (***Fz)***, respectively. Example for a force trajectory required to match cursor to target is depicted as a dashed arrow. **B)** Force target locations representative of normalized horizontal force loading directions at three different force levels. Location along the radius represents normalised effort (30%, 45%, and 60%). Target directions are separated by 30° about a circular trajectory corresponding to the horizontal loading plane.

Successful match of the cursor over the target for 0.5s triggered simultaneous recording of 3D marker trajectories, GRFs, and EMG. Twelve different target directions, evenly spaced by

30° about a circular trajectory (representative of various horizontal loading directions: 0°= lateral, 90°= anterior; 180°= medial; 270°= posterior) randomly appeared. Each direction had 3 different force magnitudes (30%, 45% and 60% max effort). This set of 36 targets was matched twice for 72 targets total (Figure 5B).

The effort required to successfully complete the force matching tasks was normalized to each participant's maximal standing force. The maximal standing force required participants to stand in the testing position, maintain equal body weight on each leg, and exert as much force on the force plate as possible with their test limb in the anterior, posterior, medial and lateral anatomical loading directions corresponding to the respective -Fy, +Fy, -Fx, +Fx force plate channels. While the subject maintained equal body weight on each leg, relaxed horizontal GRFs were also recorded and subsequently subtracted from experimental GRF readings, acting as a "zeroing" method. The normalized force required to reach each target (F_{target}) was computed based on Equation 1 presented in (Krishnan et al., 2008). This equation has been adapted to include the removal of the relaxed horizontal GRFs:

$$\boldsymbol{F_{target}} = \sqrt{\left[\left(\mathbf{cos}\,\boldsymbol{\theta} \times (\boldsymbol{F_{x_p}} - \boldsymbol{F_{x_r}}) \times \ \%\boldsymbol{maxF}\right)^{2} + \left(\mathbf{cos}\,\mathbf{90} - \boldsymbol{\theta} \times (\boldsymbol{F_{y_p}} - \boldsymbol{F_{y_r}}) \times \ \%\boldsymbol{maxF}\right)^{2}\right]}$$

where θ is the angle between the target and the + x axis, F_{x_p} and F_{x_r} are the respective peak and relaxed GRF produced along the ± X axis (medial/lateral), F_{y_p} and F_{y_r} are the respective peak and relaxed GRF produced along the ±Y axis (anterior/posterior) and $\%maxF$ is percent effort level.

Data Processing

Raw marker trajectories and GRFs were both filtered with a 2nd order 15 Hz dual-pass low-pass Butterworth filter and exported to OpenSim (v 3.2; Delp, 2007) to compute local coordinate systems of the lower limb and subsequent joint angles and internal net joint moments using inverse kinematics and dynamics. Any future mention of the term “moments” refers to internal net joint moments, unless otherwise specified.

All EMG signals were high-pass filtered at 25Hz with a 2nd order dual-pass Butterworth filter, full wave rectified, and low-pass filtered at 10Hz a with 2nd order dual-pass Butterworth filter. EMG was normalized to maximal amplitude recorded from maximum voluntary isometric contractions (EMG/EMG_{max}). EMG, kinematic and kinetic data were time averaged over the 0.5s of successful force match, ensemble averaged across repetitions, and plotted in polar coordinates. To better represent the change in GRF effort on EMG and joint moments, relaxed EMG and joint moments recorded during the system’s GRF “zeroing” step was also subtracted from corresponding trial data. In other words, the joint loads and EMG from the resting standing position were treated as a baseline and were not included in our graphs or analyses.

Group mean hip, knee, and ankle flexion angles were applied to an OpenSim musculoskeletal model (Hamner et al., 2010) to extract the flexion-extension and adduction-abduction moment arms of muscles crossing the knee or hip during these positions. To quantify the orientation of a muscle’s MAO with respect to a joint’s axis of rotation, the $\tan^{-1}$ of the Cartesian coordinates (adduction-abduction as X axis; flexion-extension as Y axis) were computed for each muscle, 0° indicating pure abduction, 90° pure extension, 180° pure adduction, and 270° a pure flexion MAO.

Data Analysis

To quantify the similarity of muscle activation patterns between participants, we used an $ICC_{(2,k)}$ following recommendations of McGraw and Wong (1996) in SPSS (v18.0, IBM, Armonk, NY). We evaluated the within-target variance with participants being the reliability measure by scaling the EMG to the maximum value recorded in the given muscle of each participant. This determined if participant activation levels varied across targets (modulated) in a similar manner and using the same neuromuscular control strategy to achieve the desired forces.

Roles of individual muscles at each effort level were classified with a symmetry analysis, mean direction of activation (Φ_M), and mean magnitude of activation (X_{EMG}) (Flaxman et al., 2012). A mean direction of moment generation (Φ_T) at 60% effort was also computed for each joint moment. *Moment actuators* were muscles whose muscle activation was asymmetrical about the polar plot origin and Φ_M was not statistically different from the extracted MAO; *general joint stabilizers* were classified as muscles with symmetrical activation about the polar plot origin; and *specific joint stabilizers* were muscles with asymmetrical activation whose Φ_M was statistically different than its extracted MAO. Significant differences between a muscle's Φ_M and their extracted MAO was tested with a one sample test for the mean angle at the $\alpha<0.05$ level (CircStat Toolbox for Matlab; Berens, 2009).

To determine if muscle activation patterns, and the roles of muscles as they relate to knee joint stability, will change with an increase in ground reaction force level (Aim 2), a one-way repeated measures ANOVA was used for each muscle to test if X_{EMG} significantly differed across the factor of effort (3 levels: 30, 45 and 60%) (SPSS, v18, IBM). The circular analog of an

ANOVA, Watson-Williams test (CircStat Toolbox for Matlab; Berens, 2009), tested if Φ_M for each muscle significantly differed across the factor of effort.

The relationship between individual muscle activations and internal net joint moments across all three loading planes (Aim 1) was evaluated with a partial least square regression (PLSR) analysis (XLSTAT, New York, NY) with cross-validation (leave-one [subject]-out method) (Wold et al., 1984; Haenlein and Kaplan, 2004). This method is appropriate when multicollinearity is present among the predicting variables (multicollinearity statistics presented in Appendix B). The predictor variables (i.e. internal net joint moments) are combined into principal components (PCs) and then regressed onto the dependent variable (muscle activation) using ordinary least squares. Results are transformed back onto the normal X scale in order to obtain estimates of each predictor's standardized coefficients (β). The number of PCs used was determined by accounting for 95% of total variance observed, or if adding another PC contributed to less than 5% VAF. Accuracy of model predictions was assessed with the variance explained (R^2) statistic. We classified a very weak prediction accuracy as $R^2<0.1$, weak as $0.1<R^2<0.3$, moderate as $0.3<R^2<0.5$, and strong as $R^2>0.5$. A predictor's standardized coefficient described the relative increase in muscle activation (EMG/EMG_{max}) with an increase in a given moment of 1 Nm/kg when all other moments are held constant. For example, a β of +0.2 for knee flexion indicates that a muscle's activation is predicted to increase by 20% of EMG_{max} when there is an increase of 1 Nm/kg of knee flexion. Only predictors with significant positive coefficients were considered meaningful. Negative βs were not considered meaningful because it indicated that as a moment increased in magnitude the activation level of the given muscle would decrease. Significance of β values was determined with 95% confidence intervals.

4.1.4 RESULTS

Based on the group mean sagittal plane hip, knee and ankle joint angles, the MAOs of muscles crossing the hip and knee joints are presented in Figure 6A and Figure 7. After removing the relaxed stance forces, group mean ± standard deviation maximum normalized force levels in the anterior, posterior, medial and lateral loading directions were 1.67 ± 0.51, 2.46 ± 0.69, 1.35 ± 0.39, and 1.37 ± 0.41 Nm/kg respectively. Group mean ± standard deviation normalized force required to move the cursor to 60% effort at 90° (anterior) was 1.05 ± 0.31 N/kg, 270° (posterior) 1.50 ± 0.43 N/kg, 0° (lateral) 0.82 ± 0.29 N/kg, and 180° (medial) 0.81 ± 0.24 N/kg (Figure 6B). Respective hip flexion, knee flexion and ankle plantarflexion mean (standard deviation) joint angles were 28.4±6.7°, 23.2±5.8°, 5.9 ±4.8° and normally distributed across participants (Shapiro-Wilk $p>0.05$).

Muscle Activations

For all muscles, a significant increase in X_{EMG} was observed with an increase in effort level (Figure 6C). EMG polar plots (Figure 7) depict individual muscle activation patterns at all 3 effort levels, group mean Φ_M at 60% effort level and extracted MAOs. $ICC_{(2,k)}$s for muscle activation patterns ranged from 0.70 – 0.99 indicating high between-subject reliability from the plotted group mean. For all muscles an increase in effort level did not significantly change asymmetry or Φ_M indicating the roles of muscles did not change (Aim 1).

Due to symmetrical activation patterns, the VL and VM were classified as general joint stabilisers. All other muscles were statistically asymmetrical. RF, TFL, ST, ADD, and GM were classified as hip moment actuators since their Φ_M did not statistically differ from their reported hip MAO ($p>0.05$). Similarly, ST, RF, and LG were classified as knee moment actuators since Φ_M

did not statistically differ from reported knee MAO ($p>0.05$). In contrast, MG and BF had Φ_Ms significantly different from their reported knee and hip MAOs ($p<0.05$), classifying them as specific joint stabilisers.

Moments

$ICC_{(2,k)}$s for internal net joint moment profiles ranged from 0.91 – 1.0 indicating very high between subject reliability from the group mean depicted in Figure 8. In general, required moment at each target location increased linearly with an increase in GRF effort. Sagittal plane moments were greatest at the hip with extension (group mean peak (GMP) 0.898 Nm/kg) magnitudes almost 4 times greater than knee flexion (GMP 0.245 Nm/kg) at posterior targets (hip extension Φ_T=267.7°; knee flexion Φ_T=270.6°). Hip flexion (GMP 0.511 Nm/kg) was approximately 2 times greater than knee extension (GMP 0.299 Nm/kg) at general anterior targets (hip flexion Φ_T=81.7°; knee extension Φ_T=84.6°). Similarly, hip adduction-abduction (GMP 0.462 and 0.474 Nm/kg) was approximately 2 times greater than knee adduction-abduction (GMP 0.218 and 0.235 Nm/kg) at general anterior-lateral (hip abduction Φ_T=28.7°; knee abduction Φ_T=39.0°) and posterior-medial (hip adduction Φ_T=213.6°; knee adduction Φ_T=218.0°) targets. Similar magnitudes of hip (internal GMP 0.218 Nm/kg; external GMP 0.202 Nm/kg) and knee (internal GMP 0.208 Nm/kg; external GMP 0.180 Nm/kg) rotation moments were observed during anterior-medial (hip internal Φ_T=164.6°; knee internal Φ_T=146.3°), and posterior-medial targets (hip external Φ_T= 340.9°; knee external Φ_T=320.8°). Participants predominantly produced dorsiflexion moments at the ankle and coupled inversion with internal ankle rotation and eversion with external ankle rotation moments.

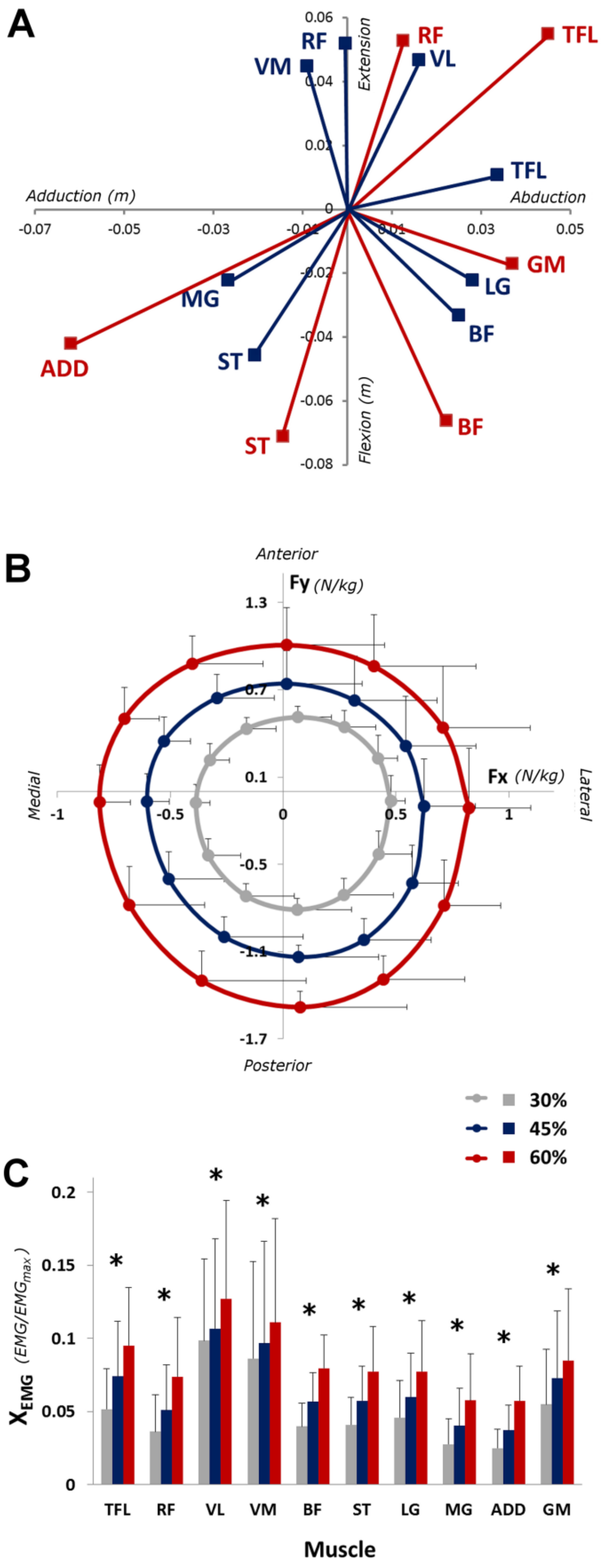

Figure 6: A) MAO of muscles crossing the knee (blue series) and hip (red series) relative to the given joints center of rotation when the knee is flexed to 25° and the hip is flexed to 28°. **B)** Group mean and standard deviation anterior-posterior and medial-lateral force magnitudes required to reach each force target. **C)** Group mean and standard deviation of X_{EMG}. Asterisks (*) indicates significant difference in between all three effort levels ($p<0.05$).

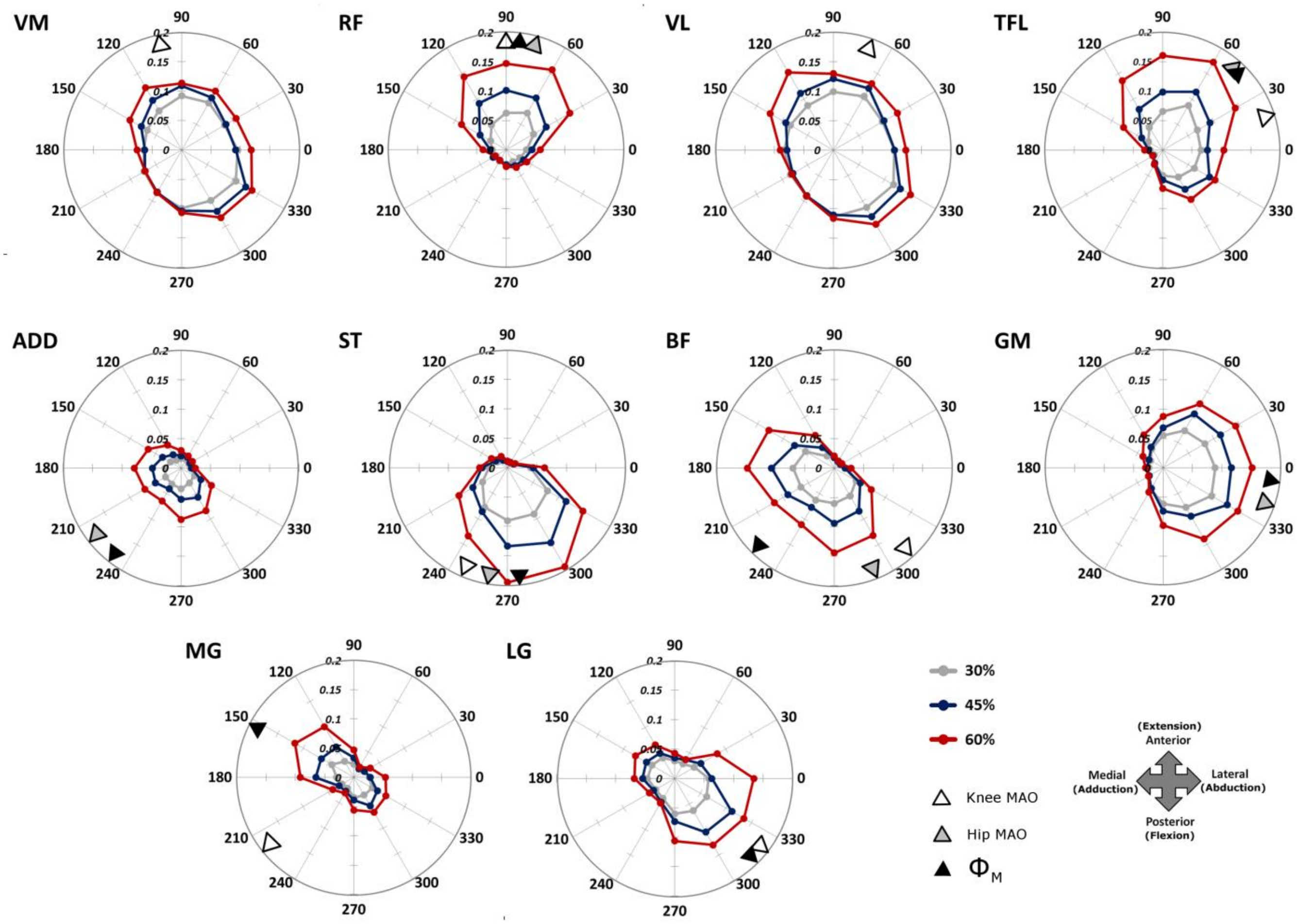

Figure 7: Group mean EMG polar plots at 30, 45 and 60% max effort level. Radii represent relative force loading direction and where the pattern intersects the radii is the normalised EMG amplitude (EMG/EMG_{max}) required to reach the force target. All plots are scaled to 0.2 EMG/EMG_{max}. Filled triangles along the circular trajectory indicate the mean direction of muscle activation (Φ_M) at the 60% effort level. Unfilled and grey triangles are the relative knee and hip moment arm orientation (MAO), respectively, as presented in Figure 6A. No triangle indicates the pattern was statistically symmetrical about its plot origin (no preferred activation direction).

Regression Models

All PLSR models showed significant associations between predictors (internal joint moments) and muscle activation ($p<0.0001$). However, the accuracy of prediction varied across muscles ($R^2=0.09-0.58$, Figure 9). The vastii had very weak prediction accuracy values ($R^2<0.10$), with knee extension as the only significant positive predictor ($\beta=0.115-0.132$). The LG and MG had moderate prediction accuracy ($R^2=0.45-0.47$) with significant knee moment predictors of internal rotation for LG (β=0.20) and external rotation for MG (β=0.25). Hip muscles, ADD (R^2=0.30) and GM (R^2=0.37), had moderate prediction accuracies. Significant predictors for ADD were hip extension (β=0.28) and hip adduction moments (β=0.17). GM had hip abduction (β=0.18) and internal hip rotation (β=0.23) as significant predictors. RF (R^2=0.58) and TFL (R^2=0.36) had strong and moderate prediction accuracies with hip flexion (RF β=0.35; TFL β=0.12) and knee extension (RF β=0.34; TFL β=0.12) as a significant predictors. TFL was also predicted by hip and knee abduction (βs=0.08). Lastly, hamstring muscles had strong prediction accuracies (R^2=0.53-0.58), with hip extension (BF β=0.10; ST β=0.23) and knee flexion (BF β=0.09; ST β=0.11) as significant predictors. ST was also predicted by internal hip (β=0.08) and knee rotation (β=0.17) while BF was also predicted by hip and knee adduction (β=0.12) and hip and knee external rotation (β=0.06-0.09).

Table 1 summarizes the classification of muscle roles and the significant moment predictors (β) for each muscle.

Table 1: Summary of variables used to classify roles of muscles acting at the hip and knee joints.

Muscle	Asymm-etrical?	Does MAO = Φ_M?		Role Classification		Prediction Accuracy	Significant Predictors (β)	
		Hip	*Knee*	*Hip*	*Knee*		*Hip*	*Knee*
BF	yes	no	no	SJS	SJS	strong	Ext, Add, IR	Flex, Add, IR
ST	yes	yes	yes	MA	MA	strong	Ext, IR	Flex, IR
TFL	yes	yes	no	MA	SJS	moderate	Flex, Abd	Ext, Abd
RF	yes	yes	yes	MA	MA	strong	Flex	Ext
VL	no		--		GJS	very weak		Ext
VM	no		--		GJS	very weak		Ext
LG	yes		yes		MA	moderate		IR
MG	yes		no		SJS	moderate		ER
ADD	yes	yes		MA		moderate	Ext, Add	
GM	yes	yes		MA		moderate	Abd, IR	

MAO= moment arm orientation; Φ_M= mean direction of muscle activation; GJS=general joint stabiliser; SJS=specific joint stabiliser; MA=moment actuator; Ext=extension; Flex= flexion; Add=adduction; Abd=abduction; IR=internal rotation; ER=external rotation moments.

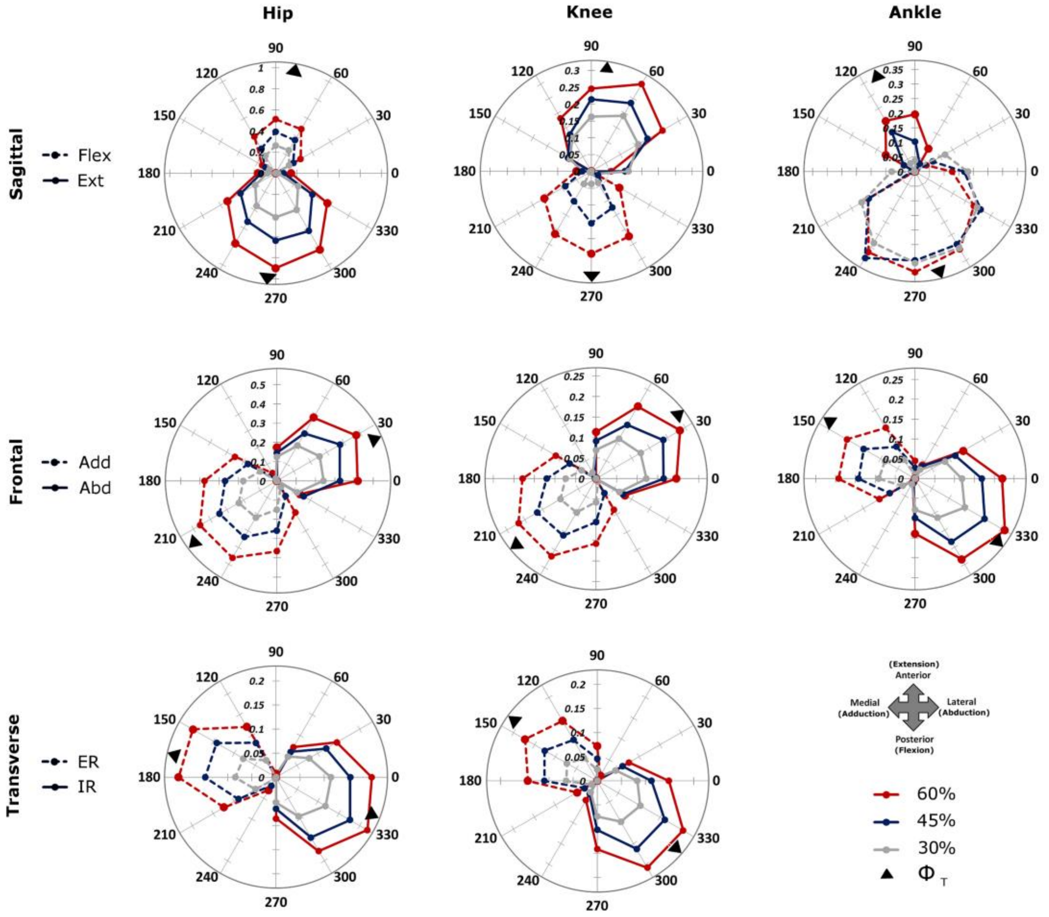

Figure 8: Group mean hip, knee and ankle joint moment polar plots at 30, 45 and 60% effort level. Radii represent relative force loading direction and where the pattern intersects the radii is the normalised internal net joint moment (Nm/kg) required to reach the force target. Triangles along circular trajectory indicate the mean direction of the given moment (Φ_T) at the 60% effort level (ext= extension/plantar flexion; flex=flexion/dorsiflexion; add=adduction/inversion; abd=abduction/eversion; IR=internal rotation; ER=external rotation moments).

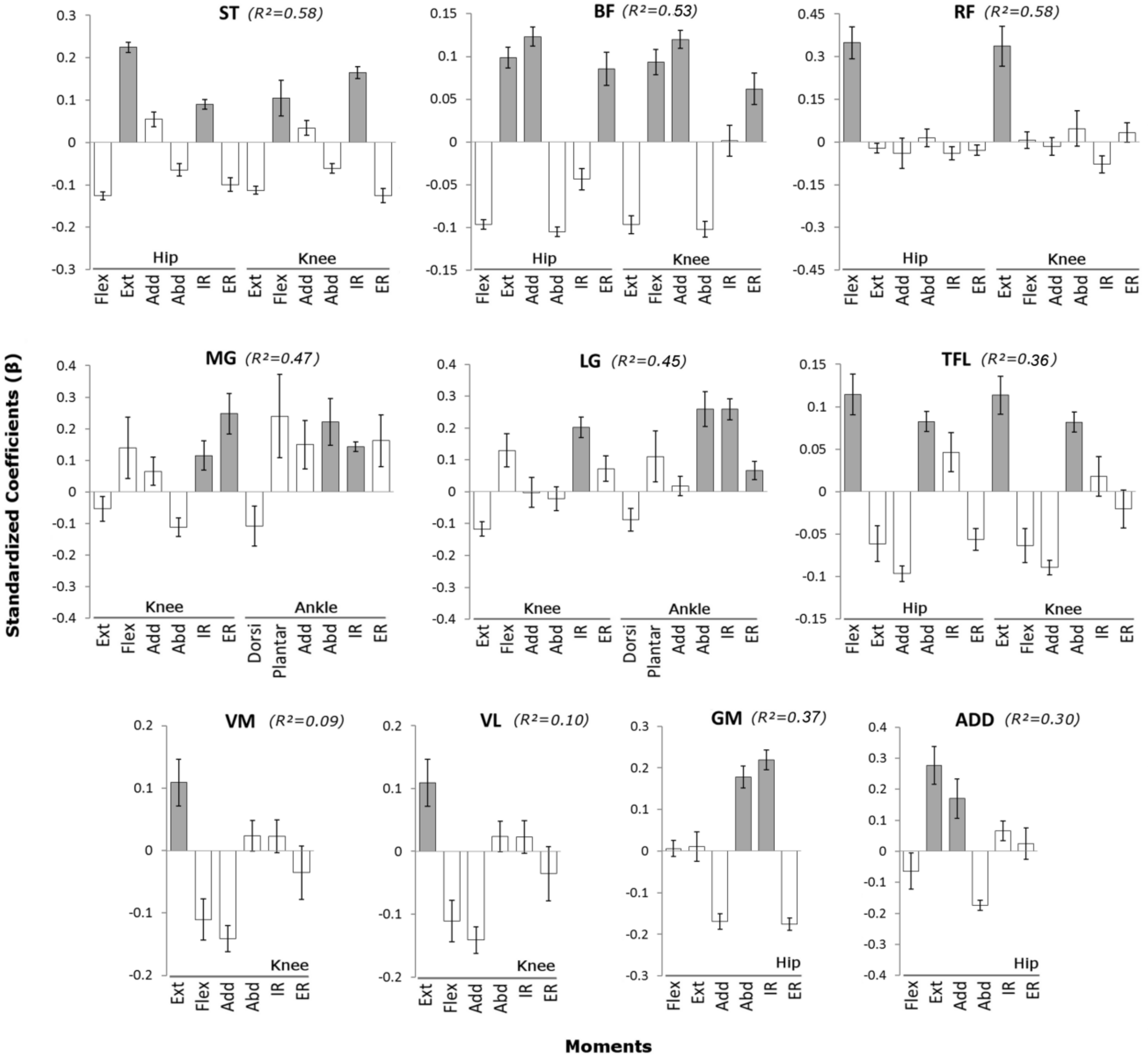

Figure 9: Standardized coefficients (β) of moments for bi- and mono-articular muscles Model goodness-of-fit indicated with R. Only significant positive β coefficients were considered meaningful. These are represented as filled data points. Error bars indicate standard error of β. Only joints over which the given muscle crossed were included in the comparisons (ext= extension; flex=flexion; add=adduction; abd=abduction; IR=internal rotation; ER=external rotation, dorsi=dorsiflexion, plantar=plantarflexion moments).

4.1.5 DISCUSSION

Our study utilized a weight-bearing isometric GRF matching protocol to classify the roles of lower limb muscle as it relates to joint stability by quantifying the relationship between individual muscle activation patterns and internal net joint moments in all three loading planes

(Aim 1) and to determine if the roles of muscles, as it relates to knee joint stability, changes with increasing force levels (Aim 2). Our results in part support our hypotheses. First, the vastii yielded symmetrical activation patterns and weak model-fit parameters classifying them to be general joint stabilisers. It was hypothesized that the activation of bi-articular muscles would be predicted by one general moment at each articulating joint; however, bi-articular muscles had more than one significant moment predictor indicating their roles to be multifactorial. Secondly, an increase in effort level did not significantly change Φ_M or asymmetry of muscle activation patterns indicating the roles of muscles did not change with an increase in force level.

To date, most research on dynamic knee joint stability has focused on how knee muscles activate as a function of their anatomical orientation (Andriacchi et al., 1984; Buchanan and Lloyd, 1997) and typically classify activation as either agonistic or antagonistic to sagittal plane motion (Granata et al., 2002; Alkjær et al., 2012). The interpretation of muscle roles is also often described from non-weight bearing tasks which limits the functional relevance of the activations (Buchanan and Lloyd, 1997; Van Deursen et al., 1998; Alkjær et al., 2012). Literature pertaining to the muscular contributions to frontal and transverse plane loads, in particular during weight bearing, is therefore very limited despite the fact that joint pain and injury occur while weight-bearing. Previously, we proposed knee definitions for the roles of knee joint muscles as it relates to stability. Our analysis is based on the modulation of muscle activation across various force directions and how this relates to MAOs (Flaxman et al., 2012; Smith et al., 2012). The addition of PLSRs to our methods has further elucidated the direct relationship between individual muscle activity and internal joint moments. For example, based on its activation pattern and MAO, the ST was classified as a moment actuator for knee flexion. Our PLSR expanded this role to include a specific stabilizing role for knee rotation because ST activation was also significantly predicated by internal knee rotation moments.

In order to generate GRFs, participants elicited various combinations of internal flexion-extension, adduction-abduction, and rotational moments at the hip, knee and ankle joints. Both the moment and muscle activation profiles were extremely similar across participants (EMG ICCs>0.7; moment ICCs>0.9) suggesting a fundamental strategy to coordinate and distribute lower limb net joint moments in order to apply a given GRF at the ground-foot interface. Unlike previous works, we were able to provide a quantifiable relationship between individual muscle EMG and lower limb internal net joint moments during weight-bearing, and thus we have the ability to isolate the functional roles of muscles.

Quadriceps Muscles

The quadriceps muscles undoubtedly function as knee extensors and due to their MAOs, activation will generate an extensor moment. Yet, this does not fully describe their functional role. Due to symmetrical muscle activation patterns we classified the vastii as general joint stabilisers. This classification was also supported by the very weak prediction accuracy values ($R^2<0.1$), indicating a change in internal joint moments will unlikely be accompanied by a change in activation of a given muscle. In contrast, RF had an asymmetrical activation with its Φ_M consistent with its MAO and significant model predictors. As such, the RF was classified as a moment actuator for hip flexion and knee extension moments. The different roles between vastii and RF demonstrate the complexity for force control in weight bearing. van Ingen Schenau et al. (1992) suggested that perhaps mono- and bi-articular muscles play different roles in lower limb force control: the mono-articular muscles provide a general gross force while bi-articular muscles "fine tune" the distribution of the net joint moments across adjacent joints. Considering the vastii are 1) primary contributors to axial knee joint force (Sasaki and Neptune, 2010), 2) can provide the greatest contribution to knee joint rotational stiffness in all three flexion-extension, varus-valgus, and transverse loading axes (Cashaback and Potvin, 2012), and 3) they actually have a protective effect on the anterior cruciate ligament (ACL) when physiological loading magnitudes are applied in vitro (Hashemi et al., 2007), we postulate that while weight bearing the vastii muscles contract to increase compressive forces, essentially bracing the knee, so bi-articular hip muscles, such as the RF, can generate and transmit the moments needed to direct the GRFs at the foot-ground interface.

Hamstrings Muscles

The role of the hamstrings as it relates to lower limb function is often associated with generating knee flexion moments. Again, anatomically if one focuses on the knee and their MAOs this is a correct observation. Since ST's knee MAO was not statistically different from its activation pattern's Φ_M we first assumed that it was the primary contributor to knee flexion. This is supported by the ST's PLSR model. However, the β for hip extension is nearly 2.5x greater than knee flexion. In contrast, the BF's knee MAO was statistically different from its $\Phi_{M.}$ Accordingly, it was classified as a specific joint stabilizer; but the PLSR model determined that knee flexion was also significantly associated with BF activity. Similar to the ST, the BF's β for hip extension is greater than knee flexion. Considering dominant hip moment strategies were elicited for generating posterior GRFs (hip extension nearly 4 times greater than knee flexion) we may assume that the hamstrings function more as hip extensors. Similarly, in a contact force control task involving all three lower limb joints, van Deursen et al. (1998) reported that the activations of the bi-articular RF and hamstring muscles had the highest correlations with changes in hip joint moments compared to knee joint moments.

In weight-bearing conditions, activation of the hamstrings is commonly interpreted as antagonist activation against the quadriceps to reduce knee extension moments and prevent anterior tibial translation (MacWilliams et al., 1999). However, moment arms of the hamstrings also possess substantial rotational components (Herzog and Read, 1993; Aalbersberg et al., 2005; Agur and Dalley, 2005). Our results also show a relatively high association of ST and BF with internal and external knee rotation, respectively, suggesting that hamstring activation is essential for stabilizing the knee against torsional loads. In fact, extension of the knee using isolated quadriceps force causes an internal rotation of the tibia relative to the femur (Victor et al., 2010) – which is linked to the ACL injury mechanism (Boden et al., 2000). This internal rotation is reduced only when hamstring loads, especially the BF, are added resulting in knee motion with a neutral alignment (Victor et al., 2010).

Interestingly, BF was also associated with hip and knee adduction moments. This may be from one of two scenarios: 1) it is generating an antagonistic force to oppose hip and knee adduction moments or 2) its activation profile reflects a bi-modal pattern encompassing external hip/knee rotation and hip extension/knee flexion. For the latter, when combined, the mean principal moment directions (Φ_T) for external hip/knee rotation and hip extension/knee flexion is equal to 211°, which is extremely close to the principle directions of hip ($\Phi_T = 213°$) and knee adduction ($\Phi_T = 218°$).

Gastrocnemius Muscles

LG and MG also demonstrated significant associations with respective internal and external knee rotation moments. In addition to its role of a knee joint flexor, the gastrocnemius has the potential to contribute to rotational knee moments (Agur and Dalley, 2005) and is commonly the first to activate during rotational perturbations (Carcia et al., 2005). Despite this and its changing activation following ACL injury (Rudolph et al., 2001) little literature is available about its contribution at the knee, possibly due to its confounding role at the ankle. Based on its anatomical alignment gastrocnemius activity has been hypothesized to create anterior shear force on the tibia, resulting in an increase in ACL loading (O'Conner, 1993). Several studies have tested this hypothesis: Durselen et al. (1995) applied a 550N gastrocnemius load in vitro and there was no effect on ACL strain at any flexion angles; Fleming et al. (2001) electrically stimulated the gastrocnemius muscle to produce a plantar-flexion moment which increased in vivo ACL strain between 0-30° knee flexion; Morgan et al. (2014) estimated in silico that elevated gastrocnemius activity is synergistic with the quadriceps to help compress the knee and lower ACL forces during single leg jump landing. Despite contradictory evidence, reported differences in gastrocnemius activity exist in populations with knee instability (Rudolph et al., 2001) and this changing activation will alter knee loads. It remains unclear whether gastrocnemius activity as it relates to knee joint stability is protective or not and further investigation is warranted.

Hip Joint Muscles

Since gluteal muscles, hip abductors and hip adductors are large determinants of femoral orientation and knee joint loads (Sasaki and Neptune, 2010), this study analyzed their contribution to hip moments. As expected, the adductor muscles and GM were predicted well with hip adduction and abduction moments, respectively. Due to the difficulty in isolating a single adductor muscle with surface EMG, our ADD EMG signal is considered a summation of inner thigh muscle activity. This could explain why its activation was also predicted by hip extension. Internal hip rotation was also deemed significant predictors for GM but we postulate it is antagonistically activating to stabilize the hip. This is a noteworthy observation since reduced hip function de-stabilizes the femur, causing an increase in frontal plane motion and knee joint moments (Powers, 2010).

Last, although the TFL is generally considered a hip joint muscle we included knee moments in its model because it attaches to the iliotibial tract and inserts on the lateral condyle of the tibia (Agur and Dalley, 2005; Winby et al., 2009). Results indicate the TFL contributes to hip flexion and knee extension. Increased knee abduction loads are linked to the ACL injury mechanism (Boden et al., 2000), so it is surprising that TFL was also the only muscle to have knee abduction moments as a significant predictor. Previous works found that the TFL contributed up to 25% of muscular loads to lateral knee compartment during gait (Winby et al., 2009) and assisted in knee extension and minor valgus moment generation (Buchanan and Lloyd, 1997). Like the gastrocnemius muscles, the role of the TFL as it relates to knee joint stability is not well understood and also warrants future examination.

General Considerations

Our protocol was conducted while weight-bearing, which we believe to be a functionally relevant task (consider bracing yourself on a moving bus, opening a heavy door or preparing to rise from a seat). However, it is important to note that the EMG-moment relationships observed in the present study and how it relates to those observed in ballistic activities remains unclear. Considering most existing studies associate a proportional increase activation to a proportional increase in joint moments based on relationships established from non-weight bearing and/or uni-axial in vivo studies (Bigland-Ritchie, 1981; Van Deursen et al., 1998), in silico investigations which may be prone to poor predictions of agonist and antagonist activations (Herzog and Binding, 1992) or in vitro anatomical investigations based on muscle lines of action and moment arms (MacWilliams et al., 1999; Zheng et al., 2014), we believe our results provide greater and novel insight into the functional role of the investigated muscles. Furthermore, the moment arms extracted from the musculoskeletal model (Hamner et al., 2010) are not subject specific. Since significant between subject differences exist in anatomical muscle paths (Brand et al., 1982), the extracted moment arms may not be representative of every participant. Last, the classification of muscle roles as it relates to knee joint stability is associated with only one bodily configuration. Especially for the bi-articular muscles, muscle activation patterns are subject to change depending on flexion angle (Buchanan and Lloyd, 1997). We opted for this position because the hip and knee flexion angles are common to several sporting maneuvers associated with ACL injury, such as side cuts, braking motions, and landing (Boden et al., 2000). We wanted to elicit as much muscular protection as possible and shallower knee flexion angle would have increased the reliance on the soft tissues and reduced mechanical advantages of the knee muscles (Lloyd and Buchanan, 1996). Nevertheless, similar activation patterns in a standing force matching have been reported with extended knees (MacLeod et al., 2013).

4.1.6 CONCLUSION

In summary, our results show that activation is not always dependent on anatomical orientation. Using PLSR models, we quantitatively associated internal moment generation with changes in individual muscle activations, thus specifying the functional contributions of muscles to maintaining knee joint stability. This can be particularly useful for delineating the roles of the bi-articular knee joint muscles. Moments required to modulate GRFs are primarily generated by the bi-articular muscles crossing the hip, while uni-articular muscles crossing the knee are responsible for producing compressive forces, essentially bracing the knee, so that proximal hip loads can be transmitted down to the foot-ground interface. Our results also emphasize the importance of the hamstrings and gastrocnemius muscles in supporting rotational torques at the knee. Functional roles of lower limb muscles need to be reconsidered when describing how muscle activity relates to moment generation, which could have implications in robotics, musculoskeletal modeling, sports sciences and rehabilitation.

4.1.7 References

Aalbersberg S, Kingma I, Ronsky JL, Frayne R, van Dieën JH (2005) Orientation of tendons in vivo with active and passive knee muscles. J Biomech 38:1780–1788.

Agur AMR, Dalley AF (2005) Grant's Atlas of Anatomy. Baltimore, MA: Lippincott Williams & Wilkins.

Alkjær T, Simonsen EB, Magnusson SP, Dyhre-Poulsen P, Aagaard P (2012) Antagonist muscle moment is increased in ACL deficient subjects during maximal dynamic knee extension. The Knee 19:633–639.

Andriacchi TP, Andersson GBJ, Ortengren R, Mikosz RP (1984) A study of factors influencing muscle activity about the knee joint. J Orthop Res 1:266–275.

Berens P (2009) CircStat: a MATLAB toolbox for circular statistics. J Stat Softw 31:1–20.

Bigland-Ritchie B (1981) EMG/force relations and fatigue of human voluntary contractions. Exerc Sport Sci Rev 9:75–117.

Boden BP, Dean CS, Feagin Jr. JA, Garrett Jr. WE (2000) Mechanisms of anterior cruciate ligament injury. Orthopedics 23:573–578.

Brand RA, Crowninshield RD, Wittstock CE, Pedersen DR, Clark CR, van Krieken FM (1982) A Model of Lower Extremity Muscular Anatomy. J Biomech Eng 104:304–310.

Brand RA, Pedersen DR, Friederich JA (1986) The sensitivity of muscle force predictions to changes in physiologic cross-sectional area. J Biomech 19:589–596.

Buchanan TS, Almdale DPJ, Lewis JL, Rymer WZ (1986) Characteristics of synergic relations during isometric contractions of human elbow muscles. J Neurophysiol 56:1225–1241.

Buchanan TS, Lloyd DG (1997) Muscle activation at the human knee during isometric flexion-extension and varus-valgus loads. J Orthop Res 151:1–17.

Carcia CR, Shultz SJ, Granata KP, Perrin DH, Martin RL (2005) Females recruit quadriceps faster than males at multiple knee flexion angles following a weight bearing rotary perturbation. Clin J Sport Med 15:167–171.

Cashaback JGA, Potvin JR (2012) Knee muscle contributions to joint rotational stiffness. Hum Mov Sci 31:118–128.

Delp SL (2007) OpenSim: Open-Source Software to Create and Analyze Dynamic Simulations of Movement. IEEE Trans Biomed Eng 54:1940–1950.

Durselen L, Claes L, Kiefer H (1995) The influence of muscle forces and external loads on cruciate ligament strain. Am J Sports Med 23:129–136.

Flaxman TE, Speirs AD, Benoit DL (2012) Joint stabilisers or moment actuators: the role of knee joint muscles while weight bearing. J Biomech 45:2570–2576.

Fleming B C, Renstrom P, Ohlen G, Johnson RJ, Peura GD, Beynnon BD, Badger GJ (2001) The gastrocnemius muscle is an antagonist of the anterior cruciate ligament. J Orthop Res 19:1178–1184.

Granata KP, Wilson SE, Padua DA (2002) Gender differences in active musculoskeletal stiffness. Part I. Quantification in controlled measurements of knee joint dynamics. J Electromyogr Kinesiol 12:119–126.

Haenlein M, Kaplan AM (2004) A Beginner's Guide to Partial Least Squares Analysis. Underst Stat 3:283–297.

Hamner SR, Seth A, Delp SL (2010) Muscle contributions to propulsion and support during running. J Biomech 43:2709–2716.

Hashemi J, Chandrashekar N, Jang T, Karpat F, Oseto M, Ekwaro-Osire S (2007) An alternative mechanism of non-contact anterior cruciate ligament injury during jump-landing: In-vitro simulation. Exp Mech 47:347–354.

Herzog W, Binding P (1992) Predictions of antagonistic muscular activity using nonlinear optimization. Math Biosci 111:217–229.

Herzog W, Read LJ (1993) Lines of action and moment arms of the major force-carrying structures crossing the human knee joint. J Anat 182:213–230.

Krishnan C, Huston K, Amendola A, Williams GN (2008) Quadriceps and Hamstrings Muscle Control in Athletic Males and Females. J Orthop Res 26:800–808.

Krishnan C, Williams GN (2009) Sex differences in quadriceps and hamstrings EMG-moment relationships. Med Sci Sports Exerc 41:1652–1660.

Levin O, Wenderoth N, Steyvers M, Swinnen SP (2003) Directional invariance during isolated-related modulations of muscle activity: evidence for motor equivalence. Exp Brain Res 148:62–67.

Lloyd DG, Buchanan TS (1996) A model of load sharing between muscles and soft tissues at the human knee during static tasks. J Biomech Eng 118:367–376.

MacLeod TD, Manal K, Silbernagel KG, Snyder-Mackler L, Buchanan TS (2013) Characteristics of human knee muscle coordination during isometric contractions in a standing posture: the effect of limb task. J Electromyogr Kinesiol Off J Int Soc Electrophysiol Kinesiol 23:1398–1405.

MacWilliams BA, Wilson DR, Desjardins JD, Romero J, Chao EYS (1999) Hamstrings cocontraction reduces internal rotation, anterior translation, and anterior cruciate ligament load in weight-bearing flexion. J Orthop Res 17:817–822.

Mantovani G, Lamontagne M (2016) How Different Marker Sets Affect Joint Angles in Inverse Kinematics Framework. J Biomech Eng. [Epub ahead of print].

McGraw KO, Wong SP (1996) Forming inferences about some intraclass correlation coefficients. Psychol Methods 1:30–46.

Morgan KD, Donnelly CJ, Reinbolt JA (2014) Elevated gastrocnemius forces compensate for decreased hamstrings forces during the weight-acceptance phase of single-leg jump landing: implications for anterior cruciate ligament injury risk. J Biomech 47:3295–3302.

O'Conner JJ (1993) Can muscle co-contraction protect knee ligaments after injury or repair? J Bone Jt Surg Br 75:41–48.

Powers CM (2010) The influence of abnormal hip mechanics on knee injury: A biomechanical perspective. J Orthop Sports Phys Ther 40:42–51.

