KNEE MUSCLE ACTIVATION CHARACTERISTICS DURING CLOSED KINETIC CHAIN DIRECTIONAL LOADING IN HEALTHY YOUNG MALES AND FEMALES

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NOTE:

This thesis is assembled in article format with two independent articles. The beginning consists of the general introduction, review of literature and methodology. The two independent articles entitled: *Joint Stabilisers or Moment Generators: the Role of Knee Joint Muscles during Closed Kinetic Chain Loading* and *Muscular Cocontraction about the Knee Joint in Healthy Young Adults during Directional Closed Kinetic Chain Loading* then follow this first section. These two articles are intended to be submitted for publication.

At the end of this document is a general discussion and conclusion section which briefly restates the results and discussion of the two articles and mentions any findings, limitations and suggestions for future research not mentioned in the articles.

The experimental protocol reported later in this document is part of a larger research program. One of the long term goals of the program is to compare the neuromuscular control strategies of healthy young and older males and females, followed by a comparison to that of age-matched osteoarthritis (OA) patients so that neuromuscular discrepancies between a healthy aging population and an OA population can be identified. Based on research discussed later in this document, it is suggested that older adults and individuals with OA have different neuromuscular control patterns compared to their young healthy counterparts, leading to the abnormal movement strategies in order to minimise risk of exposing the knee to destabilising forces. As a result, varied loads are applied to the knee joint's supporting structures which have been experimentally linked to the development and progression of OA. Thus, if deficits are determined, the development of an evidence-based training protocol will be utilised to improve neuromuscular control and increase knee joint stability for patients with OA as well as older adults who face a greater risk of developing OA. Therefore, it would be beneficial to understand the so-called normal neural abilities of young healthy individuals in hopes that neural abnormalities in an ageing population as well as an OA population can be identified. The purpose of this study was to take the first step in this program and investigate the neuromuscular activation strategies used by young healthy adult males and females to control and stabilise the knee joint.

Although this study's participant population only pertained to younger adults, a discussion on neuromuscular activation as is relates to older adults and those with joint degeneration and OA is provided in the review of literature as background information to demonstrate how this first step relates to the research program's long term goals and our main variables of interest.

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List of Acronyms

3D	Three-dimensional
Φ	Mean direction of muscle activation
ACL	Anterior cruciate ligament
ANOVA	Analysis of variance
BF	Biceps femoris
BMI	Body mass index
CI	Cocontraction index
CKC	Closed kinetic chain
EMD	Electromechanical delay
EMG	Electromyography
EMGi	Electromyography vector
$\mathbf{EMG_{Max}}$	Maximum electromyography magnitude
FE	Flexion-extension
GASTROCS	Summed gastrocnemius muscle activation
HAMS	Summed hamstrings muscle activation
LG	Lateral gastrocnemius
M	Mean
MG	Medial gastrocnemius
MU	Motor unit
MVIC	Maximum voluntary isometric contraction
OA	Osteoarthritis
OKC	Open kinetic chain
UOMAM	University of Ottawa motion analysis model
MANOVA	Multiple analysis of variance
Q :H	Quadriceps to hamstrings coactivation ratio
QUADS	Summed quadriceps muscle activation
RF	Rectus femoris
RL	Resultant load
SD	Standard deviation
SENIAM	Surface Electromyography for the Non-Invasive Assessment of Muscles
SI	Specificity index
ST	Semitendinosus
TFL	Tensor fascia lata
VL	Vastus lateralis
VM	Vastus medialis
VV	Varus-valgus
$\mathbf{X_{EMG}}$	Mean magnitude of muscle activation

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List of Operational Definitions

Active knee joint stabilisation - the application of tensial forces transmitted from the contractions of muscles crossing the knee joint, thus, compressing the joint.

Age-related remodelling theory - the theory that as an individual ages, an extensive amount of motor unit remodelling occurs whereby muscle fibres (particularly fast-twitch) become deinnervated by its neuron and either atrophy or be reinnervated by the sprouting of an adjacent neuron.

Biomechanical contributions to force production - the mechanical factors that dictate the physiological capabilities of force output such as the force-sarcomere length relationship, contraction type (eccentric versus concentric), soft tissue stiffness, contraction velocity, loading direction, loading velocity, and loading magnitude.

Biomechanical and neuromuscular uncoupling - the difficult mathematical process of separating the biomechanical and neuromuscular contributions to force production. The kinematics of joint position, movement velocity and inter-segmental relationships modulate the kinetic output such that any force generation that is not accounted for by the kinematic properties can be attributed to neuromuscular contribution to force output.

Closed kinetic chain - an isotonic exercise in which the distal segment is fixed to a surface, as in the case of a foot on the floor. An example for the quadriceps would be a simple squat extension.

Knee joint stability - (synonym: joint stiffness; antonym: joint laxity) - the biomechanical parameter(s) that regulate safe deformation of the soft tissues in response to externally applied loads. An unstable joint is one that exposes soft tissues to forces/deformations exceeding the tissue’s tolerance threshold or physiological limits.

Neuromuscular contributions to force production - the neurological factors that dictate muscle activation properties through the synergistic relationship of intentional (preparatory) and reactive (reflexive) responses. These factors include active muscle stiffness, reflexive muscular activation (latency and electromechanical delay), muscle recruitment patterns and antagonist coactivation.

Neuromuscular function/control - the ability to generate force such that a moment is applied about a joint's axis of rotation for the purpose of smooth, coordinated movement through the synergistic relationship of the skeletal muscle and neurological systems.

Open kinetic chain - an isotonic exercise in which the distal segment is unfixed to a surface and the joint are uncompressed for body weight. An example for the quadriceps would be a knee extension on a dynamometer.

Passive knee joint stabilisation - the contributions of the orientation and mechanical properties of the knee joint's soft tissues (ligaments, capsule, musculotendons, meniscii) to guide safe motion during passive loading conditions.

General Abstract

Neuromuscular control is believed to play an essential role during dynamic knee joint stabilisation. Evaluation of voluntary muscle action can be delineated as support strategies against external loading moments (Lloyd & Buchanan, 2001). The aim of this study was to determine if males and females exhibit differences in knee muscle action and cocontraction during voluntary isometric closed kinetic chain force generation in various directions in the horizontal plane representative of applied loads transverse to the long axis of the shank. Twenty-six healthy young adults (13 male, 13 female) stood with their dominant leg in a boot fixed to a force platform. A force target matching protocol required subjects to position a cursor (projected on a video screen) over a target and maintain the position for one second. To control the cursor, loads were applied against the force platform with their dominant leg to produce various combinations of anterior-posterior, medial-lateral loads while maintaining constant inferior-superior loads. A successful target match required a normalised force magnitude of equal effort for each subject and target location which triggered the recording of electromyography (EMG) for eight muscles crossing the knee joint. EMG was normalised to percent maximum voluntary isometric contraction. A mean magnitude of muscle activation, mean direction of muscle activation and a muscle specificity index was determined using EMG vectors. In addition, cocontraction indices were also computed for antagonist muscle pairs. Based on similar previous research, it was hypothesised that females would have greater quadriceps and hamstrings coactivation, greater muscle activation magnitudes, lower specificity for the quadriceps than males and no difference in hamstring characteristics. In our study, females significantly cocontracted their vastus lateralis and lateral gastrocnemius muscles to a greater degree than males ($p=0.001$). No significant differences were observed across sexes for the cocontraction of quadriceps and hamstrings or the lateral quadriceps and gastrocnemius muscles. Females displayed significantly lower specificity than males in their semitendinosus ($p=0.025$) and tensor fascia lata ($p=0.012$) activity patterns, greater magnitude of muscle activation in their lateral gastrocnemius ($p=0.002$) and tensor fascia lata ($p<0.003$) and no statistical difference in the other muscles. Furthermore, the activation patterns in our study grossly differed from previous open kinetic chain force target matching. These findings indicate that healthy young males and females have differences in their knee muscle control strategies and that knee muscle recruitment patterns differ during weight bearing and non-weight bearing tasks.

Introduction

Osteoarthritis (OA) is the most common chronic condition among older adults. The degeneration of the joint's cartilage leads to pain, swelling, and limited range of motion. In doing so, physical activity and functional movement are reduced (Health Canada, 2008). A majority of Canadians will be affected by age 70 and although OA commonly affects older adults, it is predicted that the number of 45-54 year olds diagnosed with OA will double between 1991 and 2031 (Badley, 2005).

There are many factors that have been postulated to increase one's risk for developing OA including previous joint injury, excessive joint loading through highly repetitive tasks, and obesity. In addition, age-related changes and sex-related variations in bodily function, particularly neuromuscular control, have also been theorised to increase the risk for developing OA (Health Canada, 2008; Rudolph et al., 2007; Srikanth et al., 2005). While age and sex do not directly cause OA, the condition affects a substantial number of elderly adults with increased rates in females (Srikanth et al., 2005).

A study by Rudolph et al. (2007) examined knee laxity, muscular strength and walking kinematics in young, middle aged and older healthy adults and compared these results to individuals with OA. The findings indicated that both healthy older adults and adults with OA had a loss of voluntary muscle force production compared to healthy young and middle aged adults. Interestingly though, individuals with OA possessed a significant reduction in joint stability compared to the healthy older adults. The study also showed that OA subjects, like older adults, had a reduction in knee motion and increased simultaneous activation of the hamstring and quadriceps muscles but to a greater degree during functional movement. The reduced knee motion and increased hamstring-quadriceps cocontraction was believed by Rudolph et al. (2007) to be a "knee stiffening strategy," adapted by individuals with reduced voluntary muscle control in order to maintain safe load distributions across the knee joint's soft tissues. Since individuals with OA demonstrated similar but amplified movement to their healthy age-matched counterparts, Rudolph et al. (2007) believe that the progression of the "knee stiffening strategy" 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.

In addition, several studies on sex-related differences in neuromuscular control of the knee indicate that females have a decreased ability to stabilise the knee with muscular support (Kanehisa et al., 1994; Wojtys et al., 2002; Wojtys et al., 2003). Specifically, females activate their quadriceps muscles to a greater degree in both static and dynamic tasks and even while acting as an antagonist (Krishnan et al., 2008; Krishnan & Williams, 2009; Pauda et al., 2006; Shultz et al., 2009; Sigward & Powers, 2006; Urabe et al., 2005; Youdas et al., 2007). In turn, cocontraction of the quadriceps and hamstrings is also present to a greater degree for females in dynamic tasks that induce joint instability (da Fonseca et al., 2006; Sigward & Powers, 2006). Although cocontraction is deemed to be a knee stabilisation strategy (Baratta et al., 1988; Hirokawa et al., 1991), females have been observed to possess imbalanced cocontraction between the medial and lateral knee joint compartments (Palmieri-Smith et al., 2009) which instead may increase risk for injury (Hirokawa et al., 1991).

When the above observations are taken as a whole, sex-related variations in neuromuscular control may contribute to the observed differences across sex in bone translation, joint laxity, and resistance against external loading (Wojtys et al., 2002; Wojtys et al., 2003). Now consider that females are reported to have significantly greater rates in incidence, prevalence and severity of OA compared to age-matched males (Srikanth et al., 2005). So if Rudolph et al. (2007) are valid in relating knee instability to the presence of OA, then sex-related presence of joint laxity may account for the differing rates in OA between males and females.

Accordingly, the sex-related increase in joint laxity and reduced muscle force production for females has been symptomatically linked to the presence of OA (Miura et al., 2009; Sharma et al., 1999) and in turn, joint laxity has been experimentally linked to the onset of joint degeneration (Herzog & Longino, 2007). For these reasons, joint laxity and altered neuromuscular control (i.e. reduced muscle activation) is thought by Herzog and Longino (2007) to produce more varied and abnormal internal loads that act on the articulating cartilage. Consequently, its mechanical properties change and become more prone to mircodamage.

We therefore believe that the examination of neuromuscular control as it relates to joint stabilisation must be investigated across the sexes since these findings may have the potential to increase our understanding of mechanisms underlying the onset of OA.

Previous research on neuromuscular contribution to joint stabilisation has involved functional task assessments (i.e. walking, descending, ascending and jumping) where electromyography recordings were used to determine a muscle’s role in the given movement (Hortobagyi & DeVita, 2000; Rudolph et al., 2007). However, the study of joint stabilisation during complex dynamic tasks is problematic because separating the biomechanical (joint position, movement velocity, movement acceleration, adjacent joint orientation etc.) and neuromuscular contributions (motor unit recruitment, fibre types, electromechanical delay, intrinsic stiffness properties, twitch duration, afferent feedback, reflex responses etc.) to movement is difficult (Williams et al., 2003). Examination of more “simplified” tasks, such as isometric force production and force control during a force matching protocol maybe more appropriate for evaluating the neuromuscular control strategies on joint stability because the biomechanical influences are minimised. This approach has been widely used to assess the role of muscles in providing joint stability (Buchanan et al., 1986; Krishnan et al., 2008; Lloyd & Buchanan, 1996, 2001; Patten & Kamen, 2000; Williams et al., 2003) and provides control of potentially confounding variables related to movement and muscle activity, such as adjacent joint movement or joint loading and biomechanical factors (i.e. change of moment arm, instantaneous axes of rotation etc.) that have a kinematic contribution to force production.

To date, however, these “simplified” neuromuscular assessments have only been executed in an open kinetic chain condition, whereby the foot is unfixed as well as unloaded from not bearing any weight (Buchanan et al., 1986; Krishnan et al., 2008; Lloyd & Buchanan, 1996, 2001; Patten & Kamen, 2000; Williams et al., 2003). Hence, it is unnecessary to stabilise the knee. Research has shown that muscle recruitment and activation patterns of the knee muscles vary significantly between open and closed kinetic chain (CKC) exercises (Escamilla et al., 1998; Stensdotter et al., 2003). Therefore, we believe that neuromuscular assessment through basic CKC loading of the knee joint will provide more appropriate insight into the neuromuscular contributions to joint stabilisation because any force applied against the ground by the foot must equally be applied back to the foot and will be transmitted to the knee.

Research Objective and Hypotheses

Previous research has established that an altered pattern of neuromuscular control has been experimentally and clinically linked to the development of joint degeneration (Herzog &

Longino, 2007; Slemenda et al., 1997; Slemenda et al., 1998) and there are sex-related differences in OA incidence, prevalence, and severity (Srikanth et al., 2005) as well as neuromuscular control strategies (Krishnan et al., 2008; Krishnan & Williams, 2009; Palmieri-Smith et al., 2009). We believe that further investigation into these sex-related differences of neuromuscular control will have the potential to increase our understanding of mechanisms underlying this common disease.

The purpose of this study was therefore to examine sex-related differences in neuromuscular activity about the healthy knee joint during highly controlled voluntary quasi-isometric contractions (i.e. the subject attempts to maintain a specific joint position however minor changes in joint angle, and thus muscle length, may occur) that generate various combinations of anterior-posterior-medial-lateral ground reaction forces during a CKC target matching protocol.

Based on the observations outlined above, we have postulated the following hypotheses:

1. Females would have significantly different mean direction of muscle action[1], significantly lower quadriceps muscle specificity indices[2], and significantly greater quadriceps mean magnitude of muscle activation[3] compared to males (consult Methods section and Appendix B for further explanations).

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[1] The EMG magnitude at each target location was expressed as a vector. The mean direction of muscle action is computed using the arctan of the all the vector's summed x and y components.

[2] Used to describe how variable the muscle's activation pattern is about its mean direction of muscle action. A lower specificity index indicated that the muscle is active in many directions. A higher specificity index indicates that the muscle was active in one general direction.

[3] The average of all the normalised EMG amplitudes at every target location.

2. No significant difference in hamstring activation patterns (mean direction of muscle action, muscle specificity indices and mean magnitude of muscle activation) across sexes.
3. Females would have significantly greater cocontraction of the hamstring and quadriceps muscles (defined by a cocontraction index) compared to males.

Relevancy

Instability of the knee joint is a common issue associated with older adults, individuals with OA, and females because of the altered muscular forces acting across the joint (Rudolph et al., 2007; Sharma et al., 1999). Identification of neuromuscular variations between young healthy males and females, older healthy males and females, as well as in an OA population may provide valuable insight into knee joint instability. Assessments of a young healthy adult population are however needed to understand how muscles formerly interact in response to varied joint loading conditions. This evaluation of healthy young adult neuromuscular control strategies will act as a baseline for future comparison with healthy older adults and individuals who possess OA. Also, information gained in this study will provide valuable insight for the development of preventative and rehabilitative exercise programs for those at high risk or who possess OA, respectively.

Several studies have examined neuromuscular properties of force production and force control (Hortobagyi et al., 2004; Kamen et al., 1995; Klass et al., 2008; Patten & Kamen, 2000; Zhang & Wang, 2001) and specific knee activation strategies during highly controlled isometric joint loading achieved through force matching (Buchanan et al., 1986; Buchanan & Lloyd, 1997; Krishnan et al., 2008; Krishnan & Williams, 2009; Lloyd & Buchanan, 1996, 2001; Williams et al., 2003). Yet, no study, to our knowledge, has investigated sex-related differences in neuromuscular contribution to joint stability during CKC force matching tasks where the joint is loaded from bearing weight plus the force transmitted from muscular contractions. Therefore, the use of a CKC isometric force production protocol is thought to provide more suitable evidence of neuromuscular strategies that contribute to healthy joint stabilisation.

There are many factors that regulate muscular responses to externally applied loads, such as rate of loading, preparatory response, reflexes etc., and the development of a valid and novel approach to studying functional neuromuscular control without the confounding effects of biomechanical factors is a challenge task in itself. In this study, a static approach was utilised to provide “stepping stone” for future research. Since it is believed that the muscles crossing the knee joint must active to stabilise the joint when an externally applied ground reaction force is applied (even if it is a direct result of a voluntary force), then the production of a known force during a static task can perhaps be representative of an “instant in time” and the biomechanical factors do not modulate the neuromuscular output. We are aware that a static task has minimal applicability to the functional activities because most involve the concentric and eccentric muscle actions, but further investigation into dynamic movement that generates similar ground reaction forces (i.e. forward, backward, left and right single-legged hopping) is currently being conducted. With this information, we hope to compare the dynamic and static results in hopes that the issue of external validity can be addressed appropriately.

Review of Literature

Onset of Osteoarthritis and Joint Loading

Previous research has suggested that joint degeneration is induced by forces surpassing the tolerance threshold of cartilage and other joint tissues (Maxian et al., 1995). This would occur when a single high load is transmitted to the cartilage, resulting in macrotrauma, or when lower loads are applied in a repetitive manner for prolonged periods of time, resulting in microtrauma (Maxian et al., 1995). In the healthy joint, there is continuous joint tissue remodelling such that the rate of healing exceeds the rate of injury - preventing damage progression. But in certain individuals, the rate of trauma eventually becomes unmanageable and small tissue tears progress to tissue fibrillation, deteriorating until complete degradation of the structure results (Hedlund et al., 1993). Why some people develop degenerative diseases and others do not for a given health status, physical activity level and general lifestyle, still remains unknown.

Over several years, Herzog, Clark, Suter, Wu, Hasler and colleagues (2002; 2005; 2006; 2004; 1998; 1998; 1993; 1998; 1998; 2000) have observed that joint degeneration may not be the result of general overloading of the joint but rather an alteration in load distribution across the joint. Meaning, areas that were once heavily loaded have reduced loads or no loads acting on them, and other areas that were once unloaded or lightly loaded are now overloaded. In a general sense, the joint as a whole may experience the same or even a reduced magnitude of loading but in terms of specific locations, there is an increased risk of surpassing the cartilage's tissue tolerance. This change in internal load distribution across the joint's supporting structures has been demonstrated in experimental osteoarthritis (OA) animal models where intra-articular derangements, such as anterior cruciate ligament (ACL) transections (Hasler et al., 1998; Herzog et al., 1993; Herzog et al., 1998), meniscectomies (Kamekura et al., 2005), or repetitive impulse loading (Radin et al., 1984) consistently display signs of joint degeneration post-intervention.

Load Related Changes in Articular Cartilage

Hasler and Herzog (1998) first induced the development of joint degeneration in cats via the intra-articular derangement of an ACL transection. Immediately, a variation in joint loading was observed. Patellofemoral contact forces decreased 30% which was accompanied by a 70% deficit in extensor muscle forces. In another study, Herzog's team demonstrated that although joint contact forces immediately decreased after the ACL transection, the average joint contact pressure remained relatively constant (Clark et al., 2002). This pressure regulation is a function of force over area such that area fluctuates in order to attenuate the changing forces. However, longer term observations (16 weeks post-intervention) on the experimental OA models demonstrated a 55% decrease in peak contact pressure, accompanied by a 22% increase in contact area for a given load (Herzog et al., 1998).

The increased contact area and decreased pressures four months following ACL transection can be defined by two modifications of cartilage properties. First, a mean increase in articular cartilage thickness from 48% to 102% across experimental OA joints (Herzog et al., 1998), allows for more vertical deformation, accounting for a "wrapping" effect, thus increasing contact area across articulating surfaces. The mechanisms theorised to regulate cartilage synthesis in response to altered loading is discussed later. Second, altered cartilage mechanics has been displayed in animal OA models where cartilage stiffness decreased and cartilage permeability increased compared to control. The stiffness properties have been attributed to collagen and chondrocyte composition and orientation. Healthy loaded cartilage characterises chondrocytes as ellipsoidal in shape with their symmetry axes, volume, and concentration varying as a function of depth: columnar in the deep zone, round in the middle zone, and flattened in the superficial zone. Also, collagen fibres are orthogonal to the tidemark in the deep zone, randomly oriented in the middle zone, and parallel to the surface in the superficial zone (Hedlund et al., 1993).

Inspection of loaded experimental OA models shows that the thickened cartilage contained larger chondrocytes and increased chondrocyte concentration in the middle and deep layers while smaller chondrocytes and lesser concentration was seen in the superficial layer compared to the contralateral cartilage (Clark et al., 2006). In addition, uneven proteoglycan staining (indicating uneven proteoglycan distribution) was observed throughout cartilage depth (Clark et al., 2005). Accordingly, greater vertical cartilage deformation was demonstrated in the OA modelled side (Clark et al., 2006). Thus, differences in morphological characteristics have been proposed as one factor influencing the stiffness properties of cartilage in this model (Clark et al., 2006).

The observed discrepancies in structural composition and orientation are also suggested to compromise the mechanical integrity of the articulating surfaces. Wu et al. (2000) developed a numerical simulation model which included the combined effect of reduced shear modulus (i.e. strength), increased permeability, and increased thickness of the cartilage representing osteoarthritic characteristics. Results showed a 59% increase in contact radius and 38% decrease in maximal shear stress compared to normal joint simulations. Consequently, areas of normal joint contact became unloaded and areas of little or no previous contact became overloaded in the simulated early stages of OA. Therefore, in combination with decreased shear strength, the risk for microtrauma could be increased at these newly loaded areas (Wu et al., 2000).

Wu et al. (2000) also indicated that the peak shear stress during solid phase (all fluid excluded from the tissue) was maximised at the cartilage-bone interface (tidemark) for all combinations of material properties (increased thickness, reduced shear modulus, and increased permeability) even though the overall maximal shear stresses were reduced. The findings from Wu et al. (2000) suggest that damage is more likely to occur at the cartilage-bone interface. In support, Roberts et al.’s (2003) experimental rat OA models demonstrated one week post-intervention (medial collateral ligament transection and meniscal rupture) an increase in cartilage thickness and surface fibrillations which interestingly, was accompanied by attenuation of the demarcation of the cartilage-bone interface. After three weeks, areas of the articulating cartilage was thin, had severe surface fibrillations and the cartilage-bone interface was significantly altered compared to the contralateral undisrupted leg. Roberts et al. (2003) state that the most important change in the cartilage properties was the loss of the cartilage-bone interface and this may be the initiating action of OA.

Overall, a change in cartilage properties is viewed as a response to altered joint loading. Despite the general reduction of mean and peak contract pressures, the decrease in articulating cartilage stiffness ends up dissipating a given force across a greater area (Wu et al., 2000). Due to the increase in contact area, regions become irregularly loaded (Hasler & Herzog, 1998) which may cause the onset and progression of degradation in the joint.

Chondrocyte Response to Loading

It has been shown that chondrocytes undergo deformation under compressive forces (Guilak, 1995; Guilak et al., 1995) and cellular deformations provide biomechanical stimulus that can alter biological responses (O'Hara et al., 1990). Thus a direct mechanical stimulus to the chondrocyte nucleus is signified when compression is applied to the cell resulting in decreased height and shape (Guilak, 1995). However, the direct intrinsic response to mechanical stimuli in chondrocytes is not understood.

Loaded cartilage in experimental OA models 16 weeks post-ACL transection showed similar height and volume compared to unloaded conditions, but the *unloaded* chondrocytes were more round in the superficial zone compared to control (Clark et al., 2006). Furthermore, the larger, more ellipsoidal chondrocytes from the deep layer of cartilage has been shown to synthesise more aggregating proteoglycans compared to smaller and rounder superficial chondrocytes. Supportingly, Herzog et al. (1993) revealed elevated levels of total hexuronic acid levels in experimental OA models, indicating increased proteoglycan synthesis. Hence, greater superficial cartilage cell deformation and the larger deep chondrocytes are proposed to be exposed to atypical mechanical stimuli effecting anabolic sensitivity. This may account for the increased cartilage thickness and abnormal cartilage composition observed by Clark et al. (2006).

Moreover, increased mRNA level of metalloproteinase-3 (an enzyme associated with the breakdown of collagen and extracellular matrix proteins such as proteoglycans) has also been observed during excessive extensor stimulation (Clark et al., 2004), further indicating that a change in internal joint loading conditions dictates the mechanical stimuli acting on the chondrocyte and alters the cellular intrinsic response.

Theoretically, mechanical loading is important for regulating the metabolic activity of chondrocytes and in the maintenance of healthy cartilage (O'Hara et al., 1990). However, altering contact forces between joint surfaces is speculated to change physiological responses that compromise cartilage integrity (Figure 1).