Rudolph KS, Axe MJ, Buchanan TS, Scholz JP, Snyder-Mackler L (2001) Dynamic stability in the anterior cruciate ligament deficient knee. Knee Surg Sports Traumatol Arthrosc 9:62–71.

Sasaki K, Neptune RR (2010) Individual muscle contributions to the axial knee joint contact force during normal walking. J Biomech 43:2780–2784.

Smith AJJ, Flaxman TE, Benoit DL (2012) Reliability of knee joint muscle activity during weight bearing force control. J Electromyogr Kinesiol 22:914–922.

Van Deursen RWM, Cavanagh PR, van Ingen Schenau GJ, Becker MB, Ulbrecht JS (1998) The role of cutaneous information in a contact control task of the leg in humans. Hum Mov Sci 17:95–120.

Van Ingen Schenau GJ, Boots PJM, de Groot G, Snackers RJ, van Woensel WWLM (1992) The constrained control of force and position in multi-joint movements. Neuroscience 46:197–207.

Victor J, Labey L, Wong P, Innocenti B, Bellemans J (2010) The influence of muscle load on tibiofemoral knee kinematics. J Orthop Res Off Publ Orthop Res Soc 28:419–428.

Wilkie DR (1950) The relation between force and velocity in human muscle. J Physiol 110:249–280.

Winby CR, Lloyd DG, Besier TF, Krik TB (2009) Muscle and external load contribution to knee joint contact loads during normal gait. J Biomech 42:2294–2300.

Wold S, Ruhe A, Wold H, Dunn I W (1984) The Collinearity Problem in Linear Regression. The Partial Least Squares (PLS) Approach to Generalized Inverses. SIAM J Sci Stat Comput 5:735–743.

Zheng L, Li K, Shetye S, Zhang X (2014) Integrating dynamic stereo- radiography and surface- based motion data for subject- specific musculoskeletal dynamic modeling. J Biomech 47:3217–3221.

Study 2: Protective and Adverse Muscle Activation Strategies after ACL-injury during a Weight-bearing Force Control Task

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4.2.1 ABSTRACT

Background: Anterior cruciate ligament (ACL) injury results in a loss of mechanical knee joint stability. To maintain stability, the neuromuscular system must integrate activity of all muscles that cross the knee joint and create a joint moment that effectively opposes the external load. Differences in muscle activation patterns are commonly identified between ACL deficient (ACLD) and uninjured controls (CON); however, it remains unclear how and which of these differences improve or reduce knee joint stability. **Purpose:** To quantify how differences in muscle activation are interrelated with differences in internal net joint moments between ACLD and CON groups. **Study Design:** Cross-sectional study. **Methods:** A standing force matching protocol required participants to isometrically modulate ground reaction forces to elicit various combinations of sagittal, frontal and transverse plane internal joint moments. Partial least squares regressions determined which internal moment(s) predicted the activation of 10 lower limb muscles for each group. **Results:** Compared to CON, ACLD demonstrated stronger relationships between (1) rectus femoris and knee extension, (2) semitendinosus and knee flexion, and (3) gastrocnemius and knee flexion moments. ACLD had weaker relationships between (3) biceps femoris and knee flexion, (4) gastrocnemius and external knee rotation, and (5) gluteus medius and hip abduction moments compared to CON. **Conclusions:** The relationship between individual muscle activation and internal joint moments differ between ACL and CON. We suggest neuromuscular adaptations after ACL injury increase sagittal plane stability, but reduce stability during knee abduction and external rotational loads. Results can provide insight into mechanisms of knee joint stability and contribute to rehabilitative/ preventative exercise interventions.

4.2.2 INTRODUCTION

Rupture of the anterior cruciate ligament (ACL) is the most common traumatic knee injury in active adults. ACL reconstruction is often performed to restore the joint's mechanical integrity; however, the relative effectiveness of invasive interventions over more conservative rehabilitative treatment methods is debatable. A strategy of initial rehabilitation (with the option of ACL reconstruction later) in patients with acute ACL injury yields the same subjective results judged by an outcome questionnaire as an early reconstruction plus rehabilitation

(Frobell et al., 2010). However, an approach of initial rehabilitation reduces the number of ACL reconstructions by 50% (Mikesky et al., 2006; Frobell et al., 2010), suggesting conservative approaches should be considered as a primary treatment option. Regardless of treatment strategy, only 50% of ACL injured participants regain pre-injury levels of physical activity after five years of initial treatment (Thomeé et al., 2007; Frobell et al., 2013). More work is needed to improve patient readiness for return to play and, perhaps, a new approach for understanding the relationship between neuromuscular control and knee joint stability.

During functional tasks and sporting activities, the knee is exposed to large and rapidly changing external loads. To effectively oppose these loads, the activity of all muscles that cross the knee must be integrated so that when summated, the muscular force products create a net joint moment-of-force that is opposite in direction to the external load. Neuromuscular function is compromised after an ACL injury possibly due to a loss of afferent feedback (Krogsgaard et al., 2011) and a centrally mediated inhibition of quadriceps muscle function (Palmieri-Smith et al., 2008). As compensation, ACL injured individuals typically display increased levels of quadriceps activity, earlier onsets of muscle activation, increased antagonist co-contraction, and/or overall temporal differences in activation patterns compared to controls (CON) (Sinkjaer and Arendt-Nielsen, 1991; Kvist and Gillquist, 2001; Rudolph et al., 2001; Chmielewski et al., 2005; Alkjær et al., 2012; Shanbehzadeh et al., 2015). Variations in muscle action exposes the knee to abnormal forces, essentially destabilising the joint and increasing one's risk of subsequent injuries and developing osteoarthritis (Herzog and Longino, 2007). However, there exist conflicting interpretations on how and which of these neuromuscular variations relate to loads acting on the knee (Berchuck et al., 1990; Sinkjaer and Arendt-Nielsen, 1991; Aune et al., 1997; Kvist and Gillquist, 2001; Rudolph et al., 2001; Chmielewski et al., 2005; Alkjær et al., 2012).

Knee joint function is governed by an individual's ability to actively respond and adapt to external loads applied to the knee joint. Understanding the relationship between neuromuscular control and knee loads is key in determining which adaptations and/or treatment methods actually increase knee stability and which do not. Since the ACL injury mechanism and subsequent reports of instability are related to greater tibial rotation angles, greater knee abduction angles and moments, and reduced knee flexion moments (Houck and Yack, 2001; Houck et al., 2007), a stronger relationship (evidenced by greater β values) between a given muscle and knee flexor, knee adductor, and rotational moments is suggested to increase knee joint stability (Yoo et al., 2005; Li et al., 2007). The purpose of this study was to identify muscle activation patterns that either increase or reduce joint moments related to knee instability. This was achieved by evaluating which internal net joint moments were interrelated with changes in individual muscle activations elicited using an isometric weight-bearing force matching protocol (Flaxman et al., 2012, 2016; Smith et al., 2012) in ACL deficient (ACLD) and CON participants.

4.2.3 METHODS

Participants and Equipment

Twenty-four adults with ACLD knees were recruited from Bispebjerg Hospital, Copenhagen, Denmark and participated in this study. MRI or arthroscopy confirmed ACL rupture. Inclusion criteria for ACLD were injury of the dominant limb and intention to be surgically reconstructed. Twenty-four participants with no history of significant lower limb injuries acted as CON. CON were recruited from the University of Copenhagen, Denmark and surrounding community and were matched to ACLD for sex, age, BMI and leg dominance. Experiments were completed at the University of Copenhagen. This study was approved by local ethics committee for the Capital Region of Denmark (H-3-2013-126) and the University of Ottawa Research Ethics Board (H06-14-27) with all participants providing written informed consent. Participants completed a series of subjective functional questionnaires including the Knee injury and Osteoarthritis Outcome Score (KOOS) (Roos and Lohmander, 2003), International Knee Documentation Committee (IKDC) subjective knee form (Irrgang et al., 2001), Lysholm scale (Lysholm and Gillquist, 1982), and Tegner physical activity score (Tegner and Lysholm, 1985).

Surface electromyography (EMG) signals of 10 lower limb muscles (rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), semitendinosus (ST), biceps femoris (BF), medial gastrocnemius (MG), lateral gastrocnemius (LG), tensor fascia lata (TFL), gluteus medius (GM), and adductor muscle group (ADD)) were recorded with electrodes placed in a bipolar configuration according to SENIAM guidelines (Hermens et al., 2000). EMG was sampled at 1000Hz with a 20-500Hz bandwidth and a 6dB/octave filter slope using a wireless EMG system (MQ air, Marq Medical, Farum Denmark).

Trajectories of retro-reflective markers (placed according to HMBL Cluster Marker set (Mantovani and Lamontagne, 2016)) were recorded using a ten-camera motion analysis system (6 MXF-40s and 4 T-series cameras, Oxford Metrics, Oxford, UK) sampling at 100Hz with supporting Nexus software (version 1.8.5, Oxford Metrics, Oxford, UK). Ground reaction forces

(GRFs) recorded from a force platform (AMTI-OR6, AMTI, Watertown, MA) were also collected in Nexus at 1000Hz with a gain of 1000.

Experimental Protocol

A detailed description of the protocol and its reliability has been previously reported (Flaxman et al., 2012; Smith et al., 2012; Flaxman et al., 2016). First, maximum voluntary isometric contractions (MVICs) during hip flexion, hip extension, hip abduction, hip adduction, knee extension, knee flexion, and plantarflexion exercises were completed with manual resistance from the researcher. Participants then stood in a staggered position such that their feet were spaced hip width apart and the test leg had approximate joint angles of 30° hip flexion, 30° knee flexion, and 10° ankle plantarflexion. The test leg was placed in a wakeboard boot fixed to the force platform (Figure 10A). Participants controlled a projected image of a cursor by pushing against the force platform with their foot, while maintaining equal body weight on each leg. Successful match of the cursor to a force target, for half a second, triggered data collection. Twelve force targets, evenly spaced by 30° about a circular trajectory (representing various horizontal anterior-medial-posterior-lateral loading directions) randomly appeared (Figure 10B). Each direction had three different force magnitudes (30%, 45% and 60% of previously recorded max effort trials). These 36 targets were matched twice for 72 targets total. Previous work (Flaxman et al., 2016) showed muscle activation patterns and the roles of muscles as it relates to knee joint stability does not change with an increase in force effort level. As such, only data for the 60% targets were analyzed in this study.

Data Processing

Raw marker trajectories and GRFs were conditioned with a 2nd order dual-pass 15Hz low pass Butterworth filter (Kristianslund et al., 2012) in Nexus and exported to OpenSim (v 3.2, Delp, 2007) to compute lower limb joint angles and internal net joint moments using inverse kinematics and dynamics. EMG was high-pass filtered at 20Hz with a 2nd order dual-pass Butterworth filter, full wave rectified, low-pass filtered at 10Hz with a 2nd order dual-pass Butterworth filter. Experimental EMG (EMG_{exp}) was normalized to EMG_{max} of the MVIC trials (EMG_{exp}/EMG_{max}). EMG_{max} for each muscle was computed as a 50ms mean about the maximum value in the conditioned EMG signal across all exercises. EMG, kinematic and kinetic data were time averaged over the half second of successful force target match and ensemble averaged across repetitions. Group mean EMG and moments were plotted in polar coordinates to display how activation and moments modulate as a function of force loading direction.

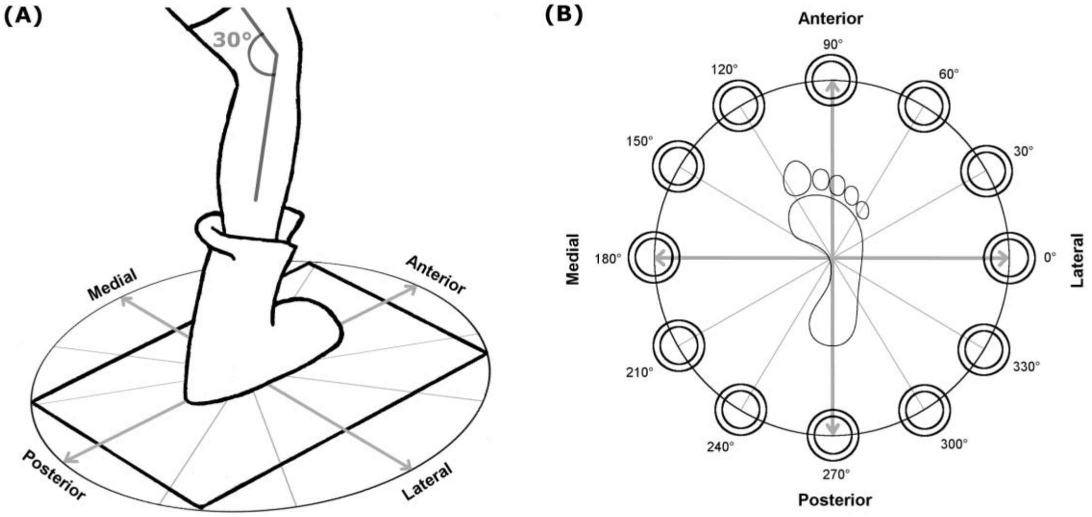

Figure 10: (A) The foot of the test limb is placed in a wakeboard boot that is fixed to a force platform. The knee of the test foot is flexed to 30°. While exposing equal weight to each leg, the participant is required to modulate various combinations of horizontal ground reaction forces (depicted as the circular plane at the ground-foot interface). **(B)** Force target locations evenly spaced about a circular trajectory. Each radii's magnitude is representative of the force level required to reach each target, normalized to 60% of participants maximal effort. Numbers about circular trajectory describe the relative force target direction in degrees (0°= pure lateral, 90°= pure anterior, 180°= pure medial, 270°= pure posterior).

Data Analyses

Partial least square regression (PLSR) models (XLSTAT, New York, NY) with cross-validation (leave-one[subject]-out method) (Haenlein and Kaplan, 2004) evaluated the relationship between individual muscle activations (dependent variable) and internal net joint moments (predictor variables) (Flaxman et al., 2016). Model goodness-of-fit was assessed with the variance explained (R^2) statistic and defined as very weak ($R^2<0.1$), weak ($0.1<R^2<0.3$), moderate ($0.3<R^2<0.5$), or strong ($R^2>0.5$). A predictor's standardized beta coefficient (β) described a muscle's relative increase in normalized muscle activation with an increase in a given moment of 1 Nm/kg when all other moments are held constant. A higher β therefore indicated a stronger relationship between that moment variable and that muscle's activity. Only significant positive coefficients (determined with 95% confidence intervals) were considered meaningful.

Significant between group differences in sagittal plane hip, knee and ankle joint angles were determined with multivariate analysis of variance (MANOVA). Significant group mean differences in target forces (at 0, 90, 180 and 270°), peak muscle activation, peak moment, R^2 and β values were determined with independent T-tests. SPSS (v20.0, IBM, Armonk, NY) was used for analyses and significance was defined at the alpha=0.05 level. Between-subject reliability of muscle activation and moment profiles were evaluated with intra-class correlation coefficients ($ICC_{(2, k)}$) (McGraw and Wong, 1996). Reliability was accepted at ICC>0.75 (Portney and Watkins, 2000).

4.2.4 RESULTS

No significant between group differences in subject demographics (sex, height, weight, age or leg dominance tested) were observed. ACLD had significantly reduced subjective functional outcome scores for KOOS, IKDC, and Lysholm ($p<0.05$; Table 2). ACLD pre-injury Tegner scores did not significantly differ from CON but were significantly reduced at time of participation (after injury) (Table 2).

Table 2: Group mean ± standard deviation of ACL deficient (ACLD) and control (CON) participant demographics and outcome scores from subjective questionnaires. *n*=number in participants; KOOS = The Knee injury and Osteoarthritis Outcome Score; IKDC = International Knee Documentation Committee. ACLD Tegner are perceived levels before (B) and at time of participation (after injury, A).

	ACLD		**CON**	
	Males	**Females**	**Males**	**Females**
n	13	11	13	11
Height (cm)	181.9±4.0	170.1±5.0	182.9±4.9	170.2±4.8
Weight (kg)	84.5±8.28	66.7±9.0	82.3±10.1	64.1±8.7
Age (years)	31.7±9.0	25.3±8.2	29.1±6.7	26.2±7.2
Time since injury (mo.)	12.9±14.3		---	
KOOS	66.1±12.9		98.6±3.18	
IKDC	65.2±16.7		99.1±2.0	
Lysholm	73.4±13.6		99.4±1.6	
Tegner	B:7.1±1.6 / A: 3.9±1.3		6.9±2.0	

Experimental Data

Respective hip flexion, knee flexion and ankle plantarflexion mean ± standard deviation joint angles were 28.4±6.7°, 23.2±5.8°, 5.9±4.8° for CON and 26.9±3.2°, 23.3± 4.91°, 6.0±3.9° for ACLD during target matching. Joint angles were normally distributed (Shapiro-Wilk $p>0.05$) and not significantly different between groups. Significantly reduced target forces were observed at 0° (lateral; ACLD=0.66±0.21, CON=0.83±0.35 N/kg), 180° (medial; ACLD=0.64±0.23, CON=0.81±0.21 N/kg), and 270° (posterior; ACLD=0.81±0.31, CON=1.23±0.24N/kg) directions in ACLD compared to CON. No statistical difference in target force at 90° (anterior; ACLD=0.94±0.19, CON=1.01±0.33 N/kg) was observed.

Significantly greater group mean peak VL, MG, and ADD EMG amplitudes were observed in ACLD compared to CON (Table 3; Figure 11). ACLD elicited significantly reduced hip and knee moments in 9/12 moment types compared to CON (Table 3; Figure 12). ICCs$_{(2,k)}$ for muscle activation and moment patterns ranged, respectively, from 0.82-0.99 and 0.92-0.99 for CON and 0.73-0.97 and 0.75-0.99 for ACLD, indicating high between-subject reliability in both groups.

Table 3: Group mean (X) and standard deviation (SD) of peak muscle activation and hip and knee joint moments during the force matching task. Asterisks (*) beside and a bolded variable name indicates significant between group differences at the p<0.05 level. (Moment acronyms: FL-flexion, EX-extension, AD-adduction, AB-abduction, IR-internal rotation, ER-external rotation).

Peak Muscle Activation *(EMG/EMG$_{max}$)*

		TFL	RF	**VL***	VM	BF	ST	LG	**MG***	**ADD***	GM
CON	X	0.21	0.19	0.18	0.17	0.20	0.21	0.17	0.14	0.12	0.21
	SD	*0.11*	*0.10*	*0.09*	*0.10*	*0.07*	*0.09*	*0.09*	*0.07*	*0.04*	*0.10*
ACL	X	0.21	0.18	0.25	0.21	0.20	0.19	0.19	0.20	0.16	0.24
	SD	*0.10*	*0.07*	*0.14*	*0.14*	*0.08*	*0.08*	*0.10*	*0.09*	*0.09*	*0.13*

Peak Internal Moments *(Nm/kg)*

		Hip						Knee					
		FL*	**EX***	AD	**AB***	**IR***	**ER***	**FL***	EX	AD	**AB***	**IR***	**ER***
CON	X	0.59	0.97	0.56	0.56	0.30	0.27	0.36	0.36	0.29	0.31	0.25	0.23
	SD	*0.21*	*0.31*	*0.14*	*0.22*	*0.09*	*0.10*	*0.18*	*0.15*	*0.10*	*0.11*	*0.08*	*0.08*
ACL	X	0.46	0.81	0.49	0.45	0.21	0.21	0.31	0.31	0.27	0.23	0.18	0.14
	SD	*0.22*	*0.20*	*0.16*	*0.14*	*0.08*	*0.09*	*0.16*	*0.16*	*0.09*	*0.06*	*0.08*	*0.08*

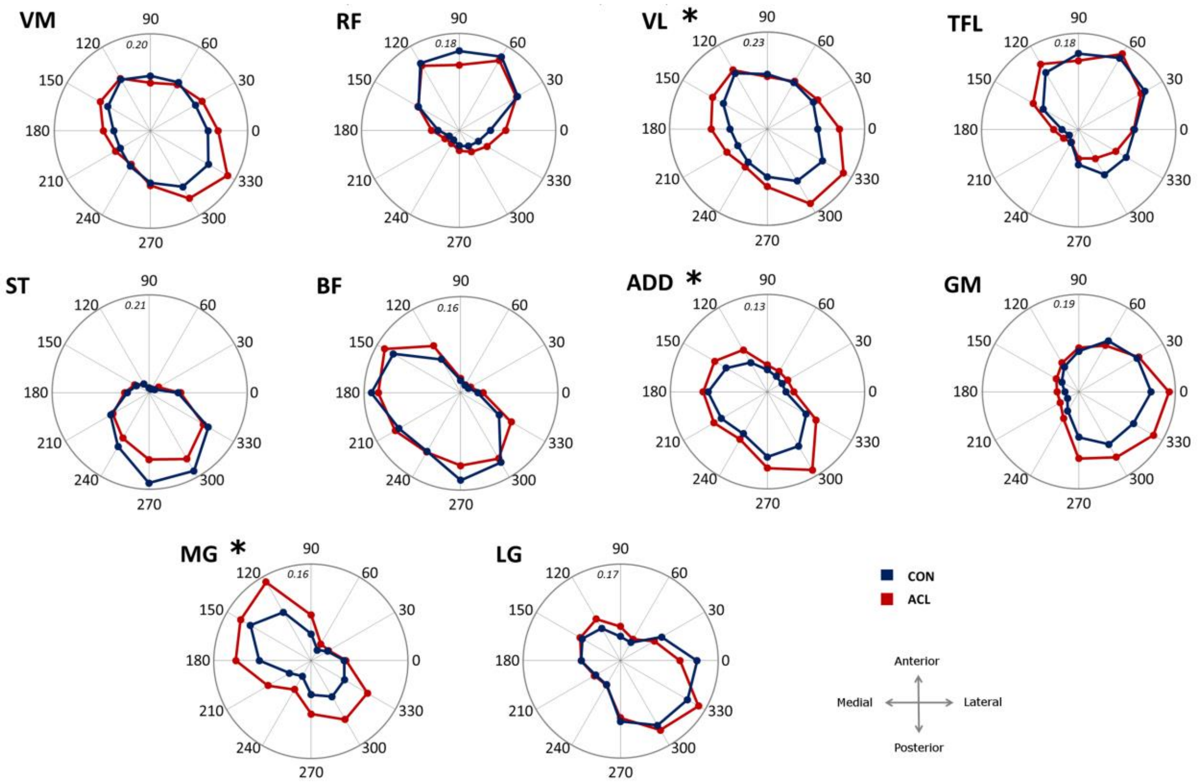

Figure 11: Group mean EMG polar plots. Numbers about the circular trajectory represents force target direction in degrees (0°= pure lateral, 90°= pure anterior, 180°= pure medial, 270°= pure posterior). Where the pattern intersects the radii is the group mean normalized EMG amplitude (EMG/EMG_{max}) required to reach the given target. Asterisks (*) indicates significant between group difference in peak EMG magnitudes at $p<0.05$ level as denoted in Table 3.

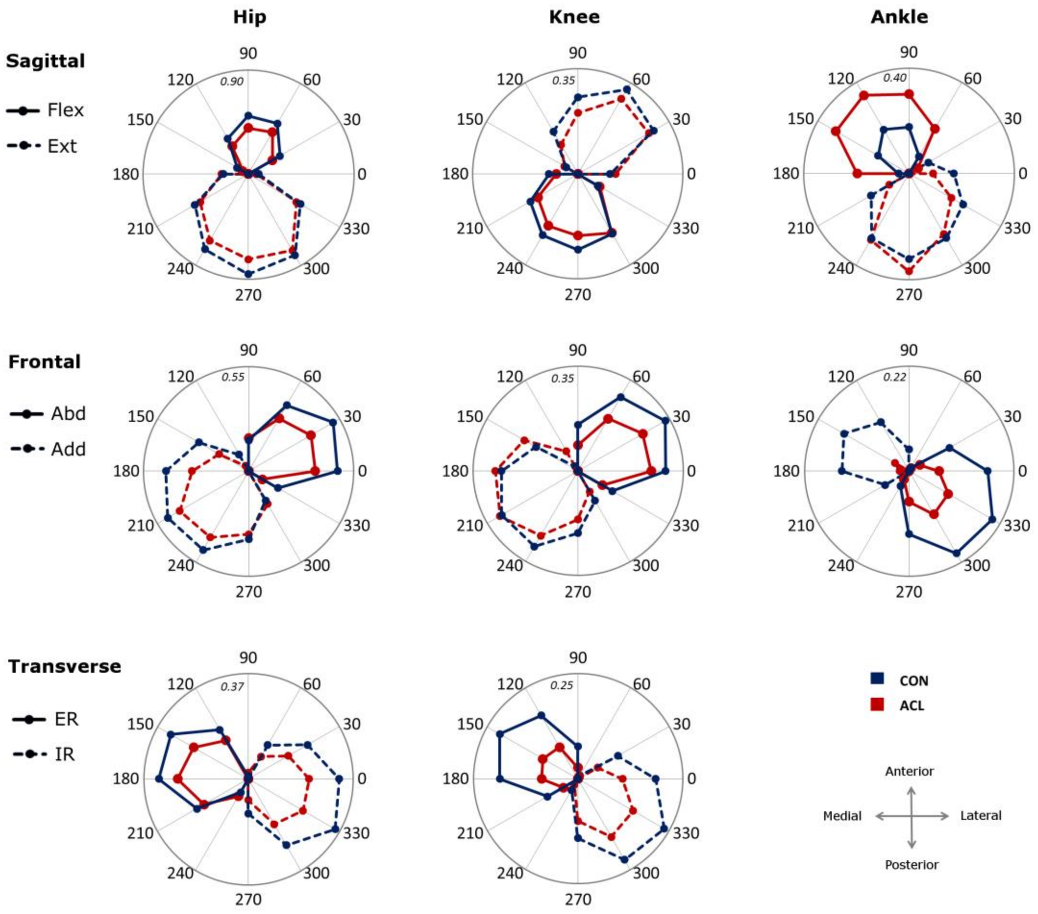

Figure 12: Group mean moment polar plots for each loading plane (X=sagittal; Y=frontal, Z=transverse). Numbers about the circular trajectory represents target force direction in degrees (0°= pure lateral, 90°= pure anterior, 180°= pure medial, 270°= pure posterior). Where the pattern intersects the radii is the normalized moment (Nm/kg) required to reach the force target (ext=extension/ dorsiflexion, flex=flexion/ plantar flexion, abd=abduction/ eversion, add=adduction/ inversion, IR=internal rotation, ER=external rotation moments).

Regression Models

All PLSR models showed significant associations between internal joint moments and muscle activation ($p<0.05$); however, goodness-of-fit (R^2) varied across muscles and groups (Figure 13). For both ACLD and CON groups, ADD and vastii (VM, VL) models showed weak (R^2=0.28-0.29) and very weak goodness-of-fit (R^2=0.06-0.10), respectively, indicating the likelihood of a reciprocal increase in muscle activity with any change in knee joint moments is poor. The LG, MG, GM, and TFL models had moderate goodness-of-fit (R^2=0.30-0.47) while RF and ST models had strong goodness-of-fit values (R^2=0.58-0.62). The BF model was significantly different between groups with moderate fit values for ACLD (R^2=0.37) compared to strong in CON (R^2=0.53). Significantly lower R^2 values for VL, MG, and GM muscles were also observed in ACLD compared to CON.

All muscles showed significant between group differences in one or more of the moment βs except the TFL (Figure 14). As muscle activations relate to knee moments, ACLD showed (1) respective increased and decreased knee extensor βs for RF and vastii; (2) respective increase and decreased knee flexion βs for ST and BF; and (3) respective greater internal knee rotation and reduced external rotation βs for LG and MG, compared to CON. Additionally, LG and MG yielded significant β values for knee flexion in ACLD where CON did not. A notable difference in hip muscles was GM's reduced βs for hip abduction in ACLD compared to CON.

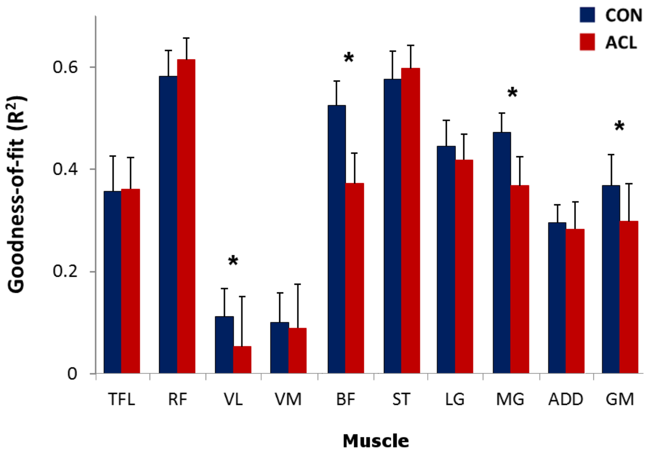

Figure 13: Model goodness-of-fit values indicated with variance explained (R^2) values. Asterisks (*) indicates significant group mean difference at $p<0.05$ level. A very weak model goodness-of-fit was classified with an $R^2<0.1$, weak as $0.1<R^2<0.3$, moderate as $0.3<R^2<0.5$, and strong as $R^2>0.5$.

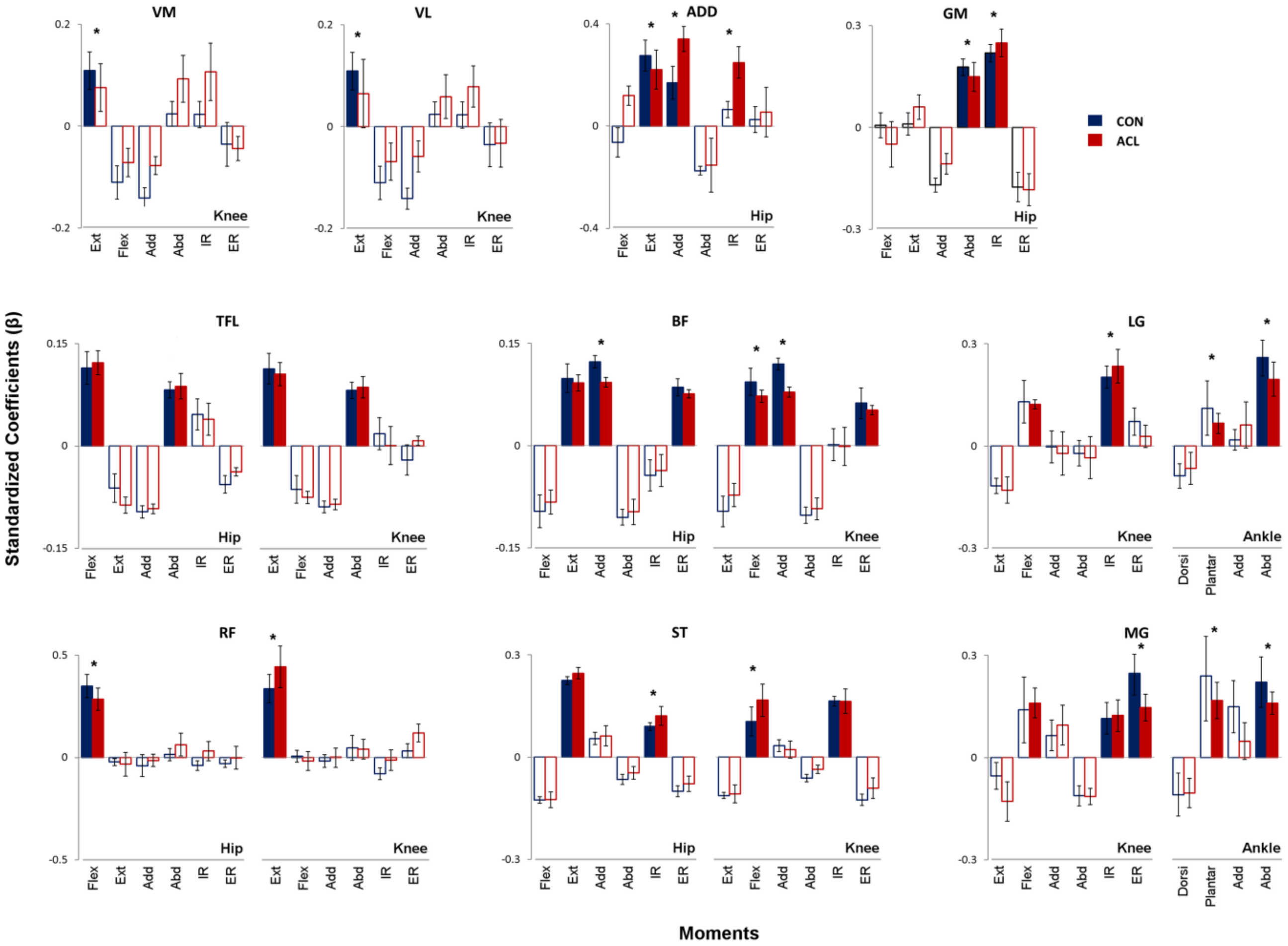

Figure 14: Standardized coefficients (β) of moments for all muscle models. Significant positive β coefficients are represented as filled data bars. Error bars indicate standard deviation of β. Only joints which the given muscle crossed were included in the comparisons. Between group differences in β coefficients are denoted by an asterisks (*) at the p<0.05 level. Moment direction: ext= extension; flex=flexion; add=adduction; abd=abduction; IR=internal rotation; ER=external rotation, Dorsi=dorsiflexion, Plantar=plantarflexion moments.

4.2.5 DISCUSSION

This study sought to identify muscle activation patterns that either increase or reduce knee joint stability in an ACLD population. To enable the link between a muscle's activation and external load to be extracted, we limited biomechanical contributions to force generation (Wilkie, 1950) by using a quasi-static task. We maintained the functional relevance of the task by ensuring it was weight-bearing (equal body weight distributed to both legs) and elicited relatively high and combined loads in the transverse and frontal planes. Moments elicited during this task were comparable in magnitude to those experienced during sport (McLean et al., 2005). We observed significantly greater muscle activation in 3/10 muscles and reduced hip and knee joint moments in ACLD. To determine how changes in muscle activation contributed to changes in internal joint moments (and thus the ability to stabilise the knee), we took a predictive approach using PLS regressions.

Since reports of ACLD knee instability are related to greater knee rotation angles, greater knee abduction angles and moments, and reduced knee flexion moments (Houck and Yack, 2001; Houck et al., 2007), we suggest a stronger relationship (evidenced by greater β values) between a given muscle and knee flexor, knee adductor, and rotational moments helps to improve knee joint stability (Yoo et al., 2005; Li et al., 2007). Compared to CON, ACLD demonstrated stronger relationships between (1) RF and knee extension moments, (2) ST and gastrocnemius to knee flexion moments and reduced relationships between (3) BF and knee flexion, (4) gastrocnemius and external knee rotation, and (5) hip muscles and hip abduction.

Quadriceps

Although there was a significant difference in the knee extension β values in the vastii models, both groups had very weak model goodness-of-fit values ($R^2<0.1$), meaning the likelihood of a reciprocal increase in vastii activity with any change in knee joint moments is poor. In other words, vastii muscle activation is independent of changes in internal net joint moments for both CON and ACLD groups, supporting our interpretation that they are general joint stabilisers (Flaxman et al., 2012, 2013, 2016) contracting to increase compressive loads and joint stiffness in all loading axes (Sasaki and Neptune, 2010; Cashaback and Potvin, 2012).

ACLD participants also demonstrated a greater relationship between RF activation and knee extension moments compared to CON. This finding is supported by previous works (Aune et al., 1997; Rudolph et al., 2001; Urbach et al., 2001). Under certain conditions increased quadriceps activation has been shown to increase ACL strain by generating excessive knee extension moments (Fleming et al., 2001; DeMorat et al., 2004; Shimokochi and Shultz, 2008). Consequently, increased quadriceps activation in populations without strength deficits is commonly interpreted as unfavourable. However, in vitro studies that simulate weight bearing tasks and impose physiological loading magnitudes demonstrates that quadriceps actually has a stabilising role such that increasing its activation increases compressive forces and reduces ACL strain (Aune et al., 1997; Hashemi et al., 2007). Furthermore, ACLD copers have increased quadriceps strength compared to non-copers and more closely resemble non-injured controls (Rudolph et al., 2001; Alkjær et al., 2002; Williams et al., 2005). Taken together, it may be time to re-evaluate the quadriceps and recognize its importance in knee stabilisation.

Hamstrings

Considering hamstring muscles resist anterior tibial translation (MacWilliams et al., 1999), reduced relationship of BF to knee flexion observed in ACLD compared to CON may also contribute to reduced stability after ACL injury. In support, ACLD non-copers show deficits in lateral hamstrings activation during weight acceptance of running/ jogging compared to copers and controls (Rudolph et al., 2001). Alternatively, the greater relationship between ST and knee flexion in the ACLD group was considered a strategy to increase knee stability. This result is noteworthy since the tendon of ST is a commonly used autograft. It is also important to note that hamstring moment arms also contribute to rotational stability (Flaxman et al., 2016).

Therefore its absence after reconstruction may provide insight into the high rates of functional dysfunction following surgical interventions (Thomeé et al., 2007; Frobell et al., 2013).

Furthermore, the inversed adaptation of the medial and lateral hamstrings to knee flexion after ACL injury aligns with previous studies reporting imbalanced antagonist activation in populations exhibiting knee instability (Hortobagyi et al., 2005; Palmieri-Smith et al., 2009; Alkjær et al., 2012). This altered hamstring activation may compromise load distribution across the knee joint, furthering the risk of injury and joint degeneration (MacWilliams et al., 1999; Zeni et al., 2010).

Gastrocnemius

Differences in gastrocnemius activation has been reported in populations with knee instability (Rudolph et al., 2001; Benoit et al., 2003; Astephen Wilson et al., 2015), but how this change impacts knee loads is unclear. Since the gastrocnemii are considered synergistic to the quadriceps, aiding to compress the knee and lower ACL forces while weight bearing (Durselen et al., 1995; Kvist and Gillquist, 2001; Morgan et al., 2014), we suggest the significant relationship between gastrocnemii and knee flexion adopted by ACLD is a protective knee stabilisation strategy.

The gastrocnemius also has potential to contribute to rotational knee moments (Agur and Dalley, 2005) and is commonly the first to activate during rotational perturbations (Carcia et al., 2005). Consider that external tibial rotation greatly affects ACL strain (Shimokochi and Shultz, 2008), and external tibial rotation is a major component of the ACL injury mechanism (McLean et al., 2005), The stronger relationship between LG and knee internal rotation moments, and the weaker relationship between MG and knee external rotation moments in

ACLD compared to CON suggests these differences are protective against external tibial rotation. Previously observed variations in ACLD gastrocnemii activity (Rudolph et al., 2001; Benoit et al., 2003; Astephen Wilson et al., 2015) may therefore be more associated with the gastrocnemii's contribution to rotational stability than to sagittal plane loads.

Hip Muscles

Abduction loads are another large determinant of knee instability and a primary contributor to ACL injury mechanism (Markolf et al., 1995; Hewett et al., 2005). To effectively oppose these loads, an internal knee adduction moment is required. BF was the only muscle that demonstrated a significant relationship with knee adduction moments. However, BF lacks sufficient mechanical advantage for generating knee adduction moment (Agur and Dalley, 2005). This result was attributed to a statistical limitation whereby BF's bi-modal pattern consisting of (1) hip extension/knee flexion and (2) hip/knee external rotation moments cannot be dissociated from that of knee adduction moment (Flaxman et al., 2016).

Interestingly, hip abductors are suggested to be the greatest contributors to internal knee adduction moments while knee spanning muscles are more related to compressing the knee joint (reflecting a stabilisation role) (Sritharan et al., 2012). Our results of reduced relationship between GM and hip abductor moment is supported by previous works that show ACLD individuals have weaker hip abductor muscles compared to CON (Thomas et al., 2013). Weakened hip abductors lead to increased knee valgus alignment and reduced knee adductor moments (Jacobs et al., 2007; Henriksen et al., 2009) In addition, our ACLD showed a greater relationship between ADD and hip adduction compared to CON. While weight bearing, increased hip adduction moment will also increase the potential for knee valgus alignment (if all other variables are held constant) (Hollman et al., 2009), suggesting changes in the relationships between hip muscle activation and hip moments after ACL injury are destabilising.

General Considerations

In addition to limitations addressed previously (Flaxman et al., 2016), we normalized our EMG signals to MVIC values. Voluntary deficits in muscle function have been observed in ACLD individuals compared to CON, and these deficits can persist for years following injury and/or reconstruction (Palmieri-Smith et al., 2008), indicating a higher activation would be needed to produce the same force. Even though our task was normalized to maximum effort, ACLD had significantly lower GRF magnitudes, reduced hip and knee joint moments, but no reductions in muscle activation levels. This suggests ACLD is weaker than CON and normalized EMG amplitudes may be overestimated. Since this deficit is most prominent in the quadriceps, this may account for several studies that also report increases in activation of the quadriceps group in ACLD populations compared to controls (Rudolph et al., 2001). It remains unclear whether increased quadriceps activation actually results in greater compressive forces (thus increasing stability) or is an up-regulation of activity to compensate for functional capacity and may not actually be a joint stiffening strategy. Furthermore, for our ACLD group, the time since injury to time of participation was not consistent across participants. Our group mean average and standard deviation was 12.9 ± 14.3 months. This could have contributed to the high variability observed in ACLD because we had acute and chronically injured participants grouped together.

4.2.6 CONCLUSION

This study sought to describe the functional contributions of muscles to maintaining knee joint stability by quantifying the relationships between lower limb muscle activation and internal net joint moments. Differences in these relationships were identified between ACL injured and healthy controls. Since the ACL injury mechanism and subsequent reports of instability are related to greater tibial rotation angles, greater knee abduction angles and moments, and reduced knee flexion moments, it is suggested that our ACLD group maintained knee stability by increasing contribution of (1) RF to knee extension moments, (2) ST and gastrocnemii to knee flexion moments and (3) differences in gastrocnemii contribution to knee rotation moments. In contrast, the ACLD also showed (1) decreased contributions of BF to knee flexion and (2) varied contributions of hip muscles to frontal plane hip moments, which may adversely affect knee joint stability.

We suggest that ACL deficient participants are able to compensate for the injury by optimizing the muscle activation that controls sagittal plane stability while their ability to stabilise the knee against external abduction and rotational loads is limited. It is therefore reasonable to question if the absence of the ACL as a joint stabiliser is paramount in reducing functional capacity in ACL deficient populations, or if knee instability originates from to neuromuscular deficits following injury. Common functional measures for evaluating return to sport readiness in ACLD or reconstructed populations are dominated by sagittal plane loads. Considering that frontal and transverse plane loads are defining factors in the ACL injury mechanism and episodes of giving way, hamstring muscle balance, hip abductor and gastrocnemius function should be emphasized in clinical assessments and rehabilitative/ preventative exercise interventions.

4.2.7 References

Adams D, Logerstedt D, Hunter-Giordano A, Axe MJ, Snyder-Mackler L (2012) Current Concepts for Anterior Cruciate Ligament Reconstruction: A Criterion-Based Rehabilitation Progression. J Orthop Sports Phys Ther 42:601–614.

Agur AMR, Dalley AF (2005) Grant's Atlas of Anatomy. Baltimore, MA: Lippincott Williams & Wilkins.

Alkjær T, Simonsen EB, Magnusson SP, Dyhre-Poulsen P, Aagaard P (2012) Antagonist muscle moment is increased in ACL deficient subjects during maximal dynamic knee extension. The Knee 19:633–639.

Alkjær T, Simonsen EB, Peter Magnusson S, Aagaard H, Dyhre-Poulsen P (2002) Differences in the movement pattern of a forward lunge in two types of anterior cruciate ligament deficient patients: copers and non-copers. Clin Biomech 17:586–593.

Astephen Wilson JL, Dunbar MJ, Hubley-Kozey CL (2015) Knee joint biomechanics and neuromuscular control during gait before and after total knee arthroplasty are sex-specific. J Arthroplasty 30:118–125.

Aune AK, Cawley PW, Ekeland A (1997) Quadriceps muscle contraction protects the anterior cruciate ligament during anterior tibial translation. Am J Sports Med 25:187–190.

Benoit DL, Lamontagne M, Cerulli G, Liti A (2003) The clinical significance of electromyography normalisation techniques in subjects with anterior cruciate ligament injury during treadmill walking. Gait Posture 18:56–63.

Berchuck M, Andriacchi TP, Bach BR, Reider B (1990) Gait adaptations by patients who have a deficient anterior cruciate ligament. J Bone Jt Surg - Ser A 72:871–877.

Besier TF, Lloyd DG, Cochrane JL, Ackland TR (2001) External loading of the knee joint during running and cutting manoeuvres. Med Sci Sports Exerc 33:1168–1175.

Carcia CR, Shultz SJ, Granata KP, Perrin DH, Martin RL (2005) Females recruit quadriceps faster than males at multiple knee flexion angles following a weight bearing rotary perturbation. Clin J Sport Med 15:167–171.

Cashaback JGA, Potvin JR (2012) Knee muscle contributions to joint rotational stiffness. Hum Mov Sci 31:118–128.

Chmielewski TL, Hurd WJ, Rudolph KS, Axe MJ, Snyder-Mackler L (2005) Perturbation training improves knee kinematics and reduces muscle co-contraction after complete unilateral anterior cruciate ligament rupture. Phys Ther 85:740–749.

Delp SL (2007) OpenSim: Open-Source Software to Create and Analyze Dynamic Simulations of Movement. IEEE Trans Biomed Eng 54:1940–1950.

DeMorat G, Weinhold P, Blackburn T, Chudik S, Garrett W (2004) Aggressive Quadriceps Loading Can Induce Noncontact Anterior Cruciate Ligament Injury. Am J Sports Med 32:477–483.

Durselen L, Claes L, Kiefer H (1995) The influence of muscle forces and external loads on cruciate ligament strain. Am J Sports Med 23:129–136.

Fitzgerald GK, Piva SR, Irrgang JJ (2004) Reports of joint instability in knee osteoarthritis: its prevalence and relationship to physical function. Arthritis Rheum 51:941–946.

Flaxman TE, Alkjær T, Simonsen EB, Krogsgaard MR, Benoit DL (Study 1; 2016) Predicting the Functional Roles of Knee Joint Muscles from Internal Joint Moments. Med Sci Sports Exerc 49:527-537.

Flaxman TE, Smith AJJ, Benoit DL (2013) Sex-Related Differences in Neuromuscular Control: Implications for Injury Mechanisms or Healthy Stabilisation Strategies? J Orthop Res 32:310–317.

Flaxman TE, Speirs AD, Benoit DL (2012) Joint stabilisers or moment actuators: the role of knee joint muscles while weight bearing. J Biomech 45:2570–2576.

Fleming B C, Renstrom P, Ohlen G, Johnson RJ, Peura GD, Beynnon BD, Badger GJ (2001) The gastrocnemius muscle is an antagonist of the anterior cruciate ligament. J Orthop Res 19:1178–1184.

Frobell RB, Roos EM, Roos HP, Ranstam J, Lohmander LS (2010) A randomized trial of treatment for acute anterior cruciate ligament tears. N Engl J Med 363:331–342.

Frobell RB, Roos HP, Roos EM, Roemer FW, Ranstam J, Lohmander LS (2013) Treatment for acute anterior cruciate ligament tear: Five year outcome of randomised trial. BMJ Online 346:f323.

Haenlein M, Kaplan AM (2004) A Beginner's Guide to Partial Least Squares Analysis. Underst Stat 3:283–297.

Hashemi J, Chandrashekar N, Jang T, Karpat F, Oseto M, Ekwaro-Osire S (2007) An alternative mechanism of non-contact anterior cruciate ligament injury during jump-landing: In-vitro simulation. Exp Mech 47:347–354.

Henriksen M, Aaboe J, Simonsen EB, Alkjaer T, Bliddal H (2009) Experimentally reduced hip abductor function during walking: Implications for knee joint loads. J Biomech 42:1236–1240.

Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G (2000) Development of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kinesiol 10:361–374.

Herzog W, Longino D (2007) The role of muscles in joint degeneration and osteoarthritis. J Biomech 40:54–63.

Hewett T, Myer G, Ford K, Heidt R, Colosimo A, McLean S, van den Bogert A, Paterno M, Succop P (2005) Biomechanical Measures of Neuromuscular Control and Valgus Loading of the Knee Predict Anterior Cruciate Ligament Injury Risk in Female Athletes. Am J Sports Med 33:492–501.

Hollman JH, Ginos BE, Kozuchowski J, Vaughn AS, Krause DA, Youdas JW (2009) Relationships between knee valgus, hip-muscle strength, and hip muscle recruitment during a single-limb step-down. J Sport Rehabil 18:104–117.

Hortobagyi T, Westerkamp L, Beam S, Moody J, Garry J, Holbert D, DeVita P (2005) Altered hamstring-quadriceps muscle balance in patients with knee osteoarthritis. Clin Biomech 20:97–104.

Houck J, Yack HJ (2001) Giving way event during a combined stepping and crossover cutting task in an individual with anterior cruciate ligament deficiency. J Orthop Sports Phys Ther 31:481-489-495.

Houck JR, De Haven KE, Maloney M (2007) Influence of anticipation on movement patterns in subjects with ACL deficiency classified as noncopers. J Orthop Sports Phys Ther 37:56–64.

Irrgang JJ, Anderson AF, Boland AL, Harner CD, Kurosaka M, Neyret P, Richmond JC, Shelborne KD (2001) Development and validation of the international knee documentation committee subjective knee form. Am J Sports Med 29:600–613.

Jacobs CA, Uhl TL, Mattacola CG, Shapiro R, Rayens WS (2007) Hip abductor function and lower extremity landing kinematics: Sex differences. J Athl Train 42:76–83.

Kristianslund E, Krosshaug T, van den Bogert AJ (2012) Effect of low pass filtering on joint moments from inverse dynamics: Implications for injury prevention. J Biomech 45:666–671.

Krogsgaard MR, Fischer-Rasmussen T, Dyhre-Poulsen P (2011) Absence of sensory function in the reconstructed anterior cruciate ligament. J Electromyogr Kinesiol 21:82–86.

Kvist J, Gillquist J (2001) Anterior positioning of tibia during motion after anterior cruciate ligament injury. Med Sci Sports Exerc 33:1063–1072.

Li G, Papannagari R, DeFrate LE, Yoo JD, Park SE, Gill TJ (2007) The effects of ACL deficiency on mediolateral translation and varus-valgus rotation. Acta Orthop 78:355–360.

Lysholm J, Gillquist J (1982) Evaluation of knee ligament surgery results with special emphasis on use of a scoring scale. Am J Sports Med 10:150–154.

MacWilliams BA, Wilson DR, Desjardins JD, Romero J, Chao EYS (1999) Hamstrings cocontraction reduces internal rotation, anterior translation, and anterior cruciate ligament load in weight-bearing flexion. J Orthop Res 17:817–822.

Mantovani G, Lamontagne M (2016) How Different Marker Sets Affect Joint Angles in Inverse Kinematics Framework. J Biomech Eng. [Epup ahead of print].

Markolf KL, Burchfield DM, Shapiro MM, Shepard MF, Finerman GA, Slauterbeck JL (1995) Combined knee loading states that generate high anterior cruciate ligament forces. J Orthop Res 13:930–935.

McGraw KO, Wong SP (1996) Forming inferences about some intraclass correlation coefficients. Psychol Methods 1:30–46.

McLean SG, Huang X, Su A, Van DB (2004) Sagittal plane biomechanics cannot injure the ACL during sidestep cutting. Clin Biomech 19:828–838.

McLean SG, Huang X, van den Bogert AJ (2005) Association between lower extremity posture at contact and peak valgus moment during sidestepping: Implications for ACL injury. Clin Biomech 20:863–870.

Mikesky A, Mazzuca S, Brandt KD, Perkins SM, Damush T, Lane KA (2006) Effects of strength training on the incidence and progression of knee osteoarthritis. Arthritis Rheum 55:690–699.

Morgan KD, Donnelly CJ, Reinbolt JA (2014) Elevated gastrocnemius forces compensate for decreased hamstrings forces during the weight-acceptance phase of single-leg jump landing: implications for anterior cruciate ligament injury risk. J Biomech 47:3295–3302.

Palmieri-Smith RM, McLean SG, Ashton-Miller JA, Wojtys EM (2009) Association of quadriceps and hamstrings cocontraction patterns with knee joint loading. J Athl Train 44:256–263.

Palmieri-Smith RM, Thomas AC, Wojtys EM (2008) Maximizing Quadriceps Strength After ACL Reconstruction. Clin Sports Med 27:405–424.

Portney LG, Watkins MP (2000) Foundations of clinical research: Applications to practice. Upper Saddle River, New Jersey: Pearson Prentice Hall.

Roos, Lohmander LS (2003) The Knee injury and Osteoarthritis Outcome Score (KOOS): from joint injury to osteoarthritis. Health Qual Life Outcomes 1:64.

Rudolph KS, Axe MJ, Buchanan TS, Scholz JP, Snyder-Mackler L (2001) Dynamic stability in the anterior cruciate ligament deficient knee. Knee Surg Sports Traumatol Arthrosc 9:62–71.

Sasaki K, Neptune RR (2010) Individual muscle contributions to the axial knee joint contact force during normal walking. J Biomech 43:2780–2784.

Shanbehzadeh S, Mohseni Bandpei MA, Ehsani F (2015) Knee muscle activity during gait in patients with anterior cruciate ligament injury: a systematic review of electromyographic studies. Knee Surg Sports Traumatol Arthrosc Off J ESSKA.

Shimokochi Y, Shultz SJ (2008) Mechanisms of noncontact anterior cruciate ligament injury. J Athl Train 43:396–408.

Sinkjaer T, Arendt-Nielsen L (1991) Knee stability and muscle coordination in patients with anterior cruciate ligament injuries: An electromyographic approach. J Electromyogr Kinesiol 1:209–217.

Smith AJJ, Flaxman TE, Benoit DL (2012) Reliability of knee joint muscle activity during weight bearing force control. J Electromyogr Kinesiol 22:914–922.

Sritharan P, Lin Y-C, Pandy MG (2012) Muscles that do not cross the knee contribute to the knee adduction moment and tibiofemoral compartment loading during gait. J Orthop Res 30:1586–1595.

Tegner Y, Lysholm J (1985) Rating systems in the evaluation of knee ligament injuries. Clin Orthop:43–49.

Thomas AC, Villwock M, Wojtys EM, Palmieri-Smith RM (2013) Lower extremity muscle strength after anterior cruciate ligament injury and reconstruction. J Athl Train 48:610–620.

Thomeé P, Währborg P, Börjesson M, Thomeé R, Eriksson BI, Karlsson J (2007) Self-efficacy, symptoms and physical activity in patients with an anterior cruciate ligament injury: A prospective study. Scand J Med Sci Sports 17:238–245.

Urbach D, Nebelung W, Becker R, Awiszus F (2001) Effects of reconstruction of the anterior cruciate ligament on voluntary activation of quadriceps femoris. J Bone Jt Surg - Ser B 83:1104–1110.

Wilkie DR (1950) The relation between force and velocity in human muscle. J Physiol 110:249–280.

Williams GN, Snyder-Mackler L, Barrance PJ, Buchanan TS (2005) Quadriceps femoris muscle morphology and function after ACL injury: a differential response in copers versus non-copers. J Biomech 38:685–693.

Yoo JD, Papannagari R, Park SE, DeFrate LE, Gill TJ, Li G (2005) The effect of anterior cruciate ligament reconstruction on knee joint kinematics under simulated muscle loads. Am J Sports Med 33:240–246.

Zeni JA, Rudolph K, Higginson JS (2010) Alterations in quadriceps and hamstrings coordination in persons with medial compartment knee osteoarthritis. J Electromyogr Kinesiol 20:148–154.

Study 3: Adaptive Muscle Activation Strategies from Experimental Muscle Pain during a Weight-bearing Force Control Task

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4.3.1 ABSTRACT

Background: Reduced voluntary quadriceps activation is a cardinal symptom in populations with knee pathologies. To maintain dynamic stability, the neuromuscular system must compensate by up/downregulating activity of other muscles that cross the knee joint. To evaluate the acute effects of reduced voluntary quadriceps function on knee joint stability, inhibition was induced with muscle pain. Changes in the interrelationship between individual muscle activation and internal net joint moments with and without pain were quantified. **Design:** Randomized cross-over study. **Methods:** 22 healthy participants performed a standing isometric force matching protocol before, during, and 20 minutes after an injection of hypertonic saline (5.8%) to the vastus medialis (VM). Isotonic saline (0.9%) was used as control. The force matching protocol elicited sagittal, frontal and transverse plane joint moments at the hip, knee and ankle. Partial least squares regressions determined which internal moment(s) predicted the activation of 10 lower limb muscles before and during injection. **Results:** Intramuscular injections to VM induced muscle pain leading to reduced VM activity and maximum voluntary knee extensor torque. Compared to baseline, (1) a stronger relationship of semitendinosus to knee flexor moments, (2) reduced relationships of rectus femoris and tensor fascia latae to knee extension moments and (3) reduced relationships of the semitendinosus and lateral gastrocnemius to knee internal rotation moments were observed during hypertonic injection. **Conclusion**: Our results indicate that neuromuscular adaptations to quadriceps muscle pain are protective responses to reduce knee extensor demand and prevent further pain. However, these neuromuscular compensations may compromise one's ability to stabilise the knee against rotational loads. Our findings provide insight into mechanisms of instability and injury in populations with quadriceps muscle weakness.

4.3.2 INTRODUCTION

Quadriceps muscle weakness is a cardinal symptom in populations with anterior cruciate ligament (ACL) injuries (Chmielewski et al., 2004; Palmieri-Smith et al., 2008). Quadriceps weakness following injury is caused by a protective reflex known as arthrogenic muscle inhibition (AMI), a neural activation deficit that reduces the ability to voluntarily activate the quadriceps muscle group (Hopkins and Ingersoll, 2000). Deficits can exceed 20% at 6 months post injury or reconstruction (Snyder-Mackler et al., 1994; Chmielewski et al., 2004), a time when many athletes are cleared to return to activity. AMI is present years after initial joint trauma (Urbach et al., 2001) and is a strong predictor of functional impairment and knee joint instability (Wojtys and Huston, 1994; Mikesky et al., 2000; Rudolph et al., 2001) and is also theorized to be a main contributor to early onset of knee osteoarthritis (Slemenda et al., 1997; Palmieri-Smith and Thomas, 2009).

Individuals with ACL deficient knees have varied movement patterns and muscle activation strategies compared to controls (Rudolph et al., 2001; Alkjaer et al., 2003; Williams et al., 2003). This makes it difficult to characterise neuromuscular compensations… The most common experimental design to elucidate mechanisms of injury and instability is to compare ACL deficient participants to healthy matched controls or uninjured limbs. Participation in such studies can range from 3 months – 7 years post-injury (Palmieri-Smith et al., 2008). At these times, other changes such as malalignment, muscle wasting from disuse, contractures, reconstruction, and joint effusion may have been progressively adapted. Therefore, it remains unclear if observed differences in neuromuscular function are a direct consequence of injury or whether they are related to other chronically related changes.

Models of experimental pain have been used to impair neuromuscular function (Palmieri et al., 2005; Henriksen et al., 2007, 2010, 2011; Arendt-Nielsen et al., 2008). Knee joint effusion (Palmieri et al., 2005) or hypertonic saline injections to the infrapatellar fat pad (Henriksen et al., 2010, 2011) and can simulate a joint injury by acting on the joint's nociceptors and reducing voluntary function (Hodges and Tucker, 2011; Bank et al., 2013) to levels similar to that observed clinically (Palmieri-Smith et al., 2008; Henriksen et al., 2010). However, experimental quadriceps muscle pain can also attenuate extensor strength without impairing the contractile apparatus and yields comparable deficits in quadriceps muscle activation (Graven-Nielsen et al., 2002).

The purpose of this study was to determine the effect of experimentally impaired quadriceps muscle function (induced by vastus medialis muscle pain) on the individual muscular contributions to joint loads as they relate to knee stability. We evaluated which internal net joint moments were interrelated with changes in individual muscle activations (Flaxman et al., 2016). This approach has been used to identify differences between ACL deficient and healthy controls (Flaxman et al., Study 2). Pain-induced changes in muscle activation are often accompanied by changes in joint dynamics (i.e. reduced knee flexion angles, reduced knee extensor moments etc.) (Henriksen et al., 2009, 2011; Bank et al., 2013). Thus, reported differences in muscle activation may be related to changes in movement strategy rather than pain itself. To better relate changes in muscle activity to changes in motor commands, we used an isometric weight-bearing force control task that does not change kinematic and force requirements between painful and non-painful conditions (Flaxman et al., 2012; 2016; Smith et al., 2012). It was hypothesized that intramuscular injections of hypertonic saline will significantly reduce vastus medialis activation and maximum voluntary knee extensor torque generating capability. Since the same mechanical output is required to be met with and without pain, it was also hypothesized that an up-regulation of non-painful synergistic muscles would be observed to compensate for reduced VM function. This change would consequently affect the interrelationship between individual muscle activation and internal knee joint moments.

4.3.3 METHODS

Participants

Twenty-two healthy active adults [12 males (height=181.3±6.3cm; weight=79.8kg; age=27.25±4.0yrs) and 10 females (height=170.9±5.4cm; weight=65.1±9.1kg; age=22.7±1.4yrs)] with no history of significant lower limb injuries were recruited from the University of Copenhagen, Denmark and the surrounding community. Experiments were completed at the University of Copenhagen and all participants provided written informed consent. This study was approved by local ethics committee for the Capital Region of Denmark (De Videnskabsetiske Komiteer for Region Hovedstaden, H-3-2013-126) and the University of Ottawa Research Ethics Board (H06-14-25).

Study Design

A cross-over placebo controlled design tested each participant on 2 days separated by one week (Figure 15). During a test day, three series of experimental tasks were performed, each under a different condition (baseline, with injection, recovery). Baseline (BASE) measurements were conducted in Series 1 and included two sets of force targets and one set of knee extension strength tests. Although the effects of experimental muscle pain are present up to 25 minutes, the perceived presence of pain only lasts 5 minutes (Henriksen et al., 2007). As such, Series 2 (with injection; INJ) was subdivided into two parts (2a and 2b). Series 2a consisted of one set of force targets while 2b consisted of one set of force targets and one set of knee extension strength tests. Immediately before Series 2a and 2b, intramuscular injections were given. Rest times between Series 1, 2a and 2b were 5 minutes. A 20 minute rest period was held between Series 2b and 3 to ensure perception of pain is gone before completing Series 3 (recovery; REC). Series 3 included one set of force targets and one set of knee extension strength tests.

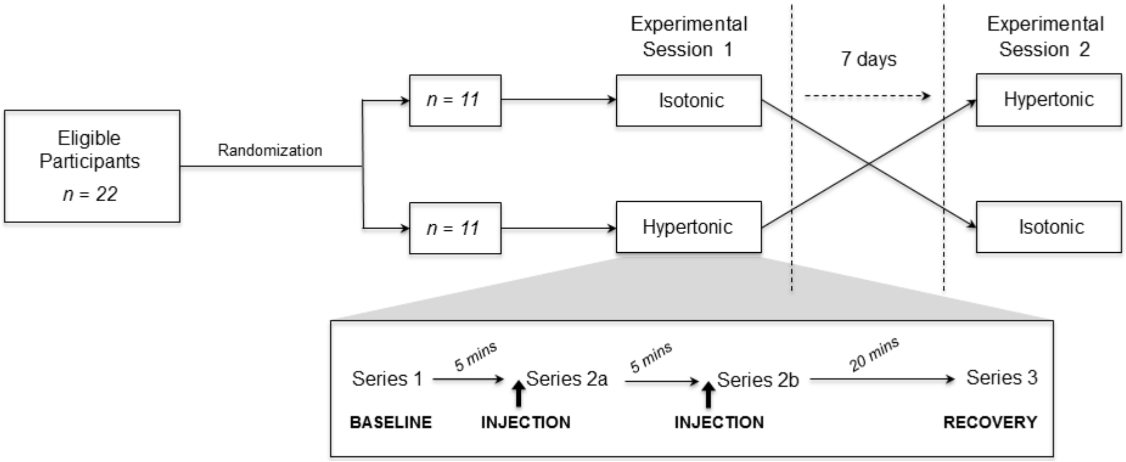

Figure 15: Crossover with placebo-control study design. Eligible participants were randomized into two groups – one group received isotonic (sham/control) injections in the first session and the other group received the hypertonic (pain inducing) injections. Participants returned one week later to repeat the experimental protocol but received the opposite injection.

Experimental Protocol

Injection Protocol

Sterile hypertonic saline (HYP; 5.8%) was used to induce muscle pain while isotonic saline (ISO; 0.9%) was used as control. Only one type of saline was given on a test day. The order of saline injections was randomized. Injections were given into the belly of the VM, approximately 5cm proximal and 5cm medial to the medial corner of the patella. Intramuscular bolus injections of 1ml were manually administered over 10 seconds with a 5-ml plastic syringe with a disposable needle (27 gauge, 40 mm) at a depth of 3.5 cm. Self-perceived pain was monitored every minute during the INJ conditions and at the beginning of the REC condition. Pain was verbally scored on a scale of 0-10, where 0 indicated "no pain" and 10 indicated "worst imaginable pain".

Force Matching Protocol

A detailed description of the protocol has been previously described (Flaxman et al., 2012, 2016; Smith et al., 2012). In short, participants stood in a staggered position such that their feet were spaced hip width apart and their test leg had approximate joint angles of 30° hip flexion, 30° knee flexion, and 10° ankle plantarflexion. The test leg was placed in a wakeboard boot fixed to a force platform. Participants controlled a projected image of a cursor by pushing against the force platform with their foot, while maintaining equal body weight on each leg. Successful match of the cursor to a force target for half a second triggered data collection. A set of 12 force target directions randomly appeared about a circular trajectory, representative of various horizontal anterior-medial-posterior-lateral loading directions. Force required to match the targets was normalized to 60% max effort while distributing equal body weight to each leg.

Participants also completed a set of squatting and lunging trials after each target series but the data was not analysed in this study. This data has been reported in a separate study (Flaxman et al., Study 5).

Knee Extension Strength Tests

To confirm the inhibitory effect of the HYP on VM function, a secondary analysis was conducted combining strength and musculoskeletal modeling data. A subset of thirteen participants (6 males; 7 females) completed knee extension strength tests during each condition using an isokinetic dynamometer (KinCom, Chattex, Chattanooga, TN). Participants sat with their hip flexed to 90° and test knee flexed to 30°. Participants ramped up their force from 0-100% over two seconds and held their maximum for three seconds. Three repetitions were conducted for each condition with a 45-second rest between reps. Visual feedback and verbal encouragement was provided. An external trigger was used to synchronize torque and EMG signals.

Equipment and Data Processing

Surface electromyography (EMG) signals of 10 lower limb muscles (rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), medial hamstrings (MH), lateral hamstrings (LH), medial gastrocnemius (MG), lateral gastrocnemius (LG), tensor fascia lata (TFL), gluteus medius (GM), and the adductor muscle group (ADD)) were recorded with bipolar electrodes, sampling at 1000Hz with a 20-500Hz bandwidth and a 6dB/octave filter slope recorded using a wireless EMG system (MQ air, Marq Medical, Farum Denmark). All EMG signals were high-pass filtered at 20Hz with a 2nd order dual-pass Butterworth filter, full wave rectified, low-pass filtered at 10Hz a with 2nd order dual-pass Butterworth filter. EMG was normalized to maximum EMG value (EMG_{max}) from previously recorded maximum voluntary isometric contraction exercises (Flaxman et al., Study 1).

A ten-camera motion analysis system (6 MXF-40s and 4 T40-series cameras, Oxford Metrics, Oxford, UK) sampling at 100 Hz with supporting Nexus software (version 1.8, Oxford Metrics, Oxford, UK) recorded trajectories of retro-reflective markers of the HMBL cluster marker set (Mantovani and Lamontagne, 2016) (Appendix A). GRFs recorded from a force platform (AMTI-OR6, AMTI, Watertown, MA) were also collected in Nexus at 1000Hz with a gain of 1000. Raw marker trajectories and GRFs were conditioned with a 2nd order 15 Hz dual-pass low pass Butterworth filter (Kristianslund et al., 2012) in Nexus and exported to OpenSim (v 3.2, (Delp, 2007)) to compute lower limb joint angles and internal net joint moments using inverse kinematics and dynamics. EMG, kinematic and kinetic data were time averaged over the half second of successful force target match and ensemble averaged across repetitions.

Torque from the knee extension strength tests were collected at 1000Hz with a custom Matlab application. Torque signals were corrected for the mass of the leg resting in the dynamometer's arm and filtered with a 2nd order 10Hz dual-pass low-pass Butterworth filter. For each condition, torque and corresponding EMG was averaged across a 50ms range about the peak torque value. EMG was normalized to EMG_{max}. These values represented the experimental torque (T_E) and EMG at peak torque used in the musculoskeletal model described below.

Data Analyses

Strength Deficit Model

A detailed description of the strength deficit framework has been reported previously (Bigham, 2016) and is provided in Appendix C. An optimization framework was used to minimise the error between experimental knee extension torque (T_E) and a simulated torque (T_M) (Figure 16). T_M was computed using an EMG driven musculoskeletal model with an ideal force actuator. Once convergence was met, the optimal scaling parameters were used to compute a theoretically ideal torque (T_I), which represented the muscles' full capacity of activation. T_I had all knee extensor muscles (RF, VL and VM) set at 100% of their potential muscle force with knee flexors (BF, ST, LG, and MG) producing no opposing force. A strength ratio, $\alpha_s = T_E/T_I$, was calculated for each participant to determine knee extensor inhibition (lower value represents greater level of inhibition).

Torque contribution ratios for RF, VL, and VM to T_E were calculated by:

$$C_j = \frac{s_j F_{0,j}^{max} r_j a_j}{\sum_{j=1}^{3} s_j F_{0,j}^{max} r_j a_j}$$

Where C_j is for contribution indices $j \in \{1,2,3\}$ referring to 3 extensor muscles; *s*, *r*, $F_0^{\max}$ and *a* designate the scale parameter, moment arm, maximum isometric force parameter, and normalized EMG of the muscles during the experimental trial. The contributions will sum to 1.0.

Two-way repeated measure ANOVAs tested the effect of injection type and condition on the maximum voluntary experimental torque (T_E), the EMG at peak T_E for RF, VL, and VM, the strength ratio (α_s), and the individual muscular contribution ratios (C_j) to extension torque. Post-hoc analysis was conducted with two planned comparisons (1) BASE to INJ and (2) BASE to REC. A Bonferroni adjustment of alpha was made to 0.025 based on the number of comparisons to be made within a session.

Relative change (RC) from BASE to INJ and from BASE to REC was quantified as:

$$RC = \left(\frac{INJ\ or\ REC - BASE}{BASE}\right) * 100$$

Significant between session differences (HYP vs ISO) in the RC across conditions were tested with dependent T-tests at the p<0.05 level.

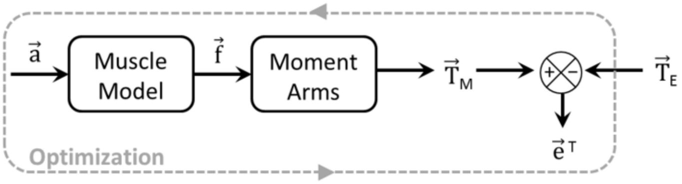

Figure 16: Schematic of EMG-driven strength tuning framework where $\vec{a}$, $\vec{f}$, $\vec{T}_M$, $\vec{e}^T$, and $\vec{T}_E$ represent activation, muscle force, muscle torque, torque error and experimentally measured isometric torque respectively (Figure adapted from Bigham (2016), page 111).

Regression Models

Group mean EMG and kinetic data elicited during the force matching protocol were plotted in polar coordinates to display how muscle activation and moments modulate as a function of GRF loading direction. One way ICCs$_{(1,k)}$ evaluated the within subject similarity of muscle activation and moment patterns from BASE to INJ conditions during the HYP session (McGraw and Wong, 1996).

Partial least square regression (PLSR) models (XLSTAT, New York, NY) with cross-validation (leave-one [subject]-out method) (Wold et al., 1984; Haenlein and Kaplan, 2004) evaluated the relationship between individual muscle activations (dependent variable) and internal net joint moments (predictor variables) (Flaxman et al, 2016). The BASE and INJ conditions of the HYP session were assessed. Model goodness-of-fit was measured with the variance explained (R^2) statistic. Very weak model goodness-of-fit was defined as $R^2<0.1$, weak as $0.1<R^2<0.3$, moderate as $0.3<R^2<0.5$, and strong as $R^2>0.5$). A predictor's standardized beta coefficient (β) described the relative increase in muscle activation (EMG/EMG_{max}) with an increase in a given moment of 1 Nm/kg when all other moments are held constant. A greater β indicates a stronger relationship between the given moment variable and muscle activity. Only significant positive coefficients (determined with 95% confidence intervals) were considered meaningful.

Significant between condition differences in sagittal plane hip, knee, and ankle joint angles, peak muscle activation, peak moment, R^2 and β values were determined with paired T-tests at the alpha=0.05 level.

4.3.4 RESULTS

Effect of Injection Type

A significant effect of injection type on self-perceived pain scores was observed (Figure 17). Dependent T-Tests showed that HYP session had significantly greater mean (standard deviation) scores during the INJ condition (~minute 0-5) than ISO session: HYP=3.45 (1.69) versus ISO=0.29 (0.55) ($p<0.001$). Pain scores of REC (~minute 25) were not significantly different between sessions.

There was a significant Session x Condition interaction for both maximum voluntary absolute (Nm) and normalized (Nm/kg) knee extension torque. Post hoc comparisons showed both sessions (HYP and ISO) had a significant decrease from BASE to INJ condition (Figure 18A). Only the HYP session showed a significant decrease in torque during REC compared to BASE. A significant Session x Condition interaction was observed for VM's EMG at peak torque; however, only the HYP session had a significant reduction from BASE to INJ (Figure 18B).

For the strength deficit model, there was a significant Session x Condition interaction for strength ratio (α_s) and the VM's contribution to the experimental torque (T_E). Post hoc comparisons showed a significant reduction in α_s and VM contribution from BASE to INJ during the HYP session (Figure 18C-D). Significant between session differences in the RC of VM contribution was also observed. No significant between condition differences were observed during the ISO session.

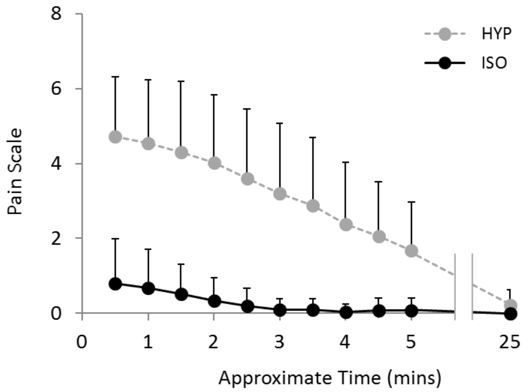

Figure 17: Perception of pain during both the HYP (pain inducing) and ISO (sham) sessions of the sets with intramuscular injection. 0 mins indicates time of injection. At minute 25 is the approximate beginning of Series 3 (recovery) tasks. Error bars are indicates standard deviation.

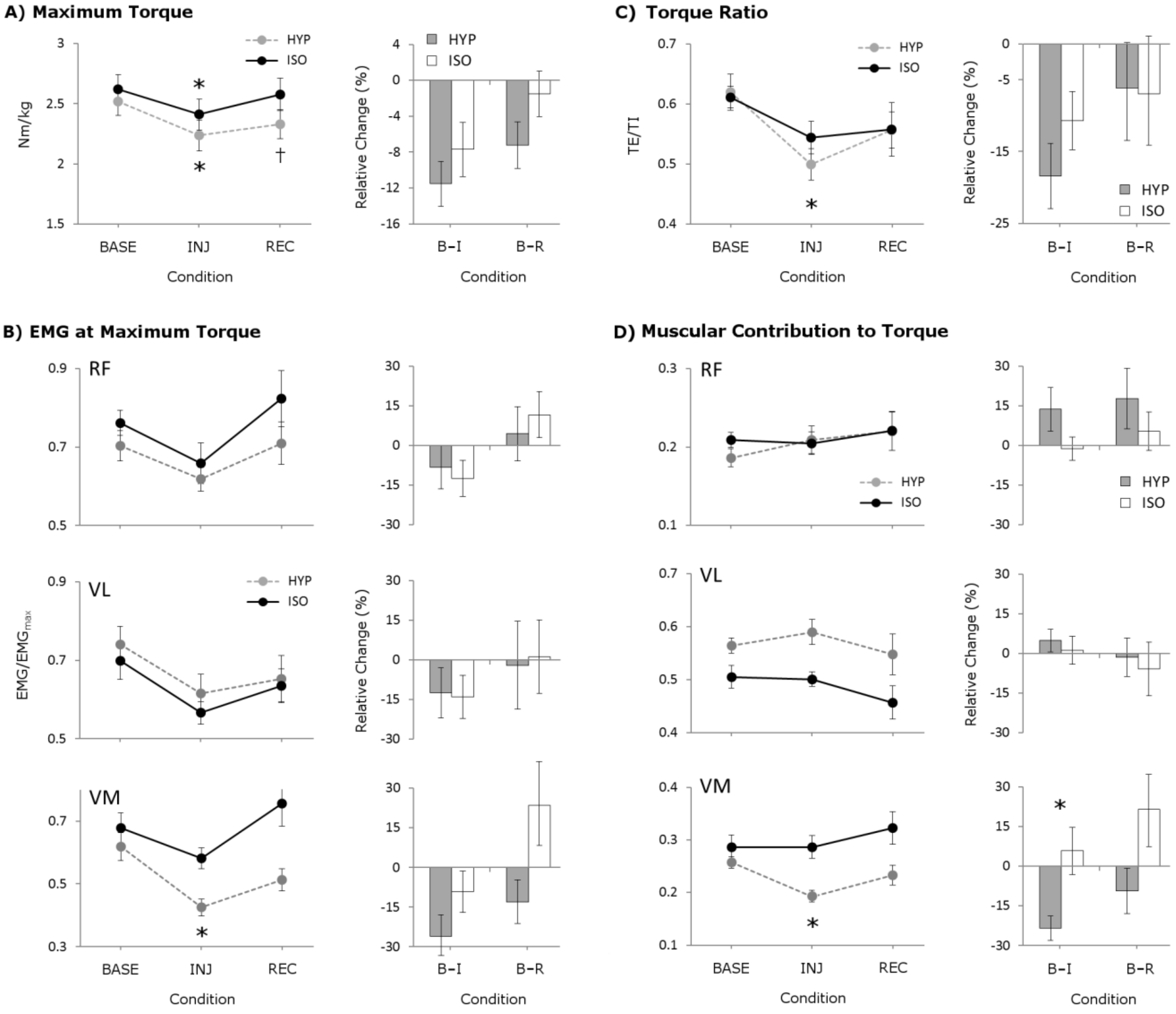

Figure 18: Group mean and standard errors during HYP and ISO sessions for: **(A)** normalized maximum knee extension torque (Nm/kg) and **(B)** normalized EMG values at time of peak torque (EMG/EMG$_{max}$) **(C)** ratio between experimental torque (TE) and ideal torque (TI) computed from a musculoskeletal model and **(D)** the contribution of each muscle to ideal torque value (TI) (note: the sum of RF, VL, and VM's contribution to each condition equals 1.0). Line graphs indicate the change in each variable across conditions where asterisks (*) indicate significant difference between baseline and injection condition and crosses (†) indicate significant difference between baseline and recovery conditions. Bar graphs indicate the relative percent (%) deficit of each variable from baseline to injection (B – I) and from baseline to recovery (B – R) conditions. Asterisks (*) indicates significant difference between sessions. All significance was denoted at the $p<0.025$ level.

Table 4: Force matching task group mean (X) and standard deviation (SD) of peak muscle activation and hip and knee joint moments for the BASE and INJ conditions during the HYP session. Asterisks (*) and a bolded variable name indicates significant between group differences at the p<0.05 level. (Moment acronyms: FL-flexion, EX-extension, AD-adduction, AB-abduction, IR-internal rotation, ER-external rotation). Intraclass correlation coefficients ($\mathrm{ICC}_{(1,k)}$) of within subject similarity of muscle activation or moment patterns from BASE to INJ.

Peak Muscle Activation *(EMG/EMG_{max})*

		TFL	RF	VL	**VM***	BF	ST	LG	MG	ADD	GM
BASE	X	0.24	0.20	0.14	**0.17**	0.16	0.20	0.13	0.15	0.12	0.21
	SD	*0.13*	*0.10*	*0.07*	***0.15***	*0.08*	*0.07*	*0.08*	*0.10*	*0.06*	*0.07*
INJ	X	0.23	0.19	0.14	**0.12**	0.17	0.19	0.12	0.14	0.12	0.19
	SD	*0.13*	*0.11*	*0.07*	***0.09***	*0.10*	*0.09*	*0.07*	*0.09*	*0.06*	*0.07*
ICC	X	0.91	0.86	0.55	**0.47**	0.88	0.93	0.78	0.70	0.84	0.91
	SD	*0.10*	*0.11*	*0.39*	***0.32***	*0.11*	*0.09*	*0.21*	*0.29*	*0.08*	*0.11*

Peak Internal Moments *(Nm/kg)*

		Hip						Knee					
		FL	EX	AD	AB	IR	ER	FL	EX	AD	AB	IR	ER
BASE	X	0.64	0.94	0.64	0.64	0.32	0.31	0.32	0.36	0.28	0.34	0.27	0.25
	SD	*0.22*	*0.27*	*0.16*	*0.17*	*0.09*	*0.07*	*0.15*	*0.18*	*0.09*	*0.12*	*0.08*	*0.06*
INJ	X	0.63	0.92	0.65	0.63	0.32	0.30	0.32	0.34	0.27	0.34	0.26	0.25
	SD	*0.21*	*0.30*	*0.15*	*0.15*	*0.09*	*0.07*	*0.16*	*0.18*	*0.08*	*0.11*	*0.09*	*0.06*
ICC	X	0.99	0.98	0.99	0.99	0.99	0.99	0.94	0.93	0.99	0.99	0.99	0.99
	SD	*0.01*	*0.02*	*0.00*	*0.01*	*0.01*	*0.01*	*0.07*	*0.08*	*0.02*	*0.02*	*0.01*	*0.01*

Force Matching Data

Significant differences between BASE and INJ conditions of the HYP session were evaluated. Differences between BASE and INJ conditions of the ISO session were not evaluated because there were no significant between condition differences in VM contribution to maximal torque (Figure 15D). Respective hip flexion, knee flexion, and ankle plantarflexion mean (standard deviation) joint angles were 28.7 (2.9°), 25.4 (3.4°), 2.9 (1.8°) at BASE and 27.1 (2.6°), 24.9 (3.2°), 2.1 (1.7°) during INJ. Joint angles were normally distributed across participants in each group (Shapiro-Wilk p>0.05) and no significant between condition differences were observed.

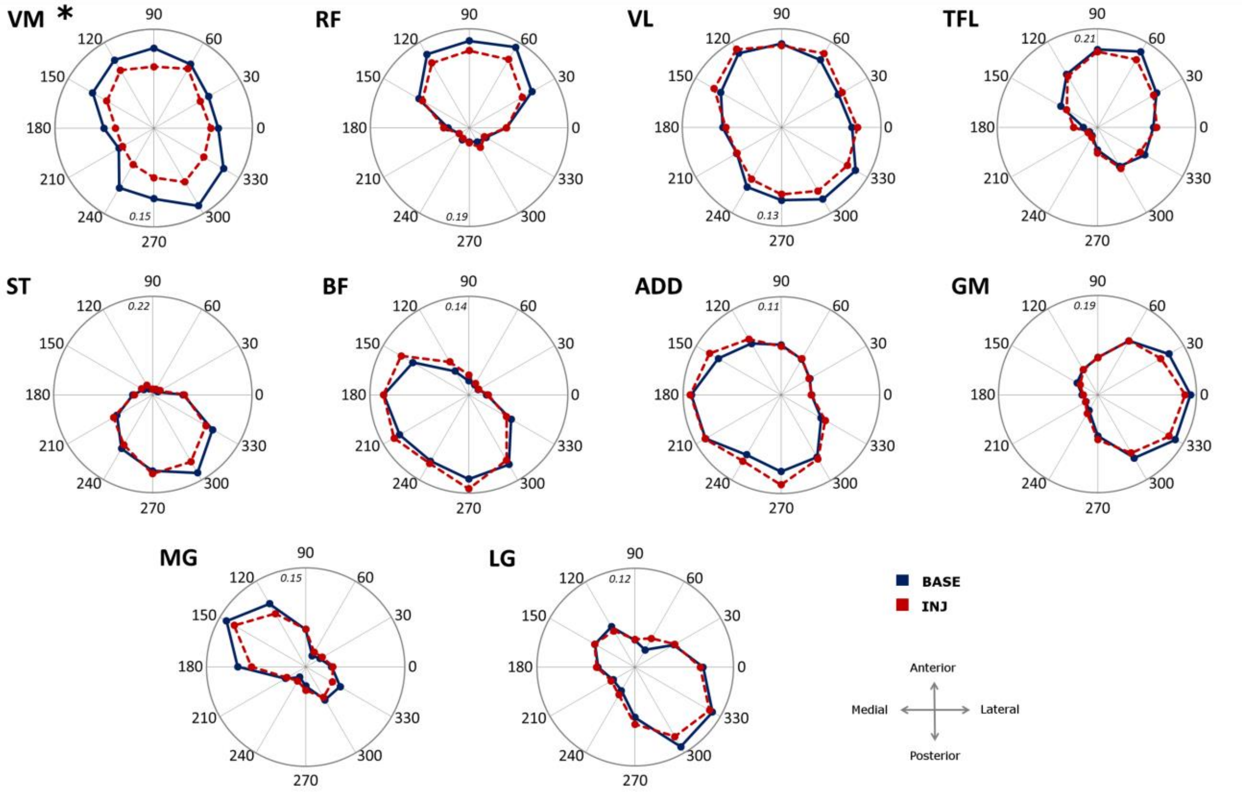

Figure 19: Group mean EMG polar plots. Numbers about the circular trajectory represents GRF target direction in degrees (0°= pure lateral, 90°= pure anterior, 180°= pure medial, 270°= pure posterior). Where the pattern intersects the radii is the group mean normalized EMG amplitude (*EMG/EMG_{max}*) required to reach the given target. Italicised numbers inside the plot is the relative scale of each muscle. Asterisks (*) indicates significant between group difference in peak EMG magnitudes at p<0.05 level as denoted in Table 4.

Mean (standard deviation) force required at 0° (lateral), 90° (anterior), 180° (medial), and 270° (posterior) target directions were respectively 1.01 (0.26), 1.15 (0.24), 0.96 (0.17), 1.53 (0.38) N/kg at BASE and 1.02 (0.27), 1.15 (0.24), 0.97 (0.17), 1.54 (0.38) N/kg during INJ. No significant between condition differences were observed.

Group mean EMG and kinetic data were plotted in polar coordinates to display how muscle activation and moments modulate as a function of GRF loading direction (Figure 19 and Figure 20, respectively). Group mean peak VM activation was significantly greater in the BASE condition compared to INJ (Table 4). No significant differences in other peak muscle activation or peak joint moments were observed (Table 4). Within-subject mean (standard deviation) ICCs ranged from 0.47-0.93 (0.08-0.39) for muscle activation profiles and 0.93- 0.99 (0.00-0.02) for moment profiles (Table 4).

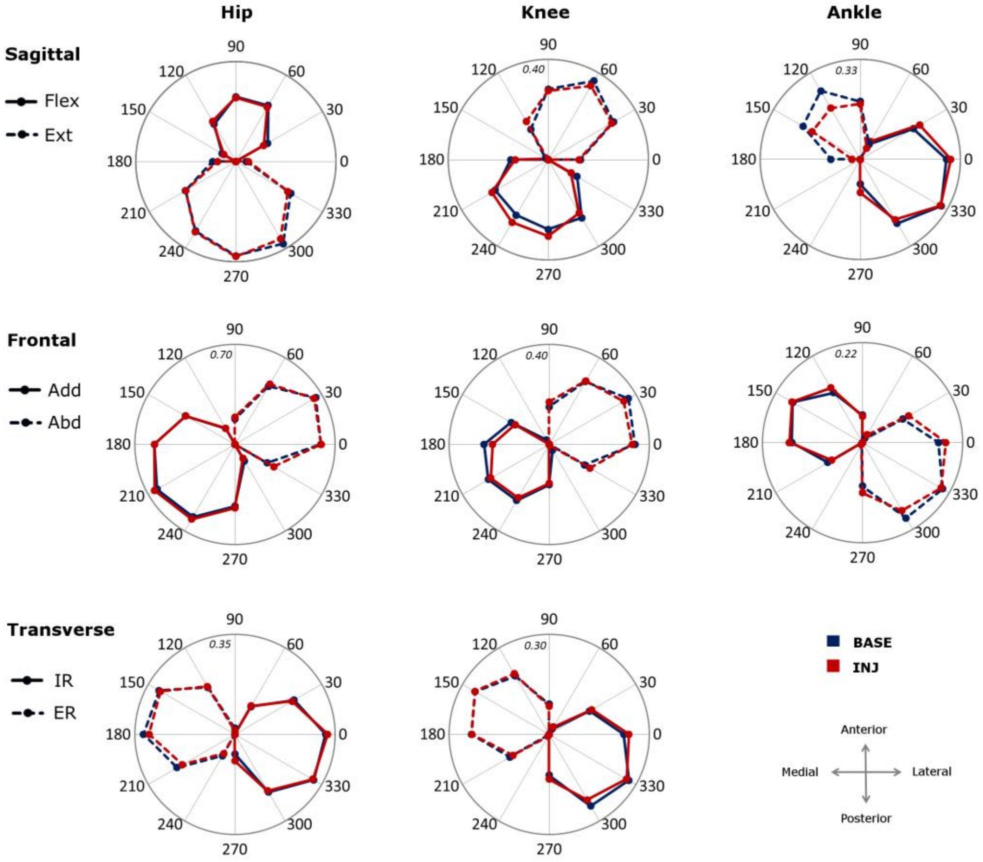

Figure 20: Group mean moment polar plots. Plots are scaled to the italicized number inside the circular trajectory with units Nm/kg. Numbers about the circular trajectory represents force target direction in degrees (0°= pure lateral, 90°= pure anterior, 180°= pure medial, 270°= pure posterior). Where the pattern intersects the radii is the normalized moment (Nm/kg) required to reach the force target (ext=extension/ dorsiflexion, flex=flexion/ plantar flexion, abd=abduction/ eversion, add=adduction/ inversion, IR=internal rotation, ER=external rotation moments). Italicised numbers inside the plot is the relative scale of each muscle. Columns are the given joint and rows are the anatomical loading planes.

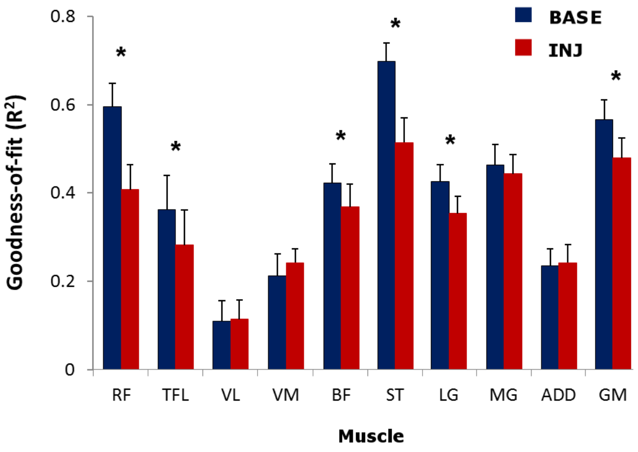

Figure 21: Model goodness-of-fit values indicated with variance explained (R^2) values. Asterisks (*) indicates significant between condition differences at $p<0.05$ level. A very weak model goodness-of-fit was classified with an $R^2<0.1$, weak as $0.1<R^2<0.3$, moderate as $0.3<R^2<0.5$, and strong as $R^2>0.5$.