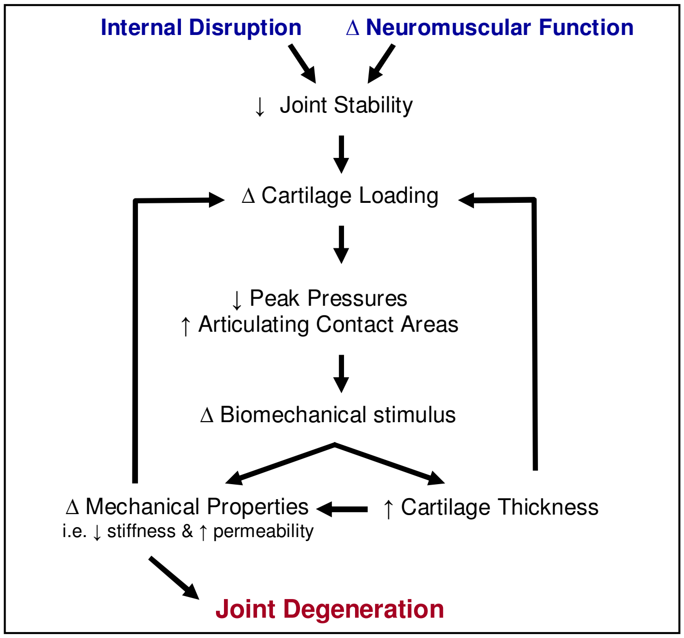

Figure 1: Chart of Herzog and colleagues` theorised paradigm for the onset of joint degeneration (Herzog, Clark, Suter, Wu, Hasler and colleagues (2002; 2005; 2006; 2004; 1998; 1998; 1993; 1998; 1998; 2000)).

Onset of Osteoarthritis and Altered Neuromuscular Function

In many cases of OA, a traumatic injury, such as an ACL rupture, is not a contributor to the presence of joint degeneration (Slemenda et al., 1997). Based on the paradigm above, a different mechanism leading to the varied joint loading must therefore be present to elicit cartilage degradation.

Previous experimental models of OA utilised the disruption of passive intra-articular structures such as ligaments, menisci, cartilage, and capsule as the intervention variable. However, the contribution of muscle action – the only dynamic and perhaps the greatest contributor to the mechanical loading of joints – has only recently been viewed as a potential risk factor for the development of OA (Fitzgerald et al., 2004; Herzog & Longino, 2007; Hortobagyi et al., 2004; Hortobagyi et al., 2005; Lewek et al., 2005; Lewek et al., 2004).

In the past, decreases in muscle mass, muscle strength, and changes in muscular activation patterns were seen as a secondary effect of OA rather than an inductive factor of the disease (Hasler & Herzog, 1998; Herzog et al., 1993). But clinical evidence (Slemenda et al., 1997) suggests that muscular weakness may be a determinant for the onset of joint degeneration. Ling et al. (2007) even stated that muscle weakness is one of the earliest symptoms in patients with OA. So if muscle weakness is a contributing factor instead of an adverse effect of OA, then it may be possible that changes in the neuromuscular system can modify the dynamic forces transmitted over the joint from muscle action and cause atypical internal (soft tissue and bone) loading conditions.

In order to examine the effects of neuromuscular control on the development and progression of OA, experimental animal muscle weakness models were developed by Herzog and Longino (2007). Muscle weakness was induced by targeted injections of Botulinum type-A toxin (BTX-A) (a neurotransmitter inhibiter at the neuromuscular junction) into the quadriceps of rabbits. This caused a substantial decrease in muscle strength but the knee functionality was maintained to enable the rabbits to continue with everyday activities such as walking, thus mimicking a clinical scenario of muscle weakness. As a result, Herzog and Longino (2007) found a significantly greater reduction in muscle mass after BTX-A injection compared to ACL transection models and in addition, two of five animals had a reddening of the articular joint margin on the tibial plateau only one month post- BTX-A injection.

Although Herzog and Longino (2007) interpreted their results as early signs of cartilage deterioration, no histological assessment of the joint surfaces or molecular biology of the cartilage was performed. Hence, Youssef et al. (2009) adapted the muscle weakness animal model and Mankin graded[4] the degenerative results of articulating surface areas based on cartilage structure, number and type of cells, matrix staining, and tidemark integrity of the

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[4] A system that grades OA according to “severity” with grades ranging between zero (normal/healthy) and 14 (severe OA) (Mankin et al., 1971).

most severe lesion present. Similar to Herzog and Longino (2007)'s findings, the experimental models showed significant muscle mass and muscle strength deficits but observations were accompanied by significantly greater Mankin scores in experimental groups compared to control, confirming histological evidence of early osteoarthritic characteristics following a muscle weakness intervention.

Age-related Changes in Neuromuscular Function

Some of the most serious consequences of ageing are its detrimental effects on skeletal muscle. Age-related reduction in muscle strength and unsteady force production has been observed in older adults compared to middle aged and younger adults (Rudolph et al., 2007; Smith et al., 1999). The majority of age-related muscle weakness appears to be directly attributed to a reduction in muscle mass, however, older muscle is also "weak" for its size (i.e. reduced force production per cross-sectional area) (Brooks & Faulkner, 1994). Therefore, sarcopenia, the age-related process of progressive muscle fibre atrophy, is characterised not only by the loss of skeletal muscle mass but also the decline in the functional capacity of muscle.

A study by Connelly et al. (1999) examined the effects of ageing on motorneuron firing rates and muscle contractile properties of the tibialis anterior. Older adults exhibited a 20% decrease in maximal muscular strength, 23% longer twitch contraction durations and mean firing rates were 30-35% lower in the old subjects compared to the young subjects.

Connelly et al. (1999) reported that firing rates are matched to twitch contraction durations and the correlation between reduced muscle force production, firing time, and contractile speeds in older adults indirectly supports the neuromuscular age-related remodelling theory. This theory states that age is accompanied by an extensive amount of

MU remodelling, whereby muscle fibres become deinnervated by its neuron and consequently either atrophy or more interestingly, be reinnervated by sprouting of an adjacent neuron. The latter fate enlarges the adjacent neuron's MU innervation ratio (or neuron-to-fibre ratio) (Doherty et al., 1993) and reduce one's ability to "fine tune" the force output. . In addition, age-related MU remodelling has demonstrated a preferential of fast-twitch MUs which are capable of generating the highest force levels (Faulkner et al., 2007; Prakash & Sieck, 1998); thus, one's maximal force output is reduced from the suggested age-related MU remodelling.

The remodelling of the MU and its neuromuscular junction also impairs the neuromuscular transmission (Delbono, 2003) such that an error in force output occurs because recruitment patterns become unfamiliar (Enoka et al., 2003). As a result, the descending motor command becomes ineffective at fully activating the desired muscles of older adults (Lewek et al., 2004).

Altered Neuromuscular Function in Osteoarthritic Individuals

A cross-sectional study by Lewek et al. (2004) used an interpolated-twitch technique to identify activation deficits (Belanger & McComas, 1981) in older adults and those with OA. Not only did subjects with knee OA possess reduced voluntary quadriceps strength compared to uninjured age-matched control subjects, this study also found that only 50% of the OA subjects could fully activate the quadriceps compared to 75% from the control group. In another study, Hortobagyi et al. (2004) used a force matching protocol to assess force production accuracy and steadiness over time. Results showed that OA participants required 67% more time to complete the functional tasks and produced 82% more errors compared to age-matched control.

One possible explanation for the irregular force productions can be illustrated with discrepancies in MU activation between healthy older adults and those with OA. Ling et al. (2007) observed that both early and late OA participants activate a greater number of large MUs compared to control for a given unit of force but early OA participants were shown to activate *fewer* large MUs per unit force compared to those with late OA. This implies that the stage of OA is correlated to the degree of MU remodelling where early OA individuals have richer muscle quality (more varied fibre type composition and larger proportion of fast twitch fibres) compared to those with later stages of OA.

When the above findings are related back to age-related MU remodelling and its impairments to normal motor unit activation, such as ineffectively activating the entire muscle, one can suggest that individuals with OA for some reason undergo age-related physiological changes in neuromuscular function but to a greater extent, thereby reducing the neural ability to produce strong, coordinated movement as compared to older adults without the disease.

Active Knee Joint Stabilisation

The monikers “joint stability” and “joint stabilisation” may have different meanings depending on the context (clinical or mechanical, for example). For the purpose of this discussion knee joint stability (synonym: joint stiffness; antonym: joint laxity) refers to the biomechanical parameter(s) that regulate safe deformation of the soft tissues in response to externally applied loads. An unstable joint is one that exposes soft tissues to forces/deformations exceeding the tissue’s tolerance threshold or physiological limits. Current methods for assessing knee instability as it relates to functional activities are limited in describing the soft tissue deformations. For example, laxity tests expose the knee to forces that do not occur in daily living (i.e. uniaxial load: no compressive forces from muscles or bearing weight); functional assessments describe how segments move relative to one another but interpretation of tissue function is extremely difficult. Ideally, directly measuring the forces acting to displace the tibia from the femur and how it deforms the supporting structures can be preformed in vivo however, this method is ethically challenging. Currently, joint stability can only be inferred based on the indirect measures mentioned as examples and those discussed below.

Typically, external loads applied to the knee are resisted by a combination of passive restraints and active muscular contractions (Hirokawa et al., 1991). The passive structures of the knee (such as bones, ligaments, menisci, cartilage, and capsule) alone only provide sufficient joint stability in modest loading conditions (Williams et al., 2001). The addition of muscular force is therefore needed to regulate safe knee joint movement when exposed to physiological loads such as those incurred through activities of daily living.

As discussed, older adults have experienced alterations in neuromuscular function that affect muscular force production and force control. By deduction, their ability to effectively stabilise the knee joint against a given external load would be reduced. However, older adults instead increase activation magnitudes of the agonist and antagonist muscles during the preparatory and loading phases of movement which reduces their joint angular displacements and the potential for abnormal tibio-femoral excursion compared to young adults (Hortobagyi & DeVita, 2000). Rudolph et al. (2007) did not see these characteristics in older adults but they did acknowledge that individuals with OA utilised a similar “knee stiffening strategy” during gait as the older adults did in Hortobagyi and Devita (2000). Even during simplified perturbations such as induced varus alignments, OA subjects showed greater coactivation of antagonist and agonist muscles prior to and during the external loading (Lewek et al., 2005).

The increase in muscle activation and decrease in knee flexion excursion is indicative of a biomechanical and neuromuscular adaptation to reduced passive and active knee joint stabilisation (Lewek et al., 2005; Rudolph et al., 2007).

Regarding neuromuscular variations as it relates to disease progression, Zeni et al. (2009) evaluated dynamic knee joint stiffness (quantified as the change in joint moment over the change in joint angle) during gait in persons with moderate and severe OA. No matter what speed the subjects walked, those with severe OA had significantly greater knee joint stiffness than moderate OA and control subjects, while interestingly no differences were observed between control and moderate OA groups. Even though this study lacked EMG data, the authors speculated that higher dynamic knee joint stiffness in severe OA groups is attributed to higher muscular resistance to external moments.

Considering the body of literature presented above and building from the aforementioned observations (individuals who develop OA experience greater neuromuscular dysfunction compared to age-matched healthy adults), we speculate that individuals with OA adapt a “knee stiffening strategy” similar to healthy older adults with the purpose of maintaining effective joint stability but to a greater degree. However, because of this population’s increased neuromuscular dysfunction, the stiffening strategy is inefficient and does not lead to increased stability. Instead, it subjects the joint’s soft tissues to adverse loads. These loads then advance or accelerate the degenerative process (Griffin & Guilak, 2005).

Thus far this discussion has demonstrated that: 1) disruption of a passive stabilisation structure (i.e. ACL, medial collateral ligament, and meniscus) alters internal loading conditions and leads to OA; 2) a deficit in neuromuscular function leads to OA; and 3) OA populations have reduced neuromuscular function and joint stability. However, the exact role that neuromuscular function plays in the development and progression of OA remains unknown. A basis for comparison is needed and thus the evaluation of young healthy neuromuscular control as it relates to knee joint stability is necessary to identify control deficits caused by age-related changes in bodily function. Once accomplished, this data can be compared to healthy older adults and an osteoarthritic population.

Sex-Related Differences

Another observation of the OA population is that females exhibit significantly greater rates of knee OA compared to their male counterparts (Srikanth et al., 2005). Females with OA also have reduced ability to develop force in both knee extensor and flexor muscles (Kanehisa et al., 1994; Slemenda et al., 1998). Investigation into healthy control strategies as well as sex-related differences can provide valuable insight into normal/healthy stabilising function. For these reasons, the remainder of this discussion will mainly pertain to young healthy neuromuscular control strategies and differences between sexes as it relates to knee joint stability.

Regulation of knee joint stability with muscular contractions is provided by the synergistic relationship of both intentional (preparatory) and reactive (reflexive) responses that are mediated by feed-forward and feedback mechanisms, respectively. Specific neuromuscular factors that contribute to knee joint stability include active muscle stiffness, reflexive muscular activation (latency and electromechanical delay), muscle recruitment patterns and antagonist coactivation (Williams et al., 2001). Hence, anything that delays or inhibits one or more of these neuromuscular factors may compromise knee stability.

Musculotendinous stiffness, the ratio of change in force to change in length, is essential for maintaining knee joint stability during the electromechanical delay period, defined as the time between the onset of EMG and force production and/or joint motion. A large portion of this time spent removing the inherent “elastic slack” (Cavanagh & Komi, 1979). So if more “slack” exists, i.e. musculotendinous stiffness is reduced, a greater electromechanical delay (EMD) results. With that said, no difference has been observed between sexes in EMD but hamstring musculotendinous stiffness is significantly greater in males compared to females and accordingly, their force production and rate of force production is greater (Blackburn, Bell, Norcross, Hudson, & Engstrom, 2009; Blackburn, Bell, Norcross, Hudson, & Kimsey, 2009; Granata et al., 2002).

Differences in reflexive muscle activations across sex has also been characterised by faster quadriceps response during unexpected perturbations in females compared to males (Carcia et al., 2005), specifically during the long latency reflexive responses (Shultz et al., 2001). Even in preparation for a voluntary perturbation, females activate their knee muscles differently than males. During single limb squats and other various jumping tasks, females demonstrate relatively greater quadriceps activation while men activate their hamstrings to a greater extent (Sigward & Powers, 2006; Urabe et al., 2005; Youdas et al., 2007). Females have also been shown to accompany the greater levels of quadriceps activity with greater levels of hamstrings and gastrocnemius activity during preparatory and loading phases of movement (but the increase is not to the same extent as the quadriceps) (Pauda et al., 2006; Shultz et al., 2009). These studies indicate significant sex-related differences in active stabilisation prior to and during functional tasks. Males perhaps are more effective at coordinating agonist and antagonist activation while females are more quadriceps dominant, thus requiring opposing forces from the hamstrings and gastrocnemius to prevent excessive anterior tibial translation during a given movement.

One possible physiological explanation of why females activate their quadriceps muscles to a greater degree is that they have a greater proportion of slow-twitch (type I) muscle fibres (Simoneau & Bouchard, 1989) and these slow-twitch muscle fibres occupy larger areas in female quadriceps compared to males (Staron et al., 2000). In addition, evidence shows that females recruit a larger number of MUs than males for a given contraction intensity and tend to preferentially recruit more MUs prior to increasing MU firing rates because of more slow-twitch muscle fibres (Cioni et al., 1994). Since muscle fibre composition, distribution and diameter differ between males and females, it is suggested that females have a reduced force production capability and thus require greater number of muscle fibres to achieve a given force output.

Cocontraction, Joint Stabilisation and Pressure Distribution

Antagonist muscle activation exists throughout the entire range of motion, producing a constant opposing torque (Baratta et al., 1988). Evidence from cadaver studies demonstrated that although rotational and linear translations can be reduced in the presence of muscular contractions, the importance of multiple muscle activities spanning the knee should not be undermined. Isolated quadriceps loads induce significant anterior displacement and internal rotation of the tibia but the addition of low-level hamstring loads significantly reduces both translations during knee extension (Hirokawa et al., 1991; MacWilliams et al., 1999).

According to Markolf et al. (1976), joint stability can be increased from the contractions of 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. The simultaneous activation, or cocontraction, of the antagonist and agonist muscles is so effective that in vitro measurements demonstrate an increase in joint stiffness of two- to five-fold and a decrease in joint laxity of 25-50% during voluntary cocontraction (Louie & Mote, 1987; Markolf et al., 1978). Thus, cocontraction is believed to be the most important factor in maintaining joint stability (Baratta et al., 1988).

Cocontraction has also been shown to regulate joint contact pressures. Using instrumented femoral head prosthesis, Hodge et al. (1986) determined intra-articular pressures during functional tasks (level walking, chair raising, and stair climbing). The in vivo peak pressures were considerably greater and more *uniform* than the estimation using external kinematic ground reaction force data and inverse Newtonian analysis. Although this study lacked supporting muscle data, the greater joint pressures experienced during functional movement was thought by Hodge et al. (1986) to be caused by the balanced agonist-antagonist muscle contractile forces acting across the joint.

Similarly, Park et al. (1999) measured intra-articular hip pressure but considered cocontraction to be present only when the intra-articular pressure magnitudes were higher than the estimated pressure magnitudes. Not only were greater pressure magnitudes observed during loading but there was also greater increase in hip loads prior to external loading. Therefore, muscle cocontraction during functional movements not only provides resistance to shear and torsional loads, it has a large contribution to the regulation of internal joint loading conditions.

Sex-related Differences in Cocontraction

As previously mentioned, females have greater simultaneous quadriceps and hamstrings activation compared to males (Pauda et al., 2006; Shultz et al., 2009; Sigward & Powers, 2006; Urabe et al., 2005; Youdas et al., 2007). This indicates greater cocontraction.

Pauda et al. (2006) evaluated sex-related differences in cocontraction, quantified with a quadriceps: hamstrings ratio, prior to, and during, ground contact in hop trials. As theorised, females significantly cocontracted the muscles about the knee more than the males. Consider that Markolf et al. (1995) believe cocontraction of the quadriceps and hamstrings leads to increased joint compression which assists knee stabilisation in all planes; then the findings of Pauda et al. (2006) indicate females to have an increased ability to maintain knee joint stability.

However, recently Palmieri-Smith et al. (2009) investigated quadriceps and hamstrings cocontraction during a functional single-limb hop test. Outcome measures included medial and lateral cocontraction as well as a ratio of medial-to-lateral cocontraction to identify imbalanced cocontraction. Females exhibited greater cocontraction levels for both the medial and lateral sides, yet, were found to have an overall lower medial-to-lateral cocontraction ratio compared to males. This was evidenced by a significant reduction in medial thigh cocontraction compared to the lateral thigh. Thus, the presence of imbalanced cocontraction may compromise the stabilising forces which may in part explain why females demonstrate greater valgus laxity as well as reduced internal and external rotational stiffness (Schmitz et al., 2008; Zhang & Wang, 2001).

In support, Wojtys and colleagues (2002; 2003) speculated that males might be able to use muscular contractions to absorb external loads acting at the joint more effectively than females. They examined the sex differences in passive and active protection of the knee against an externally applied anterior force, normalised to bodyweight, as well as a 80 N impulse force applied to the lateral aspect foot which limited the tibia to rotation motion about its long axis. Subjects were instructed to maximally contract the muscles around the knee while the perturbation was applied to the tibia. As expected, a maximum cocontraction of the knee musculature significantly decreased tibial translation and rotations in both sexes but females exhibited a significantly smaller volitional increase in shear and torsional stiffness than males.

A Reductionist Approach to Evaluation of Neuromuscular Control

Previous studies on neuromuscular function have attempted to determine what, when, to what extent, and why muscles are activated during all sorts of functional activities. These functional task assessments are however challenging to analyse because uncoupling of the biomechanical (joint position, loading rate, loading direction, contraction type (eccentric versus concentric) etc.) and neuromuscular contributions (motor unit type, musculotendinous stiffness, spinal/ intermediate/ voluntary reflex responses, afferent feedback, etc.) to movement is a complex relationship (Williams et al., 2003). Even with more simplified tasks, such as matching a force target during voluntary isometric, eccentric and concentric contractions (Hortobagyi et al., 2004; Hortobagyi et al., 2001; Patten & Kamen, 2000), the knee is a modified hinge joint which possesses three degrees of freedom (flexion/extension, adduction/abduction, internal/external rotation) (Williams et al., 2001) and muscles have been found to have different activation magnitudes depending on the direction of movement (Buchanan & Lloyd, 1997; Krishnan et al., 2008; Lloyd & Buchanan, 1996, 2001; Rudolph et al., 2001; Williams et al., 2003). So in order to better understand how the central nervous system coordinates muscle activations acting across a joint, an even simpler approach, specifically the evaluation of muscle activities over a range of isolated moment directions, must first be considered.

This approach has been applied to evaluate the nature of muscular synergies at the elbow joint during isometric contractions over a range of moment directions (Buchanan et al., 1986). It was then adapted to evaluate muscle activations over a range of isolated moment directions at the knee by Lloyd and Buchanan (1996) and Buchanan and Lloyd (1997): while seated in standardized hip and knee joint positions and the ankle was unfixed (i.e. an open kinetic chain (OKC) condition), subjects were required to match a projected cursor to a target displayed on a video screen in front of them. Cursor movement occurred in response to loads applied by the leg (shank) to a 6-axis force transducer (Figure 2A). The cursor moved such that knee extension moved the cursor up, flexion moved it down, hip abduction and adduction moved it left and right, respectively, and longitudinal forces controlled cursor size (i.e. zoom). The aim was to place the cursor over the target and once a successful match was made, EMG and force data was collected and a new target in an alternate location would appear (Figure 2B). Targets would appear randomly, one-at-a-time, about a circular trajectory in 20º increments where these locations were claimed to represent specific loading directions transverse to the long axis of the shank (Figure 3). In the 1997 study, muscle activity patterns were evaluated in polar coordinates where each muscle's EMG activity was plotted as a function of moment direction (Figure 4). A principal activation vector for each muscle was also determined by associating the direction of muscle activity to its mean magnitude of muscle activity (see Methods section and Appendix B for more details). The studies addressed in the following section utilised this protocol and so only their findings will be discussed.

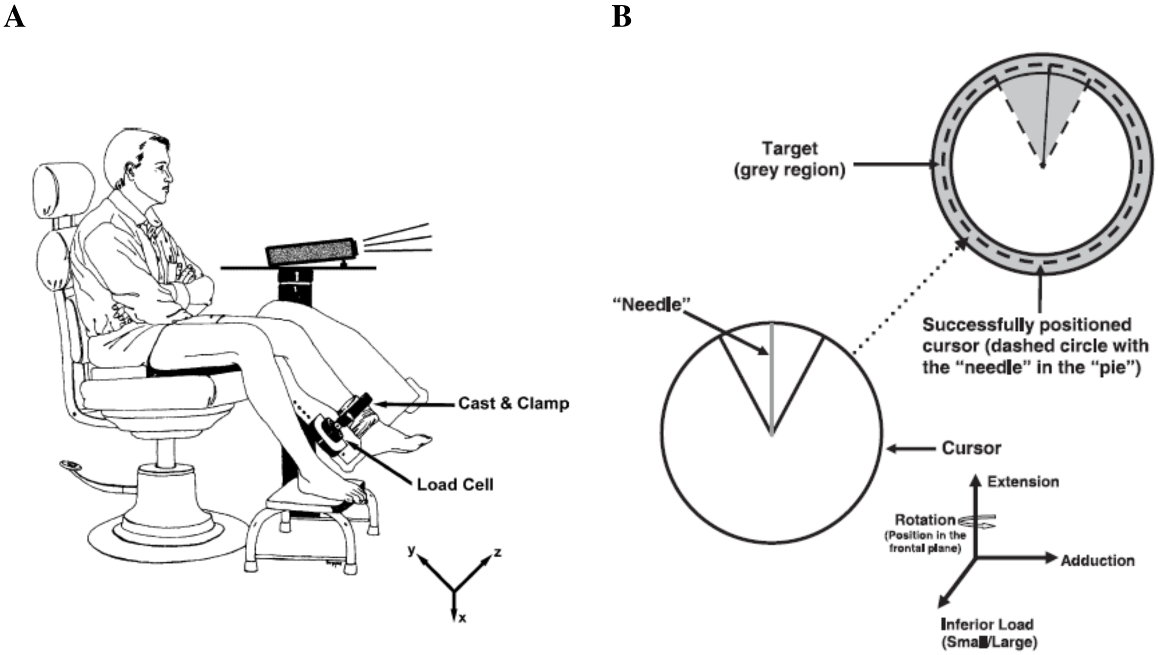

Figure 2: A) Diagram of Buchanan and colleagues' experimental set-up of their force matching protocol. Subjects were seated with the shank fixed to a 6-axis force transducer (Buchanan & Lloyd, 1997). B) The force applied against the transducer was used to control a projected cursor positioned on a screen in front of the subject. The aim was to move the cursor to various target locations which are representative of specific loading directions in the transverse plane of the shank (Williams et al., 2003).

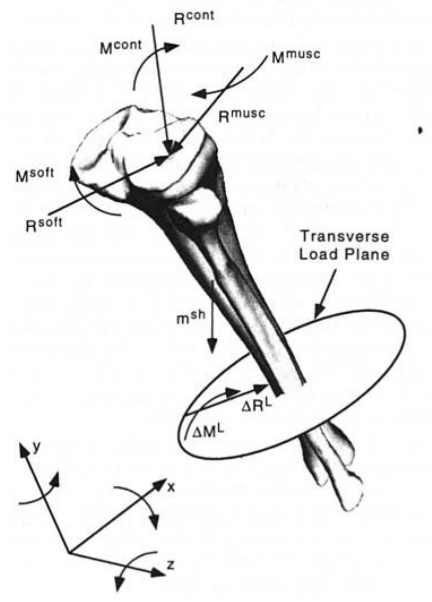

Figure 3: The free body diagram of the tibia showing all the forces and moments acting on the segment. Note the transverse load direction plane which is the plane in which the subject generated forces above the ankle on the shank (Lloyd & Buchanan, 1996).

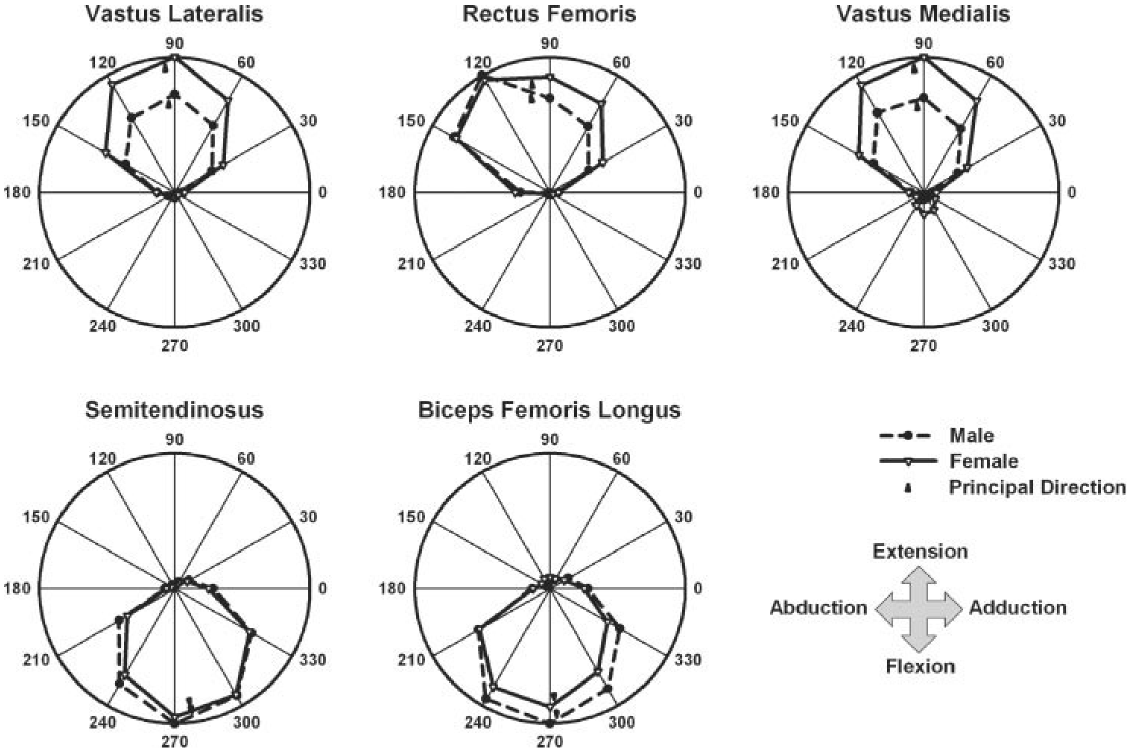

Figure 4: Example of EMG polar plots of the mean activity patterns for the male and female subjects. The numbers along the circumference of the polar plots identify target locations (in degrees). The mean normalised magnitude of activity used when matching each target is identified by the symbols that appear on the target location radius. Arrowheads denote mean directions of action (Krishnan et al., 2008). Note that the circular boundary is selected based on the location of greatest muscle activity and does not equate to percent maximum activation and no report on the scale was provided by the authors so interpretation of the magnitude is cautioned.

Isometric Muscle Activation at the Knee

Lloyd and Buchanan (1996) used the experimental EMG datum to estimate the muscular force and soft tissue contributions to external knee joint moments with a biomechanical model of the knee. Findings indicated that muscles were primarily used to support flexion and extension (FE) loads at the knee, but if the load direction slightly changed so as to have a small varus or valgus (VV) component, the muscles still produced enough force to balance the moment. However, as the VV component increased, the contribution from muscles became inadequate and soft tissue contribution was required. Soft tissues ended up supporting 83% of the external load in pure VV but muscles were still required to carry the remaining 17% of the moment.

Buchanan and Lloyd (1997; 2001) continued to quantify the strategies used to support VV loading. Again, FE moment generation had the greatest muscular contribution to knee stabilisation. This was attributed to the large FE moment arms of the quadriceps and hamstrings muscles. But unlike the quadriceps and hamstrings, muscles such as the gracilis, sartorius and tensor fascia lata which possess large VV moment arms had minimal contribution to supporting loads acting in the frontal plane. This is likely due to their low force generation capacity (Zhang & Wang, 2001). Although the contribution of muscles with large VV moment arms was considered minimal, Lloyd and Buchanan (2001) did not dismiss the fact that the quadriceps and hamstring muscles too possess VV moment arms, though undoubtedly less than that in FE. It was observed that the quadriceps and hamstring muscles were able to contribute 11–14% of the external moment in pure VV through cocontraction. In order to do so, both the hamstring and quadriceps activated together such that the hamstring and quadriceps' FE moments were neutralised and the remaining VV moments were combined to generate the VV moment (Goldfuss et al., 1973).

Since females have exhibited variable muscle recruitment patterns in response to externally applied loads and that they have a greater incidence of injury and joint degeneration, Krishnan et al. (2008) found it appropriate to evaluate the ability for males and females to produce and control isometric force in various directions. The mean EMG activity patterns and principal directions of action of the quadriceps and hamstrings are plotted in polar coordinates and are presented in Figure 4. Although muscle patterns were similar, females were observed to have significantly broader quadriceps activation patterns (increased magnitudes in all the directions with an extension component) including higher magnitude of vastus medialis and vastus lateralis muscle activity for a given VV force threshold compared to males. In contrast, males exhibited greater hamstring activity in all directions with a flexion component compared to females. Furthermore, Krishnan and Williams (2009) observed that females also had a significantly higher antagonist quadriceps activity during knee flexion compared to males, suggesting greater level of cocontraction during flexion.

What Lloyd, Buchanan, Krishnan and colleagues (1997; 2008; 2009; 1996; 2001) have observed is evidence for activation strategies dominated by the quadriceps and hamstring muscles with specific purposes to generate large FE moments and support VV moments. However, the nature of motor control strategies during these open kinetic isometric contractions is more complex than previously thought. Andriacchi et al. (1984) and Buchanan et al. (1986) argued that the peak directions of individual muscle activity at a joint would be dictated by the moment arm geometry. For example, the 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. Activation still occurs, but to a lesser degree such as to have a trigonometric effect, when a moment is required that incorporates a component of the semitendinosus' moment arm orientation: the greater the component the greater the level of activation and vice versa. If this statement were to be valid then the corresponding changes in EMG and moment arm would exhibit a causal relationship. Instead, this relationship is not as straight forward and varies across ACL deficient and sex populations (Krishnan et al., 2008; Krishnan & Williams, 2009; Williams et al., 2003). Hence, there must be other variables that govern the way muscles are activated and their roles and further investigation of these roles is warranted.

Limitation of Previous Open Kinetic Chain (OKC) Methodologies

Knee stability is reliant on geometry of the articulating surfaces, the restraints of the surrounding soft tissues, and the loads applied to the joint from muscular contractions as well as bearing weight (Williams et al., 2001). Although the aforementioned experimental approach is favoured to analyse neuromuscular control strategies, to date, however, this evidence can only be linked to OKC conditions (an isotonic exercise in which the distal segment of a limb is unfixed to a surface such that the joint is uncompressed from body weight or an external load applied to the distal segment of the limb) where the participant was required to sit in a chair and the ankle was unrestricted which in turn leaves the knee joint uncompressed from gravitational forces and applied body weight (Buchanan & Lloyd, 1997; Hortobagyi et al., 2004; Krishnan et al., 2008; Krishnan & Williams, 2009; Lloyd & Buchanan, 1996, 2001; Patten & Kamen, 2000; Williams et al., 2003).

Research comparing knee force production between OKC and closed kinetic chain (CKC) exercises has demonstrated that muscle recruitment patterns and activation characteristics vary between conditions (Escamilla et al., 1998; Stensdotter et al., 2003). Specifically, CKC exercise demonstrates greater and more simultaneous EMG activity from the quadriceps muscles compared to OKC indicating larger and faster force production abilities for individuals executing CKC exercises (Stensdotter et al., 2003). Further, CKC knee extension generates significantly greater hamstring and gastrocnemius activity compared to OKC movement, indicative of increased stabilising forces by means of cocontraction. As such, OKC muscle actions are not deemed to be representative of true joint loading and since the vast majority of physiological loading is done with the foot in contact with the ground, it would be beneficial to determine the roles of these muscles during CKC loading.

Several reasons can also be presented to explain these varied recruitment patterns which will in turn favour the utilisation of CKC exercises during experimental research on neuromuscular control strategies. First, CKC is more apt at simulating the kinematics of lower limb movement during daily activities because added ankle and hip contributions (Escamilla et al., 1998) as well as proper tibio-femoral orientation. While similar a magnitude of anterior tibial translation occurs if bearing weight or not, as soon as a knee in compressed during weight bearing, the tibia rotates: externally for females and internally for males (Shultz et al., 2009). Furthermore, torsional stiffness increases in CKC condition (Schmitz et al., 2008).

Second, proprioceptive feedback differs between OKC and CKC tasks because compressive forces from body mass and gravity is acting on joint sensory receptors and the soft tissues are properly loaded (Kiefer et al., 1998). Third, CKC is suggested to produce less shear force between tibio-femoral joint as well as properly regulate the patellofemoral joint forces and contact areas (Escamilla et al., 1998).

Furthermore, the protocol mentioned above is apt in theory but no mention of its validity was provided. We cannot assume the results are repeatable or are indicative of the reported loading conditions.

Therefore, a new method is needed that combines the benefits of muscle specificity testing with the physiological influences of gravity and joint compressive forces. When the foot of a person makes contact with the ground at heel-strike for a given movement, a force is exerted by that person to the ground and an equal an opposite force is exerted from the ground back to the person's foot. The person responds to these ground reaction forces by activating the knee muscles need to regulate these forces across the joint and maintain stability. Our protocol was built on this premises where subjects stand and are required to produce isometric ground reaction forces while maintaining standardised joint angles. Multiple ground reaction forces are produced in various directions in the horizontal plane. In doing so, we were able to evaluate and identify individual contributions of muscles the generation of the force and in turn attribute this contribution to knee joint stabilisation. This protocol is explained in detail in the following section and the computation of its intra-class validity is addressed in the General Discussion and Conclusion section (page 103).

Methodology

We developed a new target matching protocol based on the work of Buchanan and colleagues (1986) to assess participants' voluntary knee joint muscle control strategies during quasi-isometric (i.e. the subject attempts to maintain a specific joint position however minor changes in joint angle, and thus muscle length, may occur) closed kinetic chain loading.

Study Design

This was a comparative study that examined the electromyographic (EMG) patterns of healthy young male and female adults. The muscle activities of eight muscles that cross the knee joint were recorded in both legs using EMG techniques during quasi-isometric force target matching tasks conducted over a range of 360º in the horizontal plane. This design enabled the evaluation of sex-related differences in neuromuscular patterns responsible for generating direction specific forces at ground level.

Participants

G*Power (3.1.0) was used to conduct a power analysis on our primary outcome data (sex differences in mean magnitude of muscle activation (see Data Analysis for description of calculation) for the biceps femoris (mean ± standard deviation): males = 0.16 ± 0.08, females = 0.20 ± 0.12) based on the first 20 participants. The analysis determined that a sample size of 13 in each group was necessary to effectively test the research hypotheses (alpha ≤ 0.05, power ≥ 0.80).

Thirty-three healthy active (defined by participation in exercise or sport at least two times a week) young adults were recruited for this study, however, only 26 (13 male, 13 female) (mean age of 23.0 ± 1.7 years) participated in this study. Five additional participants were unable to do the experimental tasks and data collection was not completed for two other participants due to technical difficulties (for a grand total of 33 participants recruited). Exclusion criteria included the history of: 1) a significant knee or lower limb injury, defined as a life altering effect on one's functional ability, such as a ligament rupture or meniscal tear; 2) a lower limb sprain, muscle or tendon injury, or fracture within six months of participation; 3) a lower extremity motor nerve lesion, the presence of a knee joint effusion, muscle atrophy, diabetes, or any other observed physical impairment that could affect the results of the study.

Equipment and Data Collection

Muscle Activations

Muscle activities about the knee in both legs were recorded using EMG. EMG signals were collected using a 16-channel system (DS-B04, Bagnoli-16, Delsys Inc., Boston, MA). Bipolar single differential surface electrodes (SP-E04, DE 2.1, Delsys Inc., Boston, MA) with sensor contacts made from 99.9% pure silver bars measuring 10mm in length, 1mm in diameter and spaced 10mm apart were used. The reference electrode (Dermatrode HE-R, Farmadomo, BV, Nuland, Netherlands) was placed on the participant's right clavicular head. The electrodes were then connected to a portable input module (SP-N05, Bagnoli-8, DelSys Inc., Boston, MA) worn by the participant in a custom made shoulder harness. This unit was then connected to the main amplifier unit (SP-B08, Bagnoli-16, Delsys Inc., Boston, MA). EMG signals were sampled at 1000 Hz, amplified by 1000, and band-pass filtered at 20-450 Hz using a 16-bit A/D conversion board (NI PCI 6229, National Instruments Corp., Austin, TX).

Force Measurement

Ground reaction forces applied by the participant were recorded at 1000 Hz using two force platforms (FP4060-08, Bertec Corporation, Columbus, OH, USA). To reduce ankle motion contribution and allow a solid base for force transfer, the foot from the studied leg was fitted into a water ski boot (Bio, O'Brien, Redman, WA, USA). The boot's binding was then fixed to a wooden top-plate which in turn was bolted to the force plate top via four threaded holes. The other force platform was placed adjacent and slightly posterior to the first, without any extra attachments (Figure 5).

Figure 5: The test force platform (near) with the fixed water ski boot attached via a wooden top-plate and bolted with threaded holes. The support leg force platform (far) has no extra attachments and is situated adjacent and slightly posterior to the test force platform.

Data collected from the second force platform was considered secondary and was recorded to potentially describe confounding effects from the supporting limb. The ground reaction force of both force plates were compared to confirm that each leg was exerting an equal and opposite force against the force platforms. Further analysis into the kinetics and kinematics of the support leg was not conducted.

Kinematics and Kinetics Measurements

Kinematics were recorded using an infrared nine-camera motion analysis system (Vicon MX-13, Oxford Metrics, Oxford, UK) sampling at 200 Hz. Nexus software (version 1.3, Oxford Metrics, Oxford, UK) was used to record and output the three-dimensional (3D) motion capture data. The calibration of the system was conducted in two parts: first, a dynamic calibration was done with a T-shaped wand (240mm) with three reflective markers. Each camera captured at least 7000 frames with a minimum of three markers visible, allowing the position of each camera to be established relative to the others. Second, a static calibration with an L-shaped frame (ErgoCal 14mm) established the origin of the global coordinate system. The calibrated capture volume approximated 2.5m x 2.5m x 2.5m.

For 3D motion capture, participants had 45 reflective markers (14mm diameter) placed on their body following the University of Ottawa's marker placement set (refer to Appendix A). Participant weight and lower limb and pelvis anthropometric measurements were also recorded.

Similar to the data of the second force platform, the 3D motion information was collected to identify potential covariates (limb joint angles and moment of force) on the neuromuscular control strategy. Also, gait, hop, and one and two legged squat trials were recorded at the end of the experimental session but are not reported in this study's analysis.

Protocol Setup

Participant Recruitment and Introduction to Study

This study was approved by the *University Of Ottawa Research Ethics Board*. Participants for this study were recruited at the *University of Ottawa* through word of mouth and advertisements posted at the *Lees Avenue* campus. If potential participants reported no previous traumatic lower limb injuries and verbally volunteered to partake in the study, then a three hour session at the *University Of Ottawa's Human Movement Biomechanics Laboratory* was scheduled and participants were instructed to dress in appropriate clothing, bring running shoes and socks, and avoid strenuous physical activity a minimum of 12 hours before testing. On the day of the scheduled testing session, participants arrived at the lab and received an introduction to the experimental setup. They then read and signed a research consent form. After consent was provided, the researchers assisted the participants in filling out a questionnaire about their current physical activity levels, past injuries to lower limbs, and determination of dominant leg.

A minimum of six practice target matching trials were preformed. More practice trials were provided if the participant did not feel comfortable with the task or if the researcher deemed it necessary. This was to minimise the effects of learning and to ensure the participant could actually perform the protocol before continuing. Note that five of 33 participants recruited were unable to match the practice targets for unknown reasons. Live feedback of the participant's ground reaction forces during the actual experimental tasks was presented as a cursor on a projector screen. The cursor of the five subjects was "shaky" relative to the other "successful" participants. This implies that the five "unsuccessful" participants were unable to apply a controlled force against the force platform such that the cursor could be properly matched to the target. This was an unforeseen issue that was not prevalent during pilot testing thus the program had no feature to accommodate this type of situation. For this reason, data collection could not occur and this issue is currently being investigated further. Two additional participants did not complete the data collection and thus are not included in the participant count (data, if any, collected from these participants were not included in the analysis). As a result, the data from the remaining 26 subjects were analysed.

Participant Set-up

The activity of eight muscles on each leg was assessed: the rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), semitendinosus (ST), biceps femoris (BF), medial gastrconemius (MG), lateral gastrocnemius (LG), and tensor fascia lata (TFL). Muscle bellies were located using the recommendations made by SENIAM (Hermens et al., 1999) and electrodes were placed following the recommendations made by DeLuca (1997). Electrode placement sites were prepared with abrasive rubbing using alcohol prep pads to minimise surface impedance.

EMG signals were tested to ensure the visual signal to noise ratio was at a minimum. Signals were observed in real-time as participants sat in a chair and produced knee flexion/extension, plantar flexion, and hip abduction movements to isolate muscle groups as much as possible.

Before continuing data collection, an adequate warm-up on stationary bike (Monark ergometer, Monark Exercise, Sweden) was done for five minutes at a low intensity level, where subjects maintained a speed of 90 repetitions per minute with no resistance.

Maximum Voluntary Isometric Contractions (MVICs)

An isokinetic dynamometer (850-000, Biodex, New York, USA) was used to perform maximum voluntary isometric contractions (MVICs) and collect maximal EMG (EMG_{Max}) and torque of the test leg. A custom made Labview (v8.2, National Instruments Corp., Austin, TX) program was used as support software to record and evaluate the EMG signal, torque and position data at 1000 Hz.

For all MVIC trials, participants had approximately eight to ten seconds to reach maximal force production and were instructed to scale their force output such that consistent increases from a perceived zero force to maximal force could be observed. Visual feedback of torque production and verbal encouragement by the researchers were also given to help participants achieve a MVIC and hold it for approximately three seconds. Eight exercises (four for each leg), described below, involved one set of three repetitions with a 45 second rest period between repetitions. Hence, a total of 24 MVIC trials were conducted.

Firstly, participants were seated with a standardised hip flexion angle of 90º, knee flexion angle of 30º, and an ankle plantar flexion angle of 10º. Participants were instructed to plantar flex against the dynamometer attachment for the MG and LG MVICs. Participants then remained seated with same the hip and knee angles and conducted knee extension followed by knee flexion trials to obtain the RF, VL, VM, ST and BF MVICs, respectively. Finally, participants stood in a neutral position along the frontal plane for the TFL MVIC and were instructed to produce an abduction force against the thigh attachment.

Maximum Isometric Closed Kinetic Chain Loads

To define the load required for a successful target match, maximum voluntary quasi-isometric loads were applied against a force platform as a standardisation reference. The participant’s dominant foot (defined as the foot used to kick a ball as far as possible) was placed in the water-ski boot and their other leg (support limb) located atop the adjacent force platform (slightly posterior to the first) with the foot pointing forward. They were instructed to maintain their pelvis and upper body in an anteriorly facing position (i.e. no rotation about the frontal plane). Adhesive tape was placed on the second force platform to mark this location so the supporting foot's position could be consistent throughout the experiment.

The participant's dominant leg (i.e. test leg) was positioned with hip, knee, and ankle joint angles of approximately 30º, 30º, and 10º plantar flexion, respectively (see Appendix C for average joint angles of all participants). The participants were instructed before and during the experiment to try and maintain this position as best as possible. But note that the knee and hip joint positions were not physically restricted and the ankle was minimally restricted from the wakeboard boot's ankle support so there was an expected tendency for the joint angles to change. These changes in position were recorded by the Vicon motion capture system.

The maximal load trials were ten seconds long which required equal body weight distribution across each leg for the whole trial. Projected live feedback of the vertical force was provided where the participants were instructed to achieve equal weight distribution for the first five seconds and then maintain this for the remainder of the trial while performing a maximal load production in one of the anterior, posterior, medial and lateral directions. The last eighteen participants conducted these trials with the vertical force feedback, the first eight participants judged body weight distribution subjectively because the feature was unavailable at this time. Similar to the MVIC trials, the applied load was scaled from perceived zero effort to maximal effort. EMG and marker position data were also recorded during these trials.

These trials were used to standardise the resultant load (RL) required to match each target which was computed using the following equation (Krishnan et al., 2008):

$$RL = \sqrt{} \, [(\cos\theta \bullet Fx \bullet \%maxF)^{2} + (\cos(90 - \theta) \bullet Fy \bullet \%maxF)^{2}] \qquad (1)$$

Where θ is the angle between the target and the + x axis, Fx is the peak load produced along the ± X axis (medial/lateral), Fy is the peak load produced along the ±Y axis (anterior/posterior) during the above mentioned trials, and %maxF is percent maximal load recorded against the force platform. For example, if a participant produced a maximal load of 180 N in the 0º load direction (along the medial axis/ + X axis) and 300 N in the 90º load direction (along the anterior axis/ + Y axis) and 30% of the maximal load production is required for a successful target match, located at 30º, then the participant would have to produce 46.8 N in the medial direction (i.e., 30% of 180 N • cosine 30º) and 45.0 N in the anterior direction (i.e., 30% of 300 N • cosine 60º) to match the target. The RL magnitude under these circumstances would be 64.9 N. The value of the RL required for a match however was not used for data analysis. The above equation and respective discussion exists to provide the reader with information regarding the Target Match program's ability to standardise the required matching load vectors for each participant.

For this study, 50% ± 5% of body weight and 30% of maximal load production in each primary load direction (anterior, posterior, varus and valgus) were used as the target matching parameters. This load level was chosen to ensure adequate motor unit recruitment where surface EMG aptly represented whole muscle activations (DeLuca, 1997).

Target Matching

Participants remained in the same anatomical configuration from the maximal load trials (Figure 6A). A video screen with a projected image of the cursor and target was placed in front of the participant. This provided biofeedback on the participant's direction and magnitude of force production via cursor movement. The experimental task required the participant to position the projected circular cursor over the target and keep it there for one second (Figure 6B). The cursor movement occurred in response to ground reaction forces that the participant applied against the force platform. The cursor moved with three degrees-of-freedom:

1. Anterior/posterior loads moved the cursor upward/downward.
2. Varus/valgus loads moved the cursor to the right/left.
3. Inferior/superior loads generated along the long axis of the shank made the cursor smaller/larger (controlling the percent body weight exposed to the joint).

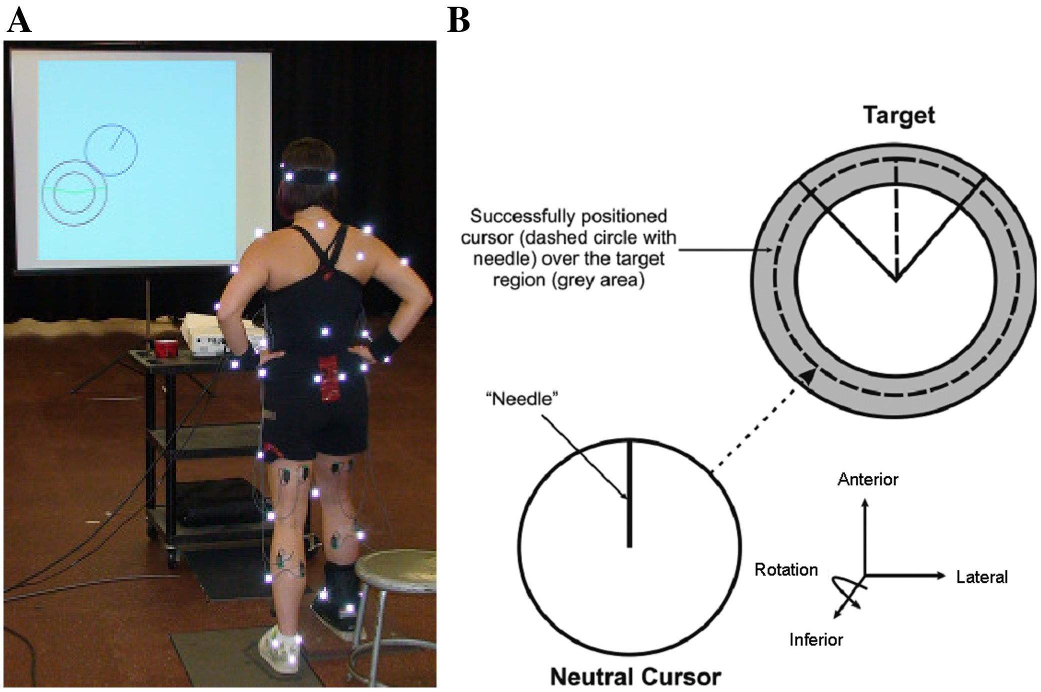

Figure 6: A) Laboratory setup and participant standing with the dominant foot on the testing force platform, fixated in a wakeboard boot. Live visual feedback from projector showed the participant's current force production direction and magnitude. B) Depiction of the cursor, target, and successful positioning of cursor over the target.

The successful matching of the cursor over the target for one second triggered the recording of EMG, force and moment information for the one second of target matching as well as data for three seconds prior to the target match, known as the “pre-match time” (total of four seconds per trial). Marker position data were also determined for these times where a successful match caused the Target Match software to produce an auditory tone that cued the researcher to record the last four seconds of marker position data within Nexus’s collection buffer. Nexus software was also used to collect the force platform data for 18 subjects in order to evaluate kinetics at a later time.

A successful match of one target was followed by a 30 second relax period then a new target in an alternate location. Targets randomly appeared one at-a-time at 12 different locations, evenly spaced by 30º, about a circular trajectory which was representative of the anterior-posterior-medial-lateral horizontal plane of the force platform (Figure 7). Each target location had to be matched three times for a total of 36 trials. Although a minimum of 30 seconds relaxation period was provided, participants were allowed to perform the trials at their own rate and if fatigue was an issue, they were encouraged to sit or take a walk for however long they felt necessary.