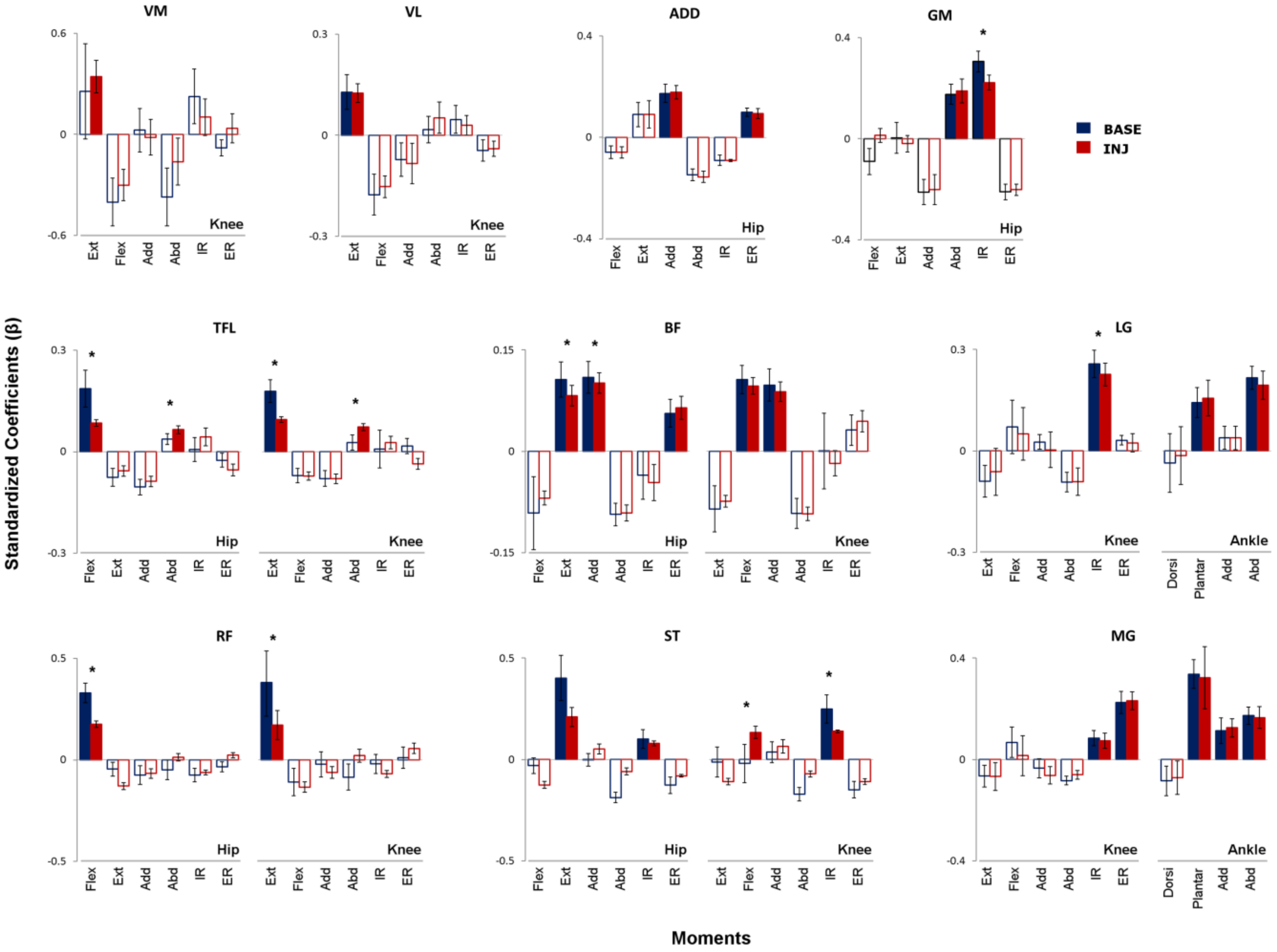

Figure 22: Standardized coefficients (β) of moments for each muscle's PLSR model during BASE and INJ conditions of the HYP session. Significant positive β coefficients are represented as solid data bars. Error bars indicate standard deviation of β. Only joints which the given muscle crossed were included in the comparisons (ext= extension; flex=flexion; add=adduction; abd=abduction; IR=internal rotation; ER=external rotation, Dorsi=dorsiflexion, Plantar=plantarflexion moments). Group mean differences in β coefficients at denoted by an asterisks (*) at the p<0.05 level.

Regression Models

PLSR models were performed for the BASE and INJ condition of the HYP session. All PLSR models showed significant associations between internal joint moments and muscle activation ($p<0.0001$). However, model goodness-of-fit varied across muscles and conditions ($R^2=0.11$-0.70) (Figure 21). The INJ condition yielded significantly reduced R^2 values compared to BASE for 6 of 10 muscles: TFL, RF, BF, ST, LG, and GM, indicating reduced variance accounted for by the model.

Muscles with significantly reduced goodness-of-fit values also showed significant between-condition differences in one or more of the moment βs (Figure 22). Note that a greater β indicates a stronger relationship between the given moment variable and muscle activity. RF and TFL had significantly reduced INJ βs for both hip flexion and knee extension compared to BASE. TFL also had significant βs for hip and knee abduction in INJ where the BASE model did not. Reduced hip extension and hip adduction βs were shown in BF's INJ model compared to BASE. The ST's INJ model showed significantly increased knee flexion and reduced internal rotation βs compared to BASE. LG also showed significantly reduced knee internal rotation βs in its INJ compared to BASE. GM presented reduced hip internal rotation βs in the INJ condition compared to BASE.

The VM, VL, ADD, and MG muscles did not show significant differences in goodness-of-fit values or β coefficients between conditions (Figure 21 and Figure 22). However, the VM model yielded significant β values for knee extension in the INJ condition where the BASE model did not.

4.3.5 DISCUSSION

The goal of this study was to identify muscle activation patterns that either increase or reduce knee joint stability during acute muscle weakness, induced with experimental muscle pain. We evaluated the effect of experimental muscle pain on (1) VM activation, (2) voluntary knee extensor strength, and (3) the neuromuscular compensations quantified as changes in the interrelationship between internal net joint moments and individual muscle activations.

Both HYP and ISO sessions showed a significant reduction in maximum voluntary isometric knee extension torque from BASE to INJ, contrasting previous studies that showed a significant reduction in voluntary torque only in a HYP session (Henriksen et al., 2007, 2011). We computed a strength ratio using a simple musculoskeletal model (Bigham, 2016) to elucidate the effect of injection type on voluntary knee extensor function. A significant strength deficit was observed from BASE to INJ condition in the HYP session while the ISO did not. This deficit was attributed to a significant decrease in muscular contribution by the VM to experimental torque in the HYP session. We did not observe a significant strength deficit during the REC condition (approximately 25 minutes after the second intramuscular injection), also contrasting previous works (Henriksen et al., 2007, 2011). Nevertheless, our results confirm the inhibitory effect of HYP on VM function and knee extensor strength.

Inhibition of the VM was also confirmed during the force matching protocol. We hypothesized that there would be an up-regulation of activation from non-painful synergistic muscles (VL and RF) and although this was not evident using group mean peak activations, our PLSR models were able to identify significant differences in the inter-relationship between muscle activation and joint moments from BASE to INJ conditions. A central premise of Hodges and Tucker's (2011) theory is that muscle pain changes the mechanical output of a task in order to reduce loads acting on the painful joint/tissue. Our study is particularly well suited to evaluate this hypothesis since it is highly reliable (Smith et al., 2012) and requires participants to generate the same mechanical output across conditions. This resulted in highly repeatable within subject moments across each condition (ICCs=0.93-0.99 with low SDs). Conversely, the muscles activations were more variable (mean ICCs=0.47-0.93 with higher SDs). We therefore attribute the pain induced changes in the activation-moment relationships to differences in muscle activation.

Although the peak activation level of VM was significantly reduced from BASE to INJ, this effect was not as obvious in the PLSR outputs. We attribute this to its weak model-fit-values indicating its activation is not dependent on loading/moment direction (Flaxman et al., 2016). However, knee extension was identified as a significant predictor in INJ but not during BASE, suggesting VM's antagonist contribution/ co-activation is reduced. This can be seen in the mean EMG plots (Figure 19) where there is relatively greater difference between BASE and INJ magnitudes during force directions that do not elicit knee extensor moments.

Compared to BASE, the INJ condition demonstrated weaker R^2 in the TFL, RF, ST, BF, GM and LG muscle models. Reduced R^2 may be attributed to increased co-contraction resulting in decreased specificity of a muscle's activation patterns as it relates to changes in moments, a common characteristic in populations with muscle weakness and knee pathologies (Alkjær et al., 2003; Williams et al., 2003; Alkjær et al., 2012). In addition, due to a redundant human neuromuscular system and multiple levels of motor modulation (cortical, spinal, or muscular), numerous options exist to increase, decrease or redistribute muscle activity (Hodges and

Tucker, 2011; Bank et al., 2013) and reduced R^2 during pain may indicate different compensation strategies across participants.

The same muscles with reduced R^2 also showed significantly different relationships between normalized muscle activation and moments. Compared to BASE, the interrelationship between RF and TFL to knee extensor moments were reduced while the interrelationship between ST and knee flexor moments was increased during INJ. While these adaptations may yield immediate benefits for pain management, similar adaptations have been observed in ACL deficient participants compared to healthy controls (Flaxman et al., Study 2), suggesting neuromuscular adaptations to acute muscle pain or muscle weakness may have long-term consequences on knee stability and joint health. This observation opens the door to explore which came first: the adaptation in ACL injured due to the pain of the injury, or the instability it incurred.

We also observed significantly reduced relationships of ST and LG to knee internal rotation moment. Considering that ACL strain is amplified with knee external rotational loads (Shimokochi and Shultz, 2008) and external rotation of the tibia with respect to the femur is a major component of the ACL injury mechanism (McLean et al., 2005), the reduced relationship between ST and LG to knee internal rotation moments implies the presence of muscle pain and/or weakness reduces muscular contribution to rotational stability.

Since gluteal muscles, hip abductors and hip adductors are large determinants of femoral orientation and knee joint loads (Sasaki and Neptune, 2010), this study included their analysis and contribution to hip moments. We have interpreted a significant relationship between GM and internal hip rotation moments as antagonistic activation to stabilize the hip

(Flaxman et al., 2016). As such, a significant reduction in this relationship during INJ is noteworthy since reduced hip function de-stabilizes the femur, causing an increase in frontal plane knee joint motion and moments (Powers, 2010).

4.3.6 CONCLUSION

This study demonstrated that experimental muscle pain inhibits voluntary muscle activation of the target quadriceps muscles and affects voluntary knee extensor strength. Using our PLSR approach, we identified several significant differences in the interrelationship between individual muscle activation and internal joint moments that were not observed with analysis of discrete variables. Our results showed a reduced relationship of RF and TFL to knee extension and a greater relationship between ST and knee flexion, supporting Hodges and Tucker's (2011) theory that neuromuscular adaptations to muscle pain are protective responses to reduce the extensor demand. However, these responses led to other neuromuscular adaptations in the ST, LG, and GM that reduce one's ability to stabilize the knee against rotational loads and increase one's risk of injury. Contrasting previous works evaluating effect of muscle pain on knee control, our participants maintained the same mechanical output across non-painful and painful conditions. As such, we believe our results provide more valid insight into the effect of muscle pain on the modulation of neuromuscular function. Our findings have implications for mechanisms of joint instability and can contribute to the development of rehabilitative and injury prevention exercise programs for populations with knee pain and quadriceps muscle weakness

4.3.7 References

Alkjær T, Simonsen EB, Jørgensen U, Dyhre-Poulsen P (2003) Evaluation of the walking pattern in two types of patients with anterior cruciate ligament deficiency: Copers and non-copers. Eur J Appl Physiol 89:301–308.

Alkjær T, Simonsen EB, Magnusson SP, Dyhre-Poulsen P, Aagaard P (2012) Antagonist muscle moment is increased in ACL deficient subjects during maximal dynamic knee extension. The Knee 19:633–639.

Arendt-Nielsen L, Sluka KA, Nie HL (2008) Experimental muscle pain impairs descending inhibition. PAIN 140:465–471.

Bank P, Peper C, Marinus J, Beek P, van Hilten J (2013) Motor consequences of experimentally induced limb pain: A systematic review. Eur J Pain 17:145–157.

Benjaminse A, Gokeler A, Fleisig GS, Sell TC, Otten B (2011) What is the true evidence for gender-related differences during plant and cut maneuvers? A systematic review. Knee Surg Sports Traumatol Arthrosc 19:42–54.

Bigham H (2016) Sex Differences in Lower Limb Muscle Activation Patterns in Participants with Knee Osteoarthritis and Healthy Controls. MSc Thesis. University of Ottawa.

Cashaback JGA, Potvin JR (2012) Knee muscle contributions to joint rotational stiffness. Hum Mov Sci 31:118–128.

Chmielewski TL, Stackhouse S, Axe MJ, Snyder-Mackler L (2004) A prospective analysis of incidence and severity of quadriceps inhibition in a consecutive sample of 100 patients with complete acute anterior cruciate ligament rupture. J Orthop Res 22:925–930.

Delp SL (2007) OpenSim: Open-Source Software to Create and Analyze Dynamic Simulations of Movement. IEEE Trans Biomed Eng 54:1940–1950.

DeMont RG, Lephart SM (2004) Effect of sex on preactivation of the gastrocnemius and hamstring muscles. Br J Sports Med 38:120–124.

Falla D, Arendt-Nielsen L, Farina D (2008) Gender-specific adaptations of upper trapezius muscle activity to acute nociceptive stimulation. PAIN 138:217–225.

Flaxman TE, Alkjær T, Simonsen EB, Krogsgaard MR, Benoit DL (Study 1; 2016) Predicting the Functional Roles of Knee Joint Muscles from Internal Joint Moments. Med Sci Sports Exerc 49:527-537.

Flaxman TE, Alkjær T, Smale KB, Simonsen EB, Krogsgaard MR, Benoit DL. (Study 2). Protective and Adverse Muscle Activation Strategies after ACL-injury during a Weight-bearing Force Control Task. Unpublished data.

Flaxman TE, Smith AJJ, Benoit DL (2013) Sex-Related Differences in Neuromuscular Control: Implications for Injury Mechanisms or Healthy Stabilisation Strategies? J Orthop Res 32:310–317.

Flaxman TE, Speirs AD, Benoit DL (2012) Joint stabilisers or moment actuators: the role of knee joint muscles while weight bearing. J Biomech 45:2570–2576.

Graven-Nielsen T, Lund H, Arendt-Nielsen L, Danneskiold-Samsøe B, Bliddal H (2002) Inhibition of maximal voluntary contraction force by experimental muscle pain: A centrally mediated mechanism. Muscle Nerve 26:708–712.

Haenlein M, Kaplan AM (2004) A Beginner’s Guide to Partial Least Squares Analysis. Underst Stat 3:283–297.

Henriksen M, Alkjaer T, Lund H, Simonsen EB, Graven-Nielsen T, Danneskiold-Samsøe B, Bliddal H (2007) Experimental quadriceps muscle pain impairs knee joint control during walking. J Appl Physiol 103:132–139.

Henriksen M, Alkjaer T, Simonsen EB, Bliddal H (2009) Experimental muscle pain during a forward lunge--the effects on knee joint dynamics and electromyographic activity. Br J Sports Med 43:503–507.

Henriksen M, Christensen R, Alkjær T, Lund H, Simonsen EB, Bliddal H (2008) Influence of pain and gender on impact loading during walking: A randomised trial. Clin Biomech 23:221–230.

Henriksen M, Graven-Nielsen T, Aaboe J, Andriacchi TP, Bliddal H (2010) Gait changes in patients with knee osteoarthritis are replicated by experimental knee pain. Arthritis Care Res 62:501–509.

Henriksen M, Rosager S, Aaboe J, Graven-Nielsen T, Bliddal H (2011) Experimental Knee Pain Reduces Muscle Strength. J Pain 12:460–467.

Hodges PW, Tucker K (2011) Moving differently in pain: A new theory to explain the adaptation to pain. Pain 152: S90-S98.

Hopkins JT, Ingersoll CD (2000) Arthrogenic muscle inhibition: A limiting factor in joint rehabilitation. J Sport Rehabil 9:135–159.

Kristianslund E, Krosshaug T, van den Bogert AJ (2012) Effect of low pass filtering on joint moments from inverse dynamics: Implications for injury prevention. J Biomech 45:666–671.

Mantovani G, Lamontagne M (2016) How Different Marker Sets Affect Joint Angles in Inverse Kinematics Framework. J Biomech Eng. [Epub ahead of print].

McGraw KO, Wong SP (1996) Forming inferences about some intraclass correlation coefficients. Psychol Methods 1:30–46.

McLean SG, Huang X, van den Bogert AJ (2005) Association between lower extremity posture at contact and peak valgus moment during sidestepping: Implications for ACL injury. Clin Biomech 20:863–870.

Mikesky AE, Meyer A, Thompson KL (2000) Relationship between quadriceps strength and rate of loading during gait in women. J Orthop Res 18:171–175.

Palmieri RM, Weltman A, Edwards JE, Tom JA, Saliba EN, Mistry DJ, Ingersoll CD (2005) Pre-synaptic modulation of quadriceps arthrogenic muscle inhibition. Knee Surg Sports Traumatol Arthrosc 13:370–376.

Palmieri-Smith RM, Thomas AC (2009) A neuromuscular mechanism of posttraumatic osteoarthritis associated with ACL injury. Exerc Sport Sci Rev 37:147–153.

Palmieri-Smith RM, Thomas AC, Wojtys EM (2008) Maximizing Quadriceps Strength After ACL Reconstruction. Clin Sports Med 27:405–424.

Powers CM (2010) The influence of abnormal hip mechanics on knee injury: A biomechanical perspective. J Orthop Sports Phys Ther 40:42–51.

Rudolph KS, Axe MJ, Buchanan TS, Scholz JP, Snyder-Mackler L (2001) Dynamic stability in the anterior cruciate ligament deficient knee. Knee Surg Sports Traumatol Arthrosc 9:62–71.

Sasaki K, Neptune RR (2010) Individual muscle contributions to the axial knee joint contact force during normal walking. J Biomech 43:2780–2784.

Shimokochi Y, Shultz SJ (2008) Mechanisms of noncontact anterior cruciate ligament injury. J Athl Train 43:396–408.

Shultz SJ, Beynnon BD, Schmitz RJ (2009) Sex differences in coupled knee motions during the transition from non-weight bearing to weight bearing. J Orthop Res 27:717–723.

Slemenda C, Brandt KD, Heilman DK, Mazzuca S, Braunstein EM, Katz BP, Wolinsky FD (1997) Quadriceps weakness and osteoarthritis of the knee. Ann Intern Med 127:97–104.

Smith AJJ, Flaxman TE, Benoit DL (2012) Reliability of knee joint muscle activity during weight bearing force control. J Electromyogr Kinesiol 22:914–922.

Snyder-Mackler L, Luca PFD, Williams PR, Eastlack ME, Bartolozzi AR (1994) Reflex inhibition of the quadriceps femoris muscle after injury or reconstruction of the anterior cruciate ligament. J Bone Jt Surg Am 76:555–560.

Urbach D, Nebelung W, Becker R, Awiszus F (2001) Effects of reconstruction of the anterior cruciate ligament on voluntary activation of quadriceps femoris. J Bone Jt Surg - Ser B 83:1104–1110.

Williams GN, Barrance PJ, Snyder-Mackler L, Axe MJ, Buchanan TS (2003) Specificity of muscle action after anterior cruciate ligament injury. J Orthop Res 21:1131–1137.

Wojtys E, Huston L (1994) Neuromuscular Performance in Normal and Anterior Cruciate Ligament-Deficient Lower Extremities. Am J Sports Med 22:89–104.

Wold S, Ruhe A, Wold H, Dunn I W (1984) The Collinearity Problem in Linear Regression. The Partial Least Squares (PLS) Approach to Generalized Inverses. SIAM J Sci Stat Comput 5:735–743.

Study 4: Effect of Quadriceps Muscle Pain on Lower Limb Muscle Synergies during a Weight-bearing Force Control Task

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4.4.1 ABSTRACT

Background: Reduced voluntary quadriceps activation is a cardinal symptom in populations with knee pathologies. The neuromuscular system must compensate by up/downregulating activity of other muscles that cross the knee joint. We tested this hypothesis by evaluating muscle synergies as they relate to internal knee joint moments and determined the effect of experimental quadriceps muscle pain (causing voluntary inhibition) on these synergies. **Study Design:** Cross-over placebo controlled. **Methods:** Concatenated non-negative matrix factorization extracted muscle and moment synergies of 22 healthy participants. Muscle pain was induced with injections of hypertonic saline (5.8%) to the vastus medialis (VM). Isotonic saline (0.9%) was used as control. Participants performed a standing isometric force matching with and without a saline injection. **Results**: Two sets of synergies were extracted for each condition. Set 1 included four synergies, each corresponding to a general anterior, posterior, medial, or lateral force direction. Set 2 included six synergies, each corresponding to a general extension, flexion, ab/adduction, internal/external rotation moment. Synergies associated with flexion and extension moments were respectively accompanied by dominant hamstring and quadriceps muscle synergies. Co-activation was observed in muscle synergies associated with frontal and transverse plane moments. Hypertonic saline reduced VM contribution to synergy weightings by 28%; however, baseline and with-injection synergies were similar across conditions. **Conclusion:** We identified muscle synergies related to internal joint moments which can provide new insight into muscular contribution to knee stability, specifically during loading conditions inducing injury. Compensatory response to muscle pain in non-painful muscles was participant-specific and a fundamental change in synergistic control was not observed. These findings provide new insights into neuromuscular function and motor adaptations to pain.

4.4.2 INTRODUCTION

Quadriceps weakness following knee injury is a neural inhibition (Graven-Nielsen et al., 2002) that reduces one’s ability to voluntarily activate the quadriceps muscle group (Hopkins and Ingersoll, 2000; Chmielewski et al., 2004; Palmieri-Smith et al., 2008). The resulting deficits in knee extensor strength are a strong predictor of functional impairment and knee joint instability (Wojtys and Huston, 1994; Mikesky et al., 2000; Rudolph et al., 2001). Several studies have associated quadriceps muscle weakness with varied movement patterns and muscle activation strategies (Rudolph et al., 2001; Alkjaer et al., 2003; Williams et al., 2003), typically inferred as compensation strategies. However, other changes after injury such as malalignment, muscle wasting from disuse, contractures, reconstruction, and joint effusion may have been progressively adapted. It is unclear if observed differences in neuromuscular function are direct consequences of injury and accompanying pain or adaptive responses to other chronically related changes.

To isolate the effect of muscle weakness, models of experimental muscle pain have been used (Palmieri et al., 2005; Henriksen et al., 2007, 2010, 2011; Arendt-Nielsen et al., 2008). There is a general consensus that experimental quadriceps pain inhibits voluntary activation of the target muscle and yields deficits in knee extensor strength comparable to levels observed clinically (Graven-Nielsen et al., 2002; Palmieri-Smith et al., 2008; Henriksen et al., 2010). However, reported differences in non-painful muscle activation and movement strategies are highly variable (Bank et al., 2013) and do not align with simple theories of motor adaptation to pain (Roland, 1986; Lund et al., 1991). Rather than uniform inhibition or excitation of non-painful muscles, Hodges and Tucker (2011) proposed a new theory of motor adaptation to pain that involves the redistribution of activity within and between muscles. Earlier studies evaluating the effect of knee muscle pain have quantified changes in individual muscles with discrete variables such as integrated electromyography (EMG), peak and mean magnitudes, onset of activation etc. (Henriksen et al., 2007, 2009; Hodges et al., 2009). By not including information of the entire EMG signal, interpreting the effect of muscle pain on the neural control of lower limb movement may be limited. A more comprehensive analysis of the redistribution of muscle activity with pain is warranted.

Several studies support the notion that the central nervous system (CNS) can control numerous degrees of freedom of the body by a reduced set of activation signals called *muscle synergies* (Ting and Macpherson, 2005; Torres-Oviedo et al., 2006; Neptune et al., 2009; Ting et al., 2015). This type of structure not only simplifies muscle activation but may also be functionally related to biomechanical variables that are important for movement control (Ivanenko et al., 2004; Torres-Oviedo et al., 2006; Neptune et al., 2009; Chvatal et al., 2011; Walter et al., 2014). For example, Ting and Macpherson (2005) demonstrated that four muscle synergies could account for the muscle activation patterns of cat postural responses elicited by surface translations in multiple horizontal directions. These synergies were able to reconstruct appropriate endpoint ground reaction forces (GRFs) required to maintain balance (Torres-Oviedo et al., 2006). Recent evidence suggests that muscle pain may modify existing motor synergies or lead to the recruitment of new ones (van den Hoorn et al., 2014; Gizzi et al., 2015) but how these changes affect human movement and associated biomechanics (i.e. knee joint kinetics) is not well understood.

This study sought to evaluate muscle synergies as they relate to the biomechanical variable of internal net knee joint moments and determined the effect of experimental quadriceps muscle pain on these synergies. Pain-induced changes in muscle activation are often accompanied by changes in motor output (i.e. reduced knee flexion angles, reduced knee extensor moments etc.) (Henriksen et al., 2009, 2011; Bank et al., 2013). Therefore, changes in muscle synergies may be related to changes in biomechanical variables rather than pain itself.

We used an isometric weight-bearing force control task to elicit muscle activation patterns. This protocol does not change the kinematic and force variables between conditions (Flaxman et al., 2016). As such, redistribution of muscle activity can be directly related to changes in motor command. We hypothesized that experimental muscle pain would reduce vastus medalis' (site of noxious stimulus) relative weightings in synergies that recruit it. Since the same mechanical outputs are required with and without pain, there would be a reorganization of muscle synergies. Data used in this study have been reported previously (Flaxman et al., Study 3).

4.4.3 METHODS

Study Design

A Cross-over placebo controlled design tested each participant on 2 days separated by one week. During a test day, three series of experimental tasks were performed, each under a difference condition (baseline-BASE, with injection-INJ, and recovery-REC). BASE measurements were first conducted and included 24 force targets. Although the physiological effects of experimental muscle pain are present up to 25 minutes, the perceived presence of pain only lasts 5 minutes (Henriksen et al., 2007). As such, INJ condition was subdivided into two parts, each including 12 force targets. Intramuscular injections were given immediately before each INJ set. Rest times between BASE and INJ sets were 5 minutes. A 20 minute rest period was held between the second INJ set to ensure perception of pain was gone before completing 12 targets in the REC condition.

Participants

Twenty-two healthy active adults [12 males (height=181.3±6.3cm; weight=79.8kg; age=27.25±4.0yrs) and 10 females (height=170.9±5.4cm; weight=65.1±9.1kg; age=22.7±1.4yrs)] with no history of significant lower limb injuries were recruited from the University of Copenhagen, Denmark and the surrounding community. Experiments were completed at the University of Copenhagen and all participants provided written informed consent. This study was approved by local ethics committee for the Capital Region of Denmark (H-3-2013-126) and the University of Ottawa Research Ethics Board (H06-14-25).

Experimental Protocol

Injection Protocol

Sterile hypertonic saline (HYP; 5.8%) induced muscle pain while isotonic saline (ISO; 0.9%) was used as control. Only one type of saline was given on each test day and the order of saline injections was randomized. Injections were given into the belly of the vastus medialis (VM), approximately 5cm proximal and 5cm medial to the medial corner of the patella. Intramuscular bolus injections of 1ml were manually administered over 10 seconds with a 5-ml plastic syringe with a disposable needle (27 gauge, 40 mm) at a depth of 3.5 cm.

Our previous work showed no significant effect of ISO injection on VM activation levels, VM's percent contribution to peak torque, or strength deficit ratio from BASE to INJ conditions (Flaxman et al., Study 3). Similarly, there was no significant difference in VM activation, VM's contribution to peak torque, or strength deficit ratio from BASE to REC conditions of the HYP session (Flaxman et al., Study 3). As such, this study limited the analysis to BASE and INJ conditions of the HYP session.

Force control task

A force matching protocol assessed muscle activation patterns of participants while they modulated GRFs with their test limb. Participants staggered their stance such that their feet were spaced hip width apart and their test leg had approximate joint angles of 30° hip flexion, 30° knee flexion, and 10° ankle plantarflexion. A projected cursor and a force target provided visual feedback of the direction and magnitude of the GRF applied to the force platform from the test leg: anterior/posterior loads (force along the ±y axis) moved the cursor upward/downward; medial/lateral loads (force along the ±x axis) moved the cursor to the left/right, and inferior/superior loads (force along the ±z axis) increased\ decreased the size of the cursor. A successful match of the cursor over the target for 0.5s required 50% body weight and a normalised force magnitude of 60% max effort. This triggered recording of 3D marker trajectories, GRFs, and EMG. Each set of 12 force targets directions were evenly spaced by 30° about a circular trajectory (representative of various horizontal loading directions: 0°= lateral, 90°= anterior; 180°= medial; 270°= posterior). The target order was randomized.

Participants also completed a set of squatting and lunging trials after each target series but the data was not analysed in this study. This data has been reported in a separate study (Flaxman et al., Study 5).

Equipment and data processing

Retro-reflective markers were placed on various anatomical landmarks on the participant's body (Mantovani and Lamontagne, 2016) (Appendix A). Trajectories were recorded using a ten-camera motion analysis system (6 MX-13 and 4F40 Vicon cameras, Oxford Metrics, Oxford, UK) sampling at 100 Hz with supporting Nexus software (version 1.85, Oxford Metrics, Oxford, UK). GRFs recorded from a force platform (AMTI-OR6, AMTI, Watertown, MA) were also collected in Nexus at 1000Hz and amplified with an internal gain of 1000. Bipolar surface electrodes collected electromyography (EMG) signals of the VM, vastus lateralis (VL), rectus femoris (RF), semitendinosus (ST), biceps femoris (LH), medial gastrocnemius (MG), lateral gastrocnemius (LG), tensor fasciae latae (TFL), gluteus medius (GM), and hip adductor (ADD) muscle group of the dominant leg (defined as leg used to kick a soccer ball as far as possible). EMG was sampled at 1000Hz and bandpass filtered at 20-500Hz bandwidth with a 6dB/octave filter slope using a wireless EMG system (MQ air, Marq Medical, Farum Denmark).

Raw marker trajectories and GRFs were filtered with a 2nd order dual 15Hz cut-off low pass Butterworth filter (Kristianslund et al., 2012) and exported to OpenSim (v 3.2, (Delp, 2007)) to compute local coordinate systems of the lower limb and subsequent joint angles and internal net joint moments using inverse kinematics and dynamics. EMG signals were high-pass filtered at 20Hz with a 2nd order dual-pass Butterworth filter, full wave rectified, low-pass filtered at 10Hz a with 2nd order dual-pass Butterworth filter. EMG were normalized to maximal amplitude recorded during maximum voluntary isometric contractions (MVIC) exercises of knee extension, knee flexion, hip abduction, hip adduction, hip extension, hip flexion, and plantarflexion. MVIC trials were conducted prior to the experimental protocol and used manual resistance from the researcher. EMG, kinematic and kinetic data were time averaged over the 0.5s of successful force match and ensemble averaged across repetitions.

Data Analysis

Muscle Synergies

Muscle synergies were extracted for each condition using a concatenated non-negative matrix factorization (CNMF) framework (Shourijeh et al., 2016; Smale et al., 2016). CNMF fixes the synergies across all participants but allows coefficient weights to fluctuate and account for the inter-participant variability. This approach is more reliable and appropriate for evaluating between condition differences in synergy variables (Shourijeh et al., 2016; Smale et al., 2016). Input for CNMF combined EMG and internal knee joint moment data (Moghadam et al., 2013). The combination of data types better map activation patterns to principal force directions and are considered more functionally relevant (Moghadam et al., 2013).

Each participant's data formed an $n\ x\ m$ matrix where n is the number of target force directions (n=12) and m is the number of muscles plus the number of knee moment directions (m=10+6=16). The knee moments at each force target direction were expressed as six values corresponding to the absolute values of the positive and negative directions (+Mx, +My, +Mz, -Mx, -My, -Mz). Such a representation of torque is a requirement of the non-negative matrix factorization algorithm. The input (A) into the CNMF framework was $N\ x\ m$ data matrix with participant data vertically concatenated such that N=264 (12 directions * 22 participants).

A solver built two output matrices: the first being a $q\ x\ m$ synergy matrix (W) where q is the number of synergies. The first 10 elements of each synergy (W_{q1}-W_{q10}) refer to the muscle synergies and last 6 elements (W_{i11}-W_{i16}) refer to the moment synergies. Each element of a given synergy specifies the relative contribution level of each muscle/moment between 0 and 1. The second output is an $N\ x\ q$ coefficient matrix (C) where each element of C is the relative scaling factor for each synergy (q) at a given target force direction of each participant. When W and C are multiplied, the original input matrix, A, can be expressed as the weighted sum of synergies $A \approx WC$.

Previous works using simulated data, participant specific synergies, or CNMF on less than 10 participants have arbitrarily used a threshold of >90-95% total variance accounted for (VAF) to determine the number of synergies that best reconstructs the experimental data

(Neptune et al., 2009; Kristiansen et al., 2013; Moghadam et al., 2013). However, we are using the concatenated data of 22 participants and we expect inter-participant variability to affect the total VAF. The addition of moment data is also assumed to reduce VAF (Steele et al., 2013). As such, the required number of synergies that best characterizes our original data was determined by (1) accounting for >80% of the total VAF and/or (2) adding another synergy will increase the VAF ≥5% (Torres-Oviedo et al., 2006; Heales et al., 2016). We also investigated the number of synergies required to account for >90% VAF, even if additional synergies contributed to <5% VAF. BASE and INJ analyses were run separately. As such, similar synergies may be produced between conditions but in a varied order. Synergies were re-sorted based on visual inspection.

Statistical Analysis

The similarity of muscle and moment synergy vectors between conditions was assessed with intraclass correlation coefficients (ICC$_{(1,k)}$) (McGraw and Wong, 1996). We considered synergy vectors to be statistically similar if ICC≥0.75 (Portney and Watkins, 2000). The percent relative change (%RC) in muscle and moment synergies were expressed as the difference in linear vector norms from BASE to INJ:

$$\%RC = \frac{\|BASE_j\| - \|INJ_j\|}{\|BASE_j\|} * 100$$

Where j is the given synergy number. Similarly, the %RC in the norm of individual element weightings across all synergies was also computed. A negative number indicates a reduction from BASE to INJ while a positive number indicates an increase.

To test the uniqueness of each condition's synergies, the experimental data of a given condition was reconstructed by means of the synergy matrix extracted from the other condition (cross-reconstructions) (Gizzi et al., 2015). Similar VAF values would be expected if synergy weightings extracted in one condition could describe the moment and muscle activation patterns of another condition.

Between condition differences in synergy activation coefficients were analysed with statistical parametric mapping (SPM) dependent T-test for circular data (Pataky, 2010). Significance was defined at the alpha=0.05 level.

4.4.4 RESULTS

Percent VAF

The number of synergies required to reconstruct >80% and >90% VAF was the same for both conditions (Figure 23). Four synergies accounted for >80% VAF and adding an additional synergy did not increase VAF >5%. Six synergies accounted for >90% VAF. The addition of S5 and S6 increased VAF by 4.88 and 3.56% for BASE and 4.53 and 3.79% for INJ, respectively. Contribution of individual synergies to total VAF is presented in Figure 24.

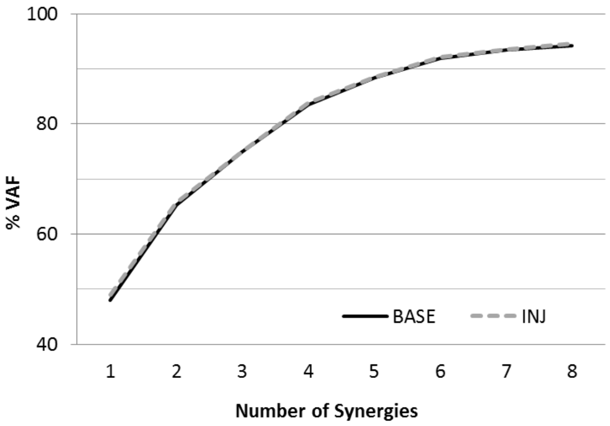

Figure 23: Number of synergies contributing to total variance accounted for (VAF) during baseline (BASE) and with injection (INJ) conditions.

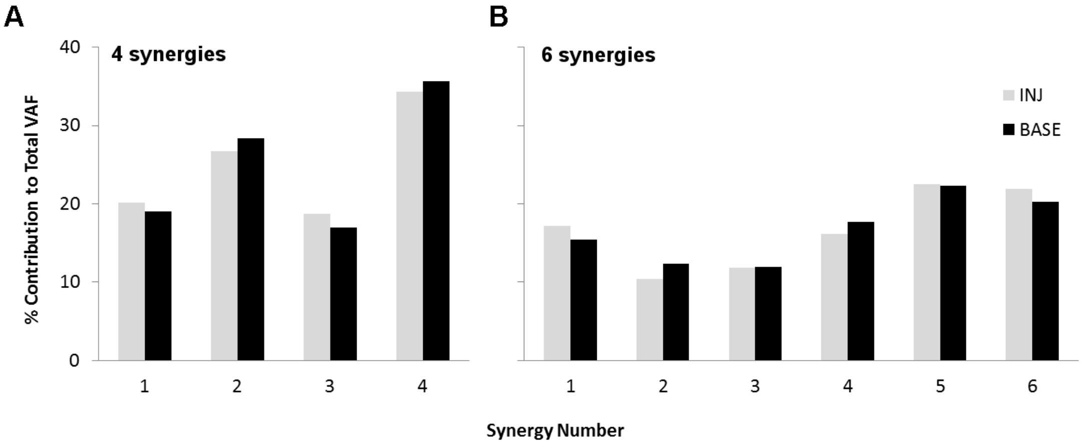

Figure 24: Individual percent (%) contribution of each synergy to total variance accounted for (VAF) in **A)** four synergy analysis and **B)** 6 synergy analysis.

Four synergy analysis

Each synergy corresponded to a general force target direction: $S1_{four}$ – anterior, $S2_{four}$ – lateral, $S3_{four}$ – posterior, and $S4_{four}$ – medial. Moment synergies of $S1_{four}$ and $S3_{four}$ were dominated by extension and flexion, respectively, and accompanied by abduction and adduction (Figure 25A). Muscle synergies of $S1_{four}$ and $S3_{four}$ were dominated by quadriceps and hamstring muscles, respectively (Figure 25B). Moment synergies of $S2_{four}$ and $S4_{four}$ were dominated by internal and external rotation moments, respectively, and accompanied by abduction and adduction. Muscle synergies of $S2_{four}$ and $S4_{four}$ were not dominated by a specific muscle group and have notable contribution of all muscles.

Six synergy analysis

Each moment synergy was dominated by a general moment direction: $S1_{six}$ – extension, $S2_{six}$ – flexion, $S3_{six}$ – abduction, and $S4_{six}$ – adduction, $S5_{six}$ – internal rotation, and $S6_{six}$ – external rotation (Figure 26A). Muscle synergies for $S1_{six}$ and $S2_{six}$ were dominated by quadriceps and hamstring muscles. $S3_{six}$ and $S4_{six}$ were dominated by GM and ADD, respectively, however, $S4_{six}$ (adduction) was accompanied by considerable contribution from hamstring and vastii muscles (Figure 26B). $S5_{six}$ and $S6_{six}$ were not dominated by a specific muscle group and have relative contribution of all muscles, except ST in $S6_{six}$.

Effect of Pain

Moment and muscle synergies were extremely similar between conditions. ICCs ranged from 0.98-1.0 and 0.90-0.99 for moment and muscle synergies, respectively, indifferent of the number of synergies used (Figure 25 and Figure 26). In the 4 synergy analysis, $S2_{four}$ had the greatest %RC in moment weightings while $S3_{four}$ had the greatest difference in muscle weightings from BASE to INJ (Figure 27A). The overall %RC in VM elements across synergies was -22.1% (Figure 27B) with noticeably reduced VM contribution to muscle synergies $S2_{four}$ and $S4_{four}$. In the 6 synergy analysis, $S6_{six}$ had the greatest %RC in moment weightings while $S2_{six}$ had the greatest difference in muscle weightings from BASE to INJ (Figure 27A). The overall %RC in VM elements across synergies was -28.1% (Figure 27B) with noticeably reduced contribution to muscle synergies $S4_{six}$, $S5_{six}$, and $S6_{six}$.

In the 4 synergy analysis, $C2_{four}$ demonstrated significantly reduced scaling coefficients from BASE to INJ at force target 300° (p=0.023, t-score=4.56, t-threshold=3.21). No other significant differences were observed (Figure 25C-D). In the 6 synergy analysis, $C4_{six}$ demonstrated significantly reduced scaling coefficients from BASE to INJ at force target 180° (p<0.0001, t-score=6.39, t-threshold=3.21). $C6_{six}$ had significantly greater scaling coefficients from BASE to INJ at targets 180°-210° (p<0.0001, t-score=4.31-5.81, t-threshold=3.21). Between conditions differences in $S4_{six}$ and $S6_{six}$ scaling coefficients may be attributed to reciprocal change of the ER element from BASE to INJ. No other significant differences were observed (Figure 26C-D).

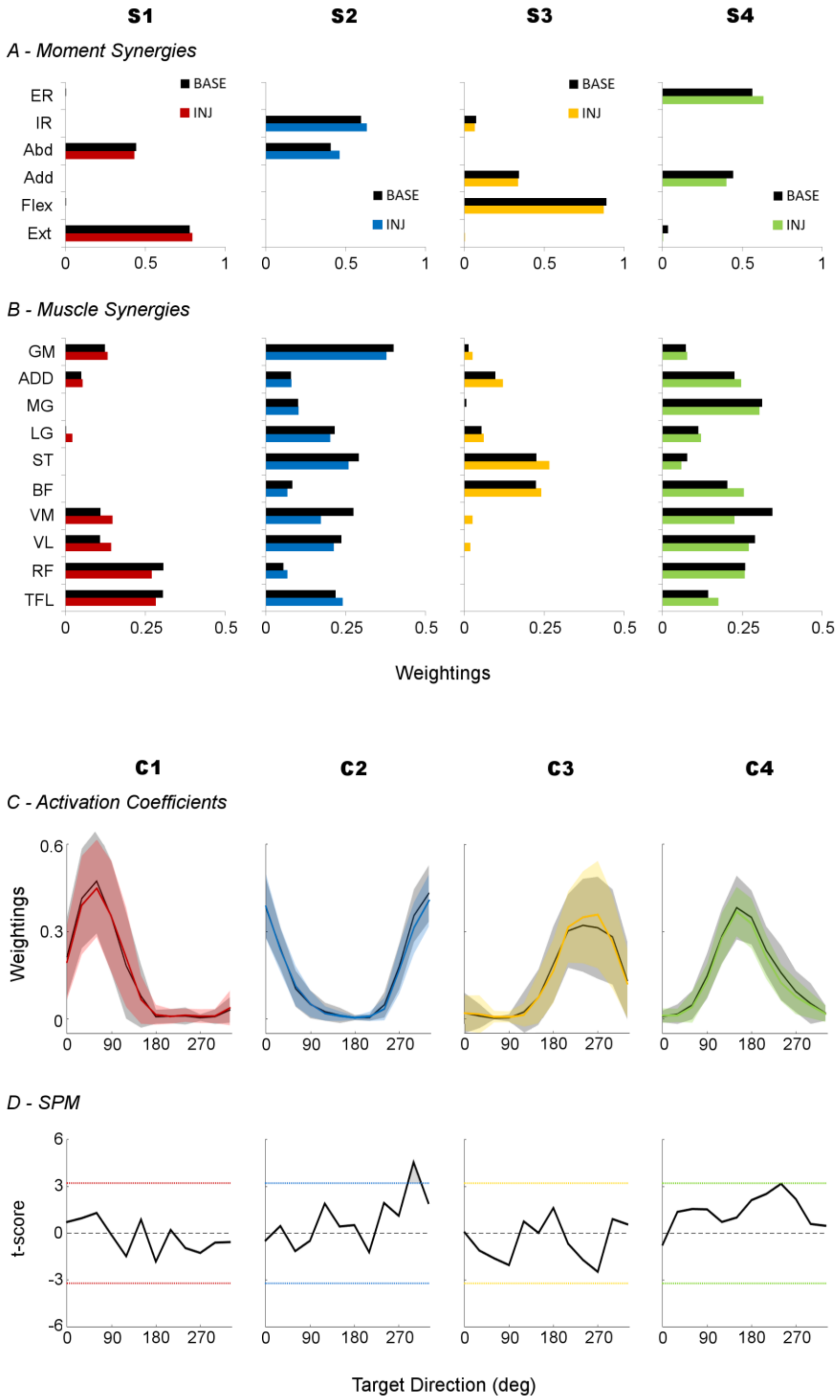

Figure 25: (A) Moment synergies and **(B)** muscle synergies, S, of BASE and INJ conditions required to reconstruct >80% variance accounted for (VAF). **(C)** Group mean and standard deviation clouds of respective synergy activation coefficients, C, across target directions. **(D)** Within-subject differences in C vectors were tested using a statistical parametric mapping (SPM) dependent T-test. Significant between condition differences in C is denoted when the t-score crosses the coloured line.

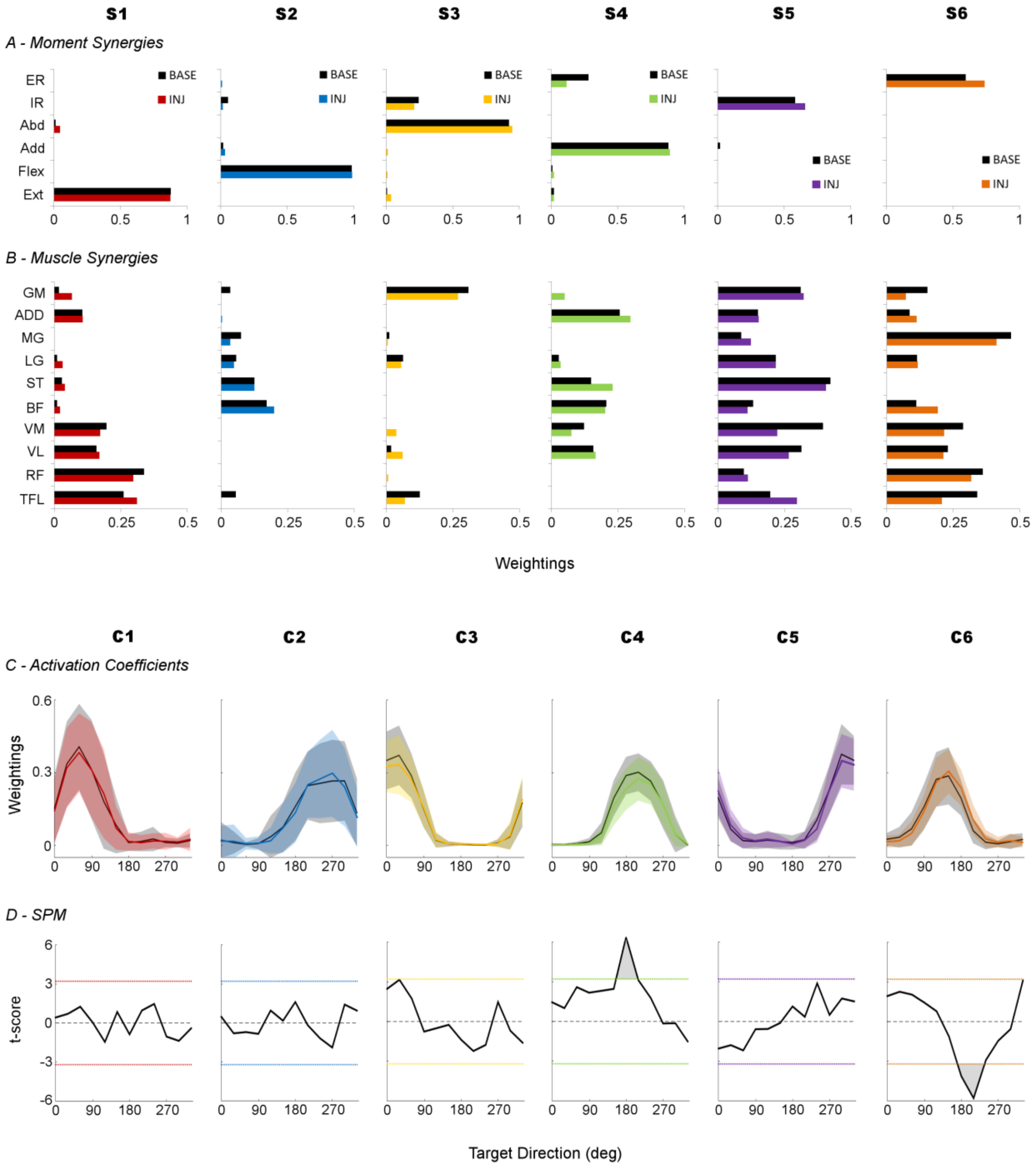

Figure 26: (A) Moment synergies and **(B)** muscle synergies, S, of BASE and INJ conditions required to reconstruct >90% variance accounted for (VAF). **(C)** Group mean and standard deviation clouds of respective synergy activation coefficients, C, across target directions. **(D)** Within-subject differences in C vectors were tested using a statistical parametric mapping (SPM) dependent T-test. Significant between condition differences in C is denoted when the t-score crosses the coloured line.