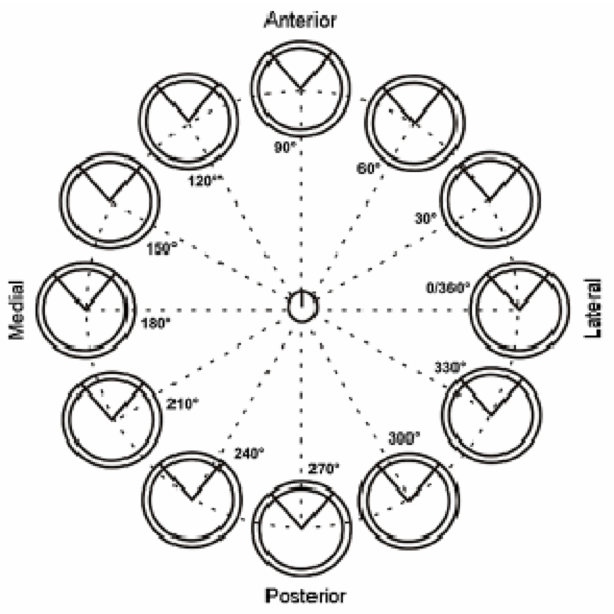

Figure 7: The target locations used in the target matching protocol: 12 targets, evenly spaced by 30º about a circular trajectory which was representative of the anterior-posterior-medial-lateral axes in the horizontal plane.

Data Analysis

Maximum Voluntary Isometric Contractions

The EMG data from the MVIC trials first had the EMG system's bias removed by taking the mean of the signal and subtracting it from the signal. This forces the signal's mean to equal zero. EMG data was full wave rectified and filtered with a 4th order Butterworth dual low-pass filter (6 Hz) using a custom made Labview (v8.2, National Instruments Corp., Austin, TX) program. The EMG_{Max} value used in normalisation was computed by averaging a ±10 ms window about each exercise's maximum data point.

Muscle Activation Patterns

There were four seconds (three seconds pre-match and one second match) of EMG, force and moment information collected but only the one second of successful target match data was analysed. The Target Match EMG data underwent a system bias removal, was full wave rectified and filtered with a 4th order Butterworth dual-pass low-pass filter (6 Hz) using the Labview program. The rest of the EMG data was processed in a Matlab (2007b) program. EMG data was normalised by dividing it by EMG_{Max}. The normalised EMG amplitude corresponded to a value between 0 and 1 where 1 represented the value of EMG_{Max}. No signal collected in this study exceeded the EMG_{Max} values. The normalised EMG for each trial was averaged over the second corresponding to the target match then ensemble averaged for the three repetitions for each muscle at each target location.

Subsequently, the normalised EMG value for each muscle at each target was represented as a vector (EMGi), where the vector's orientation corresponded to the target's location and its magnitude corresponded to the EMG_{Max}. As such, muscle activation patterns could be visually represented in EMG polar plots similar to the methods used by Buchanan et al. (1986). The results of muscles from the dominant leg were only presented and discussed in this thesis. EMG polar plots of the support leg are presented in Appendix D.

Specificity Index

Since the EMGi included a direction with respect to an arbitrary zero-location, the use of traditional linear statistical techniques is meaningless (Berens, 2009). Thus, EMGis were transformed into their corresponding Cartesian coordinates. A "mean magnitude of muscle activity" (X_{EMG}) was computed by averaging all the normalised EMGs at every target location for each muscle (Equation 2) and the "mean direction of muscle activity" (Φ) for each muscle was computed using the arctan of the vectors' summed components (x_i and y_i) (Equation 3). Together, the X_{EMG} and Φ characterise the muscle's vector mean or more appropriately termed, the muscle's resultant vector (refer to Appendix B for vector calculations) (Buchanan et al., 1986).

$$X_{EMG} = (\Sigma EMGi)/n \qquad (2)$$

$$\Phi = \arctan(\Sigma y_i/\Sigma x_i) \qquad (3)$$

Where n is the number of target locations.

Likewise, the variance of muscle activation cannot be computed in a traditional manner, therefore, an equation previously used by Williams et al. (2003) and Krishnan et al. (2008) and synonymous with ‘‘the index of spatial EMG focus’’ reported by Dewald et al. (1995) was used to describe each muscle’s “degree of focus” (i.e. variance) associated with its Φ. In other words, the degree of focus was computed to determine how concentrated the muscle’s EMG data were about its mean direction. For this, a specificity index (SI) (Equation 4) was derived from the ratio of the muscle’s resultant vector (X_{EMG} and Φ) to the sum of the all the absolute EMG magnitudes of all the vectors (refer to Appendix B for SI equation expansion and calculations).

$$SI = \surd(|\Sigma x_i|/n)^2 + (|\Sigma y_i|/n)^2 / \surd(|\Sigma x_i|/n)^2 + (|\Sigma y_i|/n)^2 \qquad (4)$$

The resultant SI was a scalar between 0 and 1. An index of 0.0 indicated that a muscle was equally active in all target directions (totally non-specific; depicted by a circular activity pattern), whereas an index of 1.0 indicated that the muscle was active at only one target (totally specific; depicted by an activity pattern that is a single radius line) (Figure 8).

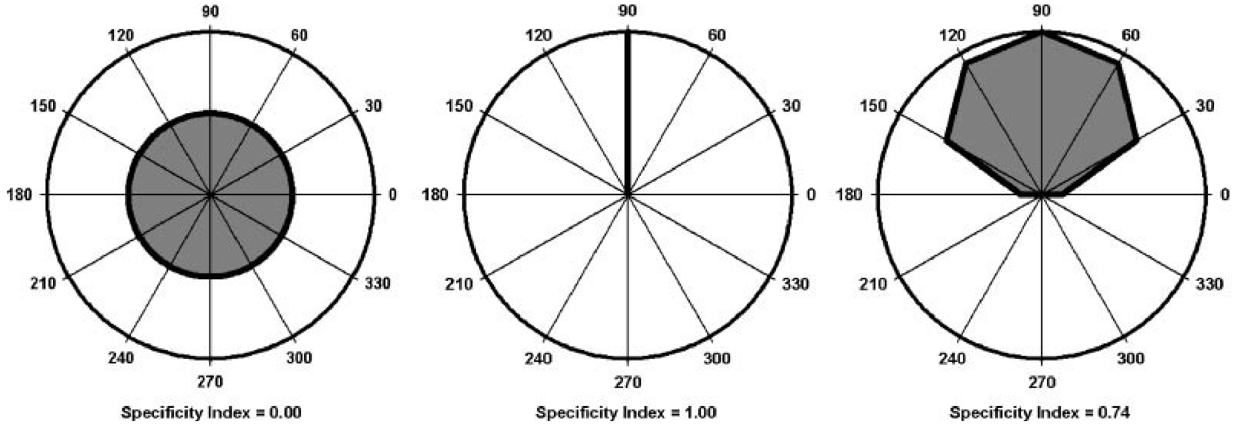

Figure 8: Polar plots demonstrating the limits of the specificity index (SI) and hypothetical patterns with a mean direction of action of 90 degrees. The lower limit of the SI is zero, indicating the muscle is equally active in all directions and depicted by a circular plot (left). The upper limit of the specificity index is 1.00, indicating the muscle is active in a single target direction and depicted by a single radius (middle). The right plot depicts a muscle activity pattern in which approximately 74% of the muscle's activity is directed toward its mean direction of action which is more representative of a physiologic pattern (Adapted from Krishnan et al. (2008)).

Cocontraction Index

Muscular cocontraction was also quantified at each target location using a cocontraction index (CI). Cocontraction was defined as the simultaneous activation of antagonistic muscles and was calculated between the vastus lateralis and biceps femoris (VL-BF), vastus medialis and semitendinosus (VM-ST), vastus lateralis and lateral gastrocnemius (VL-LG), vastus medialis and medial gastrocnemius (VM-MG), as well as a general CI of the summed quadriceps and hamstrings muscle activity (QUADS-HAMS) and quadriceps and gastrocnemius muscle activity (QUADS-GASTROCS) . The CI was defined as the ratio of the antagonist's summed activation and the agonist's summed activation at each target direction multiplied by the sum of activity in both muscles. This approach and the following equation have been previously described by Rudolph et al. (2001) and used by others (Lewek et al., 2005; Palmieri-Smith et al., 2009):

$$\text{CI} = (\text{lower EMGi}/\ \text{higher EMGi}) \bullet (\text{lower EMGi} + \text{higher EMGi}) \qquad (5)$$

EMGi referred to the muscle or muscle group's normalised EMG magnitude. The muscle or group that had the greater level of activation was chosen to be the divisor (higher EMGi). A CI of 0 is the lower limit of the index which represents no cocontraction of the antagonist muscle pairs such that the muscle with the lower EMGi value was not activated at all. The higher limit of the index is a CI of 2, meaning that both muscles are activated at 100% of maximum EMG values. This causes the activation ratio to equal 1 and the summed activation to equal 2.

This index incorporated the magnitudes of activation which provides more interpretative results of cocontraction compared to an activation ratio. For example, if the higher EMGi muscle and the lower EMGi muscle were activated to 0.15 and 0.10 EMG_{Max}, respectively, then the activation ratio would equal 0.67. The same ratio would also result if the higher EMGi muscle and the lower EMGi muscle had activation magnitudes of 0.45 and 0.30 EMG_{Max}, respectively. However, the CI equals 0.167 for the first scenario while the CI equals 0.50 of the second – indicating a more substantial degree of contraction.

Knee Joint Kinematics and Kinetics

The hip, knee, and ankle joint angles were collected for analysis as a co-variate if needed. Future analysis of the data will also include the estimation of joint forces and joint moments for each trial; however that goes beyond the scope of this thesis. In order to compute these, Vicon's supporting software, Nexus, was utilised to label markers according to the UOMAM marker set, delete unlabelled trajectories, and fill trajectory gaps with a Woltring spline filter (5 frames). Recall that the kinematic collection was cued with an audio "beep". Since all subjects relaxed immediately after the target was reached and they heard this cue, there was a corresponding change in the ground reaction forces; visual inspection of the data was used to ensure that the last frame in the motion file equated to the end of the target matching trial.

According to Bisseling and Hof (2006) and van den Bogert and de Koning (1996), both the kinematics and force platform data should be filtered using the same techniques and cut-off frequencies when computing inverse dynamics. In doing so, the "contribution of the impact peak force is sufficiently counteracted by the impact acceleration" (Bisseling & Hof, 2006). For that reason, a 4th order Butterworth dual low-pass (20Hz) filter was employed to process both signals. Data was then modelled using the modified *Plug-in-Gait*, called the UOMAM (University of Ottawa Motion Analysis Model). For each trial, the variables of interest were then exported as ASCII files and Excel was used to open and take the means of the whole file. For joint angles, the mean of all trials were computed for each subject and then ensemble averaged across all subjects.

Statistical Analysis

To test for sex-related differences on knee angles, a one-way between groups multivariate analysis of variance (MANOVA) was conducted. Sex was the independent variable and the joint angles in the sagittal, frontal and transverse planes were the dependent variables. Results for these analyses are presented in Appendix C. The main variable of interest was the knee joint flexion angle. This was not statistically significant at the $p<0.05$ level and thus the analyses below did not involve a covariate.

To test for sex-related effects on individual muscle activation characteristics the mean magnitude of activation (X_{EMG}), mean direction of activation (Φ), specificity index (SI) for each muscle, a one-way between groups MANOVA was considered because the activation characteristics are related to one another but a MANOVA was not used because the Φ is expressed in polar coordinates where the coordinate value is based on an arbitrarily chosen zero location. Thus, the use of linear statistics is inappropriate. Thus *CircStat: A MATLAB Toolbox for Circular Statistics* (Berens, 2009) was used to apply the method of Watson-Williams two-sample test of the null hypothesis, which is the circular analogue of the two-sample independent T-test. Independent T-tests of the mean X_{EMG} and mean SI data were also conducted (SPSS v15.0, SPSS Inc., Chicago, USA). The significance level was set at alpha (α) ≤ 0.05

To specify where differences across sex are located in the loading axes, a mixed within-between factorial (2x12) analysis of variance (ANOVA) was used to examine whether the amount of EMG activity significantly differed as a result of sex and target direction. Post hoc analysis using independent T-tests for each direction was conducted when a significant main effect for sex or interaction of sex and direction was reported to determine if EMG magnitude significantly differed at specific locations between sexes. Alpha was adjusted to 0.004 to protect against type I error due to the 12 related variables of force target directions.

Similarly, a mixed within-between factorial (2x12) ANOVA was used to test for significant sex-related and directional effects on the cocontraction index (CI) for each synergistic muscle pair. When a significant main effect for sex or interaction of sex and direction on CI was reported, appropriate post hoc evaluation using independent T-tests were conducted to determine at what location the CI significantly differed across sex. Alpha was adjusted to 0.004 to protect against type I error.

All statistical results are reported in full in Appendix E.

ARTICLE 1: JOINT STABILISERS OR MOMENT GENERATORS: THE ROLE OF KNEE JOINT MUSCLES IN HEALTHY YOUNG ADULTS DURING CLOSED KINETIC CHAIN FORCE CONTROL

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Running Title: The Stabilising Role of Knee Joint Muscles

Abstract

Neuromuscular control plays a critical role in dynamic knee stability. The aim of this study was to determine if males and females have different neuromuscular control patterns during voluntary isometric closed kinetic chain force generation tasks requiring directional loading. Twenty-six healthy young adults (13 male, 13 female) stood with their dominant leg in a boot fixed to a force platform. A force target matching protocol required subjects to position a cursor (projected on a video screen) over a target and maintain the position for one second. To control the cursor, loads were applied against the force platform with their dominant leg to produce various combinations of anterior-posterior, medial-lateral loads in the horizontal plane while maintaining constant inferior-superior loads. A successful target match required a normalised force magnitude of equal effort for each subject and target location which triggered the recording of electromyography (EMG) for eight muscles crossing the knee joint. EMG was normalised to percent maximum voluntary isometric contraction. A mean magnitude of muscle activation, mean direction of muscle activation and a muscle specificity index was determined using EMG vectors. Females displayed significantly lower specificity than males in their semitendinosus ($p=0.025$) and tensor fascia lata ($p=0.012$) muscle activity patterns, greater magnitude of muscle activation in their lateral gastrocnemius ($p=0.002$) and tensor fascia lata ($p<0.003$) and no statistical difference in the other muscles. Furthermore, the activation patterns in our study grossly differed from previous open kinetic chain force target matching. These findings indicate that healthy young males and females have differences in their knee muscle control strategies and knee muscle recruitment patterns differ during weight bearing and non-weight bearing tasks.

Key words: Knee, muscle activity, joint stability, closed kinetic chain, isometric, sex differences

INTRODUCTION

Neuromuscular control plays a critical role in dynamic joint stabilisation. Control deficits reduce one's ability to properly counteract externally applied loads which in turn, increases the risk for joint injury (Fitzgerald et al., 2004; Williams et al., 2001). Females have a greater prevalence and incidence for knee injuries (Gwinn et al., 2000). In addition, several studies on sex-related differences in neuromuscular control of the knee indicate that females have a decreased ability to stabilise the knee with muscular support (Kanehisa et al., 1994; Wojtys et al., 2002; Wojtys et al., 2003). Studying neuromuscular function as it relates to sex can provide valuable insight into knee joint stability and the mechanisms underlying joint injury and disease.

Evaluations of neuromuscular function with respect to knee joint stabilisation have typically involved functional task assessments (i.e. walking, jumping, perturbations etc.) (Hortobagyi et al., 2004; Hortobagyi et al., 2001; Patten & Kamen, 2000). It may be unrealistic to assume that these results are representative of isolated muscle function because assessing the roles muscles play in movement is challenging due to the uncoupling of the biomechanical and neuromuscular contributions to force generation in an anatomically complex system (Williams et al., 2003). It is important to limit the number of contributing factors to force generation so the muscle activity patterns can be more clearly measured. To do this, a protocol that restricts the kinematic observations can be devised with the utilisation of isometric contractions. Many studies have used such an approach to evaluate specific muscle function (Buchanan & Lloyd, 1997; Hortobagyi et al., 2004; Krishnan et al., 2008; Krishnan & Williams, 2009; Lloyd & Buchanan, 1996, 2001; Patten & Kamen, 2000; Williams et al., 2003), however, these studies have only been conducted in an open kinetic chain (OKC) condition where the distal shank is unfixed and the ankle joint is not restricted. As such, the force contributions of proper lower limb kinematics and proprioceptive feedback due to compressive forces from gravity and body mass are absent. Since muscle activation differs significantly between open and closed kinetic chain (CKC) conditions (Escamilla et al., 1998; Shultz et al., 2009; Shultz et al., 2001; Stensdotter et al., 2003), a new approach is needed that combines the benefits of muscle specificity testing with the physiological influences of gravity and joint compressive forces.

Based on Newtonian physics, when a person plants the foot at heel-strike during gait, or does a cutting or side-step movement, the force exerted by the subject is equal and opposite to the force exerted by the ground to the person. The person responds to these forces by contracting knee joint musculature to stabilise the joint and accommodate these forces. We have developed a CKC target matching protocol which requires subjects to maintain approximately 50% bodyweight on the test leg while producing forces against the ground via isometric contractions. Our protocol required subjects to actively produce ground reaction forces in a controlled manner in multiple directions in the horizontal plane and the activation strategy produced by the subjects was evaluated. In this way, we gain the advantages of muscle specificity testing (i.e.: the ability to identify the specific contributions of individual muscles in stabilising the knee) with the advantages of CKC loading.

The purpose of this study was therefore to evaluate sex-related differences in neuromuscular control of the muscles about young healthy adult knee joints during a CKC target matching protocol which we believe to provide more realistic evidence of physiological neuromuscular control strategies. Based on previous research (Krishnan et al., 2008; Krishnan & Williams, 2009), it was hypothesised that females would have significantly greater mean muscle activation magnitudes and significantly different mean direction of muscle action of the quadriceps muscles compared to males as well as no significant difference in hamstring activation characteristics between sexes.

METHODS

Participants

A power analysis (G*Power 3.1.0) on our primary outcome measure (sex differences in mean magnitude of muscle activation for the biceps femoris (mean ± standard deviation): males = 0.16 ± 0.08, females = 0.20 ± 0.12) determined that a sample size of 13 in each group was necessary to effectively test the research hypotheses (alpha ≤ 0.05, power ≥ 0.80). Twenty-six (13 males, 13 females) healthy active (defined by participation in exercise or sport at least two times a week) young adults (mean age of 23.0 ± 1.8 years) participated in this study. Exclusion criteria included a history of: a significant knee or lower limb injury, such as a ligament rupture; knee surgery; or a lower limb sprain, muscle or tendon injury, or fracture within six months of participation; a lower extremity motor nerve lesion; the presence of a knee joint effusion; muscle atrophy, diabetes, or any other observed physical impairment that could affect the results of the study. Prior to data collection, all participants read and signed an informed consent form approved by the University of Ottawa Ethics Committee.

Experimental Protocol

A force target matching protocol based on the work by Buchanan and colleagues (1986) was modified to assess the participant's ability to contract the muscles about the knee joint and apply isometric force against a force platform in multiple directions in the horizontal plane.

Prior to testing, a minimum of six practice target matching trials were preformed to minimise effects of learning and to ensure the participant could perform the tasks. Note that 33 participants were recruited for this study but five were unable to complete the practice trials and data collection was not completed for 2 other participants. Bipolar surface EMG electrodes (SP-E04, DE 2.1, Delsys Inc., Boston, MA) connected to a 16-channel EMG system (DS-B04, Bagnoli-16, Delsys Inc., Boston, MA) were placed over the muscle bellies of the rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), semitendinosus (ST), biceps femoris (BF), medial gastrconemius (MG), lateral gastrocnemius (LG), and tensor fascia lata (TFL) of each leg. Electrode placement followed the recommendations by SENIAM (Hermens et al., 1999) and DeLuca (1997). Maximal EMG (Max_{EMG}) activity was collected using an isokinetic dynamometer (850-000, Biodex, New York, USA) and analysed with custom made software (Labview 8.20; National Instruments Corp., Austin, TX, USA). Plantar flexion, knee extension, and knee flexion were recorded at relaxed hip, knee, and ankle joint angles of 90º, 30º, and 10º, respectively. Hip abduction was recorded in a standing position. Participants were allotted 10 seconds to scale perceived force from a zero level to their maximal and hold it for approximately three seconds. Verbal encouragement was provided by researchers. EMG signals for Max_{EMG} and the target trials were sampled at 1000 Hz, amplified by a gain of 1000, and band-pass filtered at 20-450 Hz using a 16-bit A/D conversion board (NI PCI 6229, National Instruments Corp., Austin, TX). Subjects then performed a five minute warm-up on a Monarch stationary bike (speed of 90 repetitions per minute with no resistance).

Participants stood with the dominant foot (defined by leg used to kick a ball as far as possible) in a water-ski boot (Bio, O'Brien, Redmond, WA, USA) attached to a force platform (FP4060-08, Bertec Corporation, Columbus, OH, USA). The support leg was atop another force platform situated adjacent to and slightly posterior to the test plate (Figure 9A). This positioning resulted in joint angles of approximately 30º, 30º, and 10º flexion for the hip, knee, and ankle, respectively. The participants were instructed before and during the experiment to try to maintain this position as best as possible and the foot location of the support leg was marked with tape and maintained throughout testing.

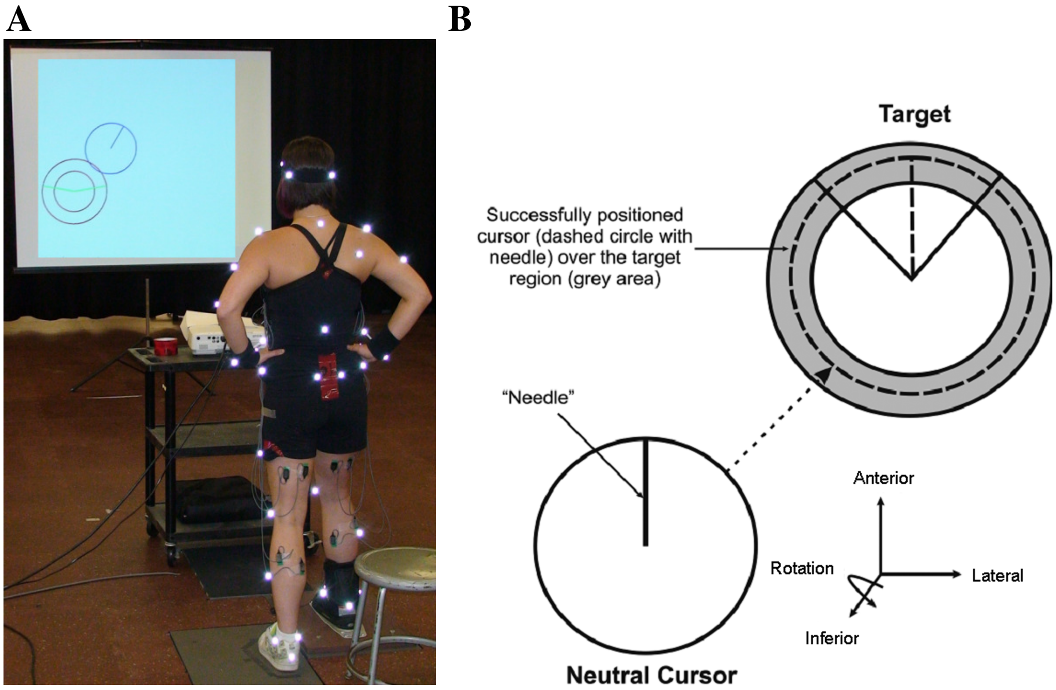

Figure 9: A) Laboratory setup and participant standing with the dominant foot on the testing force platform, fixed in a wakeboard boot. Live visual feedback from the projector showed the participant's current force production direction and magnitude. B) Depiction of the cursor, target, and successful positioning of the cursor over the target.

A video screen with a projected image of the cursor and target was placed in front of the participant (Figure 9A). This provided biofeedback on the participant's direction and magnitude of ground reaction forces applied to force platform of the dominant leg. This leg was used to control a projected cursor during the target matching. 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, 3) inferior/superior loads (force along the ±z axis) made the cursor smaller/larger (controlling the percent body weight exposed to the leg). Successful matching of the cursor over the target (Figure 9B) for one second triggered data collection for the one second of target matching and three seconds prior to the target match (pre-match) using a custom made Matlab (The Mathworks, Natick, MA, USA) application. This application also provided the biofeedback and defined the experimental parameters: targets randomly appeared one at-a-time at 12 different locations, evenly spaced by 30º, about a circular trajectory which was representative of the anterior-posterior-medial-lateral horizontal plane of the force platform. Each target location had to be matched three times for a total of 36 trials. A minimum 30 second relax period was provided between trials and participants were allowed to perform the trials at their own rate.

The magnitude of force required to reach the target locations was determined through maximal effort trials: with the foot in the boot, subjects were required to exert a maximal effort in the anterior/posterior (±y) and medial/lateral (±x). Targets locations were normalised to 30% of the peak load recorded during these maximal effort trials (see Krishnan et al. (2008) for a discussion on resultant force calculations). This load level was chosen to ensure adequate motor unit recruitment where surface EMG aptly represented whole muscle activations (DeLuca, 1997). The load required to maintain the correct cursor size over the target (i.e.: load along the long axis of the leg) was set at 50% ± 5% of body weight.

Data Processing

A custom made application written in Labview 8.20 was used to process Max_{EMG} and a custom made Matlab program was used to process the Target Match data. The EMG signals were conditioned with a bias removal, full wave rectification and a 4th order Butterworth dual-pass low-pass filter at 6 Hz to obtain a linear envelope. The one second of successful target match EMG data was analysed in this study and was normalised to percent Max_{EMG}. Each trial's normalised EMG amplitude was averaged over this second and ensemble averaged for each target location's three repetitions. These steps were then carried out for each muscle group.

The ensemble averaged normalised EMG value was represented as a vector (EMGi), where the vector's orientation corresponded to the target's location and its magnitude corresponded to the percent Max_{EMG}. As such, muscle activation patterns were visually represented in EMG polar plots similar to the methods used by Buchanan et al. (1986). Since the EMGi included a direction between zero and 360 degrees, the use of traditional linear statistics is inappropriate. Thus, EMGis were transformed into their corresponding Cartesian coordinates and a "mean magnitude of muscle activity" (X_{EMG}) was computed by averaging all the normalised EMGs at every target location for each muscle (Equation 2). The "mean direction of muscle activity" (Φ) for each muscle was computed using the arctan of the vectors' summed components (x_i and y_i) (Equation 3). Together, the X_{EMG} and Φ characterise the muscle's vector mean or more appropriately termed, the muscle's resultant vector.

$$X_{EMG} = (\Sigma EMGi)/n \qquad (2)$$

$$\Phi = \arctan (\Sigma y_i/\Sigma x_i) \qquad (3)$$

Likewise, the variance of muscle activation cannot be computed in a traditional manner. Therefore, a specificity index (SI) determined how concentrated the muscle's EMG data were distributed about its Φ. This was derived from the ratio of the muscle's resultant vector (X_{EMG} and Φ) to the sum of the all the absolute EMG components of all the vectors (Dewald et al., 1995; Williams et al., 2003):

$$SI = \sqrt{}(|\Sigma x_i|/n)^2 + (|\Sigma y_i|/n)^2 / \sqrt{}(|\Sigma x_i|/n)^2 + (|\Sigma y_i|/n)^2 \qquad (4)$$

The resultant SI was a scalar between 0 and 1. An index of 0.0 indicated that a muscle was equally active in all target directions (totally non-specific; depicted by a circular activity pattern); whereas an index of 1.0 indicated that the muscle was active at only one target (completely specific; activity pattern depicted by a single radius).

Statistical Analysis

Since the Φ was expressed in polar coordinates, *CircStat: A MATLAB Toolbox for Circular Statistics* (Berens, 2009) was used to apply the method of Watson-Williams two-sample test of the null hypothesis to determine if sex-associated differences in mean direction of muscle activation exist. Sex differences in X_{EMG} and SI were determined using independent t-tests (SPSS version 15.0, SPSS Inc., Chicago, USA). To determine if the magnitude of EMG significantly varied between sex as a function of direction, a between-within analysis of variance (ANOVA) was conducted. If significant main effects for sex or direction, or if a significant interaction was found, then post hoc evaluations were conducted with independent T-tests for each direction to determine if specific directions significantly differ across sex. Alpha was adjusted to 0.004 to protect against type I error due to 12 related variables (i.e. the number of target locations).

RESULTS

Subject characteristics are presented in Table 1; all but one subject was right leg dominant as defined by the leg used to kick a ball. The EMG polar plots of mean activation patterns in male and female participants as well as the Φ for each muscle are presented in Figure 10 and Figure 11. Independent of sex, the VL, VM, and LG had low group mean SIs (0.134, 0.142, and 0.163) which correspond to similar activation magnitudes in all directions and the circular activation patterns. The RF and TFL had moderate specificities, 0.210 and 0.236, meaning substantial activation was present in all directions but greater in the general anterior and lateral directions, respectively. The muscles with relatively high specificities, BF (0.379), ST (0.370), and MG (0.396), were primarily activated in the general directions of medial, posterior and anterior-medial, respectively, and little activation was present elsewhere. In addition, the BF and MG exhibited unexpected activation patterns in general directions opposite to their reported primary lines of action in Buchanan and Lloyd (1997). There was no significant difference between males and females with regards to Φ.

Table 1: Demographics of male and female participants.

Variables	Sex	Mean	SD	*p* Value
Age	Males	23.31	1.84	0.439
	Females	22.77	1.64	
Height (m)	Males	1.77	0.07	0.000*
	Females	1.67	0.05	
Weight (kg)	Males	79.35	11.19	0.000*
	Females	63.95	7.13	
BMI (kg/m^2)	Males	25.20	2.70	
	Females	22.82	2.02	

Abbreviations: Body mass index (BMI); standard deviation (SD). Asterisks (*) identifies significant differences at the p<0.05 level.

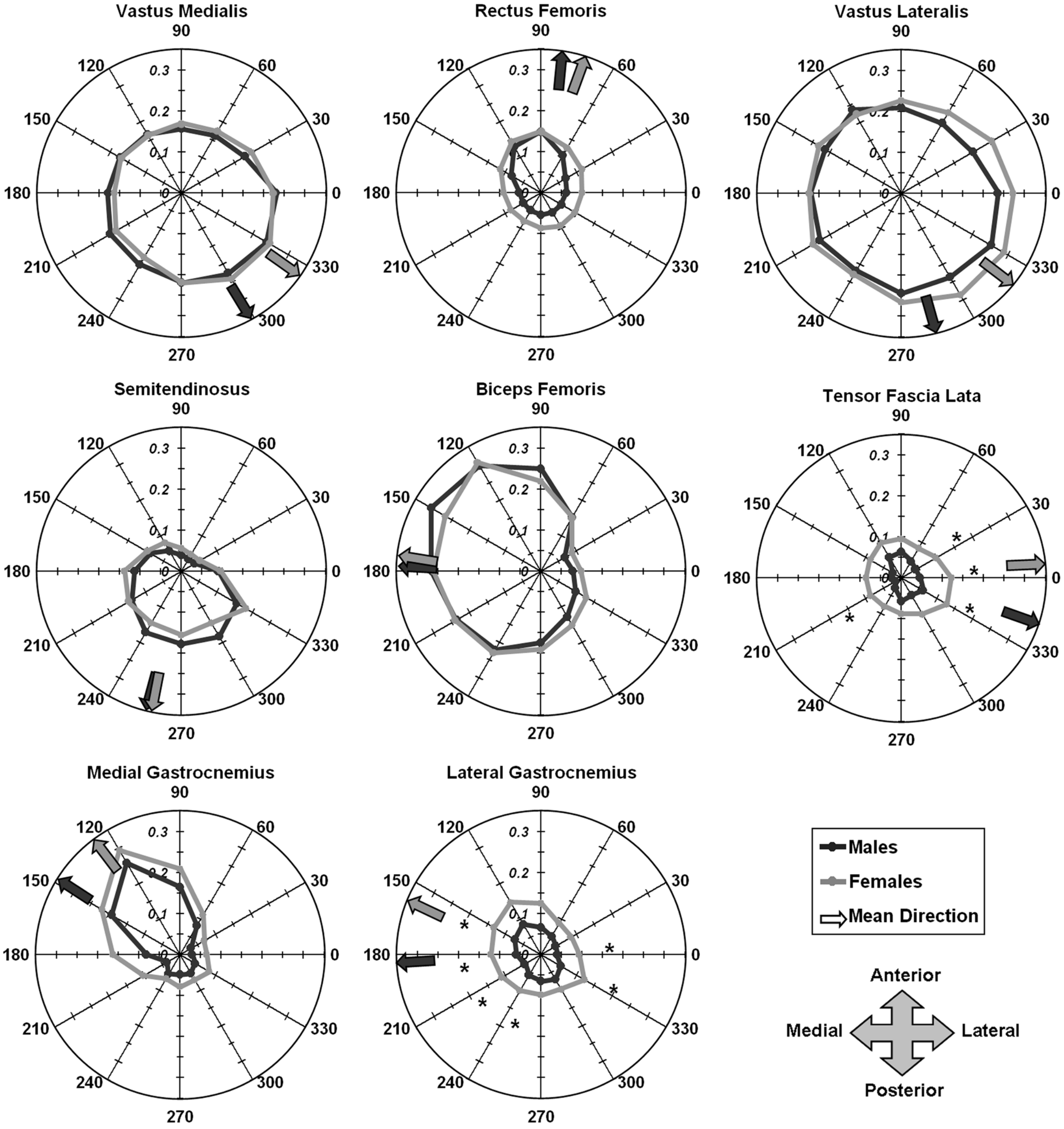

Figure 10: EMG polar plots of mean activation patterns in male and female participants. Outer numbers along the circular trajectory represent the target location angle in degrees. Inner numbers along each axis represent normalised EMG magnitude as a percentage of MVIC. Where the pattern on the target location's radius intersects represents the mean normalised EMG utilised to match that target. All plots are scaled from 0 to 0.35 (or 35% MVIC EMG level). Mean direction of muscle activation (Φ) of all eight muscles recorded for the males and females are indicated by the arrows along the circular trajectory. Asterisks (*) identify significant EMG magnitude differences for the identified target locations ($p<0.004$) between males and females.

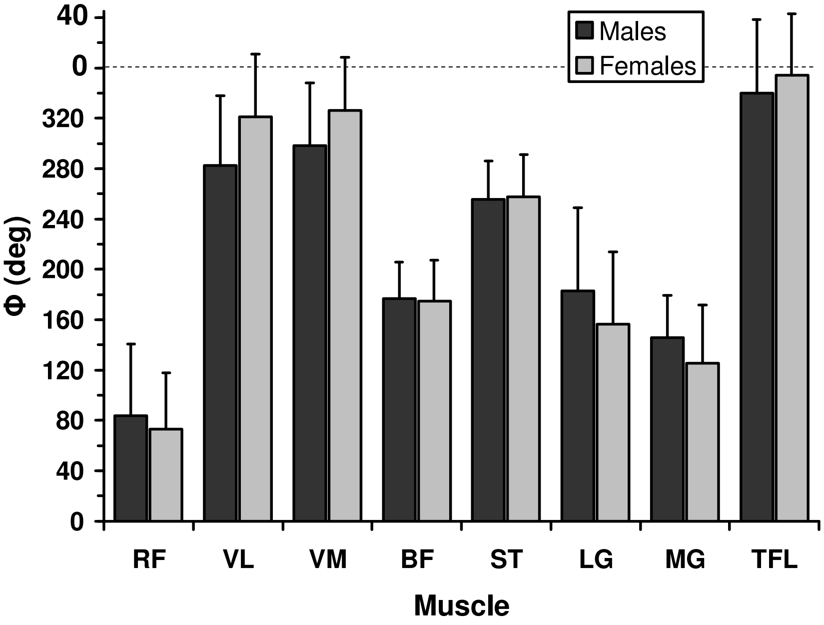

Figure 11: Mean direction of activation (Φ) of males and females. Error bars indicate standard deviation. Directions are in polar coordinates (0 to 360 degrees).

With regards to the X_{EMG}, the LG and TFL had significantly greater X_{EMG} in females compared to males (p=0.003 and p=0.002, respectively) (Figure 12). Post hoc tests revealed that females significantly activated their TFL and LG to a greater degree in the general lateral direction and medial and lateral directions, respectively, compared to males (Figure 10). The RF and MG exhibited noteworthy trends towards greater X_{EMG} in females compared to males ($p<0.10$). Furthermore, both the ST (p=0.025) and TFL (p=0.012) had significantly higher SIs in males compared to females (Figure 13).

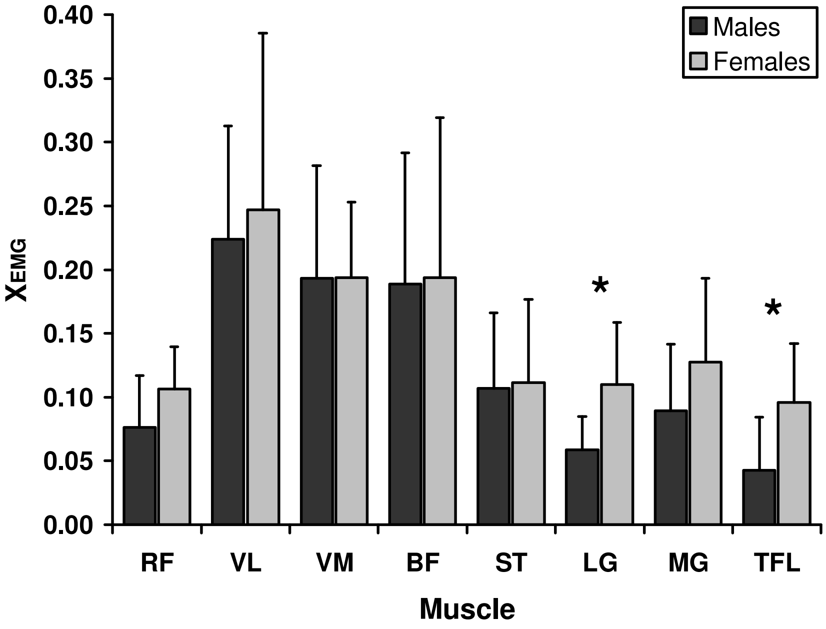

Figure 12: Mean resultant magnitude (X_{EMG}) of males and females. EMG values are normalised so they range from 0 to 1 with 1 being the maximal recorded EMG value for that muscle. Asterisks (*) identify muscles with a significant difference in X_{EMG} between sexes ($p<0.05$).

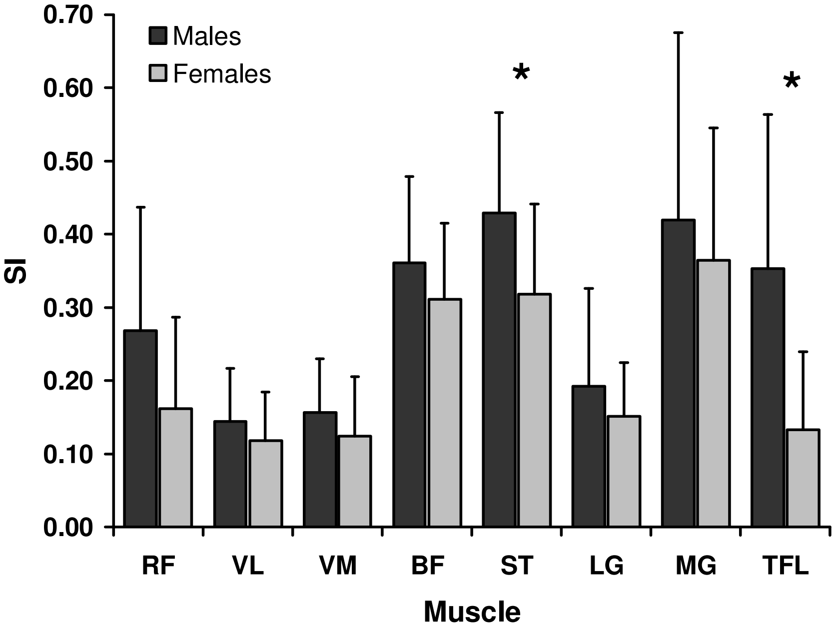

Figure 13: Mean specificity indices (SI) of males and females. Error bars indicate standard deviation. Asterisks (*) identify muscles with a significant difference in SI between sexes ($p<0.05$).

DISCUSSION

The aim of this study was to compare the voluntary knee muscle control strategies used by young healthy males and females during a CKC force target matching protocol. We did this by comparing the mean activation level of knee joint muscles used by males and females to generate normalised ground reaction forces in multiple directions. We then evaluated which muscles were most active in each direction and whether certain muscles had differing activation characteristics between males and females in response to the force generated and applied against the ground. It was hypothesised that females would have lower specificity and greater activation levels at every direction for the quadriceps muscles compared to males. Although the general activation patterns were similar and the Φ had no significant differences across sex, our results showed that females had significantly lower specificity of muscle action in the ST and TFL and a noteworthy trend towards significance for the RF: this is a direct result of broader activation patterns about the Φ as well as greater activity when the muscles act as an antagonist. In addition, females demonstrated significantly greater X_{EMG} for the LG and TFL compared to males, which for both muscles were significantly greater in the medial and lateral loading directions.

Our hypotheses were based on previous research that used a similar force matching protocol involving OKC loading (Krishnan et al., 2008; Krishnan & Williams, 2009). In these studies, females exhibited significantly lower SIs for the quadriceps and required a greater mean magnitude of VL and VM muscle activity than males to successfully match the targets. No significant differences in hamstring activation characteristics (SI, X_{EMG}, and Φ) were observed between sexes. The female characteristic of greater quadriceps activity compared to males has also been observed during dynamic functional tasks (Patten & Kamen, 2000;

Shultz et al., 2009; Sigward & Powers, 2006; Youdas et al., 2007). Our results however, contrast these studies and do not support our hypotheses. The observed differences in our results compared to the previous works may be attributed to a number of factors, including the statistical procedures used.

Firstly, Krishnan et al. (2008) evaluated group mean differences in the dependent variables (principle direction of muscle action, mean magnitude of muscle activity and SI) with a multivariate analysis of variance (MANOVA). This test was not appropriate when evaluating the principle direction of muscle action (the analogue of our Φ). The direction corresponds to an arbitrary zero-location so the use of traditional linear statistics is meaningless (Berens, 2009). Consider for example the EMG activity occurring at the 330º target location and the EMG activity occurring at 30º target location. The principle direction of muscle action would be linearly computed as 180º (pure medial), a direction opposite of the target locations rather than the true mean of 0º (pure lateral). Thus, the use of traditional linear statistics provides misleading results when analysing vector datum. In addition, the means and standard deviations of the principle direction of muscle actions were never reported in the previous study which leads one to question the validity of these results. In our study we evaluated group mean differences of Φ with the circular statistical test analogue of an independent T-test which is believed to provide more meaningful results.

Secondly, Krishnan et al. (2008) used dependent T-tests as post-hoc analysis for force direction. Yet there was no indication of an alpha adjustment for multiple related variables (i.e. number of force directions). This may have introduced type I error because the *p* value was too liberal. We were more conservative in our analysis and used a Bonferroni adjustment (alpha = 0.05/12 = 0.004) during our post hoc analysis. This way our results are more likely to represent true differences.

More importantly, on the other hand, are the modifications of the OKC force target matching protocol into a CKC protocol. Like other studies comparing OKC and CKC exercise (Escamilla et al., 1998; Shultz et al., 2009; Stensdotter et al., 2003), muscle recruitment and activation characteristics significantly varied between conditions. CKC exercise is deemed to be more apt at simulating lower limb kinematics and kinetics during functional activities (Escamilla et al., 1998; Schmitz et al., 2008; Shultz et al., 2009; Shultz et al., 2001).

When focus is not on the sex-related differences, our results as a whole presented grossly different patterns in comparison to the prior OKC target matching studies (Buchanan & Lloyd, 1997; Krishnan et al., 2008; Krishnan & Williams, 2009; Lloyd & Buchanan, 2001; Williams et al., 2003). Previously, a muscle's activation was generally thought to be dependent on its moment arm orientation, regardless of testing populations (Andriacchi et al., 1984; Buchanan & Lloyd, 1997). For example, the VL activation magnitude should have been the greatest during pure extension and decreased when a varus or valgus component was combined with extension. Negligible activation magnitudes should occur during pure varus/valgus or when a flexion component is involved. Accordingly, the VL as well as the other reported muscles should exhibit high specificities, indicating activation in one general direction. Compared to previous works (Krishnan et al., 2008; Williams et al., 2003), our muscle specificities were surprisingly much lower (averages of 0.36-0.76 versus 0.13-0.42) indicating that muscles crossing the knee joint are less likely to activate in one general direction during weight bearing tasks. For five of eight muscles (RF, VL, VM, LG, and MG), we found average extremely low SIs of 0.13 - 0.24 corresponding to their non-specific circular patterns and similar activation magnitudes in all directions.

Of the three muscles (BF, ST and MG) with the highest SIs (0.34, 0.37, and 0.39, respectively), the BF and the MG exhibited activation patterns that were *not* dependent on their moment arm's orientation. According to Buchanan and Lloyd (1997), the BF and MG's moment arm orientations at 50º knee flexion are 330º and 225º, respectively. Meaning, the BF and MG's activation patterns should occur about the posterolateral and posteromedial directions, respectively, that is if muscle activation is dependent on moment arm orientation. Yet our study yielded group mean Φ of 176º and 135º for the BF and MG, respectively. Meaning, the BF primarily activated when a medial loading component was present and the MG primarily activated with a combination of anterior and medial loading components.

According to the Linked-Segment Method of inverse dynamics, the forces acting at one end of a segment are equal and opposite to the forces acting at the other end of the segment. In our study, the medial and lateral loads could only be generated by hip abduction or adduction, respectively. Without opposition, these hip abductions and adductions would induce respective varus and valgus alignments at the knee joint; but since the subjects had to maintain constant joint positions, muscular activation must be present to balance this. We believe this opposing force is from the BF.

The sartorius and gracilis are the only muscles with substantial varus moment arms (Buchanan & Lloyd, 1997) but only contribute 5.5% of varus moment generation (Lloyd & Buchanan, 2001). The quadriceps and hamstring muscles possess components of varus moment arms as well (Buchanan & Lloyd, 1997) and is commonly thought that the cocontraction of these antagonist muscle pairs would provide sufficient support against a valgus load (da Fonseca et al., 2006; Lloyd & Buchanan, 2001; Zhang et al., 2001). However, the quadriceps-hamstrings cocontraction was calculated to only contribute 14% to a varus moment generation (Lloyd & Buchanan, 2001) and may not be efficient enough to stabilise the knee against the large valgus loads during functional activity (Kowalk et al., 1993). Furthermore, hamstring-quadriceps cocontraction is believed only to influence anterior tibial shear force and internal rotation and not even significantly affect the medial translation or valgus alignment (MacWilliams et al., 1999). Therefore, we believe that because the BF was activated primarily during medial loading, it opposed the hip adduction forces and prevented valgus alignment. Similarly, several studies have shown that the valgus alignment during weight bearing tasks (drop jumps or cutting) is largely dependent on the activation levels of the lateral hamstrings, lateral quadriceps and lateral gastrocnemius (Hanson et al., 2008; Palmieri-Smith et al., 2009; Shultz et al., 2009). Deficits in any or all of these muscles facilitated valgus deviation and external rotation (Hanson et al., 2008; Palmieri-Smith et al., 2009; Shultz et al., 2009) which is a major biomechanical factor in knee joint injury (McLean et al., 2005).

While the BF is suggested to be a general valgus stabiliser, the vastii muscles exhibited activation patterns and X_{EMG} grossly similar to each other and their circular activation patterns, with very low specificities (VL = 0.13 and VM = 0.14), suggests major stabilisation roles no matter which direction the knee joint was being loaded. In partial support of our results, studies show that there are no significant differences in the VM and VL EMG activity when an adduction or abduction factor is added to a CKC knee extension exercise (Earl et al., 2001; Hertel et al., 2004). However, greatest quadriceps activation is reported to be during planar knee extension (Hertel et al., 2004); so why the vastii muscles in our study demonstrated similar activation magnitudes during the anterior and posterior loading directions remains unknown and is unsupported by literature.

Regarding the gastrocnemius, we observed that the MG and LG’s activation patterns varied greatly which emphasises the importance of evaluating these muscles separately. The

LG, like the vastii muscles, is suggested to be a knee joint stabiliser, especially for females who significantly activated their LG greater than males in all directions. In contrast, the MG exhibited the highest muscle specificity in this study which occurred about the anterior-medial loading directions (SI = 0.39). Interestingly, this activity is opposite of its reported moment arm orientation (Buchanan & Lloyd, 1997) and there was little activity during posterior loading even though it is a flexor of the knee joint. This suggests a major stabilisation role during a knee extension moment generation.

The RF and ST had activation patterns, with higher muscle specificities (RF = 0.21 and ST = 0.37), about their reported moment arm orientations (Buchanan & Lloyd, 1997), suggesting their roles primarily consist of moment generation rather than joint stabilisation. In addition, the RF and ST demonstrated similar activation characteristics, Φ, and activation shapes to the previous OKC force matching studies (Buchanan & Lloyd, 1997; Krishnan et al., 2008; Krishnan & Williams, 2009; Lloyd & Buchanan, 2001; Williams et al., 2003). We suggest that the ST and RF's respective roles as flexion and extension moment generators are independent of kinetic chain condition. With that said, we found females to have significantly diminished specificity of their ST, suggesting that they had difficulty regulating a posterior force production (i.e. flexion). This in turn may affect the risk for knee joint injury because the ability to produce a flexion moment that opposes anterior tibial translation may be reduced.

Like all studies trying to isolate a complex system using simplified tasks, our study has limitations that warrant discussion. Firstly, we required participants to be involved in physical activity a minimum of two times a week, indifferent of activity type. Also no limitations were specified with respect to experience level or frequencies. This may account for the large statistical variability observed in our results because previous research has shown that knee muscle activation varies as a function of physical activity level (da Fonseca et al., 2006; Sigward & Powers, 2006) and physical activity type (Lattier et al., 2003).

Also, we excluded people with a history of prior knee injuries, thus introducing a potential selection bias such that those tested represented a population who have “found” successful neuromuscular control that allows them to maintain knee stability despite regular participation in activities that put them at risk for knee injuries. Thus, the application of our results to the understanding of mechanisms relating to knee joint injury may not be appropriate. However, the neuromuscular control strategies of healthy young males and females may be used as a baseline for future comparison to populations with joint instability, such as individuals with ACL ruptures. Identification of neuromuscular variations between young healthy males and females and these other populations may provide valuable insight into knee joint stability. Also, if information gained in this study and future studies utilising this protocol will provide valuable insight for the development of preventative and rehabilitative exercise programs for those at high risk of knee joint injury.

Furthermore, we only chose to examine eight muscles that cross the knee joint based on accessibility to surface EMG. Hence, any contribution to moment generation and corresponding support was assumed to be attributable to only these eight muscles. Although most research concentrates on the quadriceps and hamstring roles in knee function, research has shown that gluteal muscles, hip abductors and hip adductors are large determinants of femoral orientation (Geiser et al., 2010; Hanson et al., 2008; Hollman et al., 2009; Jacobs et al., 2007). Moreover, the smaller muscles: vastus intermedius, semimembranosus, short head of the biceps femoris, sartorius, or gracilis, have been shown to have significant contributions to moment generation (Buchanan et al., 1997) but are only accessible through indwelling EMG. Examination of a more complete set of the muscles acting at the knee joint and those contributing to the bony orientations would help provide a more accurate picture of the muscle activation strategies used by males and females.

Lastly, we recorded EMG at 30% maximum voluntary effort during the target calibration trials. This level was chosen based on recommendations made by DeLuca (1997) to ensure adequate motor unit recruitment that aptly represented whole muscle activations. Some argue that recruitment patterns alter when greater isometric force is needed while others report that the same muscle activations patterns occur but just with greater magnitudes for both knee and elbow muscles (Buchanan et al., 1986; Cioni et al., 1994; Krishnan & Williams, 2009; Levin et al., 2003). The effect of increasing activation levels with our protocol warrants further investigation.