Cross-reconstruction of experimental BASE data using the 4 PAIN synergies and 6 PAIN synergies accounted for 82.8% and 89.3% VAF, respectively. Cross-reconstruction of experimental PAIN data using the 4 BASE synergies accounted for 83.0% and 88.9%, respectively.

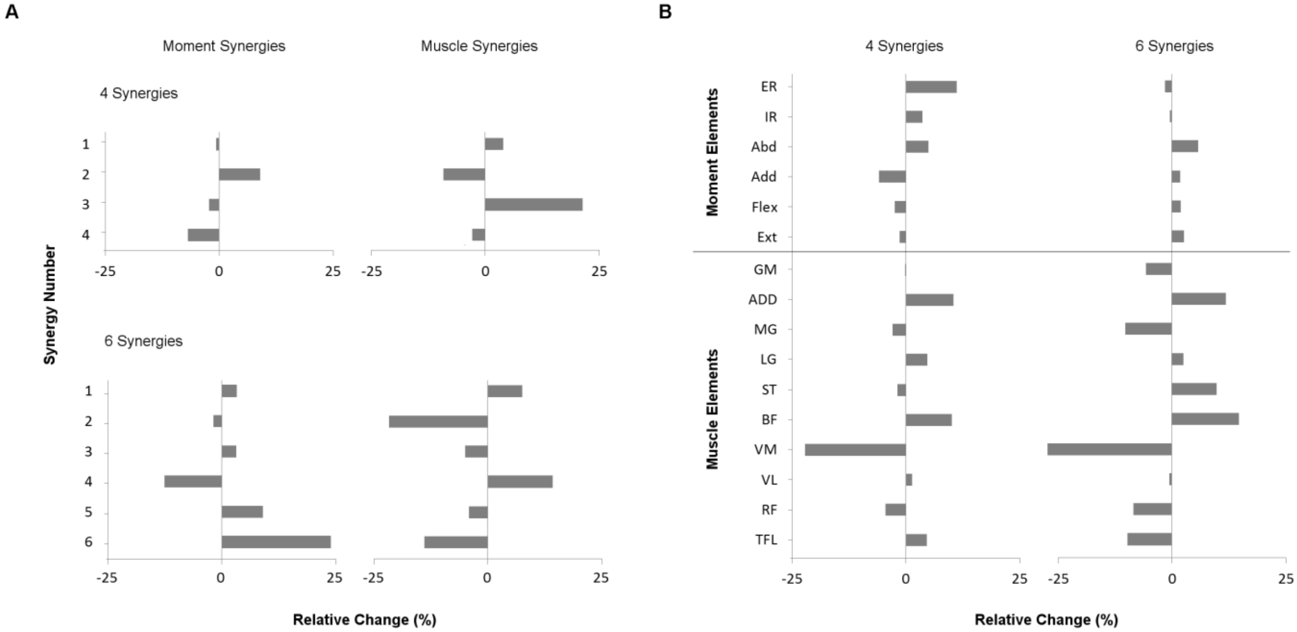

Figure 27: Percent relative change (%) in (**A**) synergy vector norms and (**B**) the norm of individual element weightings across all synergies. Positive number indicates overall increase; negative is decrease from BASE to INJ conditions.

4.4.5 DISCUSSION

This study evaluated muscle synergies as they relate to internal knee joint moments and determined the effect of experimental quadriceps muscle pain on these synergies. Muscle activation and moment data used to extract synergies were elicited during an isometric weight-bearing force control task. This approach limits biomechanical contributions to force generation such that muscle activation can be more directly attributed to force generation (Wilkie, 1950). Our task is also unique in that it elicits internal knee joint moments that are comparable in magnitude in all six loading directions (Figure 20, Flaxman et al., Study 3), whereas dynamic tasks are usually dominated by one or two moment types. This allowed us to separate the specific roles muscles play in generating each internal joint moment.

Muscle synergies related to biomechanical variables

To reconstruct >90% total VAF in our data, six synergies were required, even if adding an additional synergy contributed <5% to total VAF. These synergies corresponded to the six general moment directions: extension, flexion, abduction, adduction, internal rotation, and external rotation. Although synergies have been functionally related to global biomechanical variables such as GRF directions (Ting and Macpherson, 2005; Torres-Oviedo et al., 2006), center of mass shifts (Chvatal et al., 2011), limb kinematics (Ivanenko et al., 2004), and limb acceleration (Neptune et al., 2009), to our knowledge, no study has identified moment specific synergies in the lower limb. This is particularly important when trying to relate muscular contributions to injury inducing moments at the knee, such as externally applied abduction and rotation moments (Mills and Hull, 1991; 2004).

Not surprisingly, moment synergies dominated by knee extension and flexion were accompanied by muscle synergies with high knee extensor and flexor muscle weightings. In contrast, moment synergies dominated by knee abduction and adduction were accompanied by muscle synergies with high hip GM and ADD muscle weightings, respectively. Since knee muscles that possess large frontal plane moment arms (i.e. gracilis, sartorius, and tensor fascia latae) have minimal contribution to opposing frontal plane external loads (Brand et al., 1982; Buchanan and Lloyd, 1997), we assume the hip musculature is responsible for generating the frontal plane loads required to modulate the forces at the foot-ground interface. This is supported by greater frontal plane moment magnitudes at the hip compared to the knee, even though the moment profiles are similar for both joints (Figure 20, Flaxman et al., Study 3). Furthermore, although the gluteal muscles do not directly cross the knee, hip abductor and hip adductor muscles are large determinants of femoral orientation and knee joint loads (Sasaki and Neptune, 2010) and their relationship to knee joint kinetics should not be disregarded. Interestingly, only the adduction moment synergy was accompanied by a muscle synergy with contributions from other musculature, specifically the vastii and hamstrings. Previous works suggest medial and lateral musculature differentially contract to support externally applied abduction and adduction loads, respectively (Buchanan et al., 1996; Zhang et al., 2001). However, such investigations have been performed while not-weight bearing. Our work suggests that a more global co-activation strategy is used to support the knee against externally applied abduction loads while weight bearing.

Pivoting maneuvers are common in non-contact episodes of ACL injury (Boden et al., 2000). These pivots couple internal or external rotations moments at the knee that must be resisted by a network of muscles that are mechanically related to the task when considering their moment arm orientations. We found that rotational moment synergies were accompanied by notable contributions of all muscles, except the ST to external rotation. Global co-activation of the major muscles crossing the knee can increase torsional stiffness by increasing compression and contribute to rotational knee stability (Shultz et al., 2001; Wojtys et al., 2003; Carcia et al., 2005; Yoo et al., 2005). A co-activation strategy associated with rotational moments is extremely noteworthy considering ACL strain is greatly affected by rotational loads (Shimokochi and Shultz, 2008) and rotation is a major component of the ACL injury mechanism (McLean et al., 2005). Therefore, identification of this co-activation synergy has important clinical implications because variations in muscular co-activation have been identified in populations with unstable knees and can expose the knee to adverse loads (Palmieri-Smith et al., 2009; Zeni et al., 2010). To reconstruct >80% total VAF in our data, four synergies were required. Interestingly, these synergies corresponded to the four general force loading directions at the ground: anterior, posterior, medial, and lateral directions. This finding is similar to those reported previously where four synergies reconstructed postural responses of cats and each synergy corresponded to a principle perturbation direction (Ting and Macpherson, 2005; Torres-Oviedo et al., 2006).

Muscle synergies of $S1_{four}$ and $S3_{four}$ were dominated by extensor and flexor muscles, respectively. This is not surprising since corresponding moment synergies had high sagittal plane weightings. In contrast, muscle synergies of $S2_{four}$ and $S4_{four}$ were not dominated by a single muscle group, but rather a general co-activation of all muscles. The moment synergies of $S2_{four}$ and $S4_{four}$ coupled the frontal and transverse moments ($S2_{four}$ coupled IR and ABD; $S4_{four}$ coupled ER and ADD). Even during a controlled task, these coupled moments emphasize that while weight bearing loads acting on the knee are multifactorial and indicate which loads must be combined. This may explain the reduced muscle specificity we have previously observed during weight-bearing (Flaxman et al., 2012) as opposed to non-weight bearing tasks where force targets are achieved with far less co-activation (Williams et al., 2003). This load coupling has also been observed in vivo where the application of pure transverse moments caused frontal plane rotations and vice versa whereby applying ab/adduction moments causes transverse rotation (Mills and Hull, 1991). Based on the 6 synergy analysis, when the load coupling is broken down with each load direction attributed its own synergy, we find that general co-activation for $S2_{four}$ (IR and ABD) and $S4_{four}$ (ER and ADD) are then attributed to the rotational components of the moment synergies $S5_{six}$ (IR) and $S6_{sx}$ (ER), while the greater contribution of hamstring and quadriceps to $S4_{four}$ (ER and ADD) is associated with the adductor moment synergy $S4_{six}$ (ADD). Nevertheless, this interaction between transverse and fontal plane loads is significant since it is an important contributor to ACL injury mechanism (Mills and Hull, 1991; MacLean et al., 2004).

Effect of Pain

VM's contributions to synergy weights were reduced by 22% and 28% in the four and six synergy analyses, respectively. This aligns with our previous observation that experimental muscle pain reduces VM activity by 26% and its contribution to peak torque by 24% from baseline values during maximum knee extensor strength tests (Flaxman et al., Study 3). We expected synergy analysis to allow us to identify how the central nervous system redistributed these VM reductions across the other muscles since the task demand with and without pain was maintained.

Pain-induced reductions in quadriceps muscle activation are often accompanied by decreased knee extensor moments and peak flexion angles (Henriksen et al., 2009, 2011; Bank et al., 2013). Therefore, changes in muscle activation may be related to changes in joint dynamics rather than pain itself. Our task controlled for this by requiring participants to maintain the same mechanical outputs (GRFs) and joint positions between conditions. As such, we were able to verify reduced VM activity and any redistribution of muscle activation was directly attributed to the presence of pain. It was hypothesized that there would be a compensatory change in weights or reorganization of the synergies. Despite observed changes in individual moment and muscle synergy compositions up to 25%, as well as significant between condition differences in the coefficient weights, the muscle and moment synergies were extremely similar between conditions (ICCs>0.9). We cross-reconstructed the experimental data of a given condition with the synergies from other conditions to determine if the between condition differences in synergies were unique. However, similar reconstruction quality was observed, indicating the synergies recruited in the painful condition are not specific. This contrasts previous studies investigating the effect of experimental muscle pain on muscle synergies of the upper limb (Muceli et al., 2014) and neck (Gizzi et al., 2015).

A key aspect in Hodges and Tucker's (2011) theory of muscular adaptation to pain is that pain leads to spatial redistribution of muscle activity within and between muscles. Since the muscles studied are large in volume, redistribution of activity within a muscle may not have been detected with a single surface electrode. In addition, our experimental peak EMG levels ranged between 12-24% MVIC (Flaxman et al., Study 3). Bank et al. (2013) suggest traditional parameters derived from surface EMG (e.g. amplitude) do not significantly differ with pain during contractions at relatively low intensity (i.e. <25% MVIC). These issues may account for our lack of between condition uniqueness in synergy compositions compared to other studies (Muceli et al., 2014; Gizzi et al., 2015).

Furthermore, recent studies have reported participant-specific response to painful stimuli (van den Hoorn et al., 2014; Muceli et al., 2014; Gizzi et al., 2015). As such we sought to look at participant-specific changes in muscle activation. Table 5 shows individual variation of EMG amplitude and moment magnitude change from BASE to INJ conditions**.** A general decrease of the EMG amplitude in the VM is observed but note that the redistribution (decrease/increase) of activation of other muscles is participant-specific and no two participants showed the same strategy. Since our task elicits reliable within-subject activation patterns (Smith et al., 2012), these changes are associated with pain and is likely a reflection of the redundancy built into the neuromuscular control system of the lower limb.

Table 5: Individual variation of EMG amplitude and moment magnitude change for each of the 22 participants showing the direction of change between baseline (BASE) and injection (INJ) conditions of the hypertonic (pain inducing) session. Blue (+) indicates an increase, red (-) indicates a decrease, and white (empty) indicates no change in the INJ condition compared to BASE. The threshold used to define a change was set to ±10% of the baseline average EMG amplitude or moment over all target directions. Note that no two participants showed the same strategy.

	Muscles										Moments					
	TFL	*RF*	*VL*	*VM*	*BF*	*ST*	*LG*	*MG*	*ADD*	*GM*	*Ext*	*Flex*	*Abd*	*Add*	*IR*	*ER*
F1	-	-	-	-			+	+		-	+	-	-	+	-	+
F2	+	+	+		+			+				+			+	-
F3	-	-	-	-				+				-	-	+		
F4		+	-	-		-		+			+		+			
F5	+	-	-	-		-	+		+	-		-	+			+
F6	+	-		-			-	-	+	+			+	-		-
F7	-			-							+	-			+	
F8		-	-	-	+	+			-				+		-	-
F9	-			-		-	+	-				+	-	+	-	+
F10		+	+	-	+	+					-	+	+	-		-
M1		-		-	+	+						-		+		+
M2	-	+		-	+			+								-
M3	+			-		-		-	-	+	-	+		-		
M4	+		+	+	+		-	+	+		-					+
M5		-	-	-	+	+	+	+	+	+		-	-	+		
M6	-	-	-	-		-			-	-	+					-
M7				-	+	-		-				-	+	-	-	-
M8		+		-		-		-			+	+		-	+	
M9	-	-		-	-	-		-	-		+	-	+	-	-	
M10	+			-	+		-	-	+		-	+	+	-	+	
M11		-	-	-	+			-				+	+		-	
M12	+	-	+	-							-	+	+			

Even though previous studies have identified inconsistent responses to pain across participants, unique changes in muscle synergies were still reported (van den Hoorn et al., 2014; Muceli et al., 2014; Gizzi et al., 2015). We opted to use a CNMF approach which we believe is more appropriate for evaluating between group/condition differences in synergy variables (Shourijeh et al., 2016; Smale et al., 2016). This framework fixes the synergies across all participants but allows coefficient weights to fluctuate and account for the inter-participant variability. As such, our participant-specific responses to pain may not be represented in the synergy vectors but rather the coefficient weightings. However, due to the variation in individual responses, a systematic within-participant change in coefficient weights was not apparent.

4.4.6 CONCLUSION

Our study is the first to investigate the effects of pain on lower limb muscle synergies. Pain caused a general decrease of the VM contribution to muscle synergy weights were detected together with redistribution (decrease/increase) of the activation of other muscles in a given synergy. Although the changes in our moment and muscle synergies may show some distinct characteristics, we did not observe a significant pain specific response in synergistic control. This may due to highly variable participant-specific changes in muscle activations in response to pain.

Although previous works have related muscle synergies to mechanical performance and movement, to our knowledge, no study has identified synergies important for maintaining joint stability or resisting moments associated with injury mechanisms. To do so we combined the muscle activations with the internal net joint moment data in our analysis and extracted muscle synergies related to their role in supporting the knee. We observed muscle synergies related to individual moment directions from six synergies and coupled moment directions from four synergies. Synergies related to knee adduction and rotational moments were accompanied by substantial co-activation of the major knee joint muscles and since valgus alignment coupled with rotation is a major mechanism of ACL injury, we consider these synergies to be important stabilizing components, co-contracting to increase compressive forces and joint stiffness. Our findings therefore provide new insights for the roles of muscles in maintaining knee joint stability, mechanisms of knee joint injury, and neuromuscular responses to muscle pain and muscle weakness.

4.4.7 References

Alkjaer T, Simonsen EB, Jørgensen U, Dyhre-Poulsen P (2003) Evaluation of the walking pattern in two types of patients with anterior cruciate ligament deficiency: Copers and non-copers. Eur J Appl Physiol 89:301–308.

Arendt-Nielsen L, Sluka KA, Nie HL (2008) Experimental muscle pain impairs descending inhibition. PAIN 140:465–471.

Bank P, Peper C, Marinus J, Beek P, van Hilten J (2013) Motor consequences of experimentally induced limb pain: A systematic review. Eur J Pain 17:145–157.

Boden BP, Dean CS, Feagin Jr. JA, Garrett Jr. WE (2000) Mechanisms of anterior cruciate ligament injury. Orthopedics 23:573–578.

Brand RA, Crowninshield RD, Wittstock CE, Pedersen DR, Clark CR, van Krieken FM (1982) A Model of Lower Extremity Muscular Anatomy. J Biomech Eng 104:304–310.

Buchanan TS, Kim AW, Lloyd DG (1996) Selective muscle activation following rapid varus/valgus perturbations at the knee. Med Sci Sports Exerc 28:870–876.

Buchanan TS, Lloyd DG (1997) Muscle activation at the human knee during isometric flexion-extension and varus-valgus loads. J Orthop Res 151:1–17.

Carcia CR, Shultz SJ, Granata KP, Perrin DH, Martin RL (2005) Females recruit quadriceps faster than males at multiple knee flexion angles following a weight bearing rotary perturbation. Clin J Sport Med 15:167–171.

Chmielewski TL, Stackhouse S, Axe MJ, Snyder-Mackler L (2004) A prospective analysis of incidence and severity of quadriceps inhibition in a consecutive sample of 100 patients with complete acute anterior cruciate ligament rupture. J Orthop Res 22:925–930.

Chvatal SA, Torres-Oviedo G, Safavynia SA, Ting LH (2011) Common muscle synergies for control of center of mass and force in nonstepping and stepping postural behaviors. J Neurophysiol 106:999–1015.

Delp SL (2007) OpenSim: Open-Source Software to Create and Analyze Dynamic Simulations of Movement. IEEE Trans Biomed Eng 54:1940–1950.

Flaxman TE, Alkjaer T, Simonsen EB, Krosgaard MR, Benoit DL (Study1; 2016) Predicting the functional roles of knee joint muscles from internal joint moments. Med Sci Sports Exer 49:527-527.

Flaxman TE, Alkjaer T, Shourijeh MS, Krogsgaard MR, Benoit DL. (Study 3). Adaptive Muscle Activation Strategies from Experimental Muscle Pain during a Weight-bearing Force Control Task. Unpublished data.

Gizzi L, Muceli S, Petzke F, Falla D (2015) Experimental Muscle Pain Impairs the Synergistic Modular Control of Neck Muscles. PLoS ONE 10 Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4575045/ [Accessed June 28, 2016].

Graven-Nielsen T, Lund H, Arendt-Nielsen L, Danneskiold-Samsøe B, Bliddal H (2002) Inhibition of maximal voluntary contraction force by experimental muscle pain: A centrally mediated mechanism. Muscle Nerve 26:708–712.

Heales LJ, Hug F, MacDonald DA, Vicenzino B, Hodges PW (2016) Is synergistic organisation of muscle coordination altered in people with lateral epicondylalgia? A case–control study. Clin Biomech 35:124–131.

Henriksen M, Aaboe J, Simonsen EB, Alkjaer T, Bliddal H (2009) Experimentally reduced hip abductor function during walking: Implications for knee joint loads. J Biomech 42:1236–1240.

Henriksen M, Alkjaer T, Lund H, Simonsen EB, Graven-Nielsen T, Danneskiold-Samsøe B, Bliddal H (2007) Experimental quadriceps muscle pain impairs knee joint control during walking. J Appl Physiol 103:132–139.

Henriksen M, Graven-Nielsen T, Aaboe J, Andriacchi TP, Bliddal H (2010) Gait changes in patients with knee osteoarthritis are replicated by experimental knee pain. Arthritis Care Res 62:501–509.

Henriksen M, Rosager S, Aaboe J, Graven-Nielsen T, Bliddal H (2011) Experimental Knee Pain Reduces Muscle Strength. J Pain 12:460–467.

Hodges PW, Mellor R, Crossley K, Bennell K (2009) Pain induced by injection of hypertonic saline into the infrapatellar fat pad and effect on coordination of the quadriceps muscles. Arthritis Care Res 61:70–77.

Hodges PW, Tucker K (2011) Moving differently in pain: A new theory to explain the adaptation to pain. Pain 152: S90-S98.

Hopkins JT, Ingersoll CD (2000) Arthrogenic muscle inhibition: A limiting factor in joint rehabilitation. J Sport Rehabil 9:135–159.

Hsieh HH, Walker PS (1976) Stabilizing mechanisms of the loaded and unloaded knee joint. J Bone Jt Surg 58:87–93.

Ivanenko YP, Poppele RE, Lacquaniti F (2004) Five basic muscle activation patterns account for muscle activity during human locomotion. J Physiol 556:267–282.

Kristiansen M, Madeleine P, Hansen EA, Samani A (2013) Inter-subject variability of muscle synergies during bench press in power lifters and untrained individuals. Scand J Med Sci Sports 25:89–97.

Kristianslund E, Krosshaug T, van den Bogert AJ (2012) Effect of low pass filtering on joint moments from inverse dynamics: Implications for injury prevention. J Biomech 45:666–671.

Lund JP, Donga R, Widmer CG, Stohler CS (1991) The pain-adaptation model: a discussion of the relationship between chronic musculoskeletal pain and motor activity. Can J Physiol Pharmacol 69:683–694.

Mantovani G, Lamontagne M (2016) How Different Marker Sets Affect Joint Angles in Inverse Kinematics Framework. J Biomech Eng.

McGraw KO, Wong SP (1996) Forming inferences about some intraclass correlation coefficients. Psychol Methods 1:30–46.

McLean SG, Huang X, Su A, Van DB (2004) Sagittal plane biomechanics cannot injure the ACL during sidestep cutting. Clin Biomech 19:828–838.

Mikesky AE, Meyer A, Thompson KL (2000) Relationship between quadriceps strength and rate of loading during gait in women. J Orthop Res 18:171–175.

Mills OS, Hull ML (1991) Rotational flexibility of the human knee due to varus/valgus and axial moments in vivo. J Biomech 24:673–690.

Moghadam MN, Aminian K, Asghari M, Parnianpour M (2013) How well do the muscular synergies extracted via non-negative matrix factorisation explain the variation of torque at shoulder joint? Comput Methods Biomech Biomed Engin 16:291–301.

Muceli S, Falla D, Farina D (2014) Reorganization of muscle synergies during multidirectional reaching in the horizontal plane with experimental muscle pain. J Neurophysiol 111:1615–1630.

Neptune RR, Clark DJ, Kautz SA (2009) Modular control of human walking: A simulation study. J Biomech 42:1282–1287.

Palmieri RM, Weltman A, Edwards JE, Tom JA, Saliba EN, Mistry DJ, Ingersoll CD (2005) Pre-synaptic modulation of quadriceps arthrogenic muscle inhibition. Knee Surg Sports Traumatol Arthrosc 13:370–376.

Palmieri-Smith RM, Thomas AC, Wojtys EM (2008) Maximizing Quadriceps Strength After ACL Reconstruction. Clin Sports Med 27:405–424.

Palmieri-Smith RM, McLean SG, Ashton-Miller JA, Wojtys EM (2009) Association of quadriceps and hamstrings cocontraction patterns with knee joint loading. J Athl Train 44:256–263.

Pataky TC (2010) Generalized n-dimensional biomechanical field analysis using statistical parametric mapping. J Biomech 43:1976–1982.

Portney LG, Watkins MP (2000) Foundations of clinical research: Applications to practice. Upper Saddle River, New Jersey: Pearson Prentice Hall.

Roland MO (1986) A critical review of the evidence for a pain-spasm-pain cycle in spinal disorders. Clin Biomech Bristol Avon 1:102–109.

Rudolph KS, Axe MJ, Buchanan TS, Scholz JP, Snyder-Mackler L (2001) Dynamic stability in the anterior cruciate ligament deficient knee. Knee Surg Sports Traumatol Arthrosc 9:62–71.

Sasaki K, Neptune RR (2010) Individual muscle contributions to the axial knee joint contact force during normal walking. J Biomech 43:2780–2784.

Shimokochi Y, Shultz SJ (2008) Mechanisms of noncontact anterior cruciate ligament injury. J Athl Train 43:396–408.

Shourijeh MS, Flaxman TE, Benoit DL (2016) An approach for improving repeatability and reliability of non-negative matrix factorization for muscle synergy analysis. J Electromyogr Kinesiol 26:36–43.

Shultz SJ, Perrin DH, Adams JM, Arnold BL, Gansneder BM, Granata KP (2001) Neuromuscular response characteristics in men and women after knee perturbation in a single-leg, weight bearing stance. J Athl Train 36:37–43.

Smale KB, Shourijeh MS, Benoit DL (2016) Use of muscle synergies and wavelet transforms to identify fatigue during squatting. J Electromyogr Kinesiol 28:158–166.

Smith AJJ, Flaxman TE, Benoit DL (2012) Reliability of knee joint muscle activity during weight bearing force control. J Electromyogr Kinesiol 22:914–922.

Steele KM, Tresch MC, Perreault EJ (2013) The number and choice of muscles impact the results of muscle synergy analyses. Front Comput Neurosci.

Ting LH, Chiel HJ, Trumbower RD, Allen JL, McKay JL, Hackney ME, Kesar TM (2015) Neuromechanical Principles Underlying Movement Modularity and Their Implications for Rehabilitation. Neuron 86:38–54.

Ting LH, Macpherson JM (2005) A limited set of muscle synergies for force control during a postural task. J Neurophysiol 93:609–613.

Torres-Oviedo G, Macpherson JM, Ting LH (2006) Muscle synergy organization is robust across a variety of postural perturbations. J Neurophysiol 96:1530–1546.

van den Hoorn W, Hodges PW, Dieën JH van, Hug F (2014) Effect of acute noxious stimulation to the leg or back on muscle synergies during walking. J Neurophysiol:jn.00557.2014.

Walter JP, Kinney AL, Banks SA, D'Lima DD, Besier TF, Lloyd DG, Fregly BJ (2014) Muscle synergies may improve optimization prediction of knee contact forces during walking. J Biomech Eng 136.

Wilkie DR (1950) The relation between force and velocity in human muscle. J Physiol 110:249–280.

Williams GN, Barrance PJ, Snyder-Mackler L, Axe MJ, Buchanan TS (2003) Specificity of muscle action after anterior cruciate ligament injury. J Orthop Res 21:1131–1137.

Wojtys E, Huston L (1994) Neuromuscular Performance in Normal and Anterior Cruciate Ligament-Deficient Lower Extremities. Am J Sports Med 22:89–104.

Wojtys EM, Huston LJ, Schock HJ, Boylan JP, Ashton-Miller JA (2003) Gender differences in muscular protection of the knee in torsion in size-matched athletes. J Bone Jt Surg 85 A:782–789.

Yoo JD, Papannagari R, Park SE, DeFrate LE, Gill TJ, Li G (2005) The effect of anterior cruciate ligament reconstruction on knee joint kinematics under simulated muscle loads. Am J Sports Med 33:240–246.

Zeni JA, Rudolph K, Higginson JS (2010) Alterations in quadriceps and hamstrings coordination in persons with medial compartment knee osteoarthritis. J Electromyogr Kinesiol 20:148–154.

Zhang L-Q, Xu D, Wang G, Hendrix RW (2001) Muscle strength in knee varus and valgus. Med Sci Sports Exerc 33:1194–1199.

Study 5: Synergistic and Biomechanical Adaptations to Experimental Muscle Pain during Squatting and Lunging Tasks.

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4.5.1 ABSTRACT

Background: Altered quadriceps function observed in populations with knee pathologies are often described as deficits. The musculoskeletal system has many redundancies and there are very few studies that objectively link muscle activation with its resultant functional load in vivo, making the physiological effect of these compensation strategies unclear. Muscle activation during dynamic movement can be characterised by a reduced set of activation signals called muscle synergies. This study evaluated the effect of acute pain (causing muscle inhibition) on knee joint dynamics and muscle synergies recruited during squatting and lunging tasks. **Study Design:** Cross-over placebo controlled. **Methods:** Twenty-two healthy participants performed squatting and lunging tasks before, during and after muscle pain. Muscle pain was induced with injections of hypertonic saline (5.8%) to the vastus medialis (VM). Isotonic saline (0.9%) was used as control. Concatenated non-negative matrix factorization was used to extract muscle synergies from electromyography of ten leg muscles and full body motion analysis extracted joint kinematics and kinetics. **Results**: Both tasks recruited a synergy dominated by quadriceps that correlated with knee extensor moments, and a co-activation synergy recruited throughout the cycle. Lunging recruited a third synergy associated with heel strike and push off, which is attributed to the added complexity of the task. Muscle pain significantly reduced quadriceps synergy recruitment during the concentric phase of both tasks. This was accompanied by reduced knee joint flexion angles and knee extensor moments. **Conclusion:** The consistency in muscle synergy structure and function across different tasks suggests muscle synergies represent motor modules that can be flexibly recruited to execute a variety of movements. As a response to pain, the descending neural input to the muscle synergies specific for facilitating movement is inhibited to reduce the load on the painful tissue and protect from further pain and/or injury.

4.5.2 INTRODUCTION

Altered quadriceps function is commonly observed in populations with musculoskeletal disorders involving the knee (Powers et al., 1997; Slemenda et al., 1997; Palmieri-Smith et al., 2008). It is caused by a reduced ability to voluntarily activate the muscle group resulting in significant deficits in knee extensor strength (Hopkins and Ingersoll, 2000; Hodges and Tucker, 2011). Following a traumatic knee injury quadriceps weakness is theorized to be a strategy to prevent painful and potentially detrimental movements; however, reduced voluntary muscle function can be a main contributor to decreased joint stability and physical function commonly observed in these populations (MacWilliams et al., 1999; Houck and Yack, 2001; Hashemi et al., 2007; Houck et al., 2007). To better understand the direct effect muscle inhibition has on knee joint function, models of experimental muscle pain have been used. Experimental quadriceps pain inhibits voluntary activation of the target muscle, yields deficits in knee extensor strength comparable to levels observed clinically (Graven-Nielsen et al., 2002; Palmieri-Smith et al., 2008; Henriksen et al., 2010), and alters knee joint dynamics during functional tasks (Palmieri et al., 2005; Henriksen et al., 2007, 2009, 2010, 2011; Arendt-Nielsen et al., 2008).

Although the quadriceps are important for maintaining knee stability (Hashemi et al., 2007; Sasaki and Neptune, 2010; Cashaback and Potvin, 2012), it is possible to walk with weakened quadriceps muscles (Simonsen et al., 1997; Goldberg and Neptune, 2007; Henriksen et al., 2007). This may be due to relatively low demands on the knee and compensation from other muscles that can generate moments necessary for normal walking (Simonsen et al., 1997; Goldberg and Neptune, 2007; Henriksen et al., 2007). The forward lunge and two-legged squat are functionally and clinically relevant tasks that are primarily controlled by the quadriceps, elicit relatively greater knee joint loads than walking, and challenges knee joint stability (MacLean et al., 1999; Alkjaer et al., 2011). Evaluating the effect of muscle pain on knee joint control during these tasks may therefore have important implications for prevention and rehabilitation of knee joint diseases and injuries.

Differences in muscle activation and movement strategies induced by pain are highly variable (Bank et al., 2013). To help explain these discrepancies, Hodges and Tucker (2011) proposed a theory of motor adaptation to pain that involves the redistribution of activity within and between muscles. If this theory is valid, common approaches of evaluating pain-related changes in individual muscles using discrete variables (Henriksen et al., 2007, 2009; Hodges et al., 2009) may not provide sufficient information. Several studies support the notion that the central nervous system (CNS) can control numerous degrees of freedom of the body by a reduced set of activation signals called *muscle synergies* (Ting and Macpherson, 2005; Torres-Oviedo et al., 2006; Neptune et al., 2009; Ting et al., 2015). This is not only methodologically favorable because it simplifies muscle activation, but muscle synergies may represent basic neural mechanisms that are common across different dynamic conditions such as one- versus two-legged squats (Torres-Oviedo and Ting, 2010), stepping versus non-stepping (Chvatal et al., 2011) responses during surface perturbations, mechanical constraints during pedaling (Hug et al., 2010), different speeds during gait (Ivanenko et al., 2004), voluntary versus reactive response to perturbed walking (Chvatal and Ting, 2012), and different lifting techniques (Mirakhorlo and Mahmood, 2015).

The purpose of this study was two-fold: (1) to determine if muscle synergies recruited during lunging and squatting shared characteristics or were task specific and, (2) to evaluate the effect of experimental quadriceps muscle pain (causing voluntary inhibition) on knee joint dynamics and these muscle synergies. Recent evidence suggests muscle pain modifies existing motor modules or leads to recruitment of new ones (van den Hoorn et al., 2014; Gizzi et al., 2015). Accordingly, it was shown that experimental muscle pain would reduce vastus medalis' (site of noxious stimulus) relative weightings in synergies that recruit it (Flaxman et al., Study 4), cause a reorganization of muscle synergies as compensation (Hodges and Tucker, 2011; van den Hoorn et al., 2014; Gizzi et al., 2015), and impair knee joint dynamics (Henriksen et al., 2009).

4.5.3 METHODS

Participants

Twenty-two healthy active adults [12 males (height=181.3±6.3cm; weight=79.8kg; age=27.25±4.0yrs) and 10 females (height=170.9±5.4cm; weight=65.1±9.1kg; age=22.7±1.4yrs)] with no history of significant lower limb injuries were recruited from the University of Copenhagen, Denmark and the surrounding community. Experiments were completed at the University of Copenhagen and all participants provided written informed consent. Local ethics committee for the Capital Region of Denmark (De Videnskabsetiske Komiteer for Region Hovedstaden, H-3-2013-126) and the University of Ottawa Research Ethics Board (H06-14-25) approved this study.

Study Design

A crossover study, placebo-controlled study design tested each participant on 2 days separated by 1 week. During a test day, 3 series of tasks were completed, each under a different condition (baseline-BASE, with injection-INJ, or recovery-REC). BASE and INJ conditions included 2 sets of squatting and lunging tasks while REC included one set. Immediately before each INJ set, an intramuscular saline injection was administered. Rest times between BASE and each INJ set was 5 minutes. A 20 minute rest period was held between the second INJ set to ensure any perception of pain was gone before REC was performed.

Experimental Procedure

<u>Injection Protocol</u>

Hypertonic saline (HYP; 5.8%) was used to induce muscle pain. Isotonic saline (ISO; 0.9%) was used as control. The order of saline injections was randomized and only one type of saline was given on a test day. Intramuscular bolus injections of 1ml were manually administered into the belly of the vastus medialis (VM) using a 5-ml plastic syringe with a 27 gauge, 40 mm disposable needle at a depth of 3.5 cm. Perception of pain was monitored every 30 seconds during INJ and before starting REC using a verbal scale of 0-10 (10 indicates worst imaginable pain).

Exercise Protocol

Following participant setup (below),a series of at least 10 practice trials of each task were completed to familiarise participants and serve as warm-up. Maximum voluntary isometric contractions (MVICs) in hip flexion, hip extension, hip abduction, hip adduction, knee extension, knee flexion, and plantarflexion were completed with manual resistance.

Participants then completed a set of lunges and squats with 10 repetitions in each set. A metronome helped to limit the pace of squatting and lunging cycles to 4 seconds: 1 second for descent, 1 second for transition, 1 second for ascent, and 1 second for a rest. Squatting tasks started with participants standing straight, feet hip width apart, and arms stretched out in front, parallel to the floor. Participants squatted until thighs were parallel to the floor while keeping weight on their heels and their chest up. Lunging tasks required participants to stand hip width apart, hands on the back of their head, and lunge forward with their test leg until their front knee was flexed to 90° and return to the starting position by pushing off with their test leg, all while keeping their chest up and facing forward.

Participants also completed a set of force matching trials prior to each squatting and lunging series. This data has been reported in a separate study (Flaxman et al., Study 3).

Data Collection and Processing

Retro-reflective markers were placed on various anatomical landmarks on the participant's body according to the HMBL Cluster Marker set (Mantovani and Lamontagne, 2016) (Appendix A). Trajectories were recorded with using a ten-camera motion analysis system (6 T-40 and 4 F-40 Vicon cameras, Oxford Metrics, Oxford, UK) sampling at 100 Hz using Nexus software (version 1.8, Oxford Metrics, Oxford, UK). GRFs recorded from a force platform (AMTI-OR6, AMTI, Watertown, MA) were also collected in Nexus at 1000Hz. Electrodes placed in bipolar configuration recorded surface electromyography (EMG) of VM, vastus lateralis (VL), rectus femoris (RF), semitendinosus (ST), biceps femoris (BF), medial gastrocnemius (MG), lateral gastrocnemius (LG), tensor fascia latae (TFL), gluteus medius (GM), and hip adductor (ADD) muscle group of the dominant leg (defined as leg used to kick a soccer ball as far as possible). EMG was sampled at 1000Hz with a 20-500Hz bandwidth and a 6dB/octave filter slope using a wireless system (MQ air, Marq Medical, Farum Denmark). Raw marker trajectories and GRFs were conditioned with a 2nd order dual pass 15Hz cut-off low pass Butterworth filter (Kristianslund et al., 2012) and exported to OpenSim (v 3.2, (Delp, 2007)) to compute local coordinate systems of the test limb and subsequent knee joint angles and internal net joint moments using inverse kinematics and dynamics. EMG signals were processed to obtain a linear envelope with a 2nd order dual-pass high-pass (20Hz) Butterworth filter, full wave rectified, and a 2nd order dual-pass low-pass (10Hz) Butterworth filter. Experimental EMG were normalized to each muscle's EMG$_{max.}$

Data Analysis

Muscle Synergies

Muscle synergies were extracted for each condition using a concatenated non-negative matrix factorization (CNMF) framework (Shourijeh et al., 2016; Smale et al., 2016; Flaxman et al., Study 4). Each participant's data formed an $n \times m$ matrix where n is the number of time normalised data points (n=100 for squats, 120 for lunges) and m is the number of muscles (m=10). The input (A) into the CNMF framework was $N \times M$ data matrix with participant data vertically concatenated such that N=2200 for squats (100 data points* 22 participants) or N=2640 for lunges (120 data points* 22 participants) and M=10.

Two output matrices were built with a solver: the first being a $q \times M$ synergy matrix (W) where q is the number of synergy vectors and M is the number of synergy elements (i.e. muscles). Each synergy element specified the relative contribution of each muscle between 0 and 1. The second output is an $N \times q$ coefficient matrix (C) where each element of C is the relative scaling factor for each synergy (q) at a given point in time of each participant. When W and C are multiplied, the original input matrix, A, can be expressed as the weighted sum of synergies $A \approx WC$. The required number of synergies that best characterizes our original data was determined by (1) accounting for >90% of the total variance accounted for (VAF) and/or (2) adding another synergy will increase the VAF ≤5% VAF. BASE and INJ analyses were run separately. As such, similar synergies may be produced between conditions but in a varied order. Synergies were resorted based on visual inspection.

Statistical Analysis

Synergy similarity across tasks: The similarity of BASE synergy vectors between tasks was assessed with intraclass correlation coefficients (ICC$_{(1,k)}$) (McGraw and Wong, 1996). ICCs were opted over Pearson correlations because in addition to making inferences about variance, it takes into account changes in amplitudes when quantifying similarity (McGraw and Wong, 1996). We considered synergy vectors to be statistically similar if ICC≥0.75 (Portney and Watkins, 2000). For statistically similar synergies (ICCs>0.75), the percent relative change (%RC) in muscle synergies were expressed as the difference in linear vector norms from BASE$_{\text{lunges}}$ to BASE$_{\text{squats}}$:

$$\%RC = \frac{\left\|BASE_{lunges}\right\| - \left\|BASE_{squats}\right\|}{\left\|BASE_{lunges}\right\|} * 100$$

Where *j* is the given synergy number. A negative number indicates a reduction from BASE$_{\text{lunges}}$ to BASE$_{\text{squats}}$, a positive indicates an increase.

To test the uniqueness of each task's synergies, the experimental data of a given task was reconstructed by the synergy(s) that were statistically similar in the opposite task (cross-reconstructions) (Gizzi et al., 2015; Flaxman et al., Study 4). Similar VAF values would be expected when the synergy weightings extracted in one condition could describe the muscle activation patterns of another condition.

Effect of injection type: A two-way within-participant repeated measures ANOVA tested the effect of injection type (HYP vs ISO) and condition (BASE, INJ, and REC) on peak sagittal joint angles, integrated EMG (iEMG), and angular impulses (area under the moment curve). Post-hoc analysis was conducted using dependent T-tests. Within-session post-hoc comparisons were

BASE-INJ and BASE-REC. Between-session (HYP and ISO) post-hoc comparisons were percent baseline value at INJ and REC. A Bonferroni adjustment of alpha to 0.025 was made based on two comparisons within and between sessions.

Effect of pain on synergies: Only the BASE and INJ synergies of the HYP session were used**.** The similarity of muscle synergy vectors between conditions was assessed with $\mathrm{ICC}_{(1,k)}$. The percent relative change (%RC) in muscle synergies were expressed as the difference in linear vector norms from BASE to INJ:

$$\%RC = \frac{\left\|BASE_j\right\| - \left\|INJ_j\right\|}{\left\|BASE_j\right\|} * 100$$

Where *j* is the given synergy number. Similarly, the %RC in the norm of individual element weightings across all synergies was also computed. A negative number indicated a reduction from BASE to INJ, a positive indicated an increase.

To test the uniqueness of each condition's synergies, the experimental data of a given condition was cross-reconstructed by means of the synergy matrix extracted from the other condition (Gizzi et al., 2015; Flaxman et al., Study 4).

Between condition differences in synergy activation coefficients were analysed with statistical parametric mapping (SPM) dependent T-tests (Pataky, 2010). Significance was defined at the alpha=0.05 level.

4.5.4 RESULTS

Synergy similarity across tasks

Two synergies were required to reconstruct the squat task (S_{squats}), while three synergies were needed for the lunge task (S_{lunges}). Synergies-1 ($S1_{\mathrm{lunges}}$ and $S1_{\mathrm{squats}}$) and S2 ($S2_{\mathrm{lunges}}$ and

$S2_{squats}$) of both tasks were statistically similar (Table 6). The %RC of $S1_{lunges}$-$S1_{squats}$ and $S2_{lunges}$-$S2_{squats}$ was -0.42% and 11.73%, respectively. Cross-reconstruction of squatting data using $S1_{lunges}$ and $S2_{lunges}$ accounted for 88.97% total VAF. Cross-reconstruction of lunging data using $S1_{squats}$ and $S2_{squats}$ and $S3_{lunges}$ accounted for 89.32% total VAF.

Table 6: Intraclass correlation coefficients (ICC$_{(1,k)}$) comparing similarity of synergy vectors between squatting and lunging tasks. Asterisks (*) indicates statistical similarity at ICC>0.75.

		Squats	
	S#	1	2
Lunges	1	0.99*	-0.55
	2	-0.38	0.79*
	3	-0.72	-0.42

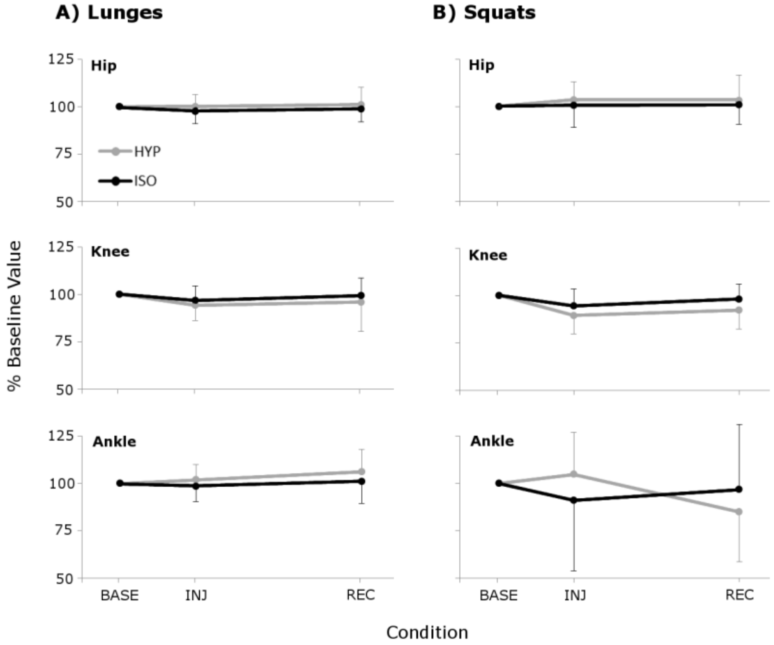

Figure 28: Group mean **(A)** lunge and **(B)** squat hip, knee and ankle sagittal angular impulses (Nm/kg*s) of the INJ and REC conditions normalised to baseline values of both HYP (grey) and ISO (black) sessions. There are no statistically significant differences between ISO and HYP injections at INJ and REC conditions.