Conclusion

Dynamic instability of the knee joint is associated with neuromuscular deficits (Hirokawa et al., 1991; Hortobagyi & DeVita, 2000; Pauda et al., 2006; Williams et al., 2001; Wojtys et al., 2002). It is important to understand how muscles interact in response to varied joint loading conditions. Analysing sex differences in neuromuscular control may provide insight into why females are more prone to functional disability and in turn shed light into injury and degenerative mechanisms as they relate to neuromuscular function. Considering the results of this study, voluntary muscle control strategies about the knee joint are far more complex than previously theorised. We have demonstrated that the hamstrings, quadriceps and gastrocnemius muscles do not function as a group and we emphasise the need to assess muscles independently. We have also demonstrated that females activate their knee lateral gastrocnemius and tensor fascia lata muscles to a greater degree in order to achieve a force target of equal difficulty in various directions about a horizontal plane. Our results differ from previous OKC research thus emphasising the importance of evaluating muscle function during CKC loading when studying knee joint stabilisation and injury mechanisms. Furthermore, we have identified that certain muscles act predominately as joint stabiliser (VL, VM and LG) and others act as moment generators (RF and ST) while the MG's role in knee joint stability is opposite of what was theorised. These stabilisers seem to act to increase joint compressive forces and create a stable mechanical system from which the moment generators can initiate directed forces.

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ARTICLE 2: MUSCULAR COCONTRACTION ABOUT THE KNEE JOINT IN HEALTHY YOUNG ADULTS DURING DIRECTIONAL CLOSED KINETIC CHAIN LOADING

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Running Title: Knee Muscle Cocontraction in Males and Females

Abstract

An individual's risk for knee joint injury is proposed to decrease when cocontraction of antagonist muscle groups is present such that the stabilisation forces acting across the joint are increased. The purpose of this study was to evaluate the sex-related differences in cocontraction during a force target matching protocol. Twenty-six healthy young adults (13 male, 13 female) stood with their dominant leg in a boot fixed to a force platform. Subjects were required to position a cursor (projected on a video screen) over a target and maintain the position for one second. To control the cursor, loads were applied against the force platform with their dominant leg to produce various combinations of anterior-posterior, medial-lateral loads in the horizontal plane while maintaining constant inferior-superior loads. A successful target match required a normalised force magnitude of equal effort for each subject and target location which triggered the recording of electromyography (EMG) for seven muscles crossing the knee joint. EMG was normalised to percent maximum voluntary isometric contraction. Muscular cocontraction was quantified at each target location using a cocontraction index (CI) between antagonist muscle groups crossing the knee joint. A two-way ANOVA determined that females had significantly higher cocontraction of the vastus lateralis and lateral gastrocnemius ($p= 0.001$) and a trend ($p<0.10$) towards increased activation of the summed quadriceps and gastrocnemius muscles. These results suggest that the gastrocnemius muscle plays an important role in knee joint stabilisation. Future research is needed to better understand its behaviour and provide important insight to knee injury prevention and rehabilitation.

Key words: sex differences, closed kinetic chain, cocontraction, knee joint, stability

INTRODUCTION

The passive structures of the knee such as bones, ligaments, menisci, cartilage, and capsule, alone only provide sufficient joint stability in modest loading conditions (Williams et al., 2001). The addition of muscular force is therefore needed to control the applied external loads and maintain safe joint movement (Hirokawa et al., 1991).

Previously evaluations of neuromuscular function with respect to knee joint stabilisation have typically involved functional task assessments (Hortobagyi et al., 2004; Hortobagyi et al., 2001; Patten & Kamen, 2000). However, due to an anatomically complex system, the biomechanical and neuromuscular contributions to force generation are dependent on one another so it may not be viable to assume that the neuromuscular results are directly representative of isolated muscle function (Williams et al., 2003). Our protocol restricts the kinematic contributions (movement velocity/acceleration, joint angle, contraction type, contraction velocity etc) to force generation where subjects are required to produce isometric ground reaction forces while maintaining standardised joint angles. Multiple ground reaction forces are produced in various directions in the horizontal plane. In doing so, we can identify individual contributions of muscles to knee joint stabilisation because when a person's foot contacts the ground because the ground reaction force is equal and opposite to the force applied by the person. The person then contracts their muscles in order to stabilise the joint and regulate the force transmitted to the knee.

Research indicates that females are at a much higher risk for anterior cruciate ligament (ACL) rupture (Gwinn et al., 2000). Although this discrepancy is most likely multifactorial, the biomechanical and neuromuscular contributions to injury may provide the greatest clinical implications because they are believed to be the most modifiable non-invasive factors related to movement. Research on sex-related differences in neuromuscular control indicates that females activate their quadriceps muscles to a greater degree in both static and dynamic tasks (Pauda et al., 2006; Shultz et al., 2009; Sigward & Powers, 2006; Urabe et al., 2005; Youdas et al., 2007). This increase is thought to pre-dispose females to greater anterior tibial translation, thus exposing the supporting soft tissues, especially the ACL, to greater strain and increasing risk of injury (Fleming, Renstrom, Beynnon et al., 2001; Palmieri-Smith et al., 2009; Sigward & Powers, 2006).

Females have been reported by Krishnan et al. (2008) and Pauda et al. (2006) to accompany the greater quadriceps activation levels with greater hamstring activation levels such that cocontraction of antagonist muscle groups is present more so than their male counterparts. These simultaneous contractions of muscles spanning both sides of the joint is said to increase joint stiffness under shear and torsional loading by driving the femoral condyles deeper into the tibial plateau (Baratta et al., 1988; Markolf et al., 1976). Therefore, according to Krishnan et al. (2008) and Pauda et al. (2006) females should have a reduced susceptibility to joint injury. However, other studies argue that there are no sex-related differences in hamstring activation levels during either static or dynamic tasks (da Fonseca et al., 2006; Kong & Burns, 2010) or greater cocontraction is present in females but it is not evenly balanced across the joint; instead, females have greater quadriceps-hamstrings coactivation on the lateral side compared to the medial (Palmieri-Smith et al., 2009) which instead increases the risk of injury. The current contradicting evidence demonstrates the need for further investigation of sex-related differences in neuromuscular control strategies.

Therefore the purpose of this study was to evaluate sex-related differences in knee joint cocontraction of antagonist muscle pairs during isometric CKC direction-dependant loading. Based on the findings by Krishnan and Williams (2009) it was hypothesised that females would significantly cocontract their quadriceps and hamstring muscles to a greater degree than males in all loading directions. No research hypothesis was formulated for sex-related differences in the quadriceps and gastrocnemius cocontraction magnitude because lack of supporting literature.

METHODS

Participants

Twenty-six (13 males, 13 females) healthy active (defined by participation in exercise or sport at least two times a week) young adults (mean age of 23.0 ± 1.8 years) participated in this study. A power analysis (G*Power 3.1.0) on our primary outcome measure (sex differences in mean magnitude of muscle activation for the biceps femoris (mean ± standard deviation): males = 0.16 ± 0.08, females = 0.20 ± 0.12) was used to determine that this sample size was effective enough to test the research hypotheses (alpha ≤ 0.05, power $\geq$ 0.80). Note that 33 participants were recruited for this study but five were unable to complete the practice trials and data collection was not completed for 2 other participants. Prior history of a significant knee or lower limb injury, such as a ligament rupture; knee surgery; a lower limb sprain, muscle or tendon injury, or fracture within six months of participation; a lower extremity motor nerve lesion, the presence of a knee joint effusion, muscle atrophy, diabetes, or any other observed physical impairment that could affect the results of the study excluded the participant for the study. An informed consent form, approved by the University of Ottawa Ethics Committee, was read and signed by all participants before data collection.

Experimental Protocol

An adapted target matching protocol from Buchanan and colleagues (1986) was developed by our research team to examine the participant's muscle activation characteristics while isometrically applying a closed kinetic chain load against a force platform in multiple directions in the horizontal plane. Learning effects were minimised and by performing a minimum of six practice target matching trials which also ensured the participant could perform the task. Once practice was completed, bipolar surface EMG electrodes (SP-E04, DE 2.1, Delsys Inc., Boston, MA) were placed according to recommendations made by SENIAM (Hermens et al., 1999) and DeLuca (1997) on eight muscles of each leg: rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), semitendinosus (ST), biceps femoris (BF), medial gastrocnemius (MG), and lateral gastrocnemius (LG) of each leg. Electrodes were then connected to a 16-channel EMG system (DS-B04, Bagnoli-16, Delsys Inc., Boston, MA) that sampled the signal at EMG at 1000 Hz, amplified by a gain of 1000, and band-pass filtered at 20-450 Hz using a 16-bit A/D conversion board (NI PCI 6229, National Instruments Corp., Austin, TX). Subjects then performed a five minute warm-up on a Monarch stationary bike (speed of 90 repetitions per minute with no resistance). Maximal EMG (Max_{EMG}) activity was collected using an isokinetic dynamometer (850-000, Biodex, New York, USA) and analysed with custom made software (Labview 8.20, National Instruments Corp., Austin, TX, USA). Plantar flexion, knee extension, and knee flexion were recorded at relaxed hip, knee, and ankle joint angles of 90º, 30º, and 10º, respectively. Hip abduction was recorded in standing position. Participants were provided 10 seconds, with verbal encouragement by researchers, to scale perceived force from a zero level to their maximal and hold it for approximately three seconds.

For the target matching protocol, participants stood with dominant leg (defined by leg used to kick a ball as far as possible) joint angles of approximately 30º, 30º, and 10º flexion for the hip, knee, and ankle, respectively. The dominant foot was fixed in a water-ski boot (Bio, O'Brien, Redmond, WA, USA) attached to a force platform (FP4060-08, Bertec Corporation, Columbus, OH, USA) (Figure 14A). The other/support foot was atop another force platform situated adjacent to and slightly posterior to the test plate and its position was marked with tape. Participants were instructed before and during the experiment to try and maintain this orientation as best as possible throughout testing.

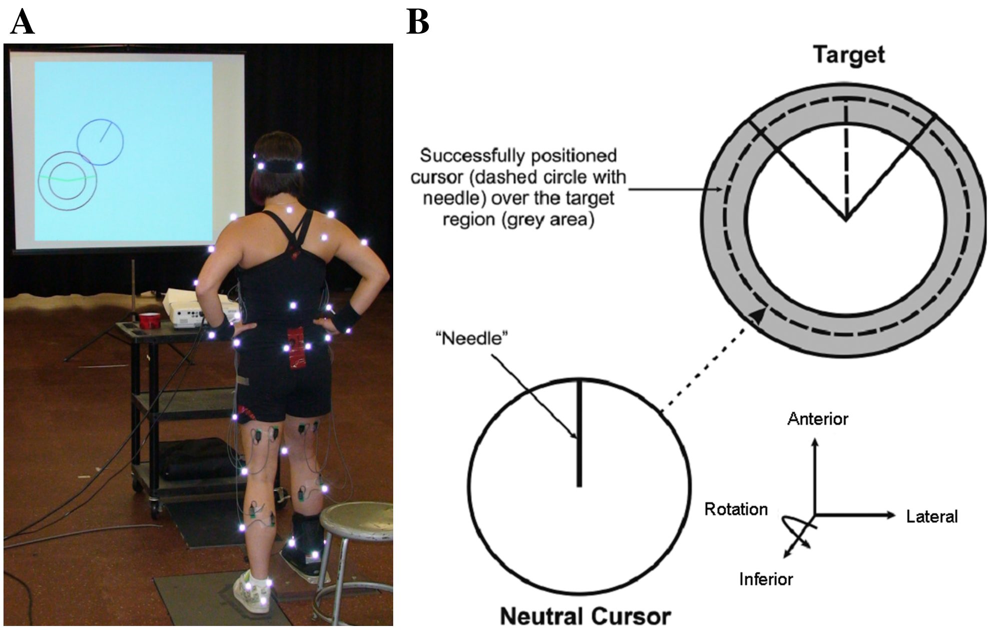

Figure 14: A) Laboratory setup and participant standing with dominant foot on the testing force platform, fixed in a wakeboard boot. Live visual feedback from the projector showed the participant's current force production direction and magnitude. B) Depiction of the cursor, target, and successful positioning of the cursor over the target.

Biofeedback of the direction and magnitude of the dominant leg's applied force against the force platform with a projected image of a cursor on a video screen placed in front of the participant (Figure 14A). The cursor was controlled 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, 3) inferior/superior loads (force along the ±z axis) made the cursor smaller/larger (controlling the percent body weight applied by the leg). The participant was required to position the cursor over a projected target and maintain its position for one second (Figure 14B). The successful match triggered data collection for the one second of target matching and three seconds prior to the target match (pre-match) using a custom made Matlab (2007b, Mathworks, Natick, MA, USA) application. This application was also used for biofeedback and defines experimental parameters. After a minimum 30 second relax period, a new target in an alternate location appeared. Targets randomly appeared one at-a-time at 12 different locations, evenly spaced by 30º, about a circular trajectory which was representative of the anterior-posterior-medial-lateral horizontal plane of the force platform (Figure 15). Each target location had to be matched three times for a total of 36 trials.

Maximal effort trials required subjects to exert a maximally perceived force in the anterior/posterior (±y) and medial/lateral (±x) directions in order to normalise the magnitude of force required to reach the target locations to 30% of peak loads (see Krishnan et al. (2008) for a discussion on the resultant force calculations). This load level was chosen to ensure adequate motor unit recruitment where surface EMG aptly represented whole muscle activation (DeLuca, 1997). The inferior load required to maintain the cursor over the target was set at 50% ± 5% of body weight.

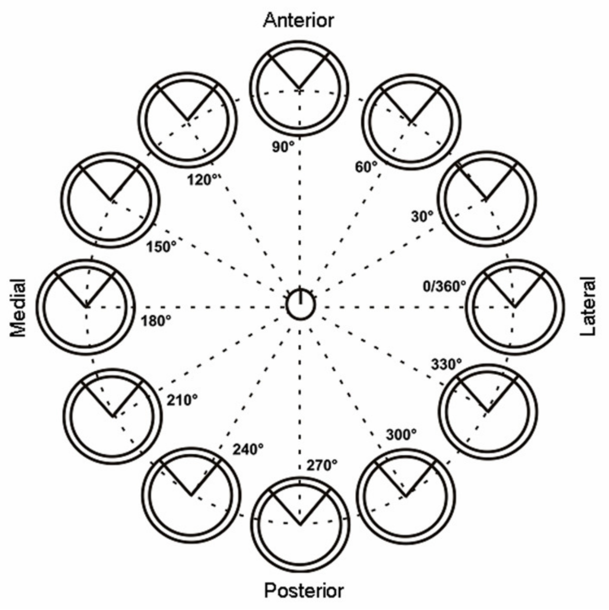

Figure 15: The target locations used in the target matching protocol: 12 targets, evenly spaced by 30º about a circular trajectory which was representative of the anterior-posterior-medial-lateral axes of the horizontal plane.

Data Processing

The EMG signals were conditioned with a bias removal, full wave rectification and a 4th order Butterworth dual-pass low-pass filter at 6 Hz to obtain a linear envelope. The Max_{EMG} signals were conditioned with a custom made application written in Labview 8.20 and the Target Match data was processed with a custom made Matlab 2007b program. Only the one second of successful target match EMG data was analysed in this study and was normalised to percent Max_{EMG}. Each trial's normalised EMG amplitude was averaged over this second and then ensemble averaged for each target location's three repetitions. These steps were carried out for each muscle group.

Muscular cocontraction was quantified at each target location using a cocontraction index (CI). Cocontraction was defined as the simultaneous activation of antagonistic muscles. The CI was calculated for the vastus lateralis and biceps femoris (VL-BF), vastus medialis and semitendinosus (VM-ST), vastus lateralis and lateral gastrocnemius (VL-LG), vastus medialis and medial gastrocnemius (VM-MG), as well as a general CI of the summed quadriceps and hamstrings muscle activity (QUADS-HAMS) and the summed gastrocnemius and quadriceps muscle activity (QUADS-GASTROC). The CI was defined as the ratio of the antagonist's summed activation and the agonist's summed activation at each target direction multiplied by the sum of activity in both muscles. This equation has been previously used and described by Rudolph et al. (2001), Lewek et al. (2005), and Palmieri-Smith et al. (2009):

$$\text{CI} = (\text{lower EMGi}/\ \text{higher EMGi}) \bullet (\text{lower EMGi} + \text{higher EMGi}) \qquad (5)$$

EMGi referred to the muscle or muscle group's normalised EMG magnitude. The muscle or group that had the greater level of activation was chosen to be the divisor (higher EMGi). A CI of 0 corresponded to no cocontraction of the antagonist muscle pairs such that the muscle with the lower EMGi value was not activated at all (0% maximum EMG values). A CI of 2 meant that both muscles were equally activated to 100% of maximum EMG values.

Statistical Analysis

SPSS (version 15.0) software was used for the statistical analyses. The significance level was set at alpha (α) ≤ 0.05. A two way within-between factorial (2x12) analysis of variance (ANOVA) was used to test for significant sex-related and directional effects on cocontraction for each synergistic muscle pair. When a significant main effect for sex or interaction of sex and direction on CI was reported, appropriate post hoc evaluation using independent T-tests were conducted to determine at what location the CI significantly differed across sex. Alpha was adjusted to 0.004 using a Bonferroni correction to protect against type I error due to the 12 related measures of force target locations.

RESULTS

Subject characteristics are in **Table 2**. One subject was left leg dominant as defined by the leg used to kick a ball.

Table 2: Demographics of male and female participants.

Variables	Sex	Mean	SD	*p* Value
Age	Males	23.31	1.84	0.439
	Females	22.77	1.64	
Height (m)	Males	1.77	0.07	0.000*
	Females	1.67	0.05	
Weight (kg)	Males	79.35	11.19	0.000*
	Females	63.95	7.13	
BMI (kg/m^2)	Males	25.20	2.70	
	Females	22.82	2.02	

Abbreviations: Body mass index (BMI); standard deviation (SD). Asterisks (*) identifies significant differences at the $p<0.05$ level.

We examined whether the CI value significantly differed as a result of sex and target direction. Both males and females displayed similar cocontraction patterns throughout the range of target locations (Figure 16). For all muscle pairs, females displayed greater mean CIs at all locations except for the VM-ST muscle pair (Figure 16). However, only the VL-LG pair showed significant main effects for sex on CI ($p= 0.001$) (Table 3). Specifically, females increased VL-LG cocontraction when generating forces at 0º ($p<0.0005$), 30º ($p= 0.001$), 90º ($p= 0.001$), 150º ($p= 0.004$), 210º ($p= 0.003$), and 330º ($p=0.003$) (i.e. during the general medial, lateral and anterior loading directions) (Figure 16). In addition, a noteworthy trend towards significantly greater QUADS-GASTROCS cocontraction in females compared to males was observed at the $p<0.10$ level. There was no significant interaction between target direction and sex for all antagonistic muscle pairs (Table 3).

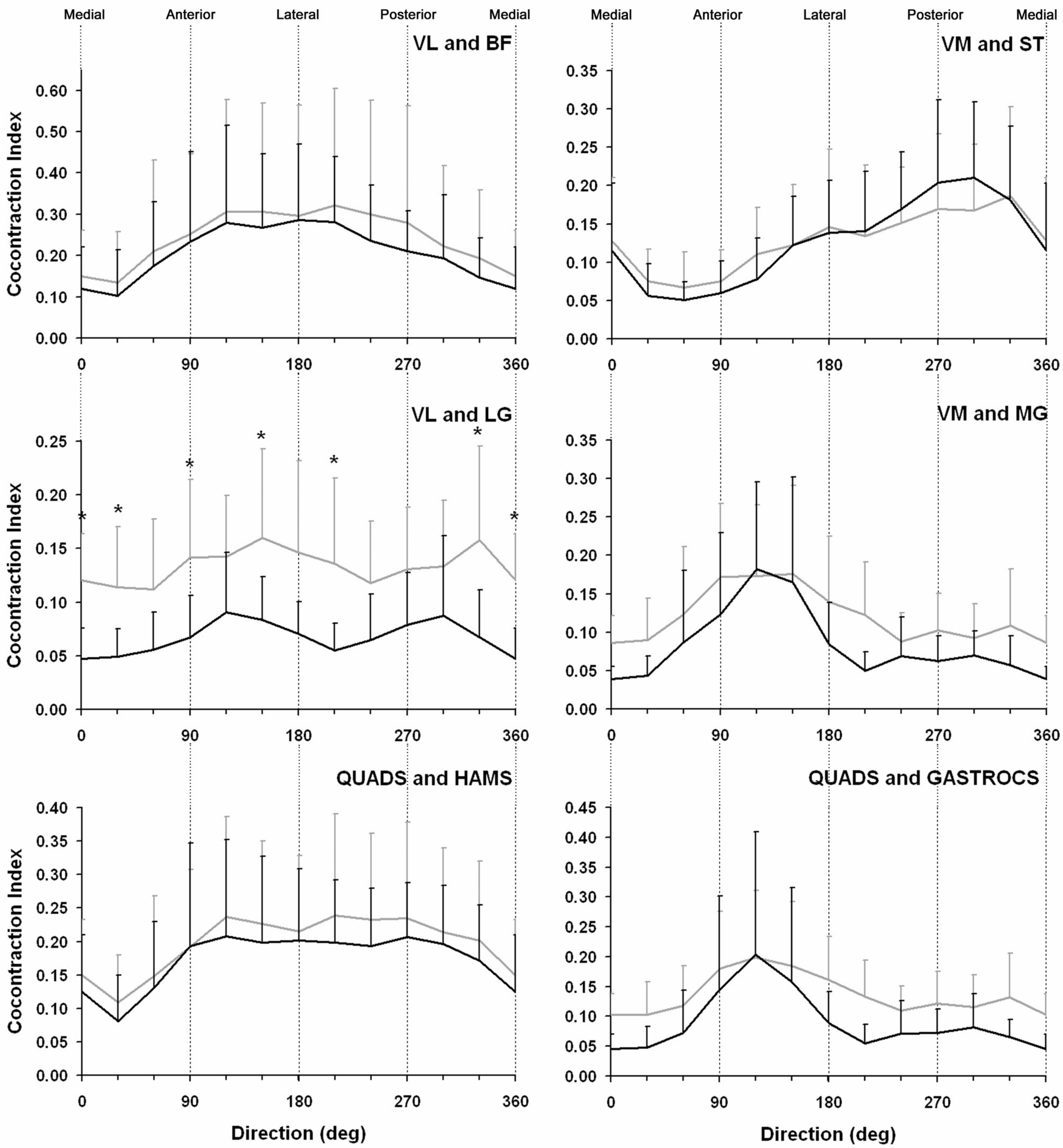

Figure 16: The cocontraction index (CI) of agonist muscle pairs displayed in line graphs for both males (dark grey) and females (light grey). The numbers along the x-axis corresponds to the target's location (circle direction angle in degrees from 0-360). Asterisks (*) identify locations where the antagonist muscles pair's CI significantly differs between sexes ($p<0.05$).

Table 3: Statistical significance of main effects of sex and target direction on cocontraction index (CI). A two way within-between factorial analysis of variance (ANOVA) was used to determine the reported *p* values.

	Main Effect of Direction	**Direction x Sex**	**Main Effect of Sex**
QUADS & HAMS	0.000*	0.823	0.407
QUADS & GASTROCS	0.000*	0.459	0.070
VL & BF	0.000*	0.712	0.409
VM & ST	0.000*	0.417	0.893
VL & LG	0.007*	0.518	0.001*
VM & MG	0.000*	0.545	0.145

Abbreviations: Rectus femoris (RF); vastus lateralis (VL); vastus medialis (VM); biceps femoris (BF); semitendinosus (ST); lateral gastrocnemius (LG); medial gastrocnemius (MG); tensor fascia lata (TFL). Asterisks (*) identifies significant differences (p<0.05).

DISCUSSION

Dynamic instability of the knee joint is associated with neuromuscular deficits (Hirokawa et al., 1991; Hortobagyi & DeVita, 2000; Pauda et al., 2006; Williams et al., 2001; Wojtys et al., 2002). It is important to understand how muscles interact in response to varied joint loading conditions. Since cocontraction is proposed to decrease an individual's risk for knee joint injury by increasing the stabilisation forces, analysing the degree of cocontraction as a function of sex may provide more insight into why females are more prone to knee joint injury. The aim of this study was to evaluate the level of coactivation, quantified with a CI, of muscles acting on the knee joint in young healthy male and female adults during a CKC force target matching protocol. It was hypothesised that females, with no prior history of knee joint injury, would exhibit a significantly greater level of cocontraction between the quadriceps and hamstrings antagonist muscle pairs (VL-BF, VM-ST, QUADS-HAMS). Our results, however, do not support our hypothesis. No significant differences in cocontraction were observed for the quadriceps and hamstrings antagonist muscle pairs (VL-BF, VM-ST, QUADS-HAMS) or the VM-MG. Instead, females significantly cocontracted their VL and LG muscles to a greater degree than their male counterparts.

Previous work by Lloyd and Buchanan (Lloyd & Buchanan, 2001) evaluated the muscular strategies of healthy young adults while producing various combinations of flexion-extension-varus-valgus moments. The subjects were seated with standardised hip and knee angles but the foot was unfixed and the knee joint was uncompressed from bodyweight or gravity. They discovered that cocontraction of the hamstrings and quadriceps muscles increased with an increase in magnitude of varus and valgus moments and that this cocontraction contributed the most to generating these moments.

However, when considering our results as a whole (i.e. not focusing on the sex-related differences but rather considering the males and females as one group), QUADS-HAMS CI is high in anterior, posterior and lateral loading directions and low during medial loading conditions. In our study, loads applied in the general medial and lateral directions were mainly generated by hip abduction or adduction, respectively. If muscular contractions are not present elsewhere to oppose the hip’s moments, then a deviation in the frontal plane would result at the knee joint. Yet, the subjects were required to maintain constant joint positions; thus muscular activation must have been present to balance this and prevent varus or valgus alignment. Since a primary mechanism for knee joint injury occurs during valgus alignment (McLean et al., 2005), we postulate that the lack of QUADS-HAMS cocontraction during medial loading may be a contributing factor to knee joint injury, while greater cocontraction in all other loading directions provides sufficient stabilisation forces.

Based on our observation that no significant difference between sexes for QUADS-HAMS cocontraction occurs during our CKC loading condition, it is less likely that QUADS-

HAMS cocontraction ratios are a contributing factor to the higher rates of knee joint injury in females compared to males. Instead, our results indicate that the significantly greater VL-LG cocontraction observed in females should be explored further as a contributing factor to ACL injury. Firstly, females' preferential activation of the lateral musculature indicates quadriceps-gastrocnemius cocontraction imbalance from the medial to lateral side. Although no known evidence exists on quadriceps-gastrocnemius cocontraction imbalance, Landry et al. (2007) found that females exhibit a mediolateral gastrocnemius activation imbalance that was not present in male athletes during early stance to midstance of side-cuts. In addition, Palimieri-Smith et al. (2009) evaluated the muscle activation patterns during a landing task and observed a predominant lateral coactivation in females of the quadriceps and hamstring muscles. Interestingly, this increased activation was correlated to higher knee abduction moments. The unbalanced activation strategy was suggested by Palimieri-Smith et al. (2009) to increases one's risk for an ACL rupture. Greater activation of the lateral muscles in females compared to males is also supported by earlier research involving tasks mimicking ACL injury-risk positions (Rozzi et al., 1999).

Secondly, due to its geometrical orientation, the gastrocnemius is proposed by O'Conner (1993) to cause a relative anterior tibial translation throughout the entire flexion-extension range of motion. Elias et al. (Elias et al., 2003) observed these relative anterior tibial translations when an isolated gastronomies load was applied to the knee joint in an in vitro cadaver study. Similar translations also resulted when the gastrocnemius load was accompanied by different combinations of anterior tibial loads and ACL status (intact versus cut); thus suggesting the gastrocnemius to be an ACL antagonist.

Note also that when in vivo ACL strain response from isolated contractions of the gastrocnemius was evaluated by Fleming et al. (2001), the greatest contribution of the gastrocnemius to ACL strain occurred at 5º and 15º knee flexion producing strains of 2.8% and 3.5%, respectively, however, no strain occurred over 30º knee flexion. The addition of a quadriceps contraction to the gastrocnemius contraction then significantly increased the ACL strain compared to isolated gastrocnemius cocontractions, while the addition of a hamstrings contraction did not. Furthermore, ACL deficient individuals have been reported to have varied gastrocnemius activity relative to their contralateral limb during gait (Benoit et al., 2003). Rudolph et al. (2001) found that ACL-injured non-copers (individuals with a ruptured ACL who cannot effectively stabilise their knees during activities involving hard cutting and pivoting or during activities of daily living) demonstrated significantly greater gastrocnemius activity during walking. It was also observed that non-copers had greater cocontraction of the gastrocnemius and quadriceps during jogging compared to copers and non-injured controls (Rudolph et al., 2001).

Collectively, the above studies and our results indicate that the gastrocnemius has different activation in subjects with (1) unstable knees (i.e. ACL injured) and (2) females (who have a higher incidence of ACL injuries). These results emphasise the importance of evaluating the gastrocnemius' role in knee joint stability and may help explain the sex-related discrepancy in injury rates (Gwinn et al., 2000).

Although great care was taken to control all aspects of the study, some limitations exist. Firstly, participants were required to be involved in physical activity a minimum of two times a week. However, no restrictions were specified with respect to physical activity type, experience level or frequencies. This may account for the large statistical variability observed in our results because previous research has shown that knee muscle activation varies as a function of physical activity level (da Fonseca et al., 2006; Sigward & Powers, 2006) and physical activity type (Lattier et al., 2003).

Secondly, a selection bias may have been introduced because only individuals with no prior history of knee injuries were permitted to participate. Our testing population thus consisted of those with neuromuscular activation strategies that allow them to effectively regulate the forces across the knee joint and prevent excessive loading of the soft tissues. Nevertheless, our results of healthy young males and females may be used for future comparison to populations with joint instability, such as individuals with ACL ruptures. With this, valuable insight into knee joint stability may be provided when discrepancies between injured and healthy populations are evaluated.

Thirdly, it has been reported that as the effort level increases the same muscle activation patterns occur but just with greater magnitudes for both knee and elbow muscles at higher levels (Buchanan et al., 1986; Cioni et al., 1994; Krishnan & Williams, 2009; Levin et al., 2003). However, we cannot extrapolate our results to higher magnitudes of ground reaction forces and their corresponding muscle activation levels. We recorded EMG at a single effort level of 30% maximum voluntary applied ground reaction forces which was opted to ensure that motor unit recruitment accurately represented whole muscle activations and to prevent fatigue (DeLuca, 1997). The effect of increasing activation levels with our protocol deserves further investigation.

Finally, we assumed that any contribution to moment generation was caused only by the seven muscles recorded. Research has shown that the vastus intermedius, semimembranosus, short head of the biceps femoris, sartorius, or gracilis significantly contribute to moment generation (Buchanan et al., 1996) but are only accessible through indwelling EMG. Also, gluteal muscles, hip abductors and hip adductors are large determinants of femoral orientation which may affect muscular activation patterns (Geiser et al., 2010; Hanson et al., 2008; Hollman et al., 2009; Jacobs et al., 2007). A full evaluation of the muscles acting at the knee joint and those contributing to the femoral and tibial orientations is needed to provide the most accurate insight into the muscle activation strategies used by males and females.

Conclusion

Previous research evaluating sex-related active knee joint stabilisation is limited to dynamic trials (DeMont & Lephart, 2004; Landry et al., 2007). Although females have demonstrated greater activity in both the gastrocnemius and quadriceps muscles, it is difficult to uncouple its role in knee joint stabilisation from the role of moment generation at the ankle. In addition, the gastrocnemius is generally not investigated in studies about active knee joint stabilisation. The studies that do include it as a variable generally evaluate one muscle head, sum the activity of both to equate a resultant gastrocnemius activity, or fail to discuss the varied roles in knee stabilisation (Benoit et al., 2003; Elias et al., 2003; Fleming, Renstrom, Ohlen et al., 2001; O'Conner, 1993; Rudolph et al., 2001). Our study emphasises the importance of evaluating the role of both the lateral and medial heads of gastrocnemius as they relate to knee joint stability during CKC loading. We have demonstrated that females and males do not differ in hamstring-quadriceps cocontraction but rather females significantly increase cocontraction of their VL and LG muscles compared to their male counterparts. We suggest that the greater cocontraction of the VL and LG alters joint stability and increases one’s risk for joint injury by means of anterior tibial translation. Strong evidence exists that the gastrocnemius plays a substantial role in joint stability; however, more research is needed to further understand this role during functional tasks and its interaction with hamstrings and quadriceps activation.

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General Discussion and Conclusion

Joint instability is associated with an inappropriate modulation of forces crossing the knee and has been linked to aging, individuals who develop OA, and females who are statistically more prone to knee injuries then males (Rudolph et al., 2007; Sharma et al., 1999). Identification of neuromuscular variations between young healthy males and females, older healthy males and females, as well as in an OA population may provide valuable insight into knee joint instability. Assessments of a young healthy adult population are however needed first to understand how muscles formerly interact in response to varied joint loading conditions. Previous evaluations of neuromuscular control have typically involved dynamic tasks (Hortobagyi et al., 2004; Hortobagyi et al., 2001; Patten & Kamen, 2000) such that the neuromuscular contribution to movement is dependant on the kinematics. Consequently, interpretation of these results are made difficult due to the changing conditions (i.e.: joint angle, muscle length, moment arms, velocity of contractions, etc.) which all affect muscle performance and thus make the contributions of individual muscles difficult to identify.

The purpose of this study was to evaluate sex-related differences in neuromuscular activity about the healthy knee joint during a CKC isometric force matching protocol. Subjects were required to actively produce ground reaction forces in multiple directions in the horizontal plane and the EMG recordings were evaluated. With this approach, we were able to identify specific contributions of individual muscles to force generation because the biomechanical influences of kinematic variable were limited.. According to Newtonian physics, the force exerted by the subject during dynamic tasks is equal and opposite to the force exerted by the ground to the subject. The subject responds to these forces by contracting knee joint musculature to stabilise the joint and accommodate these forces. In turn, our results have the potential to provide insight into knee joint stabilisation strategies. However, more research and further development of this protocol is warranted to determine the validity of our results to functional tasks. It was hypothesised that females would have significantly lower specificity and greater activation levels at every direction for the quadriceps muscles compared to males as well as a significantly greater level of cocontraction between the quadriceps and hamstrings antagonist muscle pairs. However, our findings did not support our hypothesis.

This study demonstrated that females activate their knee muscles to a greater degree in order to achieve a normalised force target in various directions about a horizontal plane. The LG and TFL had greater activation magnitudes and the ST and the TFL was less specific in activation as a function of loading direction in females compared to males. Furthermore, it was revealed that females and males do not differ in hamstring-quadriceps cocontraction but rather females significantly cocontract their lateral quadriceps and gastrocnemius muscles to a greater degree than their male counterparts. We suggest that this cocontraction facilitates anterior tibial translation in females and may be associated with the increased risk for knee joint injury as compared to their male counterparts.

Our results also varied from previous research that utilised a similar force target matching protocol. Instead of individual muscle activation being dependent on moment arm orientation our results showed that voluntary muscle control strategies about the knee joint is far more complex than previously theorised. We suggest that the vastii, gastrocnemius and lateral hamstrings are joint stabilisers while the RF and ST are moment generators. These observations were not apparent in the previous works and we attribute these differences to the varied kinetic chain condition. It is suggested that future investigations should only involve

CKC conditions since it provides more realistic evidence of muscle function during functional movement.

We also recorded and evaluated the muscle activations of the support leg. The polar plots of the support leg's EMG activation patterns are presented in Appendix D. However, these results were not statically tested or discussed because they go beyond the scope of this thesis. It was assumed, although not confirmed, that the support leg would generate equal and opposite ground reaction forces to the dominant leg. This assumption was based on the fact that participants were required to maintain 50% body weight on each leg while applying a ground reaction force via isometric contractions. With that said, it is interesting to note the similarities and differences in activation patterns of the support and dominant legs. The VL, VM, and LG similarly exhibit circular activation patterns to the dominant leg and are interpreted to be knee joint stabilisers. The ST and RF are primarily active in the dominant leg's anterior and posterior loading directions, respectively. Meaning, the RF and ST were primarily generating extension and flexion moments, respectively. This supports the idea that they are primary moment generators. The BF, MG, and TFL demonstrated patterns different from the dominant leg; however, since the lower limb joint angles vary between legs (Appendix C), it is believed that the activation of the BF, MG, and TFL are dependent on joint angle and provides reason to further investigate the effect of lower limb orientation on muscle activations using this protocol.

As mentioned prior, this study is the first step of a longer and larger research program. Our results can act as a baseline for future comparison against other populations but there are some limitations to this study that warrant discussion.

Firstly, participants were required to be physically active at least two times a week but there were no other specifications regarding physical activity type, experience level or frequencies. As mentioned prior, knee muscle activation varies as a function of physical activity level (da Fonseca et al., 2006; Sigward & Powers, 2006) and physical activity type (Lattier et al., 2003) and the lack of control of these factors may have contributed to the large statistical variability in our results. Consider Figure 17 for example, where the BF activation patterns of two male subjects are displayed. Male 4 activated his BF to a greater magnitude at every target location compared to Male 7. Also, Male 4’s BF had similar activation magnitudes in the anterior, medial and posterior directions where as Male 7’s BF was active primarily in the general posterior direction. With that said, Male 4 is a long distance runner who runs four times per week at moderate to high intensity levels. Male 7 is a soccer player who plays twice a week as well as bikes for approximately six hours a week at high intensity levels. It is suggested that the results of these two participants vary because their type of physical activity exposes the knee joint to different types of forces and thus their neuromuscular control strategies have adapted accordingly. Male 7 is thought to rely heavily on the BF’s contribution to generating a flexion moment because such great agility is required of soccer players and thus, they must be extremely efficient when moving their limbs. In contrast, the BF of Male 4 is implied to be more of a stabiliser because the requirement to quickly regulate a flexion moment is unnecessary. Evaluating male and female participants that engage in the same physical activities and are similarly experienced may have provided clearer differences in neuromuscular function.

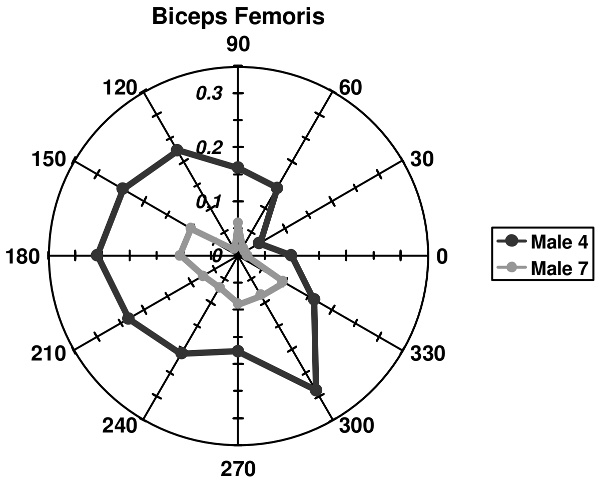

Figure 17: Biceps femoris EMG polar plots of two male participants. Outer numbers along the circular trajectory represent the target location angle in degrees. Inner numbers along each axis represent normalised EMG magnitude as a percentage of MVIC. Where the pattern on the target location's radius intersects represents the mean normalised EMG utilised to match that target. All plots are scaled from 0 to 0.35 (or 35% MVIC EMG level).

Another limitation was the methods used in the maximum voluntary isometric contraction trials. In comparison to the other quadriceps, the RF displayed low normalised EMG amplitudes in all directions. This may be due to body position during the MVIC trials: a hip flexion angle of 90º was utilised during maximum extension trials while the mean hip angle during the target matching tasks was approximately 30º. Hip angle significantly alters the EMG amplitude of a biarticular muscle during a MVIC trial such that RF activity increases as hip flexion angle increases (Maffiuletti & Lepers, 2003) but does not affect the vastii muscles (Kong & van Haselen, 2010). In contrast, more EMG activity is experienced in the hamstrings to maintain the same torque value when the hip is flexed to a lesser degree (Lunnen et al., 1981). Because the hip angles between the MVIC and target trials differ, it is suggested that our amplitudes for the RF is underestimated and our hamstrings are overestimated. Therefore, basing the importance of a muscle on the normalised EMG amplitude may be misleading and caution should be taken when interpreting our results in this manner.

Although subjects were required to maintain the same sagittal hip, knee, and ankle joint positions throughout the entire testing period, we observed that females had significantly greater plantar flexion, hip flexion and knee valgus angles throughout the entire testing period (Appendix C). We only sought out to control joint angles in the sagittal plane, with the knee flexion angle being of main interest. Thus since the knee flexion angles showed no significant difference between sexes a covariate test was not conducted. The mean difference between the hip flexion, ankle flexion and valgus angles were 6.47º, 8.55º, and 4.87º, respectively. As mentioned, hip joint angle can significantly affect the activation level of biarticular quadriceps and hamstring muscles, although these differences were observed between 0º, 45º, 90º, and 135º of hip flexion (Kong & van Haselen, 2010; Lunnen et al., 1981; Maffiuletti & Lepers, 2003). The difference of a 6.47º is unlikely to significantly affect our results but may warrant further investigation. At the ankle, Arampatzis et al. (2006) found that a change in flexion angle as little as 5º significantly affects gastrocnemius activity when other leg angles were maintained. Yet in Arampatzis et al. (2006)'s study, the varied ankle angles were combined with varied knee joint flexion angles. The effect of this angular variation can not be clearly determined because the level of gastrocnemius activity it is a collaborative result of the two joints. As for the valgus angles, it still remains unclear how the change in frontal plane alignment would affect the muscle activation seeing as the muscles recorded in our study do not possess substantial varus-valgus moment arms and thus the EMG-angle (i.e. EMG-length) relationship is difficult to interpret. Of note is that we determined joint angles using a motion analysis system that can be expected to have an error of ± 1.5º during static trials (Dorociak & Cuddeford, 1995). Clothing artefact could also have presented error. Participants in our study were permitted to sit between trials and even though spandex was worn in order to best represent the skin, the shorts and shirts used tended to migrate between sitting and standing. These same conditions generally apply to all studies using motion capture with markers placed on the skin. During dynamic trials soft tissue artefact can introduce large errors (Benoit et al., 2006); however, this is expected to be minimal given our quasi-isometric conditions.

Since our study involved novel data collection and analytical techniques, the intra-class validity must also be evaluated when interpreting the results. With respect to our study the following question needs to be addressed: does an individual activate their muscles the same way to reach a given target for each repetition? To quantify the answer, EMG data was statistically analysed using a coefficient of variance (CV): the CV of the EMG signals of all muscle groups was calculated for all 12 target locations and was computed using the following equation (Benoit et al., 2003; Giroux & Lamontagne, 1992):

$$CV = [\sqrt{} (\Sigma\sigma^2) / n) / \sqrt{} (\Sigma X^2) / n)] * 100$$

Where σ is the group ensemble average standard deviation at each target location, X is the group ensemble average of the mean EMG activity at each target location, and n is the total group sample size.

Previous research by Benoit et al. (2003) utilised this equation to determine the clinical significant of EMG normalisation techniques in subjects during treadmill walking. The CVs ranged from 28.4 to 56.7 for six muscle crossing the knee joint (RF, VL, VM, BF, ST, and MG) during gait. The CVs computed for this study are presented in Table 4. Considering that gait is a most repeatable movement, we suggest our task displays acceptable variance when the muscle's CV at the given target location is less than that reported by Benoit et al. (2003) – indicated with grey highlight in Table 4. With CV values greater than those reported during gait the data should be interpreted with caution. In our study those instances occurred at very low activation levels and thus the CV was relatively more sensitive. For example, the ST had large CVs in the general anterior loading directions, but referring back to its activation pattern (Figure 10, page 65), the activation level in this direction was lower than 0.05 MVIC.

Table 4: Coefficients of variance (CV) of each muscle for every target location. A CV of 0 means there is no variance and the task was conducted the exact same way for each repetition. The greater the CV value the more variance is present. An upper limit of CV is not known. The CVs highlighted in grey correspond to values equal to or lower than that reported in healthy young adults during gait (Benoit et al., 2003).

Target (deg)	RF	VL	VM	BF	ST	LG	MG	TFL
0	10.8	20.9	17.6	86.1	50.6	51.1	114.0	21.0
30	62.4	28.8	27.3	217.9	8.25	43.0	192.6	37.9
60	34.5	26.7	29.2	9.1	277.3	38.5	26.7	18.1
90	5.7	28.0	21.3	33.1	1337.1	46.8	24.3	23.4
120	9.4	32.5	15.1	1.2	83.5	37.0	13.0	33.2
150	10.4	31.7	26.9	15.8	162.1	15.6	18.2	11.5
180	76.2	23.8	21.8	3.8	1.4	20.0	71.6	47.5
210	110.3	21.6	17.0	13.9	2.8	42.4	151.2	47.0
240	26.7	19.2	21.6	18.2	12.1	34.6	141.2	30.0
270	14.8	21.0	16.4	19.1	72.8	51.0	47.2	27.8
300	28.8	19.7	20.3	49.3	9.8	19.4	18.7	18.3
330	64.6	15.4	15.2	7.1	0.8	15.4	102.9	25.2

In general, we consider our results to be valid with respect to intra-class variability and thus provide meaningful, repeatable data. Although the external validity of the protocol in terms of relating back to functional tasks is only theorised to be apt, future investigation is needed to confirm this and is underway.

We believe that our study has presented a novel approach to studying neuromuscular function as it relates to knee joint stability. Contrasting previous works, we have identified that certain muscles act as joint stabiliser (VL, VM and LG) and others as moment generators (RF and ST) while the MG may have an antagonistic effect on joint stabilisation. These stabilisers seem to act to increase joint compressive forces and create a stable mechanical system from which the moment generators can initiate directed forces. In addition, we found the females cocontract their lateral vastus and gastrocnemius muscles compared to males which may also have an antagonistic effect on joint stabilisation.

Considering our results, we have demonstrated the importance of 1) analysing results whereby sex is an independent variable, otherwise, grouping healthy and injured populations without accordance to sex may overlook irregular activation strategies; 2) analysing muscles in a functional group independently because they have differing roles (i.e. moment generators vs. joint stabilisers); and 3) utilising CKC conditions whereby the knee joint is compressed by gravitational forces and bearing weight, else muscle recruitment patterns vary and implications to functional movement are less valid. All the same, voluntary muscle control strategies about the knee are far more complex than previously theorised and little is known about their role in joint stabilisation. More research is necessary to understand the knee joint's neuromuscular contributions to force and moment generation in order to provide appropriate and valid information that is clinically significant.

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Appendix A – University of Ottawa’s Marker Placement Set

Figure 18: Schematic representation of University of Ottawa’s marker placement set.

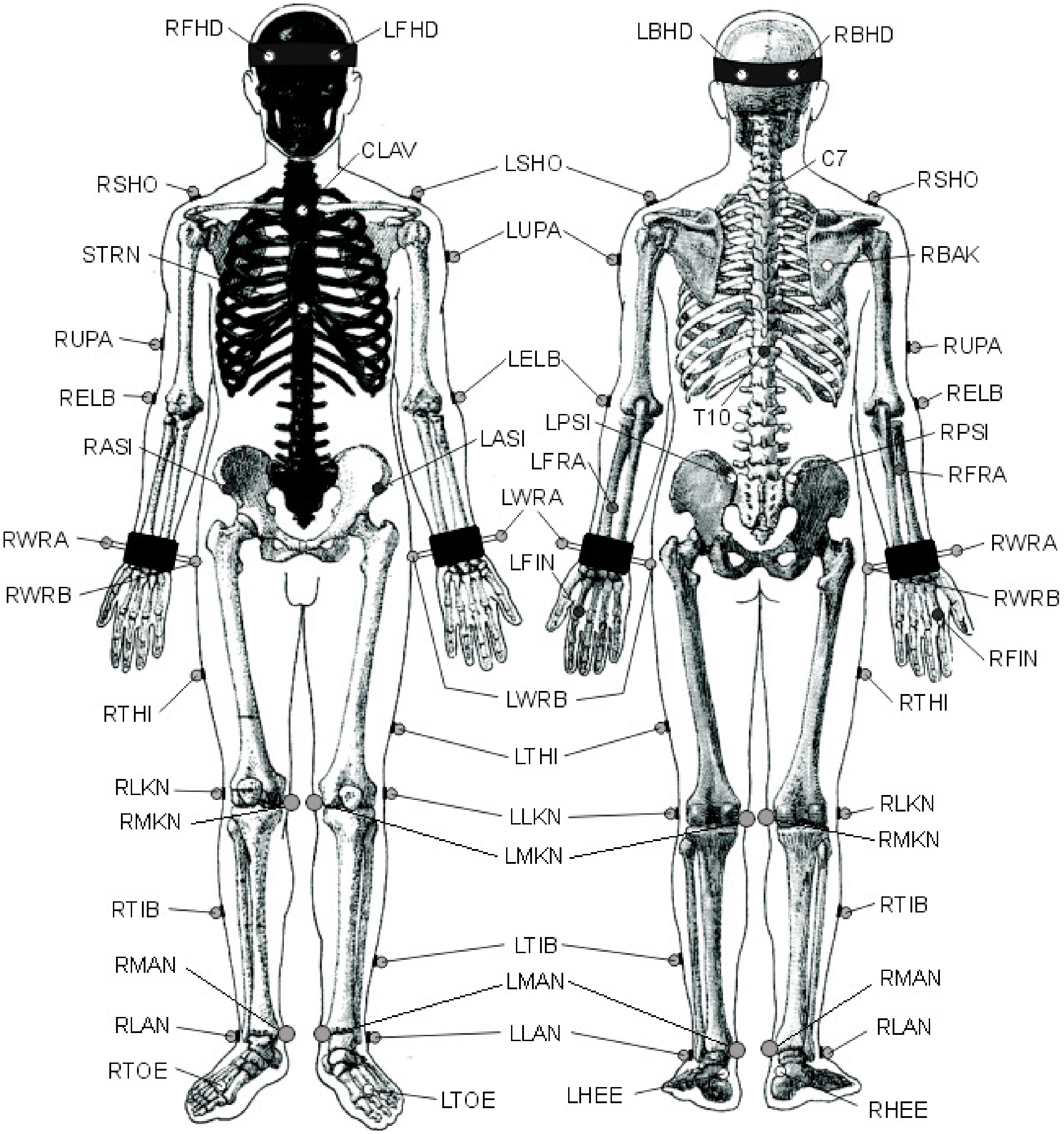

Table 5: Markers and their respective acronym descriptions that were used in this study. Each description corresponds to where the markers should be placed on the subject. Acronyms beginning with an "L" or "R" correspond to the left and right sides, respectively.

Head	
LFHD & RFHD	Left temple
LBHD & RBHD	Left back of head
Torso	
C7	7th cervical vertebrae
T10	10th thoracic vertebrae
CLAV	Jugular notch
STRN	Xiphoid process
RBAK	Middle of right scapula
Arms	
LSHO & RSHO	Acromio-clavicular joint
LUPA & RUPA	Upper arm
LELB & RELB	Lateral epicondyle
LFRA & RFRA	Forearm
LWRA & RWRA	Wrist bar thumb side
LWRB & RWRB	Wrist bar pinkie side
LFIN & RFIN	Dorsum of the hand head of 2nd metacarpal
Pelvis	
LASI & RASI	Anterior superior iliac crest
LPSI & RPSI	Posterior superior iliac spine
Legs	
LTHI & RTHI	Lateral thigh
LMKN & RMKN	Medial epicondyle of the knee
LKNE & RKNE	Lateral epicondyle of the knee
LTIB & RTIB	Lateral shank
LANK & RANK	Lateral malleolus
LMAN & RMAN	Medial malleolus
Feet	
LTOE & RTOE	2nd metatarsal head of foot
LHEE & RHEE	Posterior calcaneus

Appendix B – EMG Vector Calculations

Mean Direction of Muscle Activity: Resultant EMG Vector

In order to calculate the mean of the EMG vectors, each vector must first be resolved into its 2-dimensional horizontal (x_i) and vertical (y_i) components using the trigonometric functions sine and cosine. Consider a vector of length r_i (corresponding to the EMG amplitude) and angle theta (θ) (corresponding to the target location) that forms a right triangle with sides x and y. The EMG vector is now the hypotenuse or a right triangle.
The sine of r_i's angle θ is defined as:

$$\sin\theta = \text{length of side opposite } \theta\ (y_i)\ /\text{hypotenuse } (r_i)$$

OR

$$y_i = r_i \sin\theta$$

The cosine of the angle θ is defined as:

$$\cos\theta = \text{length of side adjacent } \theta\ (x_i)/\ \text{hypotenuse } (r_i)$$

OR

$$x_i = r_i \cos\theta$$

Using the above equations, the vertical and horizontal components of each EMG vector can be defined. These two values, x_i and y_i, are the Cartesian coordinates (x_i, y_i) that identify the point relative to the origin of the polar plot coordinate system.