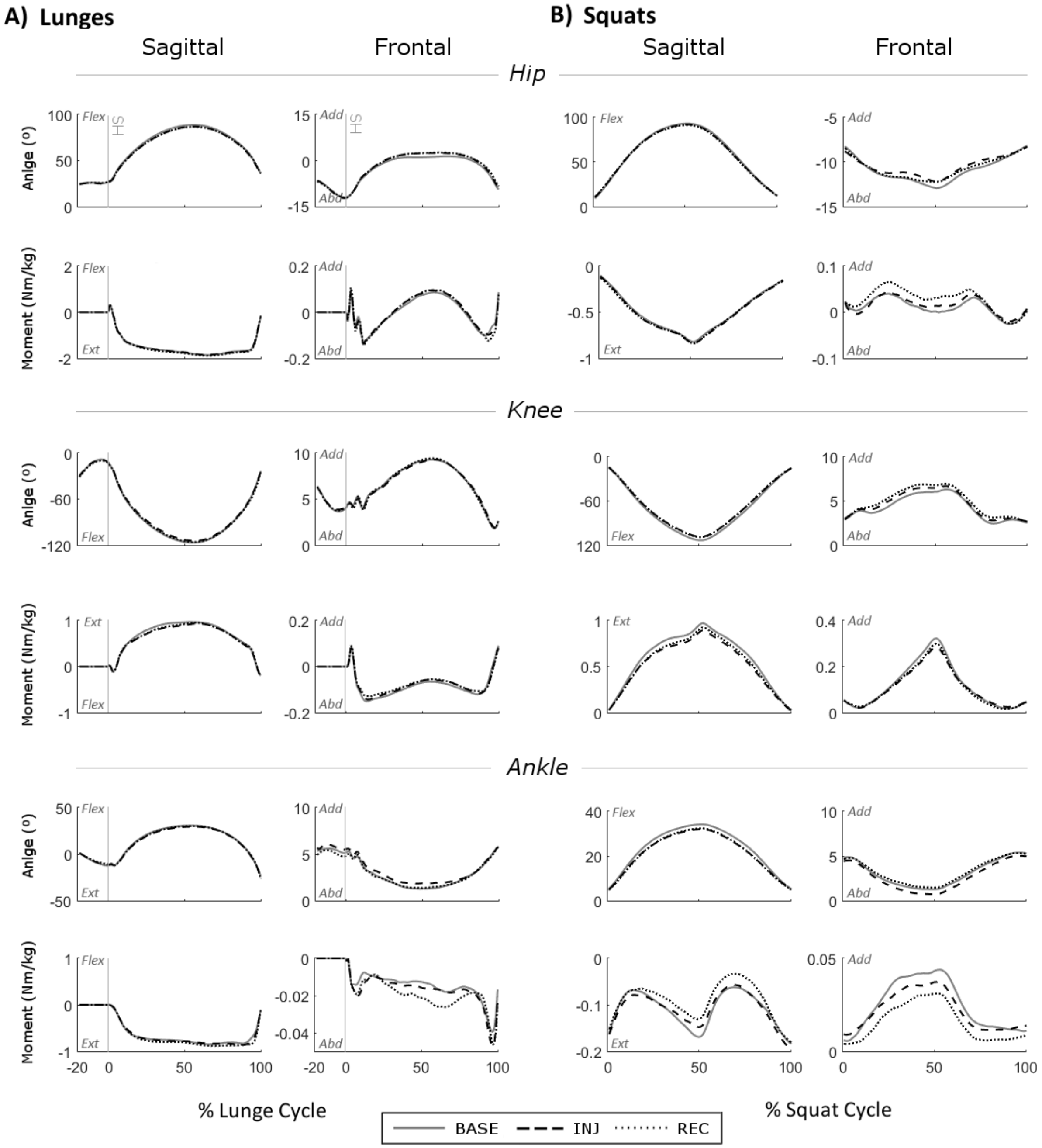

Figure 29: Group mean hip, knee and ankle joint angles and moments in the sagittal and frontal planes for (A) lunges and (B) squats during of the hypertonic session for baseline (BASE), with injection (INJ) and recovery (REC) conditions. Lunges are time normalised to 100% time spent on force plate plus 20% of this time prior to heel strike (HS). Squats are time normalised to 90% knee flexion range of motion.

Effect of injection type

Joint Dynamics

Group mean sagittal joint angles and impulse moments of the HYP session are presented in Figure 29. No significant interactions of *Injection x Condition* were observed (Figure 28). A significant main effect of *Condition* was observed during lunges for peak hip flexion angle (p=0.007), peak knee flexion angle (p=0.010), and angular impulse (p=0.030) with BASE > INJ values for both sessions. A Significant main effect of *Condition* was observed during squatting for peak knee flexion angle (p<0.001), peak ankle dorsiflexion angle (p=0.007), and knee extensor impulse moment (p<0.001) with BASE > INJ values for both sessions. Knee joint angle and extensor impulse moment also had BASE > REC values for both sessions.

Muscle Activation

For lunges, a significant *Injection x Condition* interaction for VM (p<0.001) and RF (p=0.048) iEMG were observed. Post-hoc analysis showed significantly reduced VM activity from BASE to INJ (p<0.001) and BASE-REC (p<0.001) of the HYP session and reduced activity from BASE to INJ (p=0.015) of the ISO session. RF was reduced from BASE to INJ of the HYP session (p=0.004). Significant main effect of *Condition* was observed for TFL (p=0.025), with reduced activity BASE to INJ during ISO session (p=0.008). Percent BASE value of the VM was less in the HYP session compared to the ISO session in the INJ (p<0.001) and REC (p<0.001) conditions (Figure 30 and Figure 31A).

For squats, a significant *Injection x Condition* interaction for VM iEMG (p=0.001) was observed. Post-hoc analysis showed reduced VM activity from BASE to INJ (p<0.001) and BASE-REC (p<0.001) of the HYP session and reduced activity from BASE to INJ (p=0.007) of the ISO session. A significant main effect of *Condition* was observed for the TFL (p=0.037), LG (p=0.001), and MG (p=0.005), with reduced activity BASE to -INJ for both sessions (p<0.025). Percent BASE value of the VM was less in the HYP session compared to the ISO session in the INJ (p<0.001) and REC (p<0.001) conditions (Figure 31B).

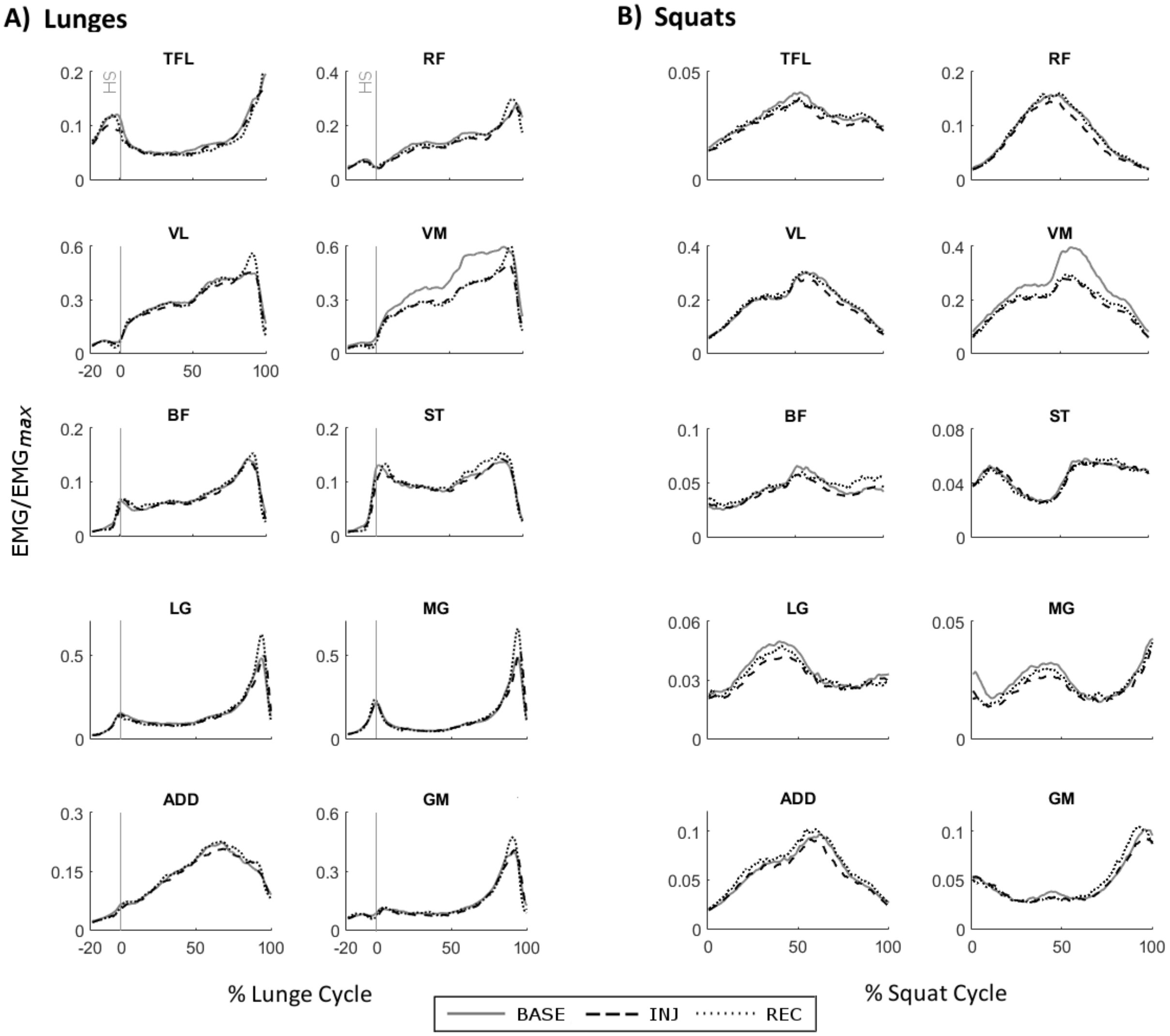

Figure 30: Group mean EMG activation patterns for (A) lunges and (B) squatting tasks of the hypertonic session. Lunges are time normalised to 100% time spent on force plate plus 20% of this time prior to heel strike (HS). Squats are time normalised to 90% knee flexion range of motion. Tasks were performed during baseline (BASE), with injection (INJ), and recovery (REC) conditions.

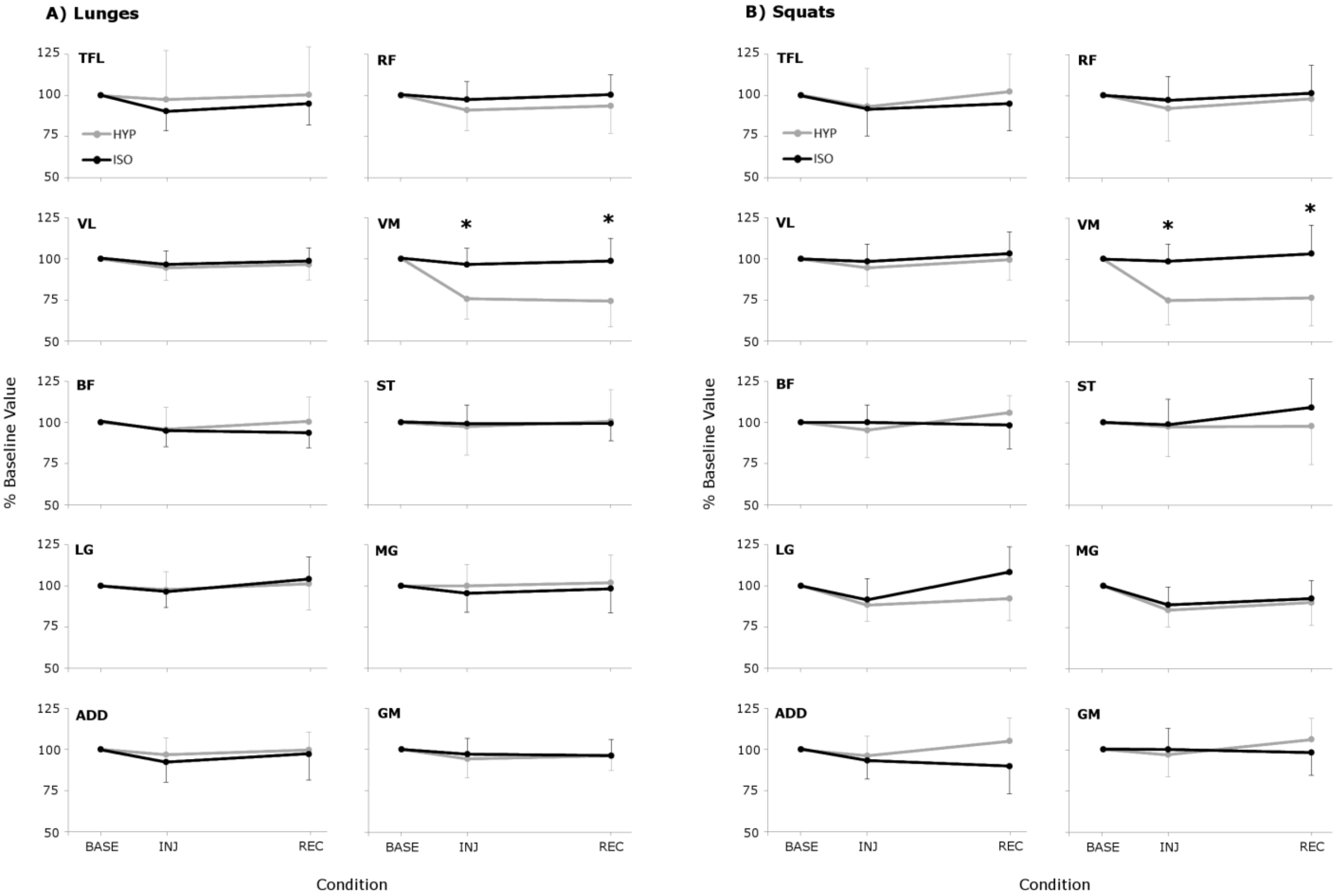

Figure 31: Group mean **(A)** lunge and **(B)** squat integrated EMG of the injection (INJ) and recovery (REC) conditions expressed as percent baseline (BASE) values for the hypertonic (HYP; grey) and isotonic (ISO; black) sessions. Only the VM had showed significant differences between ISO and HYP sessions in percent change from baseline values during the injection (INJ) and recovery (REC) conditions. Significant difference at the p<0.05 level denoted with an asterisks (*).

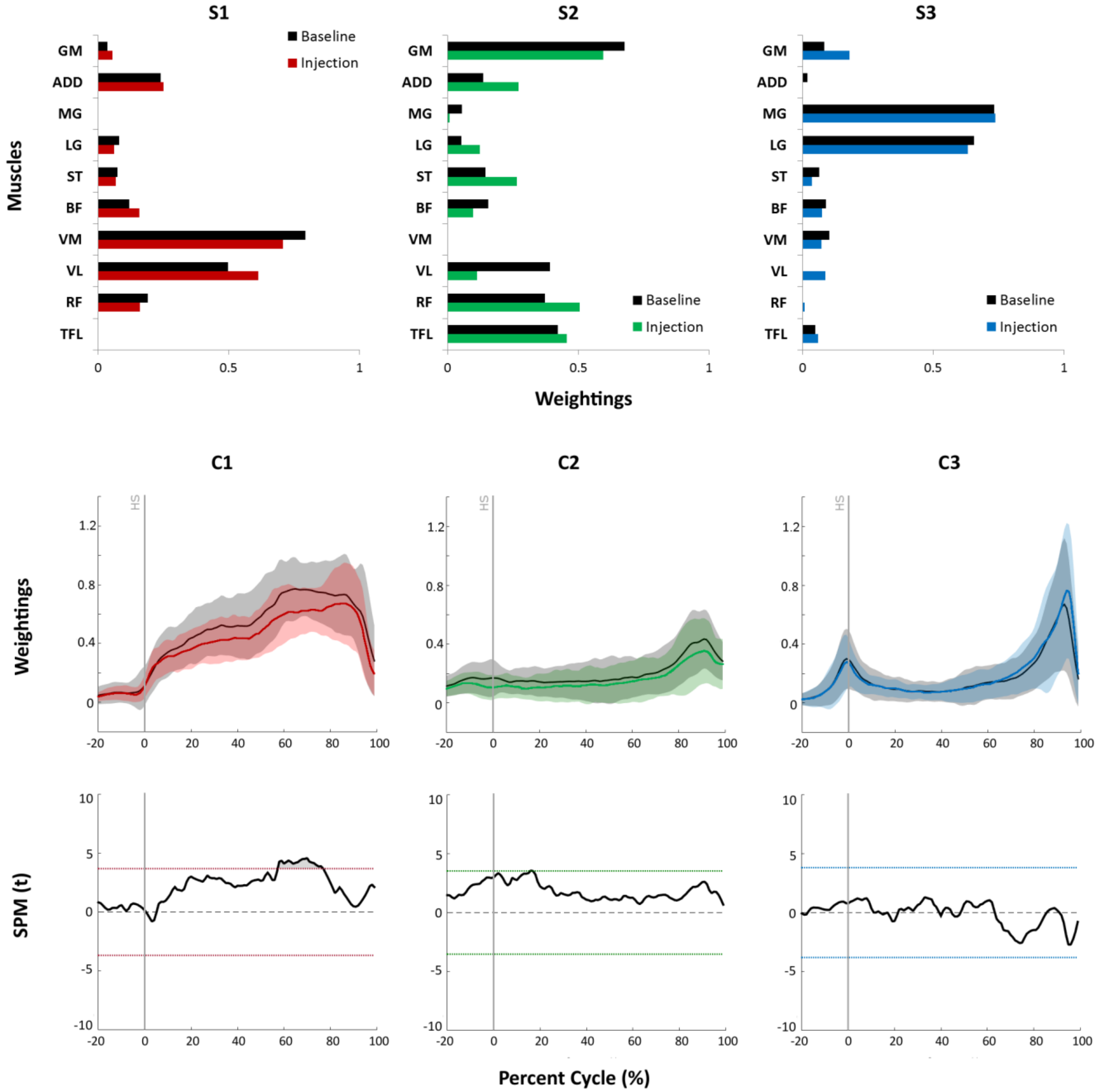

Figure 32: Muscle synergies (S) and respective scaling coefficients (C) for Baseline and Injection conditions of lunging tasks. Lunges are time normalised to 100% time spent on force plate plus -20% of this time prior to heel strike (HS). Significant differences in C tested with a SPM (t) statistic for repeated measures. A significant t value denoted by the coloured horizontal lines in the bottom graphs representative of a *p*=0.05 level. Squats are time normalised to 90% knee flexion range of motion.

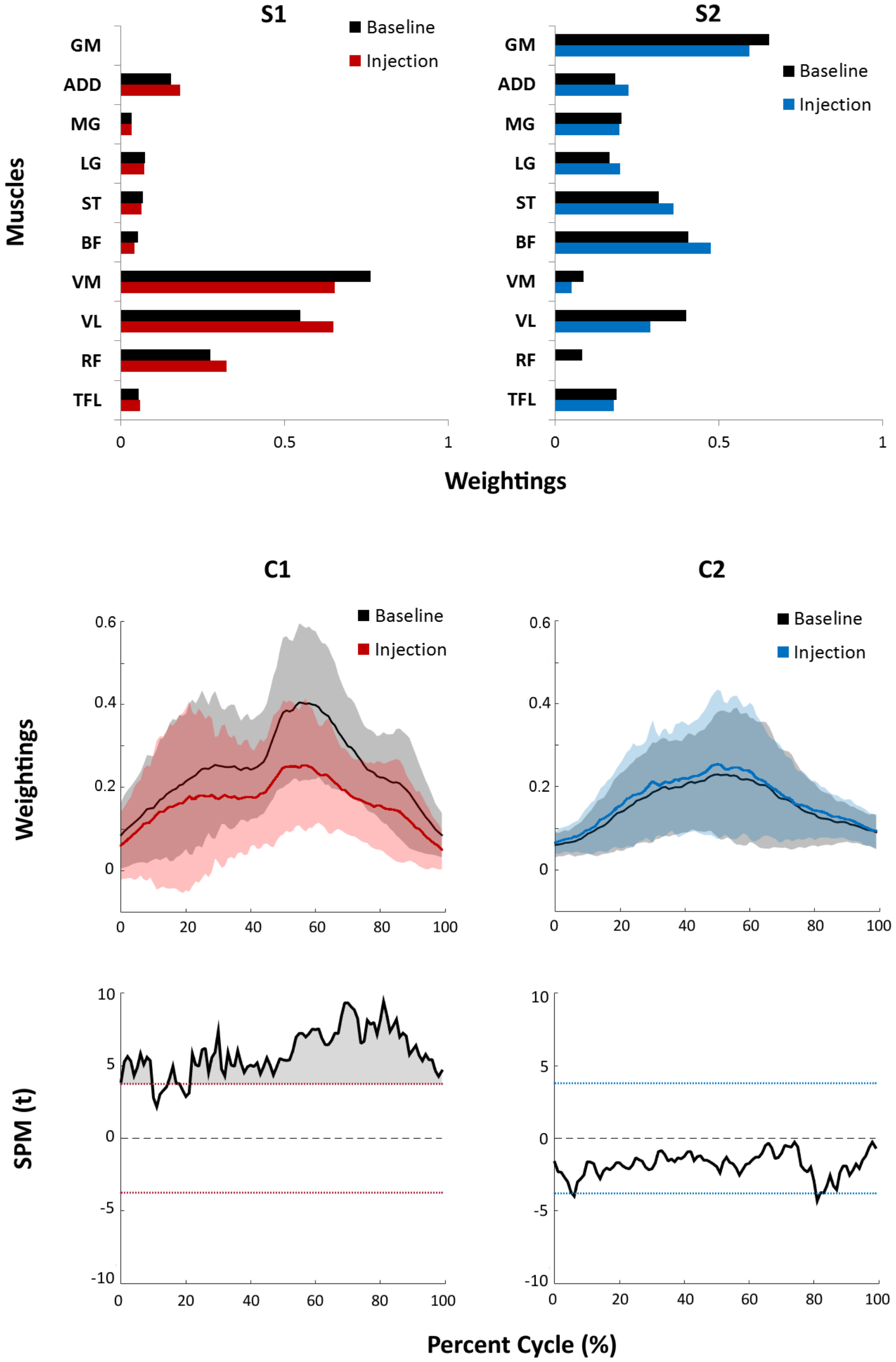

Figure 33: Muscle synergies (S) and respective scaling coefficients (C) for Baseline and Injection conditions of squatting tasks. Squats are time normalised to 90% knee flexion range of motion. Significant differences in C tested with a SPM (t) statistic for repeated measures. A significant t value denoted by the coloured horizontal lines in the bottom graphs representative of a *p*=0.05 level.

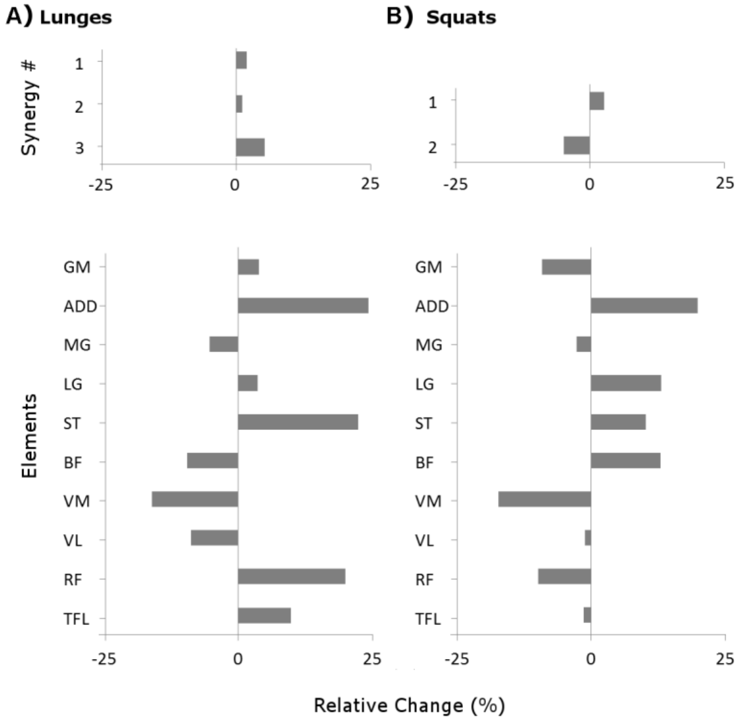

Figure 34: Percent relative change (%RC) in synergy vector norms (top) and the norm of individual element weightings across all synergies (bottom) of **(A)** lunging and **(B)** squatting tasks. Positive number indicates overall increase; negative is decrease from BASE to INJ conditions.

Effect of pain on synergies

Three synergies were required to reconstruct 93.54% and 92.77% VAF of lunging data in BASE and INJ conditions, respectively (Figure 32). $S1_{lunges}$, $S2_{lunges}$ and $S3_{lunges}$ contributed 45.3%, 32.7% and 22.0% to total VAF in BASE and 44.9%, 30.1% and 24.0%, in INJ conditions respectively. Two synergies were required to reconstruct 91.86% and 93.24% VAF of squatting data in BASE and INJ conditions, respectively (Figure 33). $S1_{squats}$ and $S2_{squats}$ contributed 61.0% and 39.0% to total VAF in BASE and 56.3% and 43.7% in INJ conditions, respectively.

Muscle synergies were extremely similar between conditions for both tasks as indicated by ICCs of 0.91-0.99 and %RC in synergy weights <5% (Figure 27). Cross-reconstruction of experimental BASE data using the INJ synergies accounted for 90.3% and 89.7% VAF in lunging and squatting, respectively. Cross-reconstruction of experimental PAIN data using the BASE synergies accounted for 91.2% and 91.0% VAF for lunging and squatting, respectively.

$C1_{lunges}$ was significantly reduced from BASE to INJ at -8 to -11% (before heel strike) and 10 to 86% lunge cycle (p-values<0.01) (Figure 32). $C2_{lunges}$ was reduced from BASE to INJ at -4 to -6% (before heel strike) (p=0.029). $C1_{squats}$ was reduced from BASE to INJ throughout the entire general squat cycle (0-10, 14-18, 22-100%; p-values<0.007) (Figure 33). $C2_{squats}$ was greater from BASE to INJ at 6% (p=0.029) and 81-82% (p=0.011) squat cycle.

4.5.5 DISCUSSION

This study identified muscle synergies recruited during lunging and squatting tasks and determined the effect of experimental quadriceps muscle pain on knee joint dynamics and these muscle synergies. Our results in part support our hypotheses: experimental muscle pain reduced VM's relative weightings in synergies that recruit it, signifying inhibition, which impaired knee joint dynamics as evidenced by reduced knee joint flexion and knee extensor moments. However, muscle pain did not significantly reorganize synergy composition as previous work suggests (van den Hoorn et al., 2014; Gizzi et al., 2015), but rather a down-regulation of quadriceps dominant synergies.

Shared Synergies

The first aim of this study was to determine if two knee extensor dominant tasks that varied in demand levels and complexity but showed similar movement profiles of the test leg could recruit a common set of muscle synergies or if synergies were task specific. Several works have observed a common set of muscle synergies recruited under different dynamic conditions (Ivanenko et al., 2004; Hug et al., 2010; Torres-Oviedo and Ting, 2010; Chvatal et al., 2011; Chvatal and Ting, 2012; Mirakhorlo and Mahmood Reza Azghani, 2015). Three muscle synergies accounted for >90% VAF in experimental lunging data and two synergies for the squatting data.

Our results show two sets of synergies ($S1_{lunges}$/$S1_{squats}$ and $S2_{lunges}$/$S2_{sqauts}$) that displayed extremely similar composition across tasks (ICCs=0.98 and 0.79) and could reconstruct the data of the opposite task. The presence of common muscle synergies in lunging and squatting may reflect a "*motor repertoire, or library of motor subtasks*", that can be flexibly recruited to execute a variety of movements (Chvatal and Ting, 2012).

S1 of both tasks ($S1_{lunges}$ and $S1_{sqauts}$) were dominated by quadriceps muscle weightings. This is not surprising considering the profiles of C1 and knee extensor moment are similar (refer to second row of Figure 29 and C1 of Figure 32 and Figure 33). This similarity was confirmed post-hoc with significant group mean (standard deviation) ICCs of 0.75 (0.09) and 0.88 (0.10) for lunging and squatting tasks, respectively. Although no known studies have investigated synergistic control during lunging or squatting tasks, several studies have identified quadriceps dominant synergies during early stance phases of gait (Ivanenko et al., 2004; Neptune et al., 2009; Chvatal and Ting, 2012), when internal knee extension moment is the greatest, suggesting $S1_{lunges}$ and $S1_{squats}$ are specific for body support and movement control.

In addition, each task recruited a more "general" muscle synergy ($S2_{lunges}$ and $S2_{sqauts}$), not dominated by a single muscle group. Co-activation synergies have been identified as important strategies for stabilizing the knee against external knee abduction and knee rotation loads (Flaxman et al., Study 4). Since the knee lacks sufficient muscular contributions to support against frontal and transverse plane loads (Brand et al., 1982; Buchanan and Lloyd, 1997; Yoo et al., 2005), these general muscle synergies may be co-activation strategies to support the knee throughout the task cycle (MacWilliams et al., 1999; Cashaback and Potvin, 2012).

An extra synergy was required to reconstruct the lunging data ($S3_{lunges}$). Recruitment of task specific muscle synergies, in addition to pre-existing ones, has been observed when tasks increase in complexity, such as stepping versus non-stepping or and one-legged versus two-legged postural responses to surface perturbations (Clark et al., 2010; Torres-Oviedo and Ting, 2010; Chvatal et al., 2011). $S3_{lunges}$ corresponded to the heel strike and push-off phases and was dominated by the gastrocnemii. This has important implications for understanding muscular contributions to knee stability and injury considering (1) knee joint injuries and or episodes of giving way occur at time of impact or immediately afterwards (Boden et al., 2000; Shimokochi and Shultz, 2008), (2) gastrocnemius activity is altered in populations with unstable knees (Rudolph et al., 2001), and (3) due to its confounding role at the ankle, little literature is available about its contribution at the knee, in particular during weight bearing, and its role as it relates to knee joint stability is often debated (Durselen et al., 1995; Fleming et al., 2001; Morgan et al., 2014).

Effect of pain on synergies

The main goal of this study was to evaluate the effect of experimental quadriceps muscle pain (causing voluntary inhibition) on knee joint dynamics and muscle synergies recruited during lunging and squatting tasks. It is generally accepted that experimental muscle pain inhibits voluntary activation of the target muscle (Graven-Nielsen et al., 2002; Palmieri-Smith et al., 2008; Henriksen et al., 2010); however, compensatory responses from non-painful muscles are highly variable (Bank et al., 2013) and do not align with simple theories of motor adaptation to pain (Roland, 1986; Lund et al., 1991). To explain inconsistent findings across studies, Hodges and Tucker (2011) theorized that pain leads to spatial redistribution of muscle activity within and between muscles. As such, we hypothesized that experimental VM pain would cause a reorganization of muscle synergies. Our results in part support our hypothesis: there was a significant reduction in VM's contribution to synergy weights; however for both tasks, BASE and INJ synergy vectors were highly correlated and similar reconstruction quality was observed, indicating the synergies recruited in the painful condition were not unique.

Another key aspect proposed by Hodges and Tucker (2011) is that muscular adaptations to pain changes movement behaviour in order to protect the body from further pain or injury. We observed significantly reduced peak knee joint flexion angles and extensor angular impulse. A decrease in quadriceps dominant synergy recruitment ($S1_{\text{lunges}}$ and $S1_{\text{squats}}$) was also observed, characterised by reduced scaling coefficients. Rather than reorganization of synergy composition, our results relate the change in joint dynamics to the inhibition of the quadriceps muscle synergy. Decreased quadriceps activation has been linked to decreased joint stiffness and ACL injury mechanism (Hashemi et al., 2007; Sasaki and Neptune, 2010; Cashaback and Potvin, 2012). Extrapolation on this, our results indicate muscle pain causes inhibition of voluntary function which may result in knee instability and increased risk of injury. In other conditions that inhibit descending motor signals, such as cortical stroke in humans, strikingly similar muscular compositions of the synergies have also been observed for both unaffected and affected arms (Cheung et al., 2009). This demonstrates the robustness of muscle synergies and supports the notion that the CNS has a pre-determined set of synergies whose activations are simply impaired by injury or pain leading to changes in motor performance.

In addition, significant reductions in quadriceps dominant synergies were observed during the concentric phase of each movement. Previous work has identified significant pain-induced strength deficits during concentric exercise (Henriksen et al., 2011); however, the effect of muscle pain during different contraction types remains unknown. Considering eccentric contraction exercise is more demanding, and more effective for treating individuals with knee pain (Jonsson and Alfredson, 2005), our results of no difference during the descent phases suggest eccentric exercise may be more beneficial at maintaining neuromuscular function in populations with muscle weakness.

Experimentally induced pain has caused significant reductions of the noxious muscle to synergies at the neck, shoulder, lower-back and calf, but the changes in the pain-free muscles surrounding the joints were participant-specific (van den Hoorn et al., 2014; Muceli et al., 2014; Gizzi et al., 2015). Similarly, we have identified a participant-specific compensatory response in non-painful muscles during an isometric, weight-bearing force control task (Flaxman et al., Study 4). In the current study, we evaluated the change in iEMG from BASE to INJ for each participant (Table 7). General decreases in VM's iEMG were observed, but the redistribution (decrease/increase) of activation in other muscles varied across participants. Our synergies were extracted using a CNMF framework, which we believe to be a more robust and appropriate approach for evaluating between group/condition differences in synergy variables (Shourijeh et al., 2016; Smale et al., 2016). However, this approach fixes synergies across all participants so if adaptive responses to muscle pain are not systematic, then pain-related changes may not be reflected in the synergy output. This may explain the lack of between condition uniqueness in synergy compositions. Nevertheless, our framework did allow the scaling coefficients to fluctuate and account for inter-participant variability. As such, participant-specific responses to pain are represented by changes in coefficient weightings, which in turn, demonstrated significant reductions in quadriceps activation that otherwise were not apparent in the synergy vectors or discrete variables.

Effect of Injection Type

The effect of injection type was evaluated using discrete variables such as peak joint angles, impulse moments, and iEMG to help facilitate comparison between our results and those previously reported (Henriksen et al., 2007, 2009). Henriksen et al. (2007, 2009) found that experimental muscle pain of the VM impairs knee joint control by attenuating knee extensor moments (Henriksen et al., 2007, 2009). Although not tested statistically, they also reported a decrease in muscle activity and knee loads during the control (ISO) session. Similarly, our results show a reduction in VM activation, peak knee flexion angles, and knee extensor impulse moments from BASE to INJ during both pain-inducing (HYP) and control (ISO) sessions, even though the HYP session yielded significantly higher perceived pain than the ISO session (scale 0-10: HYP=3.45±1.69, ISO=0.29±0.55) (Flaxman et al., Study 3). A significant main effect of INJ (indifferent of type) suggests the simple presence of an intramuscular saline bolus impairs knee control and function indicating that the aforementioned differences may not be exclusively associated with muscle pain. The significantly greater reduction in muscle activation from BASE to INJ conditions during the HYP session compared to ISO indicates pain amplifies these adaptations.

Table 7: Individual variation of integrated EMG change for each of the 22 participants showing the direction of change between baseline (BASE) and injection (INJ) conditions of the hypertonic (pain inducing) session. Blue (-) indicates an increase, red (-) indicates a decrease, and white (empty) indicates no change in the INJ condition compared to BASE. The threshold used to define a change was set to ±10% of the baseline average EMG amplitude or moment over all target directions.

	Lunges										**Squats**									
	TFL	***RF***	***VL***	***VM***	***BF***	***ST***	***LG***	***MG***	***ADD***	***GM***	***TFL***	***RF***	***VL***	***VM***	***BF***	***ST***	***LG***	***MG***	***ADD***	***GM***
F1				-	-	-														
F3				-								-		-	-	-	-	-		
F4		-	-	-	-	-	-		-		-			-						-
F8	-	-	-	-		+	-	-	-	-		-	-	-	-	-	-	-	-	
F9			-	-		-					-			-	+	+				-
F10			-	-	-	-		+	-		-	-		-	+		-	-		-
M2				-				+			-		-	-	-					-
M3				-						+				-						
M6	-	-	-	-	-	-	-		-	-	-		-	-	-		-	-		-
M7											-	+		-		-	-	-		-
M10				-	+	+						-			-			-	-	
M11						+						-		-	+	+	-			
F2	-	-		-								+			-	-	-	-		
F5	-	-		-	-			+		-	-	-		-	-	-	-	-	-	-
F6	-	-	-	-			-	-		-	-			-			-	-		-
F7	-			-			+	+			-	-	-	-	-			-	-	-
M1	-	-		-	-	-				-	-	-	-	-	-	-	-	-		-
M4	+	-		-	+	+			-		-	-	-	-	-	-	-	-		-
M5			+		+	+		+	+	+		+								
M8				-	+						+	+	+					-	+	+
M9	-	-	-	-	-					-				-	+	+				
M12	+			-		-	-	-			-	-		-	-		-	-		-

4.5.6 CONCLUSION

This study demonstrated that lower limb muscle activation during lunging and squatting tasks can be characterised by two common muscle synergies, with a third synergy needed to account for the increased task complexity of the lunge. The consistency in muscle synergy structure and function across different biomechanical contexts suggests muscle synergies represent motor modules that can be flexibly recruited to execute a variety of movements. We identified one shared synergy dominated by quadriceps activation and was highly correlated with knee extensor moment indicating its importance for controlling knee joint motion and supporting body weight. A second shared synergy was not dominated by a specific muscle group(s), suggesting a global stabilisation strategy recruited to support the knee against frontal plane and rotational loads. Lunging tasks recruited a third synergy associated with the heel strike and push off phases, highlighting that the number of recruited synergies increases with movement complexity.

We also observed that VM pain caused a general reduction in knee joint flexion angles and knee extensor moments, aligning with the basic assumption that the primary aim of altered joint dynamics is to reduce the load on the painful tissue and protect from further pain and/injury. However, this adaptation may increase risk of injury since shallow flexion angles are a common factor contributing to ACL injury mechanism. Correspondingly, we observed a general decrease in quadriceps synergy activation. Our results suggest that the CNS low-dimensionality regulates the activation profiles of specific movement facilitating synergies in response to pain only by changing the descending neural input to the muscle synergies rather than through reorganisation. This inhibition only occurred during the concentric phase of movement. Findings of this study provide novel insights into the stabilising contribution of muscle synergies and the adaptive responses to muscle inhibition.

4.5.7 References

Alkjaer T, Henriksen M, Simonsen EB (2011) Different knee joint loading patterns in ACL deficient copers and non-copers during walking. Knee Surg Sports Traumatol Arthrosc 19:615–621.

Arendt-Nielsen L, Sluka KA, Nie HL (2008) Experimental muscle pain impairs descending inhibition. PAIN 140:465–471.

Bank P, Peper C, Marinus J, Beek P, van Hilten J (2013) Motor consequences of experimentally induced limb pain: A systematic review. Eur J Pain 17:145–157.

Boden BP, Dean CS, Feagin Jr. JA, Garrett Jr. WE (2000) Mechanisms of anterior cruciate ligament injury. Orthopedics 23:573–578.

Brand RA, Crowninshield RD, Wittstock CE, Pedersen DR, Clark CR, van Krieken FM (1982) A Model of Lower Extremity Muscular Anatomy. J Biomech Eng 104:304–310.

Buchanan TS, Lloyd DG (1997) Muscle activation at the human knee during isometric flexion-extension and varus-valgus loads. J Orthop Res 151:1–17.

Cashaback JGA, Potvin JR (2012) Knee muscle contributions to joint rotational stiffness. Hum Mov Sci 31:118–128.

Cheung VCK, Piron L, Agostini M, Silvoni S, Turolla A, Bizzi E (2009) Stability of muscle synergies for voluntary actions after cortical stroke in humans. Proc Natl Acad Sci 106:19563–19568.

Chvatal SA, Ting LH (2012) Voluntary and Reactive Recruitment of Locomotor Muscle Synergies during Perturbed Walking. J Neurosci 32:12237–12250.

Chvatal SA, Torres-Oviedo G, Safavynia SA, Ting LH (2011) Common muscle synergies for control of center of mass and force in nonstepping and stepping postural behaviors. J Neurophysiol 106:999–1015.

Clark DJ, Ting LH, Zajac FE, Neptune RR, Kautz SA (2010) Merging of Healthy Motor Modules Predicts Reduced Locomotor Performance and Muscle Coordination Complexity Post-Stroke. J Neurophysiol 103:844–857.

Delp SL (2007) OpenSim: Open-Source Software to Create and Analyze Dynamic Simulations of Movement. IEEE Trans Biomed Eng 54:1940–1950.

Durselen L, Claes L, Kiefer H (1995) The influence of muscle forces and external loads on cruciate ligament strain. Am J Sports Med 23:129–136.

Fleming B C, Renstrom P, Ohlen G, Johnson RJ, Peura GD, Beynnon BD, Badger GJ (2001) The gastrocnemius muscle is an antagonist of the anterior cruciate ligament. J Orthop Res 19:1178–1184.

Flaxman TE, Alkjaer T, Shourijeh MS, Krogsgaard MR, Benoit DL. (Study 3). Adaptive Muscle Activation Strategies from Experimental Muscle Pain during a Weight-bearing Force Control Task. Unpublished data.

Flaxman TE, Alkjaer T, Shourijeh MS, Krogsgaard MR, Benoit DL. (Study 4). Effect of Quadriceps Muscle Pain on Lower Limb Muscle Synergies during a Weight-bearing Force Control Task. Unpublished data.

Gizzi L, Muceli S, Petzke F, Falla D (2015) Experimental Muscle Pain Impairs the Synergistic Modular Control of Neck Muscles. PLoS ONE 10 Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4575045/ [Accessed June 28, 2016].

Goldberg EJ, Neptune RR (2007) Compensatory strategies during normal walking in response to muscle weakness and increased hip joint stiffness. Gait Posture 25:360–367.

Graven-Nielsen T, Lund H, Arendt-Nielsen L, Danneskiold-Samsøe B, Bliddal H (2002) Inhibition of maximal voluntary contraction force by experimental muscle pain: A centrally mediated mechanism. Muscle Nerve 26:708–712.

Hashemi J, Chandrashekar N, Jang T, Karpat F, Oseto M, Ekwaro-Osire S (2007) An alternative mechanism of non-contact anterior cruciate ligament injury during jump-landing: In-vitro simulation. Exp Mech 47:347–354.

Henriksen M, Alkjaer T, Lund H, Simonsen EB, Graven-Nielsen T, Danneskiold-Samsøe B, Bliddal H (2007) Experimental quadriceps muscle pain impairs knee joint control during walking. J Appl Physiol 103:132–139.

Henriksen M, Alkjaer T, Simonsen EB, Bliddal H (2009) Experimental muscle pain during a forward lunge--the effects on knee joint dynamics and electromyographic activity. Br J Sports Med 43:503–507.

Henriksen M, Graven-Nielsen T, Aaboe J, Andriacchi TP, Bliddal H (2010) Gait changes in patients with knee osteoarthritis are replicated by experimental knee pain. Arthritis Care Res 62:501–509.

Henriksen M, Rosager S, Aaboe J, Graven-Nielsen T, Bliddal H (2011) Experimental Knee Pain Reduces Muscle Strength. J Pain 12:460–467.

Hodges PW, Mellor R, Crossley K, Bennell K (2009) Pain induced by injection of hypertonic saline into the infrapatellar fat pad and effect on coordination of the quadriceps muscles. Arthritis Care Res 61:70–77.

Hodges PW, Tucker K (2011) Moving differently in pain: A new theory to explain the adaptation to pain. Pain 152: S90-S98.

Hopkins JT, Ingersoll CD (2000) Arthrogenic muscle inhibition: A limiting factor in joint rehabilitation. J Sport Rehabil 9:135–159.

Houck J, Yack HJ (2001) Giving way event during a combined stepping and crossover cutting task in an individual with anterior cruciate ligament deficiency. J Orthop Sports Phys Ther 31:481-489-495.

Houck JR, De Haven KE, Maloney M (2007) Influence of anticipation on movement patterns in subjects with ACL deficiency classified as noncopers. J Orthop Sports Phys Ther 37:56–64.

Hug F, Turpin NA, Guével A, Dorel S (2010) Is interindividual variability of EMG patterns in trained cyclists related to different muscle synergies? J Appl Physiol 108:1727–1736.

Ivanenko YP, Poppele RE, Lacquaniti F (2004) Five basic muscle activation patterns account for muscle activity during human locomotion. J Physiol 556:267–282.

Jonsson P, Alfredson H (2005) Superior results with eccentric compared to concentric quadriceps training in patients with jumper's knee: a prospective randomised study. Br J Sports Med 39:847–850.

Kristianslund E, Krosshaug T, van den Bogert AJ (2012) Effect of low pass filtering on joint moments from inverse dynamics: Implications for injury prevention. J Biomech 45:666–671.

Lund JP, Donga R, Widmer CG, Stohler CS (1991) The pain-adaptation model: a discussion of the relationship between chronic musculoskeletal pain and motor activity. Can J Physiol Pharmacol 69:683–694.

MacLean CL, Taunton JE, Clement DB, Regan WD, Stanish WD (1999) Eccentric Kinetic Chain Exercise as a Conservative Means of Functionally Rehabilitating Chronic Isolated Insufficiency of the Posterior Cruciate Ligament. J Sport Med 9:142–150.

MacWilliams BA, Wilson DR, Desjardins JD, Romero J, Chao EYS (1999) Hamstrings cocontraction reduces internal rotation, anterior translation, and anterior cruciate ligament load in weight-bearing flexion. J Orthop Res 17:817–822.

Mantovani G, Lamontagne M (2016) How Different Marker Sets Affect Joint Angles in Inverse Kinematics Framework. J Biomech Eng.

McGraw KO, Wong SP (1996) Forming inferences about some intraclass correlation coefficients. Psychol Methods 1:30–46.

Mirakhorlo M, Mahmood RA (2015) Similarity of different lifting techniques in trunk muscular synergies. Acta Bioeng Biomech 17:21–29.

Morgan KD, Donnelly CJ, Reinbolt JA (2014) Elevated gastrocnemius forces compensate for decreased hamstrings forces during the weight-acceptance phase of single-leg jump landing: implications for anterior cruciate ligament injury risk. J Biomech 47:3295–3302.

Muceli S, Falla D, Farina D (2014) Reorganization of muscle synergies during multidirectional reaching in the horizontal plane with experimental muscle pain. J Neurophysiol 111:1615–1630.

Neptune RR, Clark DJ, Kautz SA (2009) Modular control of human walking: A simulation study. J Biomech 42:1282–1287.

Palmieri RM, Weltman A, Edwards JE, Tom JA, Saliba EN, Mistry DJ, Ingersoll CD (2005) Pre-synaptic modulation of quadriceps arthrogenic muscle inhibition. Knee Surg Sports Traumatol Arthrosc 13:370–376.

Palmieri-Smith RM, Thomas AC, Wojtys EM (2008) Maximizing Quadriceps Strength After ACL Reconstruction. Clin Sports Med 27:405–424.

Pataky TC (2010) Generalized n-dimensional biomechanical field analysis using statistical parametric mapping. J Biomech 43:1976–1982.

Portney LG, Watkins MP (2000) Foundations of clinical research: Applications to practice. Upper Saddle River, New Jersey: Pearson Prentice Hall.

Powers CM, Perry J, Hsu A, Hislop HJ (1997) Are Patellofemoral Pain and Quadriceps Femoris Muscle Torque Associated With Locomotor Function? Phys Ther 77:1063–1075.

Roland MO (1986) A critical review of the evidence for a pain-spasm-pain cycle in spinal disorders. Clin Biomech Bristol Avon 1:102–109.

Rudolph KS, Axe MJ, Buchanan TS, Scholz JP, Snyder-Mackler L (2001) Dynamic stability in the anterior cruciate ligament deficient knee. Knee Surg Sports Traumatol Arthrosc 9:62–71.

Sasaki K, Neptune RR (2010) Individual muscle contributions to the axial knee joint contact force during normal walking. J Biomech 43:2780–2784.

Shimokochi Y, Shultz SJ (2008) Mechanisms of noncontact anterior cruciate ligament injury. J Athl Train 43:396–408.

Shourijeh MS, Flaxman TE, Benoit DL (2016) An approach for improving repeatability and reliability of non-negative matrix factorization for muscle synergy analysis. J Electromyogr Kinesiol 26:36–43.