To find the length of the resultant EMG vector (REMG), the mean horizontal (Rx) and vertical (Ry) components are determined by summating the x or y components of each vector, respectively, and dividing by the number of summated vectors components (N):

$$Rx = \Sigma x_i\ /N$$

$$Ry = \Sigma y_i\ /N$$

Then the REMG is resolved by means of the Pythagorean Theorem using the mean horizontal and vertical components:

$$REMG = \sqrt{(Rx^2 + Ry^2)}$$

To find the angle of the REMG, the trigonometric function of tangent is used:

$$\tan \theta = Ry\ component/\ Rx\ component$$

OR

$$\theta = \arctan (Ry/\ Rx)$$

<u>Variance of Muscle Activation: Specificity Index</u>

In order to define a specificity index (SI), representing the variance of muscle activation about its "mean direction of muscle activity" or resultant vector (REMG), the mean *absolute* horizontal (Rx) and vertical components (Ry) is calculated by summating all the *absolute* x or y components of each vector, respectively and dividing by the number of summated vectors components (N):

$$|Rx| = |\Sigma x_i| / N$$

$$|Ry| = |\Sigma y_i| / N$$

Then an *absolute* resultant EMG (or summed EMG magnitudes of all the vectors) ($|\Sigma EMG_i|$) is determined by means of the Pythagorean Theorem using the mean *absolute* horizontal and vertical components:

$$|\Sigma EMG_i| = \sqrt{(|Rx|^2 + |Ry|^2)}$$

The specificity index (SI) is derived from the ratio of the muscle's "mean direction of muscle activity" or resultant EMG (REMG) to the *absolute* resultant EMG ($|\Sigma EMG_i|$):

$$SI = REMG / |\Sigma EMG_i|$$

Appendix C – Average Joint Angles

Table 6: Mean hip, knee and ankle joint angles of both the dominant and support leg for all participants.

Leg		**Hip:X**	**Hip:Y**	**Hip:Z**	**Knee:X**	**Knee:Y**	**Knee:Z**	**Ankle:X**	**Ankle:Y**	**Ankle:Z**
Dominant	*Mean*	28.45	-5.80	0.32	25.68	-0.90	-1.04	4.10	0.22	-1.98
	SD	7.27	3.79	12.84	6.06	6.67	13.57	5.64	1.65	10.69
Support	*Mean*	5.00	-4.93	2.92	10.02	1.23	-21.14	9.45	-1.88	12.25
	SD	6.10	4.82	9.52	6.17	3.78	14.43	4.71	1.99	9.12

* See Table 8 for descriptors.

Table 7: Mean hip, knee and ankle joint angles of the dominant leg of males and females. A one-way between groups multivariate analysis of variance (MANOVA) was conducted to see if significant differences across sex existed for lower limb kinematics. Significant differences at the p<0.05 level are indicated with an asterisks (*).

Sex		**Hip:X**	**Hip:Y**	**Hip:Z**	**Knee:X**	**Knee:Y**	**Knee:Z**	**Ankle:X**	**Ankle:Y**	**Ankle:Z**
Male	*Mean*	25.29	-6.79	4.86	23.36	3.22	-6.46	1.55	-0.25	-0.96
	SD	8.34	2.75	15.40	5.51	6.30	15.12	5.46	1.34	10.21
Female	*Mean*	31.76	-4.75	-3.12	27.92	-5.33	3.01	6.44	0.55	-1.86
	SD	4.77	4.46	8.55	5.96	4.45	10.13	4.88	1.83	11.46
***p*-Value**		*0.033**	*0.219*	*0.090*	*0.065*	*0.001**	*0.053*	*0.027**	*0.167*	*0.656*

* See Table 8 for descriptors.

Table 8: University of Ottawa Motion Analysis Model (UOMAM) output of lower body angles in the local coordinate system of the distal segment in the hierarchical kinetic chain for both the right (R) and left (L) legs and their positive and negative descriptors.

Description	**Segment Axes**	**Component**	**+ve Descriptor**	**-ve Descriptor**
Ankle X	Tibia	Dorsi/Plantarflexion	Dorsiflexion	Plantarflexion
Ankle Y	Tibia	Abduction/Adduction	Adduction	Abduction
Ankle Z	Tibia	Rotation	Internal	External
Knee X	Thigh	Flexion/Extension	Flexion	Extension
Knee Y	Thigh	Varus/Valgus	Varus	Valgus
Knee Z	Thigh	Rotation	Internal	External
Hip X	Pelvis Y	Flexion/Extension	Flexion	Extension
Hip Y	Pelvis X	Abduction/Adduction	Adduction	Abduction
Hip Z	Pelvis Z	Rotation	Internal	External

Appendix D – EMG Polar Plots of Support Leg

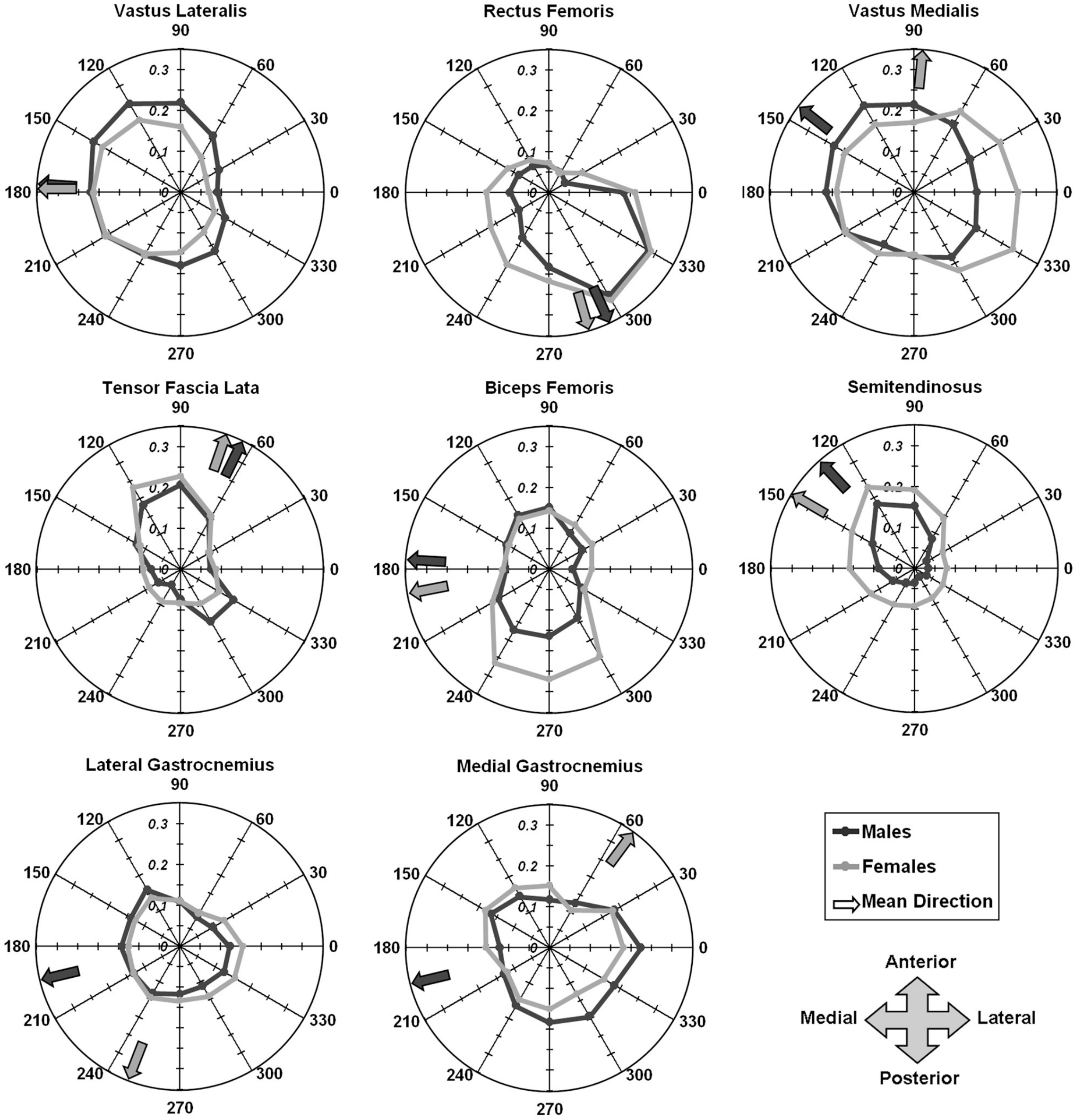

Figure 19: Support leg EMG polar plots of the mean activation patterns of male and female participants. The numbers along the circular trajectory represent the target location (angle in degrees). Where the pattern on the target location's radius intersects represents the mean normalised EMG utilised to match that target. EMG values are normalised so they range from 0 to 1 with 1 being the maximal recorded EMG value for that muscle. All plots are scaled from 0 to 0.35. Mean direction of muscle activation (Φ) of all eight muscles recorded for the males and females are indicated by the arrows along the circular trajectory. **Note that the loading axes are based on the dominant leg's loading axes and is in actuality opposite when considering the support leg.*

Appendix E – Full Report of all Statistical Results

Muscle Activation Patterns

Independent T-tests were used to test whether mean direction of activation (Φ), mean magnitude of muscle activation (XEMG), or specificity index (SI) for each muscle differed significantly across sex (Table 8). No significant differences were observed between males and females for Φ. Females had significantly lower SIs than males for the ST (M=0.31, SD=0.12 vs. M=0.43, SD=0.13, t (23) =2.931, $p<0.025$) and TFL (M=0.14, SD=0.11 vs. M=0.33, SD=0.22, t (24) =2.796, $p<0.012$). Females also displayed significantly greater XEMG than males for the LG (M=0.11, SD=0.04 vs. M=0.05, SD=0.03, t (24) =-3.437, $p<0.002$) and TFL (M=0.10, SD=0.05 vs. M=0.04, SD=0.04, t (24) =-3.365, $p<0.003$). In addition, trends were observed at the $p<0.10$ level. Females had greater XEMG than males for the RF (M=0.10, SD=0.03 vs. M=0.08, SD=0.04, t (24) =-2.001, $p<0.057$) and MG (M=0.13, SD=0.06 vs. M=0.08, SD=0.05, t (23) =-1.788, $p<0.087$). The SI for RF was also lower in females compared to males (M=0.16, SD=0.11 vs. M=0.26, SD=0.17, t (24) =1.775, $p<0.089$). No differences exist for the VL and VM.

For each muscle, a two way within-between factorial (2x12) analysis of variance (ANOVA) was utilised to examine whether the amount of EMG activity significantly differed as a result of sex and target direction (Table 9). The sample data means of EMG magnitude was simultaneously compared to the effects of the two independent factors of sex and target direction which permitted the analysis of main effects for each independent factor and the interaction of these factors. The ANOVA assumption of sphericity was violated for target location ($p<0.05$, $p=0.000$) in all the tests; therefore the Huynh-Feldt adjusted values were examined. The results displayed significant main effect for target location at the $p<0.05$ level and on EMG magnitude for all muscles. Only the ST (F (1, 24) = 11.826, p = 0.002, partial $\eta2$ = 0.330, power = 0.909) and TFL (F (1, 24) = 11.322, p = 0.003, partial $\eta2$ = 0.321, power

= 0.898) showed significant main effects for sex on EMG magnitude. The main effects of sex on RF, VL, VM, BF, LG, MG EMG activity were not statistically significant. There was no significant interaction between target direction and sex for all muscles.

Post hoc independent T test examinations yielded significant differences at specific target locations between males and females at the $p<0.004$ level (Bonferonni adjustment for repeated measures: alpha = 0.05/12 = 0.004) (Table 10). For the LG, females had significantly greater EMG magnitude at the target locations of 0º (M=0.09, SD=0.03 vs. M=0.04, SD=0.02, t(24)=-4.612, $p<0.005$), 30º (M=0.08, SD=0.04 vs. M=0.04, SD=0.02, t(20.986)= -3.699, p=0.001), 210º (M=0.11, SD=0.06 vs. M=0.04, SD=0.02, t(14.657)= -3.691, p=0.002), and 330º (M=0.12, SD=0.05 vs. M=0.05, SD=0.03, t(24)= -3.572, p=0.002). For the TFL, the EMG magnitude at 0º (M=0.13, SD=0.04 vs. M=0.05, SD=0.03, t(24)= -5.579, $p<0.005$), 150º (M=0.09, SD=0.05 vs. M=0.03, SD=0.02, t(16.532)= -4.205, p=0.001), 180º (M=0.09, SD=0.05 vs. M=0.03, SD=0.03, t(24)=-3.868, p=0.001), 210º (M=0.09, SD=0.05 vs. M=0.02, SD=0.02, t(15.423)= -4.674, $p<0.005$), 240º (M=0.09, SD=0.05 vs. M=0.03, SD=0.03, t(24)= -3.626, p=0.001), 330º (M=0.14, SD=0.07 vs. M=0.06, SD=0.05, t(24)= -3.563, p=0.002) was significantly greater in females than males.

Table 9: Descriptive statistics of the muscle activation characteristics for each muscle of males and females. Significant differences between sexes were determined with independent T-tests and indicated by *p* values.

		XEMG			**Φ**			**SI**		
Muscle	**Sex**	**Mean**	**SD**	***p* Value**	**Mean**	**SD**	***p* Value**	**Mean**	**SD**	***p* Value**
RF	Male	0.076	0.039	0.089	83.86	57.00	0.6592	0.256	0.166	0.057
	Female	0.104	0.031		72.88	45.07		0.156	0.114	
VL	Male	0.214	0.091	0.463	282.51	55.42	0.1418	0.148	0.070	0.437
	Female	0.247	0.127		321.23	49.94		0.126	0.073	
VM	Male	0.192	0.084	0.724	298.26	49.84	0.1998	0.161	0.723	0.193
	Female	0.182	0.061		326.09	42.42		0.122	0.077	
BF	Male	0.184	0.099	0.897	177.06	28.66	0.8745	0.367	0.115	0.287
	Female	0.190	0.114		174.96	32.44		0.391	0.100	
ST	Male	0.103	0.058	0.956	255.77	30.17	0.8967	0.431	0.131	0.025*
	Female	0.101	0.063		257.57	33.56		0.310	0.121	
LG	Male	0.055	0.007	0.002*	182.97	65.80	0.4356	0.187	0.129	0.269
	Female	0.106	0.012		156.41	57.40		0.140	0.074	
MG	Male	0.084	0.052	0.087	145.91	33.37	0.2772	0.409	0.246	0.769
	Female	0.126	0.063		125.67	46.13		0.384	0.178	
TFL	Male	0.044	0.040	0.003*	339.72	58.80	0.5809	0.331	0.216	0.012*
	Female	0.102	0.047		354.52	48.68		0.141	0.113	

Abbreviations: Rectus femoris (RF); vastus lateralis (VL); vastus medialis (VM); biceps femoris (BF); semitendinosus (ST); lateral gastrocnemius (LG); medial gastrocnemius (MG); tensor fascia lata (TFL); standard deviation (SD); mean magnitude of activation (X_{EMG}); mean direction of activation (Φ); specificity index (SI). Asterisks (*) identifies significant differences at the p<0.05 level.

Table 10: Statistical significance of main effects of sex and target direction on EMG amplitude. A two way within-between factorial analysis of variance (ANOVA) was used to determine the reported *p* values.

Muscle	**Main Effect of Direction**	**Direction *Sex**	**Main Effect of Sex**
RF	0.000*	0.633	0.057
VL	0.006*	0.547	0.464
VM	0.000*	0.699	0.724
BF	0.000*	0.719	0.897
ST	0.000*	0.533	0.956
LG	0.010*	0.517	0.002*
MG	0.000*	0.617	0.087
TFL	0.011*	0.296	0.003*

Abbreviations: Rectus femoris (RF); vastus lateralis (VL); vastus medialis (VM); biceps femoris (BF); semitendinosus (ST); lateral gastrocnemius (LG); medial gastrocnemius (MG); tensor fascia lata (TFL). Asterisks (*) identifies significant differences at the p<0.05 level.

Table 11: Post hoc evaluation of mean EMG amplitude differences at each target location of males and females. Independent T-tests were used to determine significance. Asterisks (*) identifies significant differences at the p<0.004 level.

		LG			TFL		
Direction	**Sex**	**Mean**	**SD**	***p* Value**	**Mean**	**SD**	***p* Value**
0º	Male Female	0.036 0.088	0.021 0.034	0.000*	0.046 0.126	0.028 0.043	0.000*
30º	Male Female	0.038 0.081	0.023 0.034	0.001*	0.040 0.100	0.056 0.043	0.006
60º	Male Female	0.047 0.087	0.037 0.049	0.029	0.468 0.837	0.078 0.045	0.154
90º	Male Female	0.061 0.122	0.044 0.077	0.022	0.623 0.101	0.089 0.064	0.217
120º	Male Female	0.080 0.140	0.057 0.088	0.500	0.057 0.103	0.070 0.057	0.082
150º	Male Female	0.069 0.127	0.041 0.066	0.013	0.027 0.094	0.023 0.052	0.001*
180º	Male Female	0.056 0.117	0.025 0.069	0.009	0.027 0.089	0.032 0.048	0.001*
210º	Male Female	0.042 0.105	0.019 0.057	0.002*	0.022 0.091	0.019 0.049	0.000*
240º	Male Female	0.053 0.096	0.049 0.048	0.034	0.032 0.089	0.031 0.046	0.001*
270º	Male Female	0.061 0.098	0.041 0.040	0.028	0.569 0.093	0.010 0.052	0.269
300º	Male Female	0.065 0.095	0.054 0.033	0.106	0.051 0.108	0.057 0.050	0.013
330º	Male Female	0.052 0.115	0.031 0.054	0.002*	0.059 0.144	0.045 0.072	0.002*

Abbreviations: Lateral gastrocnemius (LG); tensor fascia lata (TFL); standard deviation (SD). Asterisks (*) identifies significant differences at the p<0.004 level (alpha was adjusted for repeated measures).

Cocontraction Indices

For each antagonistic muscle pair, a two way within-between factorial (2x12) analysis of variance (ANOVA) was utilised to examine whether the cocontraction index (CI) value significantly differed as a result of sex and target direction (Table 11). The sample data means of CI was simultaneously compared to the effects of the two independent factors of sex and target direction which permitted the analysis of main effects for each independent factor and the interaction of these factors. The ANOVA assumption of sphericity was violated for target location (p<0.05, p=0.000) in all the tests; therefore the Huynh-Feldt adjusted values were examined. The results displayed significant main effect for target location at the $p<0.05$ level and on EMG magnitude for all antagonistic muscle pairs. Only the VL and LG (F (1, 24) = 15.586, p = 0.001, partial $\eta^2 = 0.394$, power = 0.966) showed significant main effects for sex on CI. A trend towards significance at the $p<0.10$ level was observed in the summed QUADS and GASTROCS for the main effect of sex on CCI (F (1, 23) = 3.61, p = 0.07, partial $\eta^2 = 0.136$, power = 0.445). The main effects of sex on QUADS and HAMS, VL and BF, VM and ST, VM and MG CI were not statistically significant. There was no significant interaction between target direction and sex for all antagonistic muscle pairs.

Table 12: CI means and standard deviations of antagonist muscle groups at each target direction.

	QUADS & HAMS				**QUADS & GASTROCS**				**VL & BF**			
	Males		Females		Males		Females		Males		Females	
Deg	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
0	0.12	0.08	0.15	0.08	0.05	0.02	0.10	0.03	0.11	0.10	0.15	0.11
30	0.08	0.07	0.11	0.07	0.05	0.03	0.10	0.06	0.10	0.11	0.13	0.12
60	0.13	0.10	0.15	0.12	0.07	0.07	0.12	0.07	0.17	0.15	0.21	0.21
90	0.19	0.15	0.19	0.11	0.14	0.16	0.18	0.10	0.22	0.21	0.25	0.19
120	0.20	0.14	0.25	0.14	0.20	0.21	0.20	0.11	0.27	0.23	0.33	0.25
150	0.19	0.12	0.23	0.11	0.16	0.16	0.18	0.11	0.25	0.18	0.32	0.24
180	0.20	0.10	0.23	0.10	0.09	0.05	0.16	0.07	0.27	0.18	0.32	0.25
210	0.20	0.09	0.25	0.13	0.06	0.03	0.13	0.06	0.27	0.16	0.34	0.26
240	0.19	0.08	0.25	0.11	0.07	0.06	0.11	0.04	0.23	0.13	0.32	0.25
270	0.21	0.08	0.25	0.13	0.07	0.04	0.12	0.05	0.21	0.10	0.31	0.26
300	0.19	0.08	0.22	0.12	0.08	0.06	0.12	0.05	0.19	0.15	0.24	0.18
330	0.17	0.08	0.19	0.12	0.07	0.03	0.13	0.07	0.14	0.09	0.19	0.16
	VM & ST				**VL & LG**				**VM & MG**			
	Males		Females		Males		Females		Males		Females	
Deg	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
0	0.11	0.09	0.12	0.08	0.04	0.03	0.12	0.04	0.04	0.02	0.08	0.03
30	0.05	0.04	0.07	0.04	0.05	0.03	0.11	0.05	0.04	0.03	0.08	0.05
60	0.05	0.02	0.06	0.05	0.05	0.04	0.11	0.06	0.08	0.09	0.12	0.08
90	0.06	0.04	0.07	0.04	0.06	0.04	0.14	0.07	0.12	0.11	0.16	0.09
120	0.08	0.05	0.10	0.06	0.09	0.06	0.14	0.05	0.17	0.12	0.19	0.11
150	0.12	0.06	0.12	0.08	0.08	0.04	0.16	0.08	0.16	0.13	0.17	0.11
180	0.14	0.07	0.14	0.10	0.07	0.03	0.14	0.08	0.08	0.05	0.15	0.09
210	0.14	0.07	0.13	0.09	0.05	0.03	0.13	0.07	0.05	0.02	0.12	0.06
240	0.17	0.07	0.15	0.07	0.06	0.04	0.11	0.05	0.07	0.05	0.09	0.04
270	0.20	0.10	0.16	0.10	0.07	0.05	0.13	0.06	0.06	0.03	0.10	0.05
300	0.20	0.10	0.16	0.09	0.08	0.07	0.13	0.06	0.07	0.03	0.09	0.04
330	0.17	0.10	0.17	0.12	0.06	0.04	0.15	0.08	0.05	0.04	0.10	0.07

Table 13: Statistical significance of main effects of sex and target direction on cocontraction index (CI). A two way within-between factorial analysis of variance (ANOVA) was used to determine the reported *p* values.

	Main Effect of Direction	**Direction x Sex**	**Main Effect of Sex**
QUADS & HAMS	0.000*	0.823	0.407
QUADS & GASTROCS	0.000*	0.459	0.070
VL & BF	0.000*	0.712	0.409
VM & ST	0.000*	0.417	0.893
VL & LG	0.007*	0.518	0.001*
VM & MG	0.000*	0.545	0.145

Abbreviations: Rectus femoris (RF); vastus lateralis (VL); vastus medialis (VM); biceps femoris (BF); semitendinosus (ST); lateral gastrocnemius (LG); medial gastrocnemius (MG); tensor fascia lata (TFL). Asterisks (*) identifies significant differences at the p<0.05 level.

Post hoc independent T-test examinations yielded significant differences at specific target locations between males and females at the p<0.004 level (Bonferonni adjustment for repeated measures: alpha = 0.05/12 = 0.004) (Table 12). Females had significantly greater VL and LG CI values at the target locations of 0º (M=0.11, SD=0.04 vs. M=0.04, SD=0.03, t(24)= -5.061, p<0.005), 30º (M=0.11, SD=0.05 vs. M=0.05, SD=0.03, t(17.751)= -3.880, p=0.001), 90º (M=0.14, SD=0.07 vs. M=0.06, SD=0.04, t(24)= -3.585, p=0.001), 150º (M=0.16, SD=0.08 vs. M=0.08, SD=0.04, t(24)= -3.136, p=0.004), 210º (M=0.13, SD=0.07 vs. M=0.05, SD=0.03, t(14.912)= -3.595, p=0.003), and 330º (M=0.15, SD=0.08 vs. M=0.06, SD=0.04, t(24)=-3.341, p=0.003).

Table 14: Post hoc evaluation of cocontraction differences at each target location of males and females. Independent T tests were used to determine significance. Asterisks (*) identifies significant differences at the p<0.05 level.

		VL & LG		
Direction	**Sex**	**Mean**	**SD**	***p* Value**
0º	Male Female	0.044 0.115	0.029 0.041	0.000*
30º	Male Female	0.046 0.109	0.026 0.052	0.001*
60º	Male Female	0.052 0.111	0.035 0.060	0.005
90º	Male Female	0.063 0.142	0.040 0.068	0.001*
120º	Male Female	0.085 0.143	0.056 0.053	0.013
150º	Male Female	0.078 0.156	0.042 0.078	0.004*
180º	Male Female	0.668 0.143	0.031 0.079	0.006
210º	Male Female	0.052 0.131	0.026 0.074	0.003*
240º	Male Female	0.061 0.115	0.042 0.053	0.009
270º	Male Female	0.073 0.131	0.049 0.056	0.011
300º	Male Female	0.082 0.129	0.073 0.057	0.085
330º	Male Female	0.0633 0.1503	0.044 0.082	0.003*

Abbreviations: Lateral gastrocnemius (LG); tensor fascia lata (TFL); standard deviation (SD). Asterisks (*) identifies significant differences at the p<0.004 level (alpha was adjusted for repeated measures).