Simonsen EB, Dyhre-Poulsen P, Voigt M, Aagaard P, Fallentins N (1997) Mechanisms contributing to different joint moments observed during human walking. Scand J Med Sci Sports 7:1–13.

Slemenda C, Brandt KD, Heilman DK, Mazzuca S, Braunstein EM, Katz BP, Wolinsky FD (1997) Quadriceps weakness and osteoarthritis of the knee. Ann Intern Med 127:97–104.

Smale KB, Shourijeh MS, Benoit DL (2016) Use of muscle synergies and wavelet transforms to identify fatigue during squatting. J Electromyogr Kinesiol 28:158–166.

Ting LH, Chiel HJ, Trumbower RD, Allen JL, McKay JL, Hackney ME, Kesar TM (2015) Neuromechanical Principles Underlying Movement Modularity and Their Implications for Rehabilitation. Neuron 86:38–54.

Ting LH, Macpherson JM (2005) A limited set of muscle synergies for force control during a postural task. J Neurophysiol 93:609–613.

Torres-Oviedo G, Macpherson JM, Ting LH (2006) Muscle synergy organization is robust across a variety of postural perturbations. J Neurophysiol 96:1530–1546.

Torres-Oviedo G, Ting LH (2010) Subject-Specific Muscle Synergies in Human Balance Control Are Consistent Across Different Biomechanical Contexts. J Neurophysiol 103:3084–3098.

van den Hoorn W, Hodges PW, Dieën JH van, Hug F (2014) Effect of acute noxious stimulation to the leg or back on muscle synergies during walking. J Neurophysiol:jn.00557.2014.

Yoo JD, Papannagari R, Park SE, DeFrate LE, Gill TJ, Li G (2005) The effect of anterior cruciate ligament reconstruction on knee joint kinematics under simulated muscle loads. Am J Sports Med 33:240–246.

CHAPTER 5: General Discussion

The overall goal of this thesis was to investigate the contributions of lower-limb muscles to knee joint kinetics associated with joint stabilisation strategies. During functional tasks and sporting activities, the knee is exposed to large and rapidly changing external loads. To effectively oppose these loads, the neuromuscular system must integrate the activity of all muscles that cross the knee so that when summated, the muscular force products create a net joint moment-of-force that is opposite in direction to the external load. This thesis comprised of two main objectives: **(1)** to evaluate the relationships of lower limb muscles to internal joint moments in all three loading planes, and **(2)** to determine how these relationships change in the presence of a traumatic knee joint injury or quadriceps muscle pain. Therefore, findings of this thesis can contribute to the understanding of which muscles, or group of muscles, are most important at regulating joint loads and subsequent knee joint stability.

5.1 Muscular Contributions to Stability

Objective 1 of this thesis was to evaluate the contribution of lower limb muscles to internal joint moments in all three loading planes. This was accomplished by 1) quantifying the interrelationship between individual muscle activations and internal joint moments profiles elicited during a static weight-bearing force control task (Study 1: *Predicting the Functional Roles of Knee Joint Muscles from Internal Joint Moments*, page 46) (**Objective 1-A**) and 2) determining how muscles activate collectively in a synergistic manner to generate specific moments (i.e. flexion-extension, adduction-abduction, or rotation) (Study 4: *Effect of Quadriceps Muscle Pain on Lower Limb Muscle Synergies during a Weight-bearing Force Control*

Task, page 121) (**Objective 1-B**). How these relationships are associated with certain loading conditions are described below.

Sagittal plane loads

Due to the knee's primary plane of motion and the anatomical orientation of muscles that cross it, the major muscles crossing the knee are traditionally classified as knee joint flexors (hamstrings) and extensors (quadriceps) (Buchanan and Lloyd, 1997; Li et al., 2002; Wojtys et al., 2002; Cashaback and Potvin, 2012). Therefore, it was not surprising that individual activations of quadriceps and hamstring muscles were significantly predicted by knee extensor and knee flexor moments (Study 1). Correspondingly, quadriceps and hamstring dominant muscle synergies were associated with knee extensor and flexor moment synergies (Study 4).

However, the hamstring and rectus femoris (RF) muscles also contribute to hip joint moments. By including the moments of joints that a given muscle crosses in the regression models of Study 1 we were able to elucidate at which joint a bi-articular muscle is more likely acting on. This approach determined that semitendinosus (ST) activation was more likely to increase with a reciprocal increase in hip extension compared to knee flexion, contrasting the biceps femoris (BF) and RF whose activation was equally distributed for both joints. The equal contribution to the knee and hip joint moments may be attributed to the additional contribution of the uni-articular biceps femoris short head and vastii that respectively share the same attachment site as BF and RF (Nordin and Frankel, 2001; Hamill and Knutzen, 2004; Agur and Dalley, 2005).

Study 1 was also able to differentiate the varying roles between bi-articular and uni-articular quadriceps muscles. While a significant relationship between vastii activation and knee extension moments were identified, the vastii muscles' symmetrical activation patterns, weak prediction accuracies, and significant contributions to synergies associated with knee adduction and rotation moments (Study 4) classified them as general joint stabilisers.

Considering the vastii are (1) primary contributors to axial knee joint force (Sasaki and Neptune, 2010), (2) can provide the greatest contribution to knee joint rotational stiffness in all three flexion-extension, varus-valgus, and transverse loading axes (Cashaback and Potvin, 2012), and (3) they actually have a protective effect on the anterior cruciate ligament (ACL) when physiological loading magnitudes are applied in vitro (Aune et al., 1997; Hashemi et al., 2007), we postulate that vastii muscles contract to increase compressive forces, essentially bracing the knee, so the bi-articulate muscles, such as the RF, can generate and transmit the moments needed to direct the ground reaction forces (GRFs) at the foot-ground interface. This notion coincides with that of van Ingen Schenau and colleagues who hypothesized that bi-articular muscles are responsible for controlling the distribution of moments about the joints and controlling the direction of the external force exerted on the environment (Jacobs and van Ingen Schenau, 1992; van Ingen Schenau et al., 1992; van Deursen et al., 1998).

Coupling of injury inducing loads

In addition to reasons explained in the introduction, the use of the force matching task was also beneficial because: (1) the exercise was static thus motion artefact was limited; (2) knee moments were similar in magnitude in all six axis directions, contrasting dynamic motions that are dominated by only a few moment types (i.e. side-cutting with abduction and extension); and (3) no single GRF direction elicited a pure moment type (Study 1), highlighting that even during an extremely controlled task, knee joint loads are not limited to a single axis while weight bearing.

Even though non-contact knee joint injury commonly occurs during maneuvers with shallow knee flexion angles (Boden et al., 2000), several works suggest sagittal plane loads alone does not cause injury (McLean et al., 2004; Hewett et al., 2005; Shimokochi and Shultz, 2008). Rather, the interaction between transverse and fontal plane loads are believed to place the ACL and collateral ligaments at risk of injury, particularly at shallow knee flexion angles (0° - 40°) (Mills and Hull, 1991; Besier et al., 2001; McLean et al., 2004). Interestingly, moment synergies extracted in Study 4 showed that general anterior, posterior, medial and lateral force directions respectively coupled knee moments of extension with abduction, flexion with adduction, abduction with internal rotation, and adduction with external rotation.

Muscle synergies extracted in Study 4 revealed an associated strategy of muscular co-activation with moment synergies coupling frontal and transverse plane components. Further evaluation of muscle synergies associated with individual moment types attributed this co-activation strategy to generating rotational and knee adduction moments. A co-activation strategy for supporting the knee against rotational loads is supported by Hsieh and Walker (1976) who consider the compressive forces from axial loads and muscular contractions the most important factor for rotational stability. They inferred that in order to rotate, the femur must “screw” upwards on the tibial curvatures and oppose these compressive forces. Interestingly, the co-activation strategy for generating knee adduction moments involved equal contribution from medial and lateral hamstring musculature. Since externally applied knee abduction alignment is major component of the ACL injury mechanism and episodes of giving-way (Fitzgerald et al., 2004; McLean et al., 2004; Hewett et al., 2005), the unbalanced co-activation strategies commonly observed in populations with knee pathologies (Lewek et al., 2005; Hubley-Kozey et al., 2009; Palmieri-Smith et al., 2009) may be a contributing factor to knee instability.

The muscle synergy associated with knee abduction moments lacked significant contribution from muscles crossing the knee. Rather, gluteus medius (GM) dominated the synergy. Although not a knee joint muscle, Sritharan et al. (2012) demonstrated that hip abductors were the greatest contributors to knee abduction moments, thus compressing the medial compartment of the knee and unloading the lateral while knee spanning muscles compress the whole joint (reflecting a stabilisation role). This is a noteworthy observation since acute reductions in hip function de-stabilizes the femur, causing an increase in frontal plane motion and knee joint moments (Henriksen et al., 2009a; Geiser et al., 2010; Powers, 2010).

Stabilisation synergies during dynamic motion

In Study 5 *(Synergistic and Biomechanical Adaptations to Experimental Muscle Pain during Squatting and Lunging Tasks*, page 147), we investigated the synergistic control of muscle activations during dynamic squatting and lunging activities (**Objective 1-C**). We identified two synergies that were common to both tasks. The first being a quadriceps dominant synergy for generating knee extensor moments and facilitating movement. The second synergy demonstrated a general co-activation of muscles that was recruited throughout the task. Based on the moment specific synergies presented in Study 4, this second synergy was considered an important stabilisation strategy to support rotational plane moments and contribution of GM was to support hip abduction during these tasks. Our results imply that the co-activation commonly observed in dynamic movements is more likely attributed to supporting the knee against rotational and abduction loads, rather than antagonist activation of knee extensor moments.

Results from this thesis confirm those from our previous work (Flaxman et al., 2012, 2013) that the activations of lower limb muscles are not always dependent on moment arm orientation, as previously suggested (Andriacchi et al., 1984; Buchanan et al., 1986). This is evidenced by (1) the bi-articular muscles have differing contributions at the hip compared to the knee; (2) muscles of a given functional group have varying roles when supporting the knee in the frontal and transverse plane; (3) during tasks that are not extensor dominant, activation of the uni-articular vastii is not correlated to a single moment type.

5.2 Effect of ACL injury

Rupture of the ACL compromises the mechanical integrity of the joint and results in reduced physical activity levels and overall joint function (Thomeé et al., 2007; Krogsgaard et al., 2011). However, in some cases, individuals with an ACL deficiency can effectively cope and maintain levels of physical activity to those pre-injury. Although both copers and non-copers exhibit greater levels of hamstring co-contraction compared to controls, copers exhibit neuromuscular patterns and joint dynamics similar to healthy non-injured controls (Rudolph et al., 2001). The main distinguishing factor between copers and non-copers is quadriceps weakness, increased co-activation of antagonist hamstring muscles and varied gastrocnemius activation patterns (Rudolph et al., 2001; Alkjaer et al., 2011; Alkjær et al., 2012). It is therefore reasonable to question if the absence of the ACL as a joint stabiliser is paramount in reducing functional capacity in ACL deficient populations, or if knee instability originates from to neuromuscular deficits following injury.

In Study 2 (*Protective and Adverse Muscle Activation Strategies after ACL-injury during a Weight-bearing Force Control Task,* page 74), the relationships between lower limb muscle activation and internal net joint moments were quantified in ACL deficient individuals (**Objective 2-A**). Reports of knee instability in ACL deficient populations are related to greater knee rotation angles, greater knee abduction angles and moments, and reduced knee flexion moments (Houck and Yack, 2001; Houck et al., 2007). As such, stronger relationships between a given muscle and knee flexor, knee adductor, and rotational moments were interpreted as adaptations to increase knee stability after ACL injury (Yoo et al., 2005; Li et al., 2007). Results of Study 2 suggest that neuromuscular adaptions after ACL injury help to improve sagittal plane stability (evidenced by increased contribution of RF and ST), but reduce one's ability to actively stabilise the knee when external abduction and rotational loads are applied.

One limitation of Study 2 was that EMG signals were normalised to maximum voluntary isometric contraction (MVIC) values whereas voluntary deficits in muscle function have been observed in ACL deficient individuals, and these deficits can persist for years following injury and/or reconstruction (Urbach et al., 2001; Palmieri-Smith et al., 2008; Thomas et al., 2013). However, we have since investigated the strength deficits in our ACL and CON groups using a simple musculoskeletal model (Appendix D). No significant between group differences in peak extensor or flexor torques, or strength ratios for both exercises were observed. Therefore, the normalized EMG in the ACL group may not be overestimated. However results from the musculoskeletal model did show an inverse relationship of muscular contribution to peak knee flexion torque: compared to CON, ACL had reduced BF and increased ST contribution to knee flexion (Appendix D, Figure 39). Differential hamstring response to knee exercise may be more related to their role in stabilising the knee against tibial rotation (Alkjær et al., 2012). Since tibial rotation is a major component of knee injury mechanism (Yoo et al., 2005), reduced contribution of the BF to external knee rotational moments may contribute to instability. Furthermore, increased ST contribution to knee flexion moments after ACL injury and its significant association with internal knee rotation moments identified in Study 2 is a noteworthy finding since it is commonly harvested as an ACL a graph, therefore removing it may compromise one's ability to effectively stabilise the knee after reconstruction.

Overall, results from Study 2 and Appendix D suggest that flexion and extension strength tests might not be adequate at identifying deficits in an active ACL deficient population. Since the mechanism of ACL injury and instability is multifactorial and sagittal plane loads alone does not cause injury (Besier et al., 2001; McLean et al., 2004), future works should consider voluntary strength deficits in the frontal and transverse loading planes as well as changes to relative force contributions within functional muscle groups.

5.3 Effect of Pain

Despite our lack of significant difference in maximal knee extensor torque generating abilities between ACL deficient and health controls, quadriceps weakness is a commonly observed symptom in ACL injured individuals and this deficit is more prevalent in non-copers compared to copers (Rudolph et al., 2001; Palmieri-Smith et al., 2008). As such, this thesis evaluated the acute effects of muscle inhibition on knee joint dynamics and neuromuscular adaptations in young healthy adults (**Objective 2-B**). In doing so we isolated the effect of voluntary quadriceps inhibition without other confounding effects commonly seen in ACL deficient populations, such as joint conjunctures, accompanying injury in other soft tissues, effects of rehabilitation etc.

Quadriceps muscle inhibition was experimentally induced using hypertonic saline injections causing a perception of muscle pain. Models of muscle and joint pain have been shown to reduce voluntary quadriceps activation up to 20%, similar to levels observed clinically (Palmieri-Smith et al., 2007; Henriksen et al., 2009a, 2009b; Pietrosimone et al., 2014; Rice et al., 2014). Vastus medialis (VM) was targeted because changes in muscle composition and inhibition levels after ACL injury is most profound in the VM (Konishi et al., 2007). Even though our pain was limited to a single muscle, previous works have shown experimental models of joint and muscle pain has reciprocal inhibition of the vastus lateralis (VL) and the RF (Palmieri et al., 2005; Henriksen et al., 2009b, 2010; Hodges et al., 2009).

A key aspect of the theory for muscular adaptations to pain (Hodges and Tucker, 2011) is that pain changes the mechanical output of behaviour in order to protect the body from further pain or injury. Accordingly, during both lunging and squatting tasks, significantly reduced peak knee joint flexion angles and extensor angular impulses were observed and interpreted as a protective mechanism to reduced knee extensor loads (Study 5). However, corresponding activation of knee extensor dominant muscle synergies were also reduced. It is difficult to dissociate whether the change in neuromuscular function during such tasks is caused by pain or simply to the change in knee joint dynamics and this limitation undermines many of the studies used by Hodges and Tucker (2011) to develop their theory.

To limit such confounding factors, a force matching task required participants to maintain the same joint positions and external force outputs between pain conditions. To our knowledge, no study has investigated the neuromuscular response to pain during such a controlled task that maintains the mechanical output in lower limb. This allowed us to more robustly infer causation (pain) with our dependant variables. In Study 3 (*Adaptive Muscle Activation Strategies from Experimental Muscle Pain during a Weight-bearing Force Control Task*, page 97), significant changes in individual muscle function reduced knee extensor loads, thus protecting the painful tissue; however, we also noted that the pain related changes in muscle activation may compromise one's ability to stabilise the knee against transverse plane loads.

Since another aspect of the muscular adaptions to pain theory is that pain leads to spatial redistribution of muscle activity within and between muscles (Hodges and Tucker, 2011), and results of Study 3 observed changes in individual muscle activations as it relates to sagittal and transverse plane moments, Study 4 sought to determine if there were synergistic changes in muscle activity related to the same moments. Our results did not support the pain theory since changes in synergistic control were not apparent. Although Study 3 identified significant pain-induced changes in functional roles of individual muscles, Study 4 identified a subject-specific response to muscle pain. Either the pain response is subject-specific, or our CNMF framework was not sensitive enough to detect systemic changes.

Another consideration for the lack of significant effects on synergistic function from pain was due to low loading demands. Bank et al., (2013) state that observable changes in muscle activation using parameters derived from surface EMG is not likely in tasks eliciting less than

25% MVIC. We also evaluated the effect of pain on more demanding squatting and lunging tasks and once again no significant changes in synergy composition were noted. Rather, pain-related changes were simply attributed to reduced activation of knee extensor muscle synergies. In other conditions that inhibit descending motor signals, such as cortical stroke in humans, strikingly similar muscular compositions of the synergies have also been observed for both unaffected and affected arms (Cheung et al., 2009). This demonstrates the robustness of muscle synergies and supports the notion that the CNS has a "*motor repertoire, or library of motor subtasks*", who's activations are simply reduced by injury or pain leading to changes in motor performance.

5.4 Limitations

In addition to those addressed in each of the studies, there are other limitations of the present thesis that may affect the validity of the results.

Muscles evaluated

Due to accessibility, interpretation of muscular contributions to internal joint moments and its relations to knee stability is limited to eight muscles that cross the knee and two muscles that cross the hip. Since (1) smaller muscles crossing the knee (semimembranosus, sartorius, gracilis, vastus intermedius, short head of biceps femoris) can significantly contribute to knee moment generation (Buchanan et al., 1996), and (2) hip muscles are large determinants of femoral orientation and affect knee loads (Jacobs et al., 2007; Geiser et al., 2010; Sritharan et al., 2012), and (3) ankle muscles such as the tibialis anterior and soleus muscle affect tibio-femoral motion (Elias et al., 2003), a more complete evaluation of knee joint muscles and those contributing to the femoral and tibial orientations is needed to provide valid insight into neuromuscular contributions to internal knee joint moments.

Participants

The sample population used in this thesis may bias our results. Participants were required to be physical active at least twice a week; however, no restrictions were specified with respect to physical activity type, experience level or frequencies. For our healthy control population, Tegner activity level scale ranged from 2-10 (Tegner and Lysholm, 1985). Previous research has shown that knee muscle activation varies as a function of physical activity level (da Fonseca et al., 2006; Sigward and Powers, 2006) and physical activity type (Lattier et al., 2003). This may affect results of Study 2 where ACL and CON groups were not matched for activity level at time of participation. Group mean ± standard deviation Tegner scores for ACL and CON were 3.8±1.5 and 6.7±2.9, respectively. Variations in physical activity level may also affect the perception of pain and corresponding adaptive responses (Pen and Fisher, 1994), which may contribute to inter-subject variability in muscular response to pain (Study 4 and 5). Furthermore, for our ACL deficient group, the time since injury to time of participation was not consistent across participants. Our group mean average and standard deviation was 12.9 ± 14.3 months. This could have contributed to the high variability observed in our ACL group because we had acute and chronically injured participants grouped together.

Lastly, we have previously identified sex-related differences in muscle activation patterns of healthy young adults (Flaxman et al., 2013), however, sex was not controlled for in the current thesis. Considering sex-related differences in neuromuscular function exist in ACL populations (Miranda et al., 2013), and the presence of muscle pain is thought to impair descending inhibitory responses in females but not in males (Arendt-Nielsen et al., 2008), future works should consider sex in their analysis.

Validity of Isometric Exercise

Even though our weight-bearing force matching task investigated the change in muscle activation patterns with increasing effort levels (Study 1), and these effort levels elicit horizontal GRFs that are comparable in magnitude to sporting maneuvers, such as jump landing and running initiation (McClay et al., 1994), applied forces may not reflect GRF magnitudes observed in more demanding activities associated with ACL injury, like side-cuts and braking motions (Boden et al., 2000).

EMG levels evoked during the force matching task ranged between 12-24% MVIC (Study 2). According to DeLuca (1997), a threshold of 30% MVIC is required to ensure the entire muscle is recruited. In our larger muscles, for example the RF, spatial variation in recruitment may have affected our results. This also may provide some insight into the subject-specific response to pain in the non-painful muscles, consistent with previous works (Hodges and Tucker, 2011)

In addition, our force matching task only involved one bodily configuration, as addressed in Study 1. Especially for the bi-articular muscles, muscle activation patterns are subject to change depending on flexion angle (Buchanan and Lloyd, 1997). We opted for this position because hip and knee flexion angles are common to several sporting maneuvers associated with ACL injury, such as side cuts, braking motions, and landing (Boden et al., 2000). We wanted to elicit as much muscular protection as possible and shallower knee flexion angles rely more on the soft tissues and reduces mechanical advantages of the knee muscles (Lloyd and Buchanan, 1996). Nevertheless, similar activation patterns in a standing force matching have been reported with extended knees (MacLeod et al., 2013a).

Although the force matching is considered to be functionally relevant (consider riding a bus and stabilising against a breaking or turning motion), it was kinematically isometric. As such, interpretation of results as it relates to dynamic movement is limited. Despite this, we can relate the extensor dominant synergies recruited during our dynamic tasks ($S1_{lunges}$ and $S1_{squats}$, Study 5) to the knee extensor dominant synergy recruited in the force matching task (Study 4). Two notable differences in synergy composition were the additional contribution of the TFL and reduced weighting of the vastii in the static task compared to dynamic. These differences may be attributed to varied knee kinematics: vastii is more likely to increase activation with increased knee flexion angles than the RF (Pincivero et al., 2004) while smaller knee and hip flexion angles may be more dependent on the hip flexor (TFL and RF). Future work will evaluate the similarity of synergies recruited during our target matching task and those during various dynamic tasks that produce similar knee kinematics and GRF scenarios (i.e. directional hoping, side- and cross-cutting etc.). By adding these levels of task complexity, a step-wise inductive approach can be taken by accounting for the activation dynamics of muscles and determine the dynamic relevance of our force-matching task.

Synergy Analysis

The evaluation of neuromuscular contribution to human movement using muscle synergy analysis is relatively new and several issues need to be considered. First, muscle synergy analysis is sensitive to the number of muscles included in the decomposition: a minimum of 10 muscles is recommended to obtain results similar to those if all muscles (including those that cannot be recorded with surface EMG) were included in the analysis (Steele et al., 2013). In addition, smaller muscle sets can over-estimate the variance accounted for (VAF), consequently under-estimating the number of synergies required to represent the original data set (Steele et al., 2013). Similarly, low-pass filtering of rectified EMG increases VAF so lower cut-off frequencies (i.e. 4Hz versus 10Hz) may under-estimate the number of synergies required to reconstruct the original neural drive (Hug et al., 2012).

Second, the number of synergies chosen to be extracted is based on an arbitrary threshold of a quality measure (VAF or R^2). Most studies select the number of muscle synergies that can reconstruct at least 90% VAF observed in the original data set. In contrast, the number of synergies in Study 4 was chosen based on a threshold of 80% VAF. This was originally to account for greater between-subject variability in our data set. However, lower VAF may also be attributed to the addition of the 6 moment inputs. As noted above, VAF may be overestimated in smaller muscle sets and as more variables are added to the input matrix, the VAF decreases (Steele et al., 2013).

Third, how EMG signals are normalised in the input matrix is not standardised across studies. Mean activation during a submaximal effort (Kristiansen et al., 2013), peak value during the analysed task (Hug et al., 2011; van den Hoorn et al., 2014; Steele et al., 2015), median activation magnitude (Cheung et al., 2009), and maximum voluntary isometric contraction (MVIC) (Smale et al., 2016) are examples of normalisation techniques used in muscle synergy analysis. Figure 35 shows the difference between the two sets of synergies recruited during the BASE condition of squatting, the first set has individual muscles normalised to maximum value observed during the dynamic trials while the second set normalised muscle activation to MVIC. Not only are there observable differences in the synergy compositions, there is a significant effect on the activation coefficients of S2. We chose to normalise EMG for Studies 4 and 5 to MVIC values as this method is appropriate for identifying differences in neuromuscular function across populations (Benoit et al., 2003) and to ensure synergy compositions would not be biased against low activation amplitudes. Future work needs to consider the normalisation technique used in muscle synergy analysis and its impact on output variables and subsequent interpretations.

Nevertheless, we believe the synergy analysis is a unique and powerful tool for evaluating neuromuscular control strategies during our tasks and we aimed to use the current best practices when applying them.

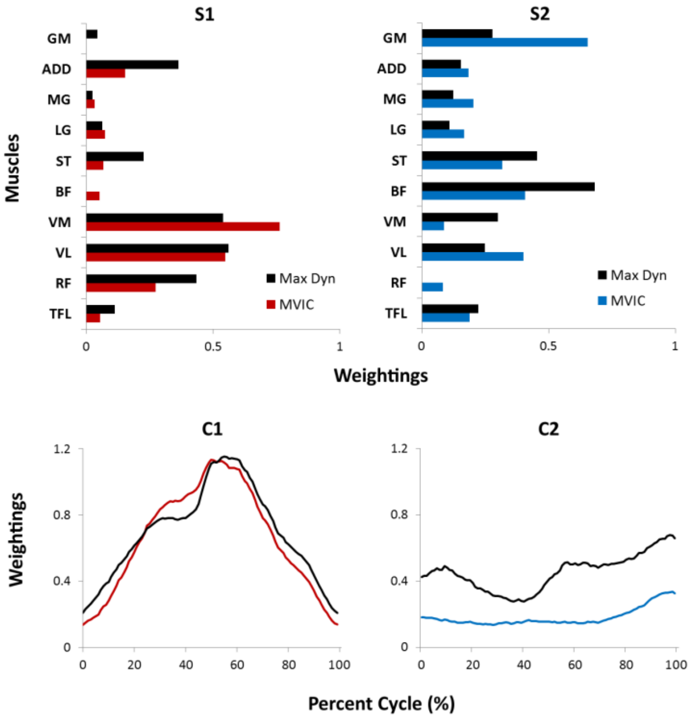

Figure 35: Two sets of synergy vectors (S) and coefficient weights (C) recruited during baseline condition of squatting. One set has EMG input into the CNMF framework normalised to peak value observed during the squatting task (Max Dyn) while the other set has EMG normalised to peak value observed in maximum voluntary isometric contractions (MVIC).

5.5 Implications/Future direction

A major finding of this thesis was that in an ACL deficient population, reductions in muscular contributions to rotational knee stability and hip abductor function exist. This has important implications for diagnostic methods and rehabilitative/ preventative exercise interventions. In recent years, evidence based clinical practice guidelines for the treatment of ACL deficient individuals has included “sport-specific activities, agility exercises, and functional hop tests” incorporating multi-directional loading (Fitzgerald et al., 2000; Adams et al., 2012). However, these exercises are (1) usually not integrated into a recovery program until 3-months post-reconstruction (a time when patients are typically transferred to a fitness facility and left to continue rehabilitation unsupervised); (2) only implemented for individuals with high levels of physical activity pre-injury; and (3) vague in description and unstandardized (Fitzgerald et al., 2000; Adams et al., 2012). Furthermore, the criteria to be met before an individual can return to sport are typically measured as knee extensor strength, forward hop tests, and subjective functional scores (Adams et al., 2012), all of which limit evaluation to loading conditions dominated by sagittal plane loads. Considering that frontal and transverse plane loads are defining factors in the ACL injury mechanism (Besier et al., 2001; McLean et al., 2004) and linked to episodes of giving way in populations with knee instability (Houck and Yack, 2001; Fitzgerald et al., 2004), the findings of this thesis emphasize that examination methods and rehabilitative/preventative exercise interventions need to include tasks that elicit frontal and transverse plane loading conditions and the associated neuromuscular strategies if mechanisms of knee instability are to be truly understood.

Since most existing studies associate a proportional increase in activation to a proportional increase in joint moments based on relationships established from non-weight bearing and/or uni-axial in vivo studies (Bigland-Ritchie, 1981; van Deursen et al., 1998), this thesis can provide valuable insight for clinical interpretations of muscle activation and moment interactions and to improve methods for musculoskeletal models. One of the major challenges in musculoskeletal modeling is the human muscle redundancy – there exists more muscles than the degrees of freedom in human movement. Therefore, in silico models using inverse dynamics are prone to poor estimates of agonist and antagonist activations compared to those observed in vivo (Herzog and Binding, 1992). The EMG-moment relationships established in Studies 1-3 may help to improve such predictions. Furthermore, muscle synergies extracted in Studies 4 and 5 can be used to reduce/simplify neuromuscular control strategies by grouping actuators. Walter et al., (2014) applied a mixed dynamic optimization technique (i.e., inverse skeletal dynamics with forward muscle activation and contraction dynamics) to walking data collected from a subject implanted with a force-measuring knee replacement and found that the use of subject-specific muscle synergies can improve knee contact force predictions. Our results can be similarly applied to further elucidate how changes in muscle synergy activation coefficients, such as those observed in the presence of muscle pain, contribute to joint contact forces and ultimately joint stability.

Lastly, this thesis is part of a larger research program aimed at identifying the direct contribution of muscle activations to in vivo knee joint contact mechanics and stability. Data collected from in this thesis will serve as input for an EMG-driven musculoskeletal model to predict internal muscle forces and these forces will then be applied to corresponding actuators of a cadaveric knee joint simulator. In vitro knee joint contact forces, areas, and pressures will be measured. This will create a direct link between in vivo muscle activations and its effects on soft tissue loading of the joint. At the end of this program, we hope to have shed light on the relationship between individual muscle function, tibio-femoral joint loading, and joint stability. Such information may uncover specific characteristics that render some muscles to be more effective at producing knee joint stability than others. If this is the case, then programs of rehabilitation and injury prevention can be tailored to target these muscles/synergies such that patient populations can increase stability and maintain activity levels.

5.6 Conclusion

This thesis sought to evaluate the effect of injury and muscle weakness on the interrelationship between muscle activation and internal joint moments. Our findings indicate muscle activation is not always dependent on its anatomical orientation, but rather on its role in maintaining knee joint stability especially in the frontal and transverse loading planes. In tasks that are dominated by sagittal plane loads, hamstring and quadriceps will differentially activate. However, when the knee is required to resist externally applied rotational and abduction loads, a strategy of global co-activation was identified. Contributions from muscles crossing the knee for supporting against knee adduction loads were not apparent. Rather hip abductors were deemed the more important regulator of varus inducing loads. This thesis suggests that commonly held descriptions of muscle roles in the lower limb need to be reconsidered in the development of rehabilitative interventions and not be overlooked in future works when interpreting neuromuscular function as it relates to knee stability.

Both muscle pain and ACL groups demonstrated changes in muscle activation that reduced rotational stability. Since frontal plane EMG-moment changes were not present during muscle pain, reduced relationships between hip muscles and abduction moments may be chronic adaptions to ACL injury that facilitate instability. Results of this thesis may be key in improving musculoskeletal models that attempt to estimate muscle activations as well as clinical interpretation of biomechanical assessments since they would need to account for this stabilising role. Our results also indicate that rehabilitative/ preventative exercise interventions should focus on neuromuscular training during tasks that elicit rotational and frontal loads (i.e. side cuts, pivoting maneuvers) as well as maintaining hip abductor and plantarflexor muscle strength in populations with knee pathologies and quadriceps muscle weakness.

General References

Adams D, Logerstedt D, Hunter-Giordano A, Axe MJ, Snyder-Mackler L (2012) Current Concepts for Anterior Cruciate Ligament Reconstruction: A Criterion-Based Rehabilitation Progression. J Orthop Sports Phys Ther 42:601–614.

Agur AMR, Dalley AF (2005) Grant's Atlas of Anatomy. Baltimore, MA: Lippincott Williams & Wilkins.

Alkjaer T, Henriksen M, Simonsen EB (2011) Different knee joint loading patterns in ACL deficient copers and non-copers during walking. Knee Surg Sports Traumatol Arthrosc 19:615–621.

Alkjær T, Simonsen EB, Magnusson SP, Dyhre-Poulsen P, Aagaard P (2012) Antagonist muscle moment is increased in ACL deficient subjects during maximal dynamic knee extension. The Knee 19:633–639.

Alkjær T, Simonsen EB, Peter Magnusson S, Aagaard H, Dyhre-Poulsen P (2002) Differences in the movement pattern of a forward lunge in two types of anterior cruciate ligament deficient patients: copers and non-copers. Clin Biomech 17:586–593.

Andriacchi TP, Andersson GBJ, Ortengren R, Mikosz RP (1984) A study of factors influencing muscle activity about the knee joint. J Orthop Res 1:266–275.

Arendt-Nielsen L, Sluka KA, Nie HL (2008) Experimental muscle pain impairs descending inhibition. PAIN 140:465–471.

Aune AK, Cawley PW, Ekeland A (1997) Quadriceps muscle contraction protects the anterior cruciate ligament during anterior tibial translation. Am J Sports Med 25:187–190.

Bank P, Peper C, Marinus J, Beek P, van Hilten J (2013) Motor consequences of experimentally induced limb pain: A systematic review. Eur J Pain 17:145–157.

Barrance PJ, Williams GN, Snyder-Mackler L, Buchanan TS (2006) Altered knee kinematics in ACL-deficient non-copers: A comparison using dynamic MRI. J Orthop Res 24:132–140.

Bennell KL, Hunt MA, Wrigley TV, Hunter DJ, McManus FJ, Hodges PW, Hinman RS (2010) Hip strengthening reduces symptoms but not knee load in people with medial knee osteoarthritis and varus malalignment: a randomised controlled trial. Osteoarthritis Cartilage 18:621–628.

Benoit DL, Lamontagne M, Cerulli G, Liti A (2003) The clinical significance of electromyography normalisation techniques in subjects with anterior cruciate ligament injury during treadmill walking. Gait Posture 18:56–63.

Berchuck M, Andriacchi TP, Bach BR, Reider B (1990) Gait adaptations by patients who have a deficient anterior cruciate ligament. J Bone Jt Surg - Ser A 72:871–877.

Besier TF, Lloyd DG, Cochrane JL, Ackland TR (2001) External loading of the knee joint during running and cutting manoeuvres. Med Sci Sports Exerc 33:1168–1175.

Bigland-Ritchie B (1981) EMG/force relations and fatigue of human voluntary contractions. Exerc Sport Sci Rev 9:75–117.

Bisseling RW, Hof AL (2006) Handling of impact forces in inverse dynamics. J Biomech 39:2438–2444.

Boden BP, Dean CS, Feagin Jr. JA, Garrett Jr. WE (2000) Mechanisms of anterior cruciate ligament injury. Orthopedics 23:573–578.

Brand RA, Crowninshield RD, Wittstock CE, Pedersen DR, Clark CR, van Krieken FM (1982) A Model of Lower Extremity Muscular Anatomy. J Biomech Eng 104:304–310.

Brand RA, Pedersen DR, Friederich JA (1986) The sensitivity of muscle force predictions to changes in physiologic cross-sectional area. J Biomech 19:589–596.

Buchanan TS, Almdale DPJ, Lewis JL, Rymer WZ (1986) Characteristics of synergic relations during isometric contractions of human elbow muscles. J Neurophysiol 56:1225–1241.

Buchanan TS, Kim AW, Lloyd DG (1996) Selective muscle activation following rapid varus/valgus perturbations at the knee. Med Sci Sports Exerc 28:870–876.

Buchanan TS, Lloyd DG (1997) Muscle activation at the human knee during isometric flexion-extension and varus-valgus loads. J Orthop Res 151:1–17.

Cashaback JGA, Potvin JR (2012) Knee muscle contributions to joint rotational stiffness. Hum Mov Sci 31:118–128.

Cheung VCK, Piron L, Agostini M, Silvoni S, Turolla A, Bizzi E (2009) Stability of muscle synergies for voluntary actions after cortical stroke in humans. Proc Natl Acad Sci 106:19563–19568.

Chmielewski TL, Hurd WJ, Rudolph KS, Axe MJ, Snyder-Mackler L (2005) Perturbation training improves knee kinematics and reduces muscle co-contraction after complete unilateral anterior cruciate ligament rupture. Phys Ther 85:740–749.

Chmielewski TL, Stackhouse S, Axe MJ, Snyder-Mackler L (2004) A prospective analysis of incidence and severity of quadriceps inhibition in a consecutive sample of 100 patients with complete acute anterior cruciate ligament rupture. J Orthop Res 22:925–930.

Chvatal SA, Ting LH (2012) Voluntary and Reactive Recruitment of Locomotor Muscle Synergies during Perturbed Walking. J Neurosci 32:12237–12250.

Chvatal SA, Torres-Oviedo G, Safavynia SA, Ting LH (2011) Common muscle synergies for control of center of mass and force in nonstepping and stepping postural behaviors. J Neurophysiol 106:999–1015.

Comins JD, Krogsgaard MR, Brodersen J (2013) Development of the Knee Numeric-Entity Evaluation Score (KNEES - ACL): A condition-specific questionnaire. Scand J Med Sci Sports 23:e293–e301.

Curray JR (1956) The analysis of two-dimensional orientation data. J Geol 64:117–131.

da Fonseca ST, Vaz DV, de Aquino CF, Brício RS (2006) Muscular co-contraction during walking and landing from a jump: Comparison between genders and influence of activity level. J Electromyogr Kinesiol 16:273–280.

Daniel DM, Stone ML, Dobson BE, Fithian DC, Rossman DJ, Kaufman KR (1994) Fate of the ACL-injured patient. A prospective outcome study. Am J Sports Med 22:632–644.

Delp SL (2007) OpenSim: Open-Source Software to Create and Analyze Dynamic Simulations of Movement. IEEE Trans Biomed Eng 54:1940–1950.

DeLuca CJ (1997) The use of surface electromyography in biomechanics. J Appl Biomech 13:135–163.

DeMont RG, Lephart SM (2004) Effect of sex on preactivation of the gastrocnemius and hamstring muscles. Br J Sports Med 38:120–124.

DeMorat G, Weinhold P, Blackburn T, Chudik S, Garrett W (2004) Aggressive Quadriceps Loading Can Induce Noncontact Anterior Cruciate Ligament Injury. Am J Sports Med 32:477–483.

Dewald JPA, Pope PS, Given JD, Buchanan TS, Rymer WZ (1995) Abnormal muscle coactivation patterns during isometric torque generation at the elbow and shoulder in hemiparetic subjects. Brain 118:495–510.

Durselen L, Claes L, Kiefer H (1995) The influence of muscle forces and external loads on cruciate ligament strain. Am J Sports Med 23:129–136.

Escamilla RF, Fleisig GS, Zheng N, Barrentine SW, Wilk KE, Andrews JR (1998) Biomechanics of the knee during closed kinetic chain and open kinetic chain exercises. Med Sci Sports Sci 30:556–569.

Fitzgerald GK, Axe MJ, Snyder-Mackler L (2000) Proposed Practice Guidelines for Nonoperative Anterior Cruciate Ligament Rehabilitation of Physically Active Individuals. J Orthop Sports Phys Ther 30:194–203.

Fitzgerald GK, Piva SR, Irrgang JJ (2004) Reports of joint instability in knee osteoarthritis: its prevalence and relationship to physical function. Arthritis Rheum 51:941–946.

Flaxman TE, Smith AJJ, Benoit DL (2013) Sex-Related Differences in Neuromuscular Control: Implications for Injury Mechanisms or Healthy Stabilisation Strategies? J Orthop Res 32:310–317.

Flaxman TE, Speirs AD, Benoit DL (2012) Joint stabilisers or moment actuators: the role of knee joint muscles while weight bearing. J Biomech 45:2570–2576.

Fleming B C, Renstrom PA, Beynnon BD, Engstrom B, Peura GD, Badger GJ, Johnson RJ (2001a) The effect of weightbearing and external loading on anterior cruciate ligament strain. J Biomech 34:163–710.

Fleming B C, Renstrom P, Ohlen G, Johnson RJ, Peura GD, Beynnon BD, Badger GJ (2001b) The gastrocnemius muscle is an antagonist of the anterior cruciate ligament. J Orthop Res 19:1178–1184.

Frobell RB, Roos EM, Roos HP, Ranstam J, Lohmander LS (2010) A randomized trial of treatment for acute anterior cruciate ligament tears. N Engl J Med 363:331–342.

Frobell RB, Roos HP, Roos EM, Roemer FW, Ranstam J, Lohmander LS (2013) Treatment for acute anterior cruciate ligament tear: Five year outcome of randomised trial. BMJ Online 346:f323.

Geiser CF, O'Conner KM, Earl JE (2010) Effects of isolated hip abductor fatigue on frontal plane knee mechanics. Med Sci Sports Exerc 42:535–545.

Glitsch U, Baumann W (1997) The three-dimensional determination of internal loads in the lower extremity. J Biomech 30:1123–1131.

Goldberg EJ, Neptune RR (2007) Compensatory strategies during normal walking in response to muscle weakness and increased hip joint stiffness. Gait Posture 25:360–367.

Goldfuss AJ, Morehouse CA, Leveau BF (1973) Effect of muscular tension on knee stability. Med Sci Sports Exerc 5:267–271.

Graven-Nielsen T, Lund H, Arendt-Nielsen L, Danneskiold-Samsøe B, Bliddal H (2002) Inhibition of maximal voluntary contraction force by experimental muscle pain: A centrally mediated mechanism. Muscle Nerve 26:708–712.

Hame SL, Oakes DA, Markolf KL (2002) Injury to the anterior cruciate ligament during alpine skiing: A biomechanical analysis of tibial torque and knee flexion angle. Am J Sports Med 30:537–540.

Hamill J, Knutzen K (2004) Biomechanical basis of human movement. Baltimore, MD: Lippincott Williams & Wilkins.

Harner CD, Höher J, Vogrin TM, Carlin GJ, Woo SL-Y (1998) The Effects of a Popliteus Muscle Load on In Situ Forces in the Posterior Cruciate Ligament and on Knee Kinematics A Human Cadaveric Study. Am J Sports Med 26:669–673.

Hashemi J, Chandrashekar N, Jang T, Karpat F, Oseto M, Ekwaro-Osire S (2007) An alternative mechanism of non-contact anterior cruciate ligament injury during jump-landing: In-vitro simulation. Exp Mech 47:347–354.

Henriksen M, Aaboe J, Simonsen EB, Alkjaer T, Bliddal H (2009a) Experimentally reduced hip abductor function during walking: Implications for knee joint loads. J Biomech 42:1236–1240.

Henriksen M, Alkjaer T, Lund H, Simonsen EB, Graven-Nielsen T, Danneskiold-Samsøe B, Bliddal H (2007) Experimental quadriceps muscle pain impairs knee joint control during walking. J Appl Physiol 103:132–139.

Henriksen M, Alkjaer T, Simonsen EB, Bliddal H (2009b) Experimental muscle pain during a forward lunge--the effects on knee joint dynamics and electromyographic activity. Br J Sports Med 43:503–507.

Henriksen M, Graven-Nielsen T, Aaboe J, Andriacchi TP, Bliddal H (2010) Gait changes in patients with knee osteoarthritis are replicated by experimental knee pain. Arthritis Care Res 62:501–509.

Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G (2000) Development of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kinesiol 10:361–374.

Herzog W, Binding P (1992) Predictions of antagonistic muscular activity using nonlinear optimization. Math Biosci 111:217–229.

Herzog W, Longino D (2007) The role of muscles in joint degeneration and osteoarthritis. J Biomech 40:54–63.

Hewett T (2000) Neuromuscular and hormonal factors associated with knee injuries in female athletes: Strategies for intervention. Sports Med 29:313–327.

Hewett T, Myer G, Ford K, Heidt R, Colosimo A, McLean S, van den Bogert A, Paterno M, Succop P (2005) Biomechanical Measures of Neuromuscular Control and Valgus Loading of the Knee Predict Anterior Cruciate Ligament Injury Risk in Female Athletes. Am J Sports Med 33:492–501.

Hinman RS, Hunt MA, Creaby MW, Wrigley TV, McManus FJ, Bennell KL (2010) Hip muscle weakness in individuals with medial knee osteoarthritis. Arthritis Care Res 62:1190–1193.

Hodges PW, Mellor R, Crossley K, Bennell K (2009) Pain induced by injection of hypertonic saline into the infrapatellar fat pad and effect on coordination of the quadriceps muscles. Arthritis Care Res 61:70–77.

Hodges PW, Tucker K (2011) Moving differently in pain: A new theory to explain the adaptation to pain. Pain 152:S90–S98.

Hopkins JT, Ingersoll CD (2000) Arthrogenic muscle inhibition: A limiting factor in joint rehabilitation. J Sport Rehabil 9:135–159.

Hortobágyi T, Devita P (2000) Muscle pre- and coactivity during downward stepping are associated with leg stiffness in aging. J Electromyogr Kinesiol 10:117–126.

Hortobagyi T, Westerkamp L, Beam S, Moody J, Garry J, Holbert D, DeVita P (2005) Altered hamstring-quadriceps muscle balance in patients with knee osteoarthritis. Clin Biomech 20:97–104.

Houck JR, De Haven KE, Maloney M (2007) Influence of anticipation on movement patterns in subjects with ACL deficiency classified as noncopers. J Orthop Sports Phys Ther 37:56–64.

Houck J, Yack HJ (2001) Giving way event during a combined stepping and crossover cutting task in an individual with anterior cruciate ligament deficiency. J Orthop Sports Phys Ther 31:481–489.

Hsieh HH, Walker PS (1976) Stabilizing mechanisms of the loaded and unloaded knee joint. J Bone Jt Surg 58:87–93.

Hubley-Kozey CL, Hill NA, Rutherford DJ, Dunbar MJ, Stanish WD (2009) Co-activation differences in lower limb muscles between asymptomatic controls and those with varying degrees of knee osteoarthritis during walking. Clin Biomech 24:407–414.

Hug F, Turpin NA, Couturier A, Dorel S (2011) Consistency of muscle synergies during pedaling across different mechanical constraints. J Neurophysiol 106:91–103.

Hug F, Turpin NA, Dorel S, Guével A (2012) Smoothing of electromyographic signals can influence the number of extracted muscle synergies. Clin Neurophysiol 123:1895–1896.

Hug F, Turpin NA, Guével A, Dorel S (2010) Is interindividual variability of EMG patterns in trained cyclists related to different muscle synergies? J Appl Physiol 108:1727–1736.

Ireland ML, Willson JD, Ballantyne BT, Davis IM (2003) Hip Strength in Females with and without Patellofemoral Pain. J Orthop Sports Phys Ther 33:671–676.

Irrgang JJ, Anderson AF, Boland AL, Harner CD, Kurosaka M, Neyret P, Richmond JC, Shelborne KD (2001) Development and validation of the international knee documentation committee subjective knee form. Am J Sports Med 29:600–613.

Ivanenko YP, Poppele RE, Lacquaniti F (2004) Five basic muscle activation patterns account for muscle activity during human locomotion. J Physiol 556:267–282.

Jacobs CA, Uhl TL, Mattacola CG, Shapiro R, Rayens WS (2007) Hip abductor function and lower extremity landing kinematics: Sex differences. J Athl Train 42:76–83.

Jacobs R, van Ingen Schenau GJ (1992) Control of an external force in leg extensions in humans. J Physiol 457:611–626.

Kennedy JC, Weinberg HW, Wilson AS (1974) The anatomy and function of the anterior cruciate ligament. As determined by clinical and morphological studies. J Bone Jt Surg - Ser A 56:223–235.

Kiefer G, Forwell. L, Kramer J, Birmingham TL (1998) Comparison of sitting and standing protocols for testing knee proprioception. Physiother Can 50:30–34.

Kim HJ, Fernandez JW, Akbarshahi M, Walter JP, Fregly BJ, Pandy MG (2009) Evaluation of predicted knee-joint muscle forces during gait using an instrumented knee implant. J Orthop Res 27:1326–1331.

Konishi Y, Ikeda K, Nishino A, Sunaga M, Aihara Y, Fukubayashi T (2007) Relationship between quadriceps femoris muscle volume and muscle torque after anterior cruciate ligament repair. Scand J Med Sci Sports 17:656–661.

Kostogiannis I, Ageberg E, Neuman P, Dahlberg L, Fridén T, Roos H (2007) Activity level and subjective knee function 15 years after anterior cruciate ligament injury: A prospective, longitudinal study of nonreconstructed patients. Am J Sports Med 35:1135–1143.

Krishnan C, Huston K, Amendola A, Williams GN (2008) Quadriceps and Hamstrings Muscle Control in Athletic Males and Females. J Orthop Res 26:800–808.

Krishnan C, Williams GN (2009) Sex differences in quadriceps and hamstrings EMG-moment relationships. Med Sci Sports Exerc 41:1652–1660.

Kristiansen M, Madeleine P, Hansen EA, Samani A (2013) Inter-subject variability of muscle synergies during bench press in power lifters and untrained individuals. Scand J Med Sci Sports 25:89–97.

Kristianslund E, Krosshaug T, van den Bogert AJ (2012) Effect of low pass filtering on joint moments from inverse dynamics: Implications for injury prevention. J Biomech 45:666–671.

Krogsgaard MR, Fischer-Rasmussen T, Dyhre-Poulsen P (2011) Absence of sensory function in the reconstructed anterior cruciate ligament. J Electromyogr Kinesiol 21:82–86.

Kvist J, Gillquist J (2001) Anterior positioning of tibia during motion after anterior cruciate ligament injury. Med Sci Sports Exerc 33:1063–1072.

Lattier G, Millet GY, Maffiuletti NA, Babault N, Lepers R (2003) Neuromuscular differences between endurance-trained, power-trained, and sedentary subjects. J Strength Cond Res 17:514–521.

Lee WA (1984) Neuromotor synergies as a basis for coordinated intentional action. J Mot Behav 16:135–170.

Lewek MD, Ramsey DK, Snyder-Mackler L, Rudolph KS (2005) Knee stabilization in patients with medial compartment knee osteoarthritis. Arthritis Rheum 52:2845–2853.

Li G, Papannagari R, DeFrate LE, Yoo JD, Park SE, Gill TJ (2007) The effects of ACL deficiency on mediolateral translation and varus-valgus rotation. Acta Orthop 78:355–360.

Li L, Landin D, Grodesky J, Myers J (2002) The function of gastrocnemius as a knee flexor at selected knee and ankle angles. J Electromyogr Kinesiol 12:385–390.

Lloyd DG, Buchanan TS (1996) A model of load sharing between muscles and soft tissues at the human knee during static tasks. J Biomech Eng 118:367–376.

Lloyd DG, Buchanan TS (2001) Strategies of muscular support of varus and valgus isometric loads at the human knee. J Biomech 34:1257–1267.

Logerstedt DS, Snyder-Mackler L, Ritter RC, Axe MJ, Godges JJ (2010) Knee stability and movement coordination impairments: knee ligament sprain. J Orthop Sports Phys Ther 40:A1–A37.

Lohmander LS, Östenberg A, Englund M, Roos H (2004) High prevalence of knee osteoarthritis, pain, and functional limitations in female soccer players twelve years after anterior cruciate ligament injury. Arthritis Rheum 50:3145–3152.

Lubowitz JH, Appleby D (2011) Cost-Effectiveness Analysis of the Most Common Orthopaedic Surgery Procedures: Knee Arthroscopy and Knee Anterior Cruciate Ligament Reconstruction. Arthrosc J Arthrosc Relat Surg 27:1317–1322.

Lund JP, Donga R, Widmer CG, Stohler CS (1991) The pain-adaptation model: a discussion of the relationship between chronic musculoskeletal pain and motor activity. Can J Physiol Pharmacol 69:683–694.

MacLean CL, Taunton JE, Clement DB, Regan WD, Stanish WD (1999) Eccentric Kinetic Chain Exercise as a Conservative Means of Functionally Rehabilitating Chronic Isolated Insufficiency of the Posterior Cruciate Ligament. J Sport Med 9:142–150.

MacLeod TD, Manal K, Silbernagel KG, Snyder-Mackler L, Buchanan TS (2013a) Characteristics of human knee muscle coordination during isometric contractions in a standing posture: the effect of limb task. J Electromyogr Kinesiol Off J Int Soc Electrophysiol Kinesiol 23:1398–1405.

MacLeod TD, Snyder-Mackler L, Buchanan TS (2013b) Differences in Neuromuscular Control and Quadriceps Morphology Between Potential Copers and Noncopers Following Anterior Cruciate Ligament Injury. J Orthop Sports Phys Ther 44:76–84.

MacWilliams BA, Wilson DR, Desjardins JD, Romero J, Chao EYS (1999) Hamstrings cocontraction reduces internal rotation, anterior translation, and anterior cruciate ligament load in weight-bearing flexion. J Orthop Res 17:817–822.

Mall NA, Chalmers PN, Moric M, Tanaka MJ, Cole BJ, Bach BR, Paletta GA (2014) Incidence and Trends of Anterior Cruciate Ligament Reconstruction in the United States. Am J Sports Med 42:2363–2370.

Mantovani G, Lamontagne M (2016) How Different Marker Sets Affect Joint Angles in Inverse Kinematics Framework. J Biomech Eng [Epub ahead of print].

Markolf KL, Burchfield DM, Shapiro MM, Shepard MF, Finerman GA, Slauterbeck JL (1995) Combined knee loading states that generate high anterior cruciate ligament forces. J Orthop Res 13:930–935.

Markolf KL, Mensch JS, Amstutz HC (1976) Stiffness and laxity of the knee - the contributions of the supporting structures. A quantitative in vitro study. J Bone Jt Surg Am 58:583–594.

McClay IS, Robinson JR, Andriacchi TP, Frederick EC, Gross T, Martin P, Valiant G, Williams KR, Cavanagh PR (1994) A profile of ground reaction forces in professional basketball. J Appl Biomech 10:222–236.

McLean SG, Huang X, Su A, Van DB (2004) Sagittal plane biomechanics cannot injure the ACL during sidestep cutting. Clin Biomech 19:828–838.

Mikesky A, Mazzuca S, Brandt KD, Perkins SM, Damush T, Lane KA (2006) Effects of strength training on the incidence and progression of knee osteoarthritis. Arthritis Rheum 55:690–699.

Mills OS, Hull ML (1991) Rotational flexibility of the human knee due to varus/valgus and axial moments in vivo. J Biomech 24:673–690.

Miranda DL, Fadale PD, Hulstyn MJ, Shalvoy RM, Machan JT, Fleming BC (2013) Knee biomechanics during a jump-cut maneuver: Effects of gender & ACL surgery. Med Sci Sports Exerc 45:942–951.

Moghadam MN, Aminian K, Asghari M, Parnianpour M (2013) How well do the muscular synergies extracted via non-negative matrix factorisation explain the variation of torque at shoulder joint? Comput Methods Biomech Biomed Engin 16:291–301.

Morgan KD, Donnelly CJ, Reinbolt JA (2014) Elevated gastrocnemius forces compensate for decreased hamstrings forces during the weight-acceptance phase of single-leg jump landing: implications for anterior cruciate ligament injury risk. J Biomech 47:3295–3302.

Neptune RR, Clark DJ, Kautz SA (2009) Modular control of human walking: A simulation study. J Biomech 42:1282–1287.

Nordin M, Frankel VH (2001) Basic Biomechanics of the Musculoskeletal System. Philadelphia, PA: Lippincott Williams & Wilkins.

O'Conner JJ (1993) Can muscle co-contraction protect knee ligaments after injury or repair? J Bone Jt Surg Br 75:41–48.

Palmieri RM, Weltman A, Edwards JE, Tom JA, Saliba EN, Mistry DJ, Ingersoll CD (2005) Pre-synaptic modulation of quadriceps arthrogenic muscle inhibition. Knee Surg Sports Traumatol Arthrosc 13:370–376.

Palmieri-Smith RM, Kreinbrink J, Ashton-Miller JA, Wojtys EM (2007) Quadriceps inhibition induced by an experimental knee joint effusion affects knee joint mechanics during a single-legged drop landing. Am J Sports Med 35:1269–1275.

Palmieri-Smith RM, McLean SG, Ashton-Miller JA, Wojtys EM (2009) Association of quadriceps and hamstrings cocontraction patterns with knee joint loading. J Athl Train 44:256–263.

Palmieri-Smith RM, Thomas AC (2009) A neuromuscular mechanism of posttraumatic osteoarthritis associated with ACL injury. Exerc Sport Sci Rev 37:147–153.

Palmieri-Smith RM, Thomas AC, Wojtys EM (2008) Maximizing Quadriceps Strength After ACL Reconstruction. Clin Sports Med 27:405–424.

Panjabi MM (1992) The stabilizing system of the spine. Part I. Function, dysfunction, adaptation, and enhancement. J Spinal Disord 5:383–389; discussion 397.

Pen LJ, Fisher CA (1994) Athletes and Pain Tolerance. Sports Med 18:319–329.

Pietrosimone B, Lepley AS, Murray AM, Thomas AC, Bahhur NO, Schwartz TA (2014) Changes in voluntary quadriceps activation predict changes in muscle strength and gait biomechanics following knee joint effusion. Clin Biomech 29:923–929.

Pincivero DM, Salfetnikov Y, Campy RM, Coelho AJ (2004) Angle- and gender-specific quadriceps femoris muscle recruitment and knee extensor torque. J Biomech 37:1689–1697.

Powers CM (2010) The influence of abnormal hip mechanics on knee injury: A biomechanical perspective. J Orthop Sports Phys Ther 40:42–51.

Powers CM, Perry J, Hsu A, Hislop HJ (1997) Are Patellofemoral Pain and Quadriceps Femoris Muscle Torque Associated With Locomotor Function? Phys Ther 77:1063–1075.

Reed-Jones RJ, Vallis LA (2008) Kinematics and muscular responses to a ramp descent in the ACL deficient knee. The Knee 15:117–124.

Reinke EK, Spindler KP, Lorring D, Jones MH, Schmitz L, Flanigan DC, An AQ, Quiram AR, Preston E, Martin M, Schroeder B, Parker RD, Kaeding CC, Borzi L, Pedroza A, Huston LJ, Harrell Jr. FE, Dunn WR (2011) Hop tests correlate with IKDC and KOOS at minimum of 2 years after primary ACL reconstruction. Knee Surg Sports Traumatol Arthrosc 19:1806–1816.

Rice DA, McNair PJ, Lewis GN, Dalbeth N (2014) Quadriceps arthrogenic muscle inhibition: the effects of experimental knee joint effusion on motor cortex excitability. Arthritis Res Ther 16:502–508.

Roland MO (1986) A critical review of the evidence for a pain-spasm-pain cycle in spinal disorders. Clin Biomech Bristol Avon 1:102–109.

Roos EM (2005) Joint injury causes knee osteoarthritis in young adults. Curr Opin Rheumatol 17:195–200.

Roos EM, Lohmander LS (2003) The Knee injury and Osteoarthritis Outcome Score (KOOS): from joint injury to osteoarthritis. Health Qual Life Outcomes 1:64.

Rudolph KS, Axe MJ, Buchanan TS, Scholz JP, Snyder-Mackler L (2001) Dynamic stability in the anterior cruciate ligament deficient knee. Knee Surg Sports Traumatol Arthrosc 9:62–71.

Rudolph KS, Axe MJ, Snyder-Mackler L (2000) Dynamic stability after ACL injury: Who can hop? Knee Surg Sports Traumatol Arthrosc 8:262–269.

Rudolph KS, Schmitt LC, Lewek MD (2007) Age-related changes in strength, joint laxity, and walking patterns:

Are they related to knee osteoarthritis? Phys Ther 87:1422–1432.

Sasaki K, Neptune RR (2010) Individual muscle contributions to the axial knee joint contact force during normal walking. J Biomech 43:2780–2784.

Shanbehzadeh S, Mohseni Bandpei MA, Ehsani F (2015) Knee muscle activity during gait in patients with anterior cruciate ligament injury: a systematic review of electromyographic studies. Knee Surg Sports Traumatol Arthrosc Off J ESSKA.

Shimokochi Y, Shultz SJ (2008) Mechanisms of noncontact anterior cruciate ligament injury. J Athl Train 43:396–408.

Shultz SJ, Beynnon BD, Schmitz RJ (2009) Sex differences in coupled knee motions during the transition from non-weight bearing to weight bearing. J Orthop Res 27:717–723.

Sigward SM, Powers CM (2006) The influence of gender on knee kinematics, kinetics and muscle activation patterns during side-step cutting. Clin Biomech 21:41–48.

Sinkjaer T, Arendt-Nielsen L (1991) Knee stability and muscle coordination in patients with anterior cruciate ligament injuries: An electromyographic approach. J Electromyogr Kinesiol 1:209–217.

Slemenda C, Brandt KD, Heilman DK, Mazzuca S, Braunstein EM, Katz BP, Wolinsky FD (1997) Quadriceps weakness and osteoarthritis of the knee. Ann Intern Med 127:97–104.

Smale KB, Shourijeh MS, Benoit DL (2016) Use of muscle synergies and wavelet transforms to identify fatigue during squatting. J Electromyogr Kinesiol 28:158–166.

Smith AJJ, Flaxman TE, Benoit DL (2012) Reliability of knee joint muscle activity during weight bearing force control. J Electromyogr Kinesiol 22:914–922.

Sritharan P, Lin Y-C, Pandy MG (2012) Muscles that do not cross the knee contribute to the knee adduction moment and tibiofemoral compartment loading during gait. J Orthop Res 30:1586–1595.

Steele JR, Brown JMM (1999) Effects of chronic anterior cruciate ligament deficiency on muscle activation patterns during abrupt deceleration task. Clin Biomech 14:247–257.

Steele KM, Rozumalski A, Schwartz MH (2015) Muscle synergies and complexity of neuromuscular control during gait in cerebral palsy. Dev Med Child Neurol 57:1176–1182.

Steele KM, Tresch MC, Perreault EJ (2013) The number and choice of muscles impact the results of muscle synergy analyses. Front Comput Neurosci.

Stensdotter AK, Hodges PW, Mellor R, Sundelin G, Hager-Ross C (2003) Quadriceps activation in closed and in open kinetic chain exercise. Med Sci Sports Exerc 35:2043–2047.

Suter E, Herzog W (2000) Does muscle inhibition after knee injury increase the risk of osteoarthritis? Exerc Sport Sci Rev 28:15–18.

Suter E, Herzog W, Leonard TR, Nguyen H (1998) One-year changes in hind limb kinematics, ground reaction forces and knee stability in an experimental model of osteoarthritis. J Biomech 31:511–517.

Tegner Y, Lysholm J (1985) Rating systems in the evaluation of knee ligament injuries. Clin Orthop NO. 198:43–49.

Thomas AC, Villwock M, Wojtys EM, Palmieri-Smith RM (2013) Lower extremity muscle strength after anterior cruciate ligament injury and reconstruction. J Athl Train 48:610–620.

Thomeé P, Währborg P, Börjesson M, Thomeé R, Eriksson BI, Karlsson J (2007) Self-efficacy, symptoms and physical activity in patients with an anterior cruciate ligament injury: A prospective study. Scand J Med Sci Sports 17:238–245.

Ting LH, Chiel HJ, Trumbower RD, Allen JL, McKay JL, Hackney ME, Kesar TM (2015) Neuromechanical Principles Underlying Movement Modularity and Their Implications for Rehabilitation. Neuron 86:38–54.

Ting LH, Macpherson JM (2005) A limited set of muscle synergies for force control during a postural task. J Neurophysiol 93:609–613.

Torres-Oviedo G, Macpherson JM, Ting LH (2006) Muscle synergy organization is robust across a variety of postural perturbations. J Neurophysiol 96:1530–1546.

Torres-Oviedo G, Ting LH (2010) Subject-Specific Muscle Synergies in Human Balance Control Are Consistent Across Different Biomechanical Contexts. J Neurophysiol 103:3084–3098.

Tresch MC, Saltiel P, Bizzi E (1999) The construction of movement by the spinal cord. Nat Neurosci 2:162–167.

Urbach D, Nebelung W, Becker R, Awiszus F (2001) Effects of reconstruction of the anterior cruciate ligament on voluntary activation of quadriceps femoris. J Bone Jt Surg - Ser B 83:1104–1110.

van den Hoorn W, Hodges PW, Dieën JH van, Hug F (2014) Effect of acute noxious stimulation to the leg or back on muscle synergies during walking. J Neurophysiol:jn.00557.2014.

van der Krogt MM, Delp SL, Schwartz MH (2012) How robust is human gait to muscle weakness? Gait Posture 36:113–119.

van Deursen RWM, Cavanagh PR, van Ingen Schenau GJ, Becker MB, Ulbrecht JS (1998) The role of cutaneous information in a contact control task of the leg in humans. Hum Mov Sci 17:95–120.

van Ingen Schenau GJ, Boots PJM, de Groot G, Snackers RJ, van Woensel WWLM (1992) The constrained control of force and position in multi-joint movements. Neuroscience 46:197–207.

Walter JP, Kinney AL, Banks SA, D'Lima DD, Besier TF, Lloyd DG, Fregly BJ (2014) Muscle synergies may improve optimization prediction of knee contact forces during walking. J Biomech Eng 136.

Weresh MJ, Gabel RH, Brand RA, Tearse DS (1997) Popliteus function in ACL-deficient patients. Scand J Med Sci Sports 7:14–19.

Wilkie DR (1950) The relation between force and velocity in human muscle. J Physiol 110:249–280.

Williams GN, Barrance PJ, Snyder-Mackler L, Axe MJ, Buchanan TS (2003) Specificity of muscle action after anterior cruciate ligament injury. J Orthop Res 21:1131–1137.

Williams GN, Chmielewski T, Rudolph KS, Buchanan TS, Snyder-Mackler L (2001) Dynamic knee stability: Current theory and implications for clinicians and scientists. J Orthop Sports Phys Ther 31:546–566.

Winby CR, Lloyd DG, Besier TF, Krik TB (2009) Muscle and external load contribution to knee joint contact loads during normal gait. J Biomech 42:2294–2300.

Winter DA (2009) Biomechanics and Motor Control of Human Movement. Hoboken, New Jersey: John Wiley & Sons Inc.

Wojtys E, Huston L (1994) Neuromuscular Performance in Normal and Anterior Cruciate Ligament-Deficient Lower Extremities. Am J Sports Med 22:89–104.

Wojtys EM, Ashton-Miller JA, Huston LJ (2002) A gender-related difference in the contribution of the knee musculature to sagittal-plane shear stiffness in subjects with similar knee laxity. J Bone Jt Surg 84-A:10–16.

Wojtys EM, Huston LJ, Schock HJ, Boylan JP, Ashton-Miller JA (2003) Gender differences in muscular protection of the knee in torsion in size-matched athletes. J Bone Jt Surg 85 A:782–789.

Yack HJ, Washco LA, Whieldon T (1994) Compressive forces as a limiting factor of anterior tibial translation in the ACL-deficient knee. Clin J Sport Med 4:233–239.

Yoo JD, Papannagari R, Park SE, DeFrate LE, Gill TJ, Li G (2005) The effect of anterior cruciate ligament reconstruction on knee joint kinematics under simulated muscle loads. Am J Sports Med 33:240–246.

Zhang L-Q, Wang G (2001) Dynamic and static control of the human knee joint in abduction–adduction. J Biomech 34:1107–1115.

Zhang L-Q, Xu D, Wang G, Hendrix RW (2001) Muscle strength in knee varus and valgus. Med Sci Sports Exerc 33:1194–1199.

Appendices

Appendix A: HMBL cluster marker set

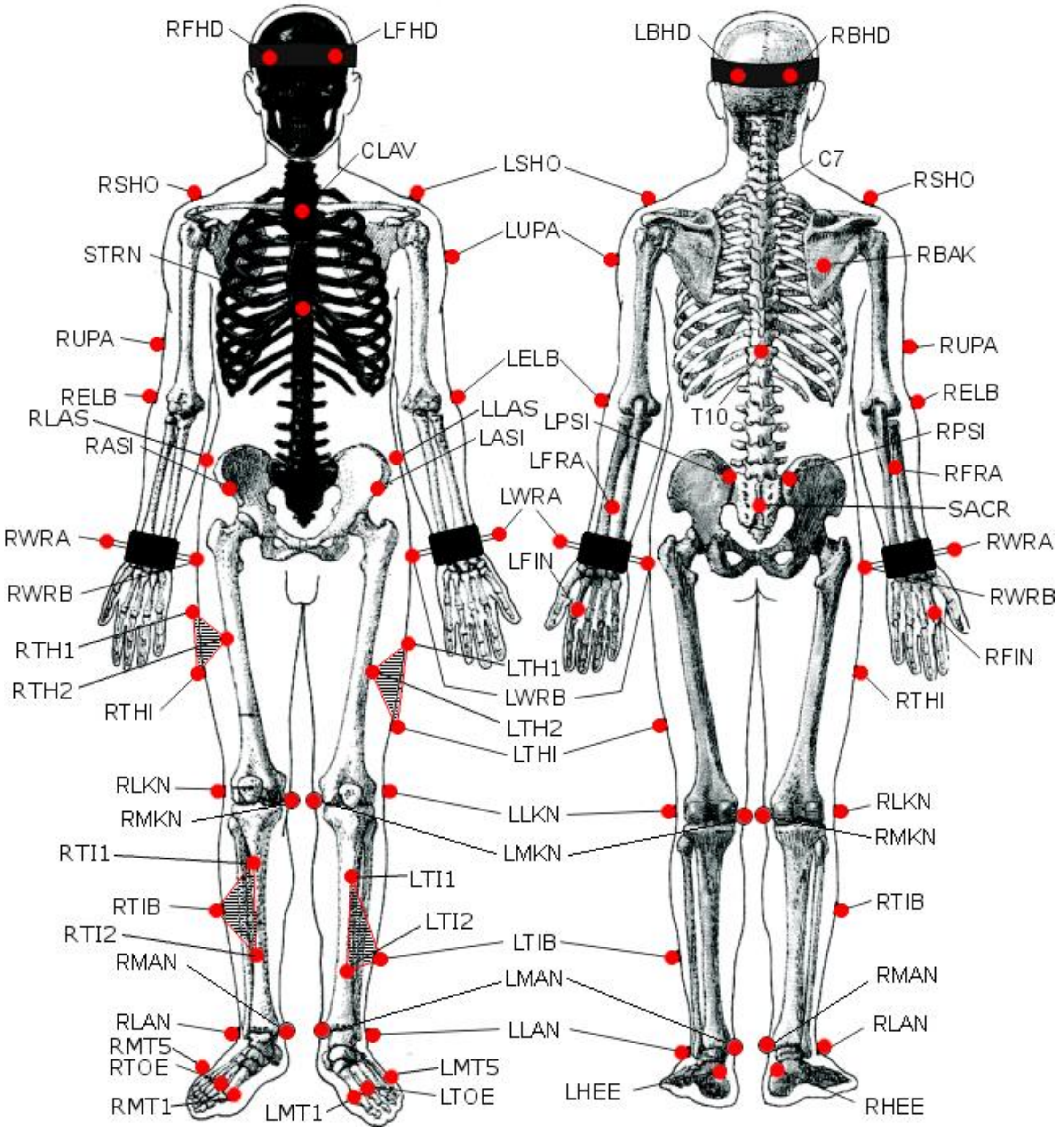

Figure 36: HMBL cluster marker set. Plug-in-Gait marker set acts as the basis of the marker set. Markers added to the set are medial knee and ankle (LMKN, RMKN, LMAN, RMAN) markers, two thigh (RTH1, RTH2, LTH1, LTH2), two tibial (RTI1, RTI2, LTI1, LTI2), and two metatarsal (RMT1, RMT5, LMT1, LMT5) markers. RTHI, LTHI, RTIB and LTIB are used as part of the cluster for right and left thigh, and right and left tibia segments, respectively (figure adapted from Mantovani and Lamontagne, 2016).

Appendix B: Multicollinearity Diagnostics

An important assumption of a multiple linear regression is the predictor variables must not show multicollinearity. This leads to problems with understanding which independent variable contributes to the variance explained in the dependent variable, as well as technical issues in calculating a multiple regression model. Multicollinearity can be assessed by examining the two diagnostic statistics of tolerance and the variance inflation factor (VIF). A predictor variable's tolerance is $1\text{-}R^2$. A small tolerance value (<0.1) indicates the given variable is almost in perfect linear combination with one or more of the independent variables already in the equation and that it should not be added to the regression (Field, 2009). VIF measures the impact of collinearity among the variables in a regression model. VIF is 1/Tolerance and is always greater than or equal to 1. Values exceeding 10 indicate multicollinearity causing instability of the b and beta coefficients.

A correlation coefficient matrix of normalised internal joint moments (Nm/kg) was computed in MatLab (*corrcoef.m*). A significant correlation was defined at the $p<0.05$ level. Tolerance and VIF values were computed using the Besley collinearity diagnostic tool in MatLab (*collintest.m*). Results show significant correlation coefficients among 104/120 comparisons ($p<0.05$). 11/16 moments had VIF >10 and tolerance <0.1 and multicollinearity was assumed present (Table 8).

Reference

Field, A (2009). Discovering Statistics Using SPSS, 3rd Edition. Thousand Oaks, CA: Sage Publications; p. 244.

Table 8: Correlation coefficients and collinearity statistics for normalized internal net joint moments of the hip, knee and ankle (kg/Nm). Data is from healthy controls during the force matching task at 60% effort level. Threshold for identifying multicollinearity was set to tolerance (Tol) values less than 0.1 and variance inflation factors (VIF) greater than 10. 104/120 moment comparisons had significant correlations (p<0.05) and 11/16 moment types showed multicollinearity (white cells). Shaded cells indicate non-significant correlation coefficients and moment types not violating the assumption of multicollinearity.

			Moments																Collinearity statistics	
			Hip						Knee						Ankle				Tol	VIF
			Flex	Ext	Add	Abd	IR	ER	Flex	Ext	Add	Abd	IR	ER	Flex	Ext	Add	Abd		
Moments	Hip	Flex	-																0.17	5.8
		Ext	-0.58	-															0.08	12.7
		Add	-0.47	0.51	-														0.09	11.1
		Abd	0.37	-0.39	-0.58	-													0.06	14.9
		IR	-0.12	0.17	-0.45	0.61	-												0.03	30.9
		ER	-0.12	-0.16	0.54	-0.45	-0.54	-											0.03	28.0
	Knee	Flex	0.82	-0.57	-0.48	0.54	0.09	-0.18	-										0.17	5.7
		Ext	-0.46	0.83	0.49	-0.33	0.02	0.00	-0.49	-									0.17	5.9
		Add	-0.44	0.46	0.93	-0.54	-0.45	0.60	-0.46	0.47	-								0.07	13.4
		Abd	0.41	-0.38	-0.59	0.95	0.62	-0.45	0.54	-0.35	-0.55	-							0.07	15.1
		IR	-0.35	0.46	-0.20	0.27	0.89	-0.51	-0.20	0.23	-0.23	0.27	-						0.02	52.7
		ER	0.12	-0.34	0.30	-0.40	-0.54	0.91	0.00	-0.17	0.34	-0.38	-0.53	-					0.02	62.2
	Ankle	Flex	-0.33	0.41	0.13	-0.02	0.25	-0.21	-0.14	0.12	0.07	0.01	0.35	-0.31	-				0.40	2.5
		Ext	0.28	-0.29	-0.09	-0.08	-0.25	0.30	0.02	-0.08	-0.05	-0.06	-0.30	0.46	-0.45	-			0.43	2.3
		Add	0.05	-0.27	0.21	-0.33	-0.35	0.62	-0.01	-0.17	0.20	-0.31	-0.34	0.70	-0.23	0.20	-		0.03	31.3
		Abd	-0.31	0.39	-0.13	0.22	0.69	-0.25	-0.20	0.27	-0.12	0.21	0.79	-0.26	0.30	-0.07	-0.54	-	0.02	48.5

Appendix C: Strength Deficit Model Description

The following is a description of the strength deficit model developed by M.S. Shourijeh and first presented in Bigham (2016). Corresponding manuscript is currently under revision (Bigham et al., 2016, unpublished data).

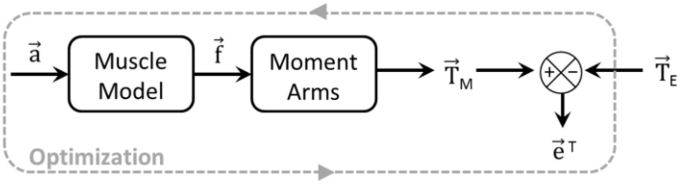

Figure 37: Schematic of EMG-driven strength tuning framework where $\vec{\boldsymbol{a}}$, $\vec{\boldsymbol{f}}$, $\vec{\boldsymbol{T}}_{\boldsymbol{M}}$, $\vec{\boldsymbol{e}}^{\boldsymbol{T}}$, and $\vec{\boldsymbol{T}}_{\boldsymbol{E}}$ represent activation, muscle force, muscle torque, torque error and experimentally measured isometric torque respectively (Figure adapted from Bigham (2016), page 111).

Model description

Model input was peak torque, normalised to body mass (Nm/kg), and experimental EMG at time of peak torque, normalised to MVIC value (*EMG/EMG$_{max}$*). Description of signal processing is provided in Study 3 (page 89).

OpenSim Gait-2354 model was used to extract moment arms of the muscles for which EMG was recorded. Moment arms around the hip, knee, and ankle sagittal plane rotations were computed by setting the hip, knee, and ankle joint angles to 90° flexion, 30° flexion, and 10° plantar flexion, respectively, corresponding to the experimental MVIC test positions.

An optimization framework (Figure 37) was used to scale maximum isometric force parameter ($F_0^{\max}$) muscles so that the error between experimental knee extension torque (T_E) and a simulated torque (T_M) was minimized. Lower and upper bounds for the scale parameters were set to 0.2 and 5.0, respectively. Initial scale values were set to unity, implying $F_0^{\max}$ equalled those of Delp et al. (1990). This simulation was performed in a *Matlab* application (2013a, Mathworks, Natick, MA) where a combination of a sequential quadratic programming (SQP), and a direct search (DS) was used to assure convergence (Sharif Shourijeh and McPhee, 2013, 2014).

T_M was computed using an EMG driven musculoskeletal model with an ideal force actuator (i.e. each muscle force is calculated as normalized EMG multiplied by the scaled $F_0^{\max}$). The model joint torque (T_M) was then computed as:

$$T_M = \sum_{i=1}^{7} s_i F_{0,i}^{\max} r_i a_i$$

where *s*, *r*, and *a* designate the scale parameter, moment arm, and normalized EMG of the muscle during the isometric trial; *i* shows the muscle index, which is from 1 to 7.

The objective function used for the optimization was defined as:

$$J = \mu \sum_{h=1}^{2} \left(\frac{T_{M,h} - T_{B,h}}{T_{B,h}} \right)^2 + (1-\mu) \sum_{i=1}^{7} (s_i - 1)^2$$

where the first and the second term account for the torque error and the muscle redundancy solution; μ is a weight factor that was set to 0.9; and index *h* is the task index.

Once convergence was met, the optimal scaling parameters were used to compute a theoretically ideal torque (T_I), which represented the muscles' full capacity of activation. T_I had all agonist muscles set at 100% of their potential muscle force with antagonist muscles producing no opposing force, i.e. $T_I^E = \sum_{j=1}^{3} \hat{s}_j F_{0,j}^{\max} r_j$ for knee extensors (RF, VL, and VM) and $T_I^F = \sum_{k=1}^{4} \hat{s}_k F_{0,k}^{\max} r_k$ for knee flexors (BF, ST, LG, and MG).

The strength ratio α_S was calculated for each participant to determine knee extensor and knee flexor inhibition (lower value represents greater level of inhibition). It was defined as:

$$\alpha_s^E = \frac{T_B^E}{T_I^E}, \text{ and } \alpha_s^F = \frac{T_B^F}{T_I^F}$$

where superscripts *E* and *F* refer to extensor and flexor-related quantities.

Individual muscular contributions to T_E were also computed. Torque contribution rations for RF, VL, and VM to peak knee extensor torque was calculated by:

$$C_j^E = \frac{s_j F_{0,j}^{\max} r_j a_j}{\sum_{j=1}^{3} s_j F_{0,j}^{\max} r_j a_j}$$

and BF, ST, LG, and MG's contribution to peak knee flexor torque were calculated as:

$$C_k^F = \frac{s_k F_{0,k}^{\max} r_k a_k}{\sum_{k=1}^{4} s_k F_{0,k}^{\max} r_k a_k}.$$

where C is for contribution; indices $j \in \{1,2,3\}$ and $k \in \{1,2,3,4\}$ refer to extensor and flexor muscles, respectively; and superscripts *E* and *F* represent Extensor and Flexor, respectively. Note that the contributions for each group will sum to 1.0.

References

Bigham H (2016) Sex Differences in Lower Limb Muscle Activation Patterns in Participants with Knee Osteoarthritis and Healthy Controls. MSc Thesis. University of Ottawa.

Delp SL, Loan JP, Hoy MG, Zajac FE, Topp EL, Rosen JM (1990) An interactive graphics-based model of the lower extremity to study orthopaedic surgical procedures. IEEE Trans Biomed Eng 37:757–767.

Sharif Shourijeh M, McPhee J (2013) Optimal Control and Forward Dynamics of Human Periodic Motions Using Fourier Series for Muscle Excitation Patterns. J Comput Nonlinear Dyn 9:021005–021005.

Sharif Shourijeh M, McPhee J (2014) Forward Dynamic Optimization of Human Gait Simulations: A Global Parameterization Approach. J Comput Nonlinear Dyn 9:031018–031018.

Appendix D: Knee Muscle Strength Deficits not Prevalent in an ACL-Deficient Population (abstract)

Teresa E. Flaxman, Kenneth B. Smale, Tine Alkjær, Mohammad S. Shourijeh, Michael R. Krogsgaard, Daniel L. Benoit

Introduction

Rupture of the anterior cruciate ligament (ACL) is the most common traumatic knee injury in active adults, causing a loss of mechanical knee joint stability and reduced functional ability (Frobell et al., 2013). Surface electromyography (EMG) is typically used to elucidate the effect of ACL injury on neuromuscular control. To facilitate comparison between individuals, EMG must be normalized to a known value, the most common being a maximum voluntary isometric contraction (MVIC) value. However, following an ACL injury, voluntary quadriceps inhibition (Palmieri-Smith et al., 2008) compromises one's ability to achieve a true MVIC value and changes in muscular composition (Williams et al., 2005) significantly affects the EMG signal (Farina et al., 2004) Consequently, experimental activation may be overestimated. The purpose of this study was to determine the knee flexor and extensor strength deficits and individual muscular contributions to peak torques in young adults with and without ACL deficiency.

Methods

Twenty-two ACL deficient adults (11 males, 11 females) and 22 matched controls (CON) completed a series of knee extension and knee flexion MVIC trials using an isokinetic dynamometer. The hip and knee was flexed to 90° and 30°, respectively. Data from 14 ACL and 17 CON has been reported previously (Flaxman et al., Study 2). Surface EMG and peak experimental torque (T_E) was used in a simple musculoskeletal model (Bigham, 2016) (Appendix C) to quantify an Ideal Torque (T_I), assuming agonist muscles are fully activated. A torque deficit ratio (T_E/T_I) and individual muscle contributions to peak T_E (C_j) was also computed. Significant between group differences in absolute (N/kg) and normalised (Nm/kg) T_E, EMG at peak T_E, α_s, and C_j were determined with Independent-tests at p<0.05 level.

Results

No significant between group differences in T_E or normalised EMG at peak $T_E$was observed (Figure 38). No significant between group differences in strength ratios were observed (Figure 39A). However, CON demonstrated significantly greater BF mean ±std contribution (CON=0.49±0.07, ACL=0.43±0.08, *p*=0.049) and a trend towards significantly lesser ST contribution (CON=0.19±0.05, ACL=0.22±0.05, *p*=0.091) to peak knee flexion torque compared to ACL (Figure 39B).

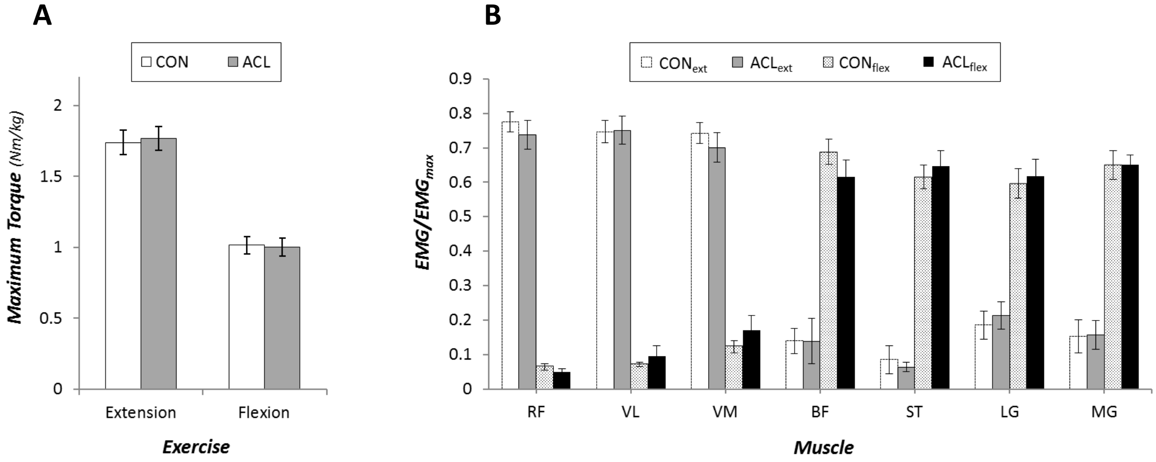

Figure 38: Group mean and standard errors of experimental (**A**) knee extension and flexion peak torques normalised to subject body mass (Nm/kg) and (**B**) normalised EMG amplitudes of muscles at peak knee extension (ext) and flexion (flex) torques.

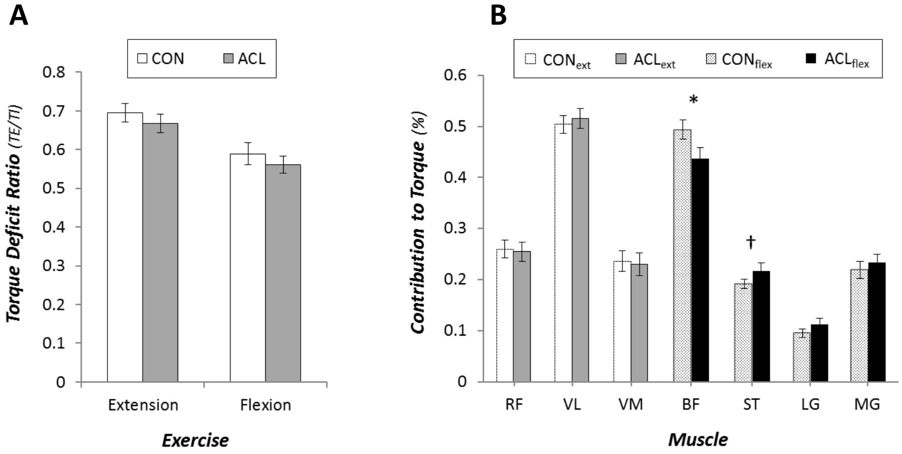

Figure 39: Group mean and standard errors of (A) knee extension and flexion torque deficit ratio and (B) percent contribution of each muscle to experimental knee extension (ext) and flexion (flex) torque. Note that the summed contribution of RF, VL and VM contribution to knee extension torque equals 1.0 and summed contribution of BF, ST, LG, and MG to knee flexion equals zero. Asterisks (*) indicate significant between condition differences at p<0.05 level. Cross (†) indicates a trend towards significance at p<0.10 level.

Discussion/Conclusion

Contrasting previous works (Palmieri-Smith et al., 2008), no significant between group differences in peak extensor or flexor torques, or strength ratios for both exercises were observed. However, an inversed relationship of muscular contribution to peak knee flexion torque was identified: compared to CON, ACL had reduced biceps femoris and increased semitendinosus contribution to knee flexion. Differential hamstring response to knee exercise may be more related to their role in stabilising the knee against tibial rotation (Alkjær et al., 2012; Flaxman et al., 2016). Since rotation is a major component of knee injury mechanism (Yoo et al., 2005), reduced contribution of the BF may be a detrimental adaptation. Furthermore, increased ST contribution to knee flexion is a noteworthy seeing as it is commonly harvested as an ACL a graph.

Results suggest that knee flexion and extension strength tests might not be adequate at identifying deficits in an active ACL deficient population. Since the mechanism of ACL injury and instability is multifactorial and sagittal plane loads alone does not cause injury (Besier et al., 2001; McLean et al., 2004), future works should consider voluntary strength deficits in the frontal and transverse loading planes as well as changes in contribution to torque within functional muscle groups.

References

Alkjær T, Simonsen EB, Magnusson SP, Dyhre-Poulsen P, Aagaard P (2012) Antagonist muscle moment is increased in ACL deficient subjects during maximal dynamic knee extension. The Knee 19:633–639.

Besier TF, Lloyd DG, Cochrane JL, Ackland TR (2001) External loading of the knee joint during running and cutting manoeuvres. Med Sci Sports Exerc 33:1168–1175.

Bigham H (2016) Sex Differences in Lower Limb Muscle Activation Patterns in Participants with Knee Osteoarthritis and Healthy Controls. uOttawa Msc Thesis: 110-112.

Farina D, Merletti R, Enoka RM (2004) The extraction of neural strategies from the surface EMG. J Appl Physiol 96:1489–1495.

Flaxman TE, Alkjaer T, Simonsen EB, Krosgaard MR, Benoit DL (Stud 1; 2016) Predicting the functional roles of knee joint muscles from internal joint moments. Med Sci Sports Exer. 49:527-537.

Frobell RB, Roos HP, Roos EM, Roemer FW, Ranstam J, Lohmander LS (2013) Treatment for acute anterior cruciate ligament tear: Five year outcome of randomised trial. BMJ Online 346:f323.

McLean SG, Huang X, Su A, Van DB (2004) Sagittal plane biomechanics cannot injure the ACL during sidestep cutting. Clin Biomech 19:828–838.

Palmieri-Smith RM, Thomas AC, Wojtys EM (2008) Maximizing Quadriceps Strength After ACL Reconstruction. Clin Sports Med 27:405–424.

Williams GN, Snyder-Mackler L, Barrance PJ, Buchanan TS (2005) Quadriceps femoris muscle morphology and function after ACL injury: a differential response in copers versus non-copers. J Biomech 38:685–693.

Yoo JD, Papannagari R, Park SE, DeFrate LE, Gill TJ, Li G (2005) The effect of anterior cruciate ligament reconstruction on knee joint kinematics under simulated muscle loads. Am J Sports Med 33:240–246.

Appendix E: Ethics Approval Notices

Data collection 1: "Knee joint stability and neuromuscular control of the healthy and injured knee"
File Number: H06-14-27

Data collection 2: "Neuromuscular adaptations to experimentally reduced quadriceps muscle function"
File Number: H06-14-25

File Number: H06-14-27

Date (mm/dd/yyyy): 12/12/2014

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

Ethics Approval Notice

Health Sciences and Science REB

Principal Investigator / Supervisor / Co-investigator(s) / Student(s)

First Name	**Last Name**	**Affiliation**	**Role**
Daniel	Benoit	Health Sciences / Physiotherapy	Principal Investigator
Tine	Alkjaer Eriksen	Others / Others	Co-investigator
Erik	Bruun Simonsen	Others / Others	Co-investigator
Teresa	Flaxman	Health Sciences / Others	Co-investigator
Michael	Rindom Krogsgaard	Others / Others	Co-investigator
Brent	Smale	Health Sciences / Human Kinetics	Co-investigator

File Number: H06-14-27

Type of Project: Professor

Title: Knee joint stability and neuromuscular control of the healthy and injured knee

Approval Date (mm/dd/yyyy)	**Expiry Date (mm/dd/yyyy)**	**Approval Type**
12/12/2014	12/11/2014	Ia

(Ia: Approval, Ib: Approval for initial stage only)

Special Conditions / Comments:

This project is taking place in Denmark, is lead by a Danish researcher and received ethics approval from the University of Copenhagen in September 2013. Due to confusion about process, Professor Benoit who was on sabbatical in Denmark, submitted the project for review in summer 2014 instead of submitting it immediately following the Danish approval. The University of Ottawa REB accepts that this was an honest error and following ethics review of the project has agreed to approve the submission.

File Number: H06-14-25 **Date (mm/dd/yyyy):** 12/12/2014

Université d'Ottawa University of Ottawa
Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

Ethics Approval Notice

Health Sciences and Science REB

Principal Investigator / Supervisor / Co-investigator(s) / Student(s)

First Name	Last Name	Affiliation	Role
Daniel	Benoit	Health Sciences / Physiotherapy	Principal Investigator
Teresa	Flaxman	Health Sciences / Others	Co-investigator
Brent	Smale	Health Sciences / Human Kinetics	Co-investigator
Tine	Alkjaer Eriksen	Others / Others	Project Coordinator
Erik	Bruun Simonsen	Others / Others	Project Coordinator
Michael	Rindom Krogsgaard	Others / Others	Project Coordinator

File Number: H06-14-25

Type of Project: Professor

Title: Neuromuscular adaptations to experimentally reduced quadriceps muscle function

Approval Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
12/12/2014	12/11/2015	Ia

(Ia: Approval, Ib: Approval for initial stage only)

Special Conditions / Comments:

This project is taking place in Denmark, is lead by a Danish researcher and received ethics approval from the University of Copenhagen in March 2014. Due to confusion about process, Professor Benoit who was on sabbatical in Denmark, submitted the project for review in summer 2014 instead of submitting it immediately following the Danish approval. The University of Ottawa REB accepts that this was an honest error and following ethics review of the project has agreed to approve the submission